The Human Microbiome

Edited by Sunil Thomas

CRC Press
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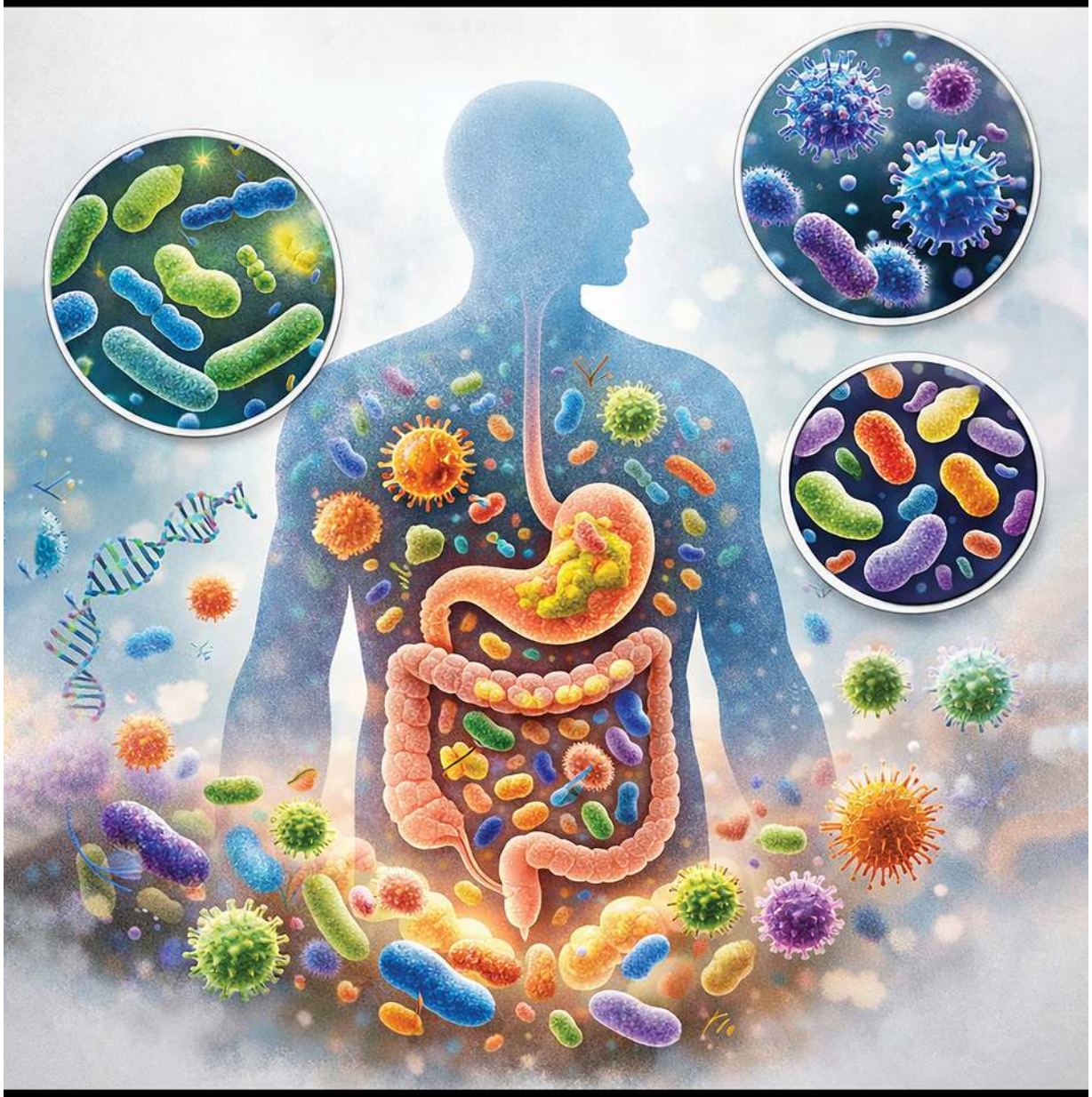

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The Human Microbiome

The relationship between humans and their resident microbiomes is complex and influences many aspects of health and disease. Microbes cover all surfaces inside and outside the human body. Diabetes, obesity, food allergies, and many other conditions can be exacerbated or ameliorated by the host microorganisms. This volume includes chapters devoted to the myriad aspects of the microbiome of humans.

Sunil Thomas is a Research Professor at the Lankenau Institute for Medical Research in Wynnewood, Pennsylvania, where he leads interdisciplinary work spanning cell biology, microbiology, and translational medicine. His research focuses on next-generation immunotherapies for chronic conditions such as inflammatory bowel disease and Alzheimer's disease, with particular emphasis on how the human microbiome influences disease progression and treatment response.

Over his career, he has contributed to infectious disease research, vaccine development, and viral structural biology, helping advance diagnostics, therapeutics, and preventive strategies. He earned his Ph.D. in Environmental Biotechnology from Cochin University of Science and Technology in India and completed postdoctoral training at the Mount Sinai School of Medicine. He has authored over 70 peer-reviewed publications, contributed to books on vaccines and infectious diseases, and holds multiple patents.

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Preface

Microorganisms represent the earliest and most enduring forms of life on Earth. Long before the emergence of complex multicellular organisms, microbial life shaped the planet's atmosphere, soils, waters, and biogeochemical cycles. Even today, all eukaryotic life—including plants, animals, and humans—remains fundamentally rooted in microbial ancestry. Modern eukaryotic cells themselves are conserved amalgamations of ancient microbial partnerships, a testament to the central role microorganisms have played in the evolution of life.

Higher organisms cannot exist in isolation from microorganisms. From plants drawing nutrients through symbiotic soil microbes to animals relying on gut bacteria for digestion, microorganisms are indispensable partners in survival. The collective community of microorganisms that inhabit a host or environment is known as the microbiome. These microbial communities are not passive inhabitants; rather, they form dynamic, interactive systems that profoundly influence the health, development, and resilience of their hosts.

The microbiome—comprising trillions of bacteria, archaea, fungi, viruses, and protozoa—acts much like a vital organ. In humans and animals, it supports digestion, synthesizes essential vitamins, regulates metabolism, trains the immune system to distinguish friend from foe, and influences inflammation, behavior, mood, and disease susceptibility. In plants, the microbiome enhances nutrient uptake, improves stress tolerance, and protects against pathogens. Microbial diversity is a defining feature of a healthy microbiome, conferring stability and adaptability to changing conditions.

Beneficial microorganisms produce a wide array of secondary metabolites that are critical for maintaining physiological balance and overall well-being. When this balance is disrupted—a condition known as

dysbiosis—the consequences can be severe. Dysbiosis has been linked to infections, allergies, autoimmune diseases, metabolic disorders, reduced crop productivity, and even neurological and behavioral conditions. Understanding the mechanisms that govern microbial balance is therefore essential for advancing health, agriculture, and environmental sustainability.

Recent breakthroughs in high-throughput sequencing, metagenomics, and bioinformatics have revolutionized microbiome research. These technologies allow rapid identification and functional analysis of microbial communities associated with different hosts, diets, environments, and lifestyles. What once took decades to uncover can now be achieved in a fraction of the time, opening new frontiers in medicine, nutrition, agriculture, ecology, and biotechnology.

The six interconnected volumes listed below present a comprehensive exploration of microbial life across biological systems and ecosystems:

Volume 1: The Human Microbiome

Volume 2: Diet and the Microbiome

Volume 3: Animal Microbiomes

Volume 4: Plant Microbiomes

Volume 5: Insect Microbiomes

Volume 6: The Environmental Microbiome

Together, these volumes provide an integrated perspective on how microbial communities shape life on Earth.

I extend my sincere and heartfelt gratitude to all the authors who contributed chapters to these books. Their expertise, dedication, and scholarly insight form the foundation of these volumes. Each contribution reflects a commitment to scientific rigor and a shared passion for advancing knowledge in the rapidly evolving field of microbiome research.

The collective efforts of these authors have enriched the depth, breadth, and quality of these books, transforming it into a comprehensive and authoritative resource. Their willingness to share ideas, collaborate across

disciplines, and invest their time and intellect has been invaluable. This work stands as a testament to their contributions, and I am deeply thankful for their role in bringing these books to fruition.

This year marks the 100th anniversary of the Lankenau Institute for Medical Research. The anniversary represents decades of unwavering commitment to scientific inquiry, education, and the advancement of medical knowledge. The Lankenau Institute for Medical Research has played a pivotal role in shaping and supporting my scientific endeavors. Its culture of intellectual rigor, encouragement, and dedication to research excellence has provided an environment in which curiosity could thrive, and ideas could evolve into meaningful scientific contributions.

This six-volume collection is presented as a tribute to the Lankenau Institute for Medical Research. It stands as a testament to the Institute's enduring legacy, its profound impact on generations of researchers, and its mission to advance science for the betterment of human health and society. Through this work, I honor its past achievements, acknowledge its present influence, and celebrate its continued inspiration for future scientific discovery.

Contributors

Sumit Aggarwal
Division of Epidemiology and Communicable Diseases
Indian Council of Medical Research, DHR, Ministry of Health and Family Welfare
New Delhi, India

Alexander Aksenov
Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Amedeo Amedei
Department of Experimental and Clinical Medicine
University of Florence
Florence, Italy

Catherine J. Andersen
Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Anna Antony
Department of Microbiology
All India Institute of Medical Sciences
Bhopal, Madhya Pradesh, India

Natarajan Aravindan
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma, USA
OU Health Stephenson Cancer Center
Oklahoma City, Oklahoma, USA

Sheeja Aravindan
OU Health Stephenson Cancer Center
Oklahoma City, Oklahoma, USA

Simone Baldi
Department of Experimental and Clinical Medicine
University of Florence
Florence, Italy

Jyoti Batgire
Department of Molecular Immunology and Microbiology
ICMR-National Institute for Research in Reproductive and Child Health (ICMR-NIRRCH)
Mumbai, Maharashtra, India

Camilla Anna Kristina Berg
Department of Food Science
University of Copenhagen
Frederiksberg, Denmark

Vikrant M. Bhor
Department of Molecular Immunology and Microbiology
ICMR-National Institute for Research in Reproductive and Child Health (ICMR-NIRRCH)

Mumbai, Maharashtra, India

S. Bikram Singh
Department of Microbiology
Assam University
Silchar, Assam, India

Robert D. Burk
Department of Microbiology & Immunology
Albert Einstein College of Medicine
Bronx, New York, USA
Department of Pediatrics (Genetic Medicine)
Albert Einstein College of Medicine
Bronx, New York, USA
Department of Epidemiology and Population Health
Albert Einstein College of Medicine
Bronx, New York, USA
Department Obstetrics and Gynecology and Women's Health
Albert Einstein College of Medicine
Bronx, New York, USA

Niteeka Chandel
Department of Molecular Immunology and Microbiology
ICMR-National Institute for Research in Reproductive and Child Health (ICMR-NIRRCH)
Mumbai, Maharashtra, India

Caroline T. Cheung
INSERM/Nantes University/UMR1302/INCIT
Nantes, France

Jyoti Kant Choudhari

School of Sciences
Sanjeev Agrawal Global Educational University
Bhopal, Madhya Pradesh, India
Department of Microbiology
All India Institute of Medical Sciences
Bhopal, Madhya Pradesh, India

Yashmin Choudhury
Department of Biotechnology
Assam University
Silchar, Assam, India

Ock K. Chun
Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Francesca Cuffaro
Department of Experimental and Clinical Medicine
University of Florence
Florence, Italy

Prerna Diwan
Department of Microbiology
Ram Lal Anand College
University of Delhi South Campus
New Delhi, India

Brigitte Dréno
INSERM/Nantes University/UMR1302/INCIT
Nantes, France

Ashvin Dumadiya
Department of Biosciences
Saurashtra University
Rajkot, Gujarat, India

Maria Fernanda Ebs
Laboratory of Translational Psychiatry—Depression Research Unit
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil
Laboratory of Experimental Neurobiology
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil

Shymaa Enany
Department of Microbiology and Immunology
Faculty of Pharmacy
Galala University
Suez, Egypt

Jessica L. Fernandes
Laboratory of Translational Psychiatry—Depression Research Unit
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil

Harshita Gupta
Department of Microbiology
Ram Lal Anand College
University of Delhi South Campus
New Delhi, India

Department of Microbiology
University of Delhi South Campus
New Delhi, India

Rakesh Kumar Gupta
Department of Microbiology
Ram Lal Anand College
University of Delhi South Campus
New Delhi, India

Vishal L. Handa
Department of Biosciences
Saurashtra University
Rajkot, Gujarat, India

Afsana Parveen Jahir Hussain
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma, USA

Dhyey R. Kothari
Department of Biosciences
Saurashtra University
Rajkot, Gujarat, India

Ramesh K. Kothari
Department of Biosciences
Saurashtra University
Rajkot, Gujarat, India

Elaine Choung-Hee Lee

Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Ulrike Löber
Max Delbrück Center Berlin-Buch
Max-Delbrück-Centrum für Molekulare Medizin in der Helmholtz-Gemeinschaft
Berlin, Germany

Olivia Lutz
Lankenau Institute for Medical Research
Wynnewood, Pennsylvania, USA

Pavithra Madamarandawala
University of Missouri-Saint Louis
St. Louis, Missouri, USA

Dhammika Magana-Arachchi
Molecular Microbiology & Human Diseases Programme
National Institute of Fundamental Studies
Kandy, Sri Lanka

Nour Magdeldin
Department of Microbiology and Immunology
Faculty of Pharmacy
Galala University
Suez, Egypt

Sreenidhi Mohanvelu
Department of Physiological Sciences
College of Veterinary Medicine

Oklahoma State University
Stillwater, Oklahoma, USA

Gabriel S. Mondo
Laboratory of Translational Psychiatry—Depression Research Unit
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil

Alex Muppasani
Lankenau Institute for Medical Research
Wynnewood, Pennsylvania, USA

Sivasubramani Narayanan
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma, USA

Kalimuthusamy Natarajaseenivasan
Rowan-Virtua School of Osteopathic Medicine
Stratford, New Jersey, USA

Elena Niccolai
Department of Experimental and Clinical Medicine
University of Florence
Florence, Italy

Briana M. Nosal
Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Giulia Ospite
Department of Food Science
University of Copenhagen
Frederiksberg, Denmark

Shreya Peddakolmi
Department of Molecular Immunology and Microbiology
ICMR-National Institute for Research in Reproductive and Child Health (ICMR-NIRRCH)
Mumbai, Maharashtra, India

Lucas C. Pedro
Laboratory of Translational Psychiatry—Depression Research Unit
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil

Loganayaki Periyasamy
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma, USA

Shashank Purwar
Department of Microbiology
All India Institute of Medical Sciences
Bhopal, Madhya Pradesh, India

Madhi Oli Ramamurthy
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University

Stillwater, Oklahoma, USA

Torben Sølbeck Rasmussen
University of Copenhagen
Department of Food Science
Frederiksberg, Denmark

Gislaine Z. Réus
Laboratory of Translational Psychiatry—Depression Research Unit
Graduate Program in Health Sciences
University of Southern Santa Catarina (UNESC)
Criciúma, SC, Brazil

Sumana Saseevan
Department of Medical Laboratory Sciences
Faculty of Allied Health Sciences
University of Jaffna
Sri Lanka

Abdelrahman M. Sedeek
Department of Microbiology and Immunology
Faculty of Pharmacy
Galala University
Suez, Egypt

Sarah Shabayek
Department of Microbiology and Immunology
Faculty of Pharmacy
Galala University
Suez, Egypt

Indu Sharma

Department of Microbiology
Assam University
Silchar, Assam, India

Shubham Sharma
Department of Microbiology
Ram Lal Anand College
University of Delhi South Campus
New Delhi, India
Department of Microbiology
University of Delhi South Campus
New Delhi, India

Akanksha Singh
Department of Microbiology
All India Institute of Medical Sciences
Bhopal, Madhya Pradesh, India

Samar M. Solyman
Department of Microbiology and Immunology
Faculty of Pharmacy
Suez Canal University
Ismailia, Egypt

Poorvi Subramanian
Department of Physiological Sciences
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma, USA

Amal Tahri
Department of Experimental and Clinical Medicine

University of Florence
Florence, Italy

Sunil Thomas
Lankenau Institute for Medical Research
Wynnewood, Pennsylvania, USA
Department of Gastroenterology
Lankenau Medical Center
Wynnewood, Pennsylvania, USA

Nevena Todorovic
Department of Experimental and Clinical Medicine
University of Florence
Florence, Italy

Marie Ève Tremblay
School of Medical Sciences
University of Victoria
Victoria, British Columbia, Canada

Abhi Tyagi
Department of Microbiology
All India Institute of Medical Sciences
Bhopal, Madhya Pradesh, India

Mykhaylo Usyk
Department of Microbiology & Immunology
Albert Einstein College of Medicine
Bronx, New York, USA

Cameo Volk
School of Medical Sciences

University of Victoria
Victoria, British Columbia, Canada

B.R.M. Vyas
Department of Biosciences
Saurashtra University
Rajkot, Gujarat, India

Prerna Yadav
Department of Microbiology
Ram Lal Anand College
University of Delhi South Campus
New Delhi, India
Department of Microbiology
University of Delhi South Campus
New Delhi, India

Hanna Yumnam
Department of Microbiology
Assam University
Silchar, Assam, India

Yanjiao Zhou
Department of Nutritional Science
University of Connecticut
Storrs, Connecticut, USA

Section I

Introduction

1

Human Microbiome

Abdelrahman M. Sedeek, Sarah Shabayek, Nour Magdeldin, Shymaa Enany, and Samar M. Solyman

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1.1 Introduction

The human body is colonized by a diverse and dynamic ecosystem of microorganisms, including bacteria, archaea, fungi, and viruses, that together constitute the human microbiome (Aggarwal et al. 2023; Leonard and Toro, 2023). These microbial communities inhabit nearly all environmentally exposed surfaces of the body, from the skin and oral mucosa to the gastrointestinal tract, thriving within distinct habitats defined by their distinct physiological and biochemical properties (Figure 1.1).

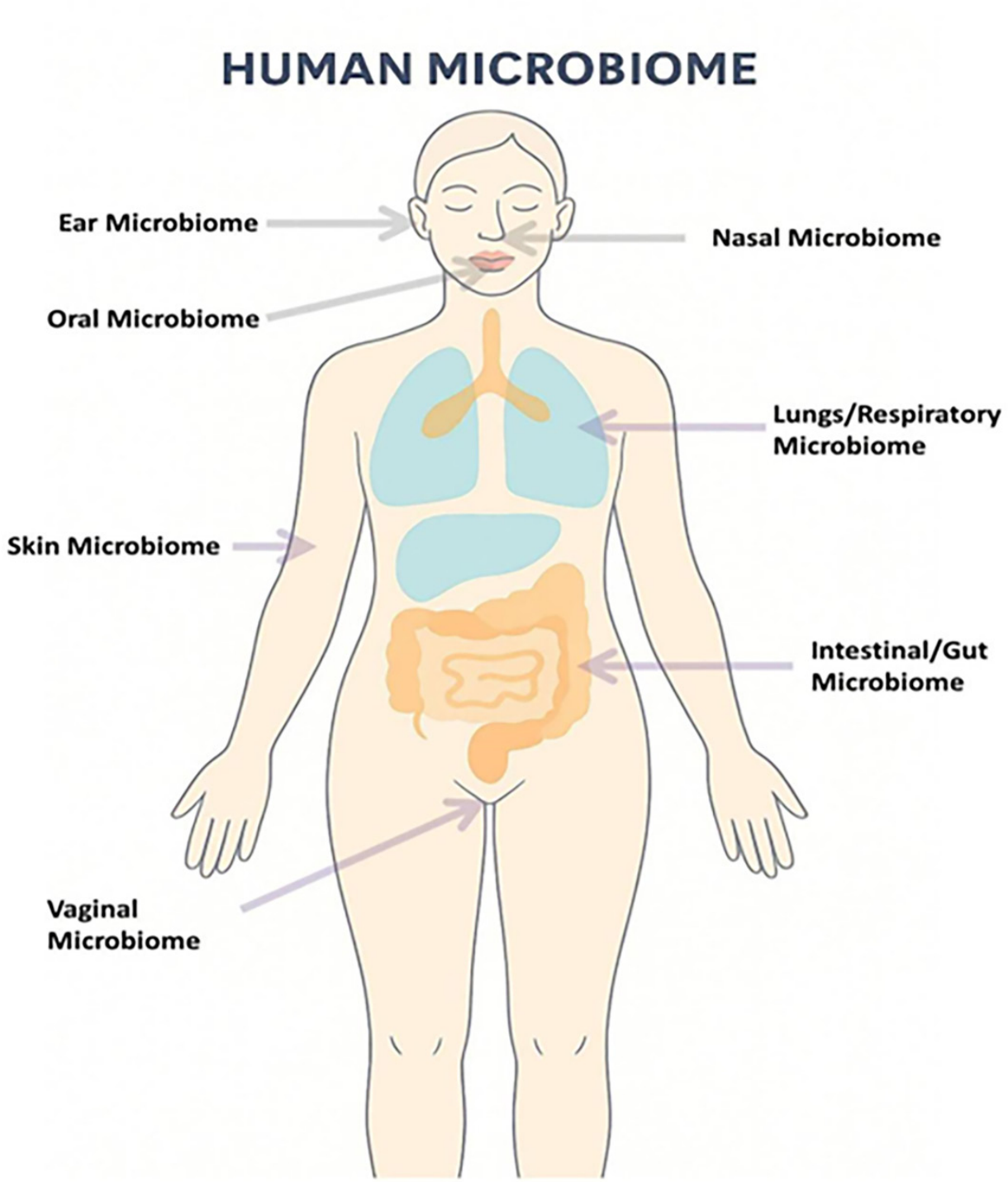

FIGURE 1.1 The major human microbiome colonizing sites. ⏎

With the advent of next-generation sequencing, understanding of the microbiome's heterogeneity has expanded dramatically (Mousa et al. 2022). Microbial diversity, taxonomic and functional, varies widely across body

sites and among individuals, shaped by factors including ethnicity, age, health status, and temporal dynamics. Perturbations in the microbiome's equilibrium, commonly referred to as dysbiosis, have been implicated in disrupted mutualistic host–microbe interactions and linked to a range of disease states. This imbalance also has implications for treatment efficacy and the risk of undesirable therapeutic outcomes (Mousa et al. 2022; Reynoso-García et al. 2022; Leonard and Toro 2023; Ma et al. 2024).

Clinically, the recognition that the indigenous microbiota confers vital ecosystem services, including nutrient bioconversion, metabolite production, and defense against pathogens, has shifted paradigms in biomedical science. Disturbances in these functions predispose hosts to disease, whereas restoring or modulating the microbiota opens new therapeutic avenues (Gilbert et al. 2018; Aggarwal et al. 2023; Ellis et al. 2024). Consequently, contemporary research seeks to elucidate not only compositional changes but also functional microbial roles in health and disease (Gilbert et al. 2018; Aggarwal et al. 2023; Ellis et al. 2024).

Overall, these insights reflect a rapidly evolving field that increasingly recognizes the microbiome's critical role in sustaining health and its potential as a therapeutic target. This chapter aims to deliver an integrative overview of the human microbiome across anatomical sites, examine the implications of microbial dysbiosis, and outline emerging microbiome-informed strategies for diagnostics, personalized medicine, and therapy.

1.2 Skin Microbiome

1.2.1 Skin Is More than Just a Barrier

For centuries, human skin was primarily viewed as a simple, vital, physical barrier, protecting the internal organs from the harsh external environment. Its role was understood to be largely passive: a waterproof, elastic shield, an impermeable boundary between the host and the outside world (Elias and Feingold 2005). However, in recent decades, a revolutionary paradigm shift

has occurred in our understanding of this remarkable organ. We now recognize the skin as a dynamic, interactive ecosystem, teeming with life—a complex community of microorganisms collectively known as the skin microbiome (Grice and Segre, 2011). These invisible residents, comprising bacteria, fungi, viruses, and mites as shown in Figure 1.2, play a profoundly active role in maintaining skin health, influencing our immune system, and even impacting our susceptibility to various diseases (Byrd et al. 2018). In the next lines, we will delve into the fascinating world of the skin microbiome, exploring its composition, the factors that shape its diversity, its crucial functions, and the implications of its dysbiosis for human health.

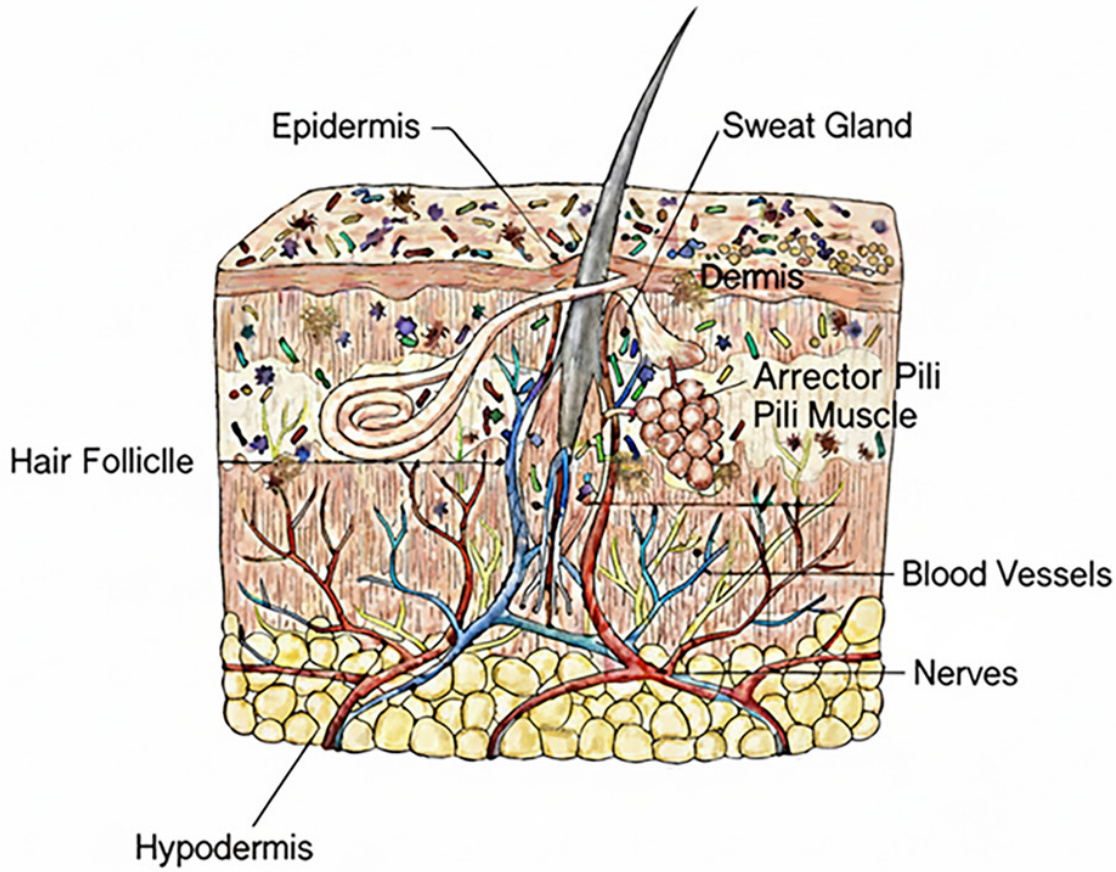

FIGURE 1.2 Schematic diagram of skin layers where different types of microorganisms (bacteria, fungi, viruses, and mites) reside on epidermis and in specific sites of dermis layer. ⏎

1.2.2 Composition and Distribution of Skin Microbiome

The human skin, with its varied topography and environmental conditions, offers a diverse range of habitats for microbial colonization. Different anatomical sites, such as oily (i.e., sebaceous) areas like the forehead and

back, moist areas like the armpits and groin, and dry areas like the forearms and legs, harbor distinct microbial communities. This heterogeneity is driven by variations in factors such as pH, temperature, moisture levels, sebum production, and oxygen availability (Grice et al. 2009; The Human Microbiome Project Consortium 2012).

1.2.3 Bacterial Dominance: A Closer Look at the Key Players

While the skin microbiome is polymicrobial, bacteria are by far the most abundant and well-studied inhabitants. Traditional culture-based methods, which could only identify a small fraction of the resident microbes, have been largely superseded by culture-independent techniques, primarily 16S rRNA gene sequencing (Scharschmidt and Fischbach 2013). The dominant bacterial phyla found on healthy human skin include:

1. *Actinobacteria:* This phylum is often prevalent in sebaceous areas. Genera like *Cutibacterium* (formerly *Propionibacterium*), especially *Cutibacterium acnes* (*C. acnes*), are common inhabitants of hair follicles and sebaceous glands. While *C. acnes* is well known for its association with *acne vulgaris*, it is also a normal commensal, playing a role in maintaining skin pH and producing beneficial fatty acids (Fitz-Gibbon et al. 2013). Some strains of *C. acnes* are protective, while others are inflammatory (2019).
2. *Proteobacteria:* This is a diverse phylum found across various skin sites, with genera like *Pseudomonas* and *Acinetobacter*. While some species within this phylum can be opportunistic pathogens, particularly in immunocompromised individuals or on damaged skin, many are harmless commensals. Proteobacteria present the highest diversity in Antecubital Fossa (AF) and Popliteal Fossa (PF), including *Klebsiella, Bacillus, Pseudomona*s, and *Escherichia* (Ramadan et al. 2016).
3. *Firmicutes:* Particularly abundant in moist areas, this phylum includes genera such as *Staphylococcus* and *Streptococcus*.

Staphylococcus epidermidis is a highly prevalent and key commensal, contributing significantly to host defense by producing antimicrobial peptides (AMPs) like phenol-soluble modulins and inhibiting the growth of pathogens, including *Staphylococcus aureus* (*S. aureus*) and Group A Streptococcus (Iwase et al. 2010; Pastar et al. 2021). *S. aureus*, on the other hand, is an opportunistic pathogen often associated with skin infections like impetigo and cellulitis, though it can also be a transient or persistent colonizer on healthy skin (Miller and Cho 2011).

4. *Bacteroidetes:* Less abundant than the other major phyla, but still present on various skin sites, their specific roles on the skin are less thoroughly characterized compared to gut Bacteroidetes (Ramadan et al. 2016). The relative proportions and specific species within these phyla vary significantly between individuals and across different body sites of the same individual. This highlights the highly personalized nature of the skin microbiome, which is often described as a "fingerprint" unique to each person (Grice et al. 2009; The Human Microbiome Project Consortium 2012).
5. *Beyond Bacteria: Fungi, Viruses, and Mites*: While bacteria are the most numerous, other microorganisms also contribute to the complexity of the skin microbiome: *Fungi (Mycobiome):* The most common fungal genera on the skin include *Malassezia*, particularly *Malassezia globosa* and *Malassezia restricta*. These are lipophilic yeasts that thrive in sebaceous areas, utilizing skin lipids for growth (Wu et al. 2015). While generally commensal, overgrowth of *Malassezia* can contribute to conditions like dandruff (*Pityriasis capitis*) and seborrheic dermatitis (Hou et al. 2022). Other fungi, such as Candida species, can also be found, particularly in moist intertriginous areas (Beardsley et al. 2024). *Viruses (Virome):* The skin is home to a diverse array of viruses, including bacteriophages and eukaryotic viruses. The skin virome is less well understood than the bacterial and fungal components, largely due to technical challenges in detection and characterization. However, emerging

research suggests its potential role in modulating both the bacterial microbiome (via bacteriophages) and host immunity (via eukaryotic viruses like human papillomaviruses (HPVs) and polyomaviruses, many of which are harmless residents) (Hannigan et al. 2015; Chen et al. 2022). *Mites:* Microscopic arthropods, such as Demodex mites (*Demodex folliculorum* and *Demodex brevis*), reside in hair follicles and sebaceous glands. They are typically considered commensals and are ubiquitous in adult skin (Rather and Hassan 2014). While generally harmless, their overgrowth has been implicated in certain skin conditions like rosacea, where their presence may trigger an inflammatory response in susceptible individuals (Jarmuda et al. 2012).

1.2.4 Factors Influencing the Composition of Skin Microbiome

The composition and diversity of the skin microbiome are not static; they are dynamically shaped by a complex interplay of intrinsic and extrinsic factors throughout an individual's life (Nørreslet et al. 2020).

1.2.4.1 Intrinsic Factors

Host genetics play a role in determining inherent skin characteristics, including epidermal barrier function, sebum production, pH, and the production of AMPs, all of which influence microbial colonization and growth (Moitinho-Silva et al. 2022). For example, genetic predispositions to conditions like atopic dermatitis (AD) can influence the skin's microbial landscape (Kong et al. 2012). As we age, the skin microbiome undergoes significant shifts from birth through infancy, childhood, adolescence, adulthood, and old age. Infant skin is initially colonized by maternal microbes during birth (vaginal vs. C-section), and the microbiome diversifies as the child develops, interacting with its environment (Howard et al. 2022). Hormonal changes during puberty influence sebum production, leading to increased abundance of lipophilic bacteria like *C. acnes*. In later

life, changes in skin integrity, immune function, and lifestyle can again alter the microbial profile (Borrego-Ruiz and Borrego, 2024).

Skin anatomy profoundly influences the composition and diversity of the microbiome residing on different body sites. The human skin is not a uniform surface; rather, it presents a highly varied landscape of microenvironments, each with distinct physical and chemical characteristics that favor the colonization of specific microbial communities. This concept is often referred to as the biogeography of the skin microbiome (Grice et al. 2009; The Human Microbiome Project Consortium 2012).

1.2.4.2 Moisture Levels

Skin sites vary significantly in their moisture content, which is a primary determinant of microbial growth. Moist Sites: Areas like the armpits (axillae), groin, between toes, and the AF (inner elbow) are characterized by high humidity due to sweat accumulation and skin folds (Leyden et al. 1981; Elias and Feingold, 2005; Ramadan et al. 2016). These environments support the growth of moisture-loving bacteria. *Characteristic Microbes: Corynebacterium* species and certain *Staphylococcus* species (e.g., *Staphylococcus hominis*, *S. aureus*) thrive here. Gram-negative bacilli can also be found in these areas. The high bacterial load in these moist regions often contributes to body odor due to microbial metabolism of sweat components (Leyden et al. 1981; Grice et al. 2009). *Dry Sites:* Areas like the forearms, legs, and less occluded parts of the torso are relatively dry and experience more fluctuations in temperature and exposure to the environment (Elias and Feingold 2005; Ramadan et al. 2016; Byrd et al. 2018). *Characteristic Microbes:* These areas tend to have the lowest microbial density but exhibit the highest diversity. They host a more mixed population, often including Beta-Proteobacteria (e.g., *Variovorax*, *Methylophilus*) and *Flavobacteriales*, in addition to some *Firmicutes* and *Actinobacteria* (Grice et al. 2009; The Human Microbiome Project Consortium 2012). Sebaceous gland density and sebum production sebaceous glands produce sebum, an oily, lipid-rich substance that lubricates the skin and hair. The density of these glands varies greatly

across the body, creating distinct "oily" niches. *Sebaceous Sites:* Areas like the face (forehead, nose), scalp, chest, and upper back have a high density of sebaceous glands (Elias and Feingold 2005; Grice and Segre 2011). Sebum provides a rich nutrient source, particularly lipids, for specific microbial groups. *Characteristic Microbes: Cutibacterium* species (formerly *Propionibacterium*), especially *C. acnes*, are highly abundant in these areas because they are lipophilic and anaerobic/facultative anaerobic, thriving in the oxygen-poor environment of hair follicles where sebum is secreted (McLaughlin et al. 2019). *Malassezia* species, which are lipophilic yeasts, also dominate in these regions (Wu et al. 2015).

Impact: The breakdown of sebum by these microbes influences skin pH and produces free fatty acids, which in turn can affect other microbial populations and host immunity (Lambers et al. 2006). The presence of hair follicles and hair follicle glands, sweat glands (eccrine and apocrine), and sebaceous glands forms micro-invaginations and appendages that provide unique sheltered niches for microbes (Lambers et al. 2006). *Hair Follicles:* These offer a relatively anaerobic environment below the skin surface, favorable for *C. acnes*. The hair shaft itself can also harbor bacteria due to its grooved surface (The Human Microbiome Project Consortium 2012). *Eccrine Glands:* Found almost universally, eccrine glands produce watery sweat primarily for thermoregulation. While sweat itself is largely sterile, its evaporation can leave behind salts and other compounds, influencing the local environment. The constant flow of sweat can also help flush away transient microbes (Byrd et al. 2018). *Apocrine Glands:* Located in specific areas like the axillae, groin, and around the nipples, these glands produce milky, viscous secretions that are largely odorless until metabolized by bacteria. This provides a rich nutrient source for certain specialized bacteria (Ho and Kupper 2019). *Skin Thickness and Barrier Integrity:* The thickness of the epidermis, particularly the stratum corneum, and the overall integrity of the skin barrier vary across the body. *Thick Skin:* Areas like the palms of the hands and soles of the feet have a much thicker stratum corneum, offering enhanced physical protection but also potentially creating different microclimates compared to thinner skin (Grice et al. 2009; The Human

Microbiome Project Consortium 2012). *Barrier Function:* The stratum corneum, composed of corneocytes embedded in a lipid matrix, is the primary physical barrier. Its continuous shedding (desquamation) helps remove surface microbes (Elias and Feingold 2005). However, microbes that can adhere strongly or colonize deeper layers can persist. Anatomical locations with compromised barrier function (e.g., skin folds, areas prone to friction) can be more susceptible to dysbiosis and opportunistic infections. Skin folds and occlusion areas with skin folds (e.g., axillae, groin, inframammary folds) are naturally occluded, leading to higher temperature and humidity, which favor the growth of microorganisms that thrive in moist, warm conditions. This occlusion also reduces air exposure, promoting anaerobic or facultative anaerobic species (Leyden et al. 1981; Byrd et al. 2018; Ho and Kupper 2019).

1.2.4.3 Immune System

The skin immune system, comprising keratinocytes, Langerhans cells, dermal dendritic cells, and various T cells, constantly interacts with the skin microbiome. This intricate dialogue helps maintain immune homeostasis, distinguishing between beneficial commensals and potential pathogens. Commensals can educate immune cells, promote tolerance, and prevent excessive inflammatory responses to harmless stimuli (Leyden et al. 1981; Byrd et al. 2018; Ho and Kupper 2019). *Skin pH:* The acidic pH of healthy skin (typically 4.5–5.5), often referred to as the "acid mantle," acts as a natural antimicrobial barrier. This acidity favors the growth of certain commensals while inhibiting the proliferation of many pathogens, including *S. aureus* (Lambers et al. 2006). Disruption of the acid mantle can lead to dysbiosis and increased susceptibility to infections (Lambers et al. 2006). *Sebum Production:* Sebum, an oily substance produced by sebaceous glands, is a crucial nutrient source for lipophilic microbes like *Cutibacterium* and *Malassezia*. Variations in sebum quantity and composition significantly influence the abundance of these specific microbial groups (Łuczaj et al. 2020; Nørreslet et al. 2020).

1.2.4.4 Extrinsic Factors: Environmental Influences, Lifestyle

While less direct, lifestyle choices can indirectly influence skin health and, consequently, the microbiome. For instance, diet can impact systemic inflammation and nutrient availability, potentially influencing skin conditions. Stress can also alter skin barrier function and immune responses, affecting microbial balance (Ahn and Hayes 2021). *Geographic Location and Climate*: Environmental factors like humidity, temperature, and UV radiation can impact skin barrier function, moisture levels, and microbial growth patterns. Individuals living in different climates may exhibit variations in their dominant skin microbial species (D'Orazio et al. 2013). *Hygiene Practices:* Frequent washing, use of soaps, and topical antimicrobial products can disrupt the natural balance of the skin microbiome. Harsh cleansers can strip beneficial lipids, alter pH, and indiscriminately remove commensal microbes, potentially creating opportunities for opportunistic pathogens to colonize (Lee et al. 2018). *Occupation:* Exposure to specific environmental agents, chemicals, or frequent contact with certain materials due to occupation can influence microbial communities on the skin (Chen et al. 2022). *Cosmetics and Skincare Products:* The ingredients in these products (e.g., preservatives, emollients, fragrances, pH adjusters) can directly impact the skin microbiome by altering pH, providing nutrients, or introducing antimicrobial agents (Nakatsuji and Gallo 2019). The concept of "microbiome-friendly" skincare is emerging to address this influence. *Medications:* Antibiotics, both systemic and topical, can significantly alter the skin microbiome by indiscriminately killing susceptible bacteria, leading to a reduction in diversity and potential overgrowth of resistant strains or fungi (Lee et al. 2025). Immunosuppressants can also impact microbial communities by affecting the host immune response and increasing susceptibility to opportunistic infections. *Diet:* The "gut–skin axis" highlights the intricate connection between gut health and skin conditions. Dietary patterns can influence the gut microbiome, which in turn can affect systemic inflammation, nutrient absorption, and immune

responses that impact skin health and its microbiome (De Pessemier et al. 2021). *Exposure to Animals and Nature:* Contact with diverse environments, soil, and animals can introduce a wider range of microbes to the skin, potentially increasing microbiome diversity and fostering a more robust immune system, especially in early life (Haahtela et al. 2013).

1.2.5 Functions of the Skin Microbiome

The relationship between the host and its skin microbiome is largely symbiotic, with the microbial inhabitants providing numerous benefits that contribute to overall skin health and host well-being (Grice and Segre 2011).

1.2.5.1 Pathogen Defense (Colonization Resistance)

Commensal skin microbes act as a natural defense system against invading pathogens through several mechanisms (Byrd et al. 2018). *Competitive Exclusion:* They occupy ecological niches, colonizing adhesion sites, and competing for limited nutrients, making it difficult for transient or pathogenic microbes to establish themselves (The Human Microbiome Project Consortium 2012). *Production of AMPs and Bacteriocins:* Many commensal bacteria produce substances that directly inhibit the growth of pathogenic microbes. For instance, *S. epidermidis* produces a range of AMPs, such as δ-toxin and phenol-soluble modulins, which can kill *S. aureus* and other Gram-positive and Gram-negative bacteria (Iwase et al. 2010; Pastar et al. 2021). They also produce bacteriocins (e.g., epidermin) that target specific competitors (Iwase et al. 2010; Miller and Cho 2011). *Modulation of Skin pH:* Commensals contribute to maintaining the acidic pH of the skin by producing organic acids (e.g., lactic acid, short-chain fatty acids (SCFAs)) through fermentation. This acidic environment is unfavorable for the growth of many pathogens (Lambers et al. 2006). *Immune System Modulation:* The skin microbiome plays a critical role in educating and shaping the host immune system (Byrd et al. 2018). *Immune Homeostasis:* Commensals interact with various skin immune cells (e.g.,

keratinocytes, Langerhans cells, T cells, innate lymphoid cells) to promote immune tolerance and prevent excessive inflammatory responses. They help the immune system distinguish between harmless foreign substances and true threats (Ho and Kupper 2019).

1.2.5.2 Maturation of Immune Responses

Early life exposure to diverse microbes is crucial for the proper development and maturation of the immune system. This "hygiene hypothesis" suggests that a lack of early microbial exposure can lead to dysregulated immune responses and increased susceptibility to allergic and autoimmune diseases (Strachan 1989). *Protection Against Allergic Diseases:* A diverse skin microbiome, particularly in early life, has been associated with a reduced risk of developing allergic conditions, such as AD and asthma (Strachan 1989; Kong et al. 2012; Nakatsuji and Gallo 2019). *Skin Barrier Maintenance:* Certain commensals contribute directly or indirectly to the integrity of the skin barrier, which is essential for preventing transepidermal water loss and protecting against environmental insults (Dréno et al. 2016). *Production of Metabolites:* Some bacteria produce beneficial metabolites, such as SCFAs, that can support keratinocyte function, promote tight junction formation, and enhance barrier repair (Kim and Leung 2018). *Lipid Metabolism:* Certain microbes may influence lipid synthesis and turnover in the epidermis, contributing to the formation of the lipid matrix that is crucial for barrier function (Łuczaj et al. 2020). *Wound Healing:* The skin microbiome can significantly influence the wound healing process. A balanced microbiome promotes faster and more efficient repair, potentially by modulating inflammation and stimulating keratinocyte migration (Misic et al. 2014). Conversely, dysbiosis, particularly the presence of opportunistic pathogens like *Pseudomonas aeruginosa* or *S. aureus*, can impede healing, lead to chronic wounds, and increase the risk of infection (Buch et al. 2019; Mohamed Mohamed Adel 2021). *Odor Production:* While sometimes perceived negatively, the breakdown of sweat components (e.g., fatty acids, amino acids) by certain skin bacteria, particularly in the axillae, contributes to

characteristic body odor. This process is a natural part of human biology and has social and evolutionary implications (Di Cicco et al. 2023). *Vitamin Synthesis:* Some skin bacteria, such as certain *Corynebacterium* species, may be involved in the synthesis of certain vitamins (e.g., B vitamins, vitamin K), although the extent of this contribution to host nutrition is not fully understood (Mladěnka et al. 2022).

1.2.6 When Balance Is Lost

The skin microbiome plays a pivotal role in maintaining skin health. When its delicate balance is disrupted a state known as *dysbiosis*; it can significantly contribute to the development, exacerbation, and persistence of various skin diseases. This involves complex interactions between the host's immune system, genetic predispositions, environmental factors, and the microbial community itself (Ogunrinola et al. 2020). Dysbiosis can involve a loss of beneficial microbes, an overgrowth of opportunistic pathogens, or a reduction in overall diversity.

1.2.6.1 Acne Vulgaris

While *C. acnes* is a normal commensal, specific phylotypes (strains) of *C. acnes* are associated with inflammatory acne (2019). Dysbiosis in acne involves an increase in these pathogenic strains, often accompanied by altered sebum composition (e.g., increased squalene) and increased inflammatory responses within the pilosebaceous unit (2019; McLaughlin et al. 2019). A reduction in *Cutibacterium* diversity and an increase in *Staphylococcus* species are also observed in acne lesions (Podwojniak et al. 2025).

1.2.6.2 Atopic Dermatitis (Eczema)

The skin microbiome in AD is characterized by reduced diversity and a significant increase in the abundance of *S. aureus*, especially during flares (Ramadan et al. 2019). *S. aureus* produces virulence factors (e.g., toxins, superantigens) that can exacerbate inflammation, impair skin barrier

function, and contribute to the intense itch and scratching cycle characteristic of eczema (Kim et al. 2019). Beneficial commensals like *S. epidermidis* are often diminished, leading to a loss of protective mechanisms (Landemaine et al. 2023). Specific bacterial signatures observed in Egyptian AD patients include an increased prevalence of *Staphylococcus* species (especially *S. aureus*), along with shifts in *Streptococcus, Cutibacterium*, and *Corynebacterium* species, which appear to be specific signatures for AD in different age groups (children, adolescents, adults) (Ramadan et al. 2019).

1.2.6.3 Psoriasis

Psoriasis is a chronic inflammatory skin disease. Emerging evidence suggests that dysbiosis of both the skin and gut microbiome may play a role in its pathogenesis, although the specific microbial signatures are still being investigated. Studies have shown altered microbial diversity and differences in specific bacterial taxa (e.g., increased *Streptococcus* and *Corynebacterium*) on psoriatic lesions compared to healthy skin (Yerushalmi et al. 2019; Celoria et al. 2023).

1.2.6.4 Rosacea

Overgrowth of Demodex mites and certain bacterial species (e.g., *Bacillus oleronius*) associated with these mites has been implicated in the inflammatory manifestations of rosacea. The immune response to Demodex-associated bacteria or mite antigens is thought to contribute to the redness, papules, and pustules (Jarmuda et al. 2012; Rather and Hassan, 2014).

1.2.6.5 Fungal Infections

Conditions like dandruff (*P. capitis*), seborrheic dermatitis, and tinea (ringworm, athlete's foot) infections are often linked to the overgrowth of specific fungal species. *Malassezia* species are the primary culprits in dandruff and seborrheic dermatitis, while dermatophytes (e.g.,

Trichophyton, Microsporum) cause tinea infections (Wu et al. 2015; Dréno et al. 2016; Hou et al. 2022).

1.2.6.6 Wound Infections

An imbalanced microbiome can predispose to chronic wounds and infections. In chronic wounds, the microbial community often shifts from a diverse commensal population to a polymicrobial biofilm dominated by opportunistic pathogens like *S. aureus* and *P. aeruginosa*, which can impede healing and increase systemic infection risk (Misic et al. 2014; Buch et al. 2019; Mohamed Mohamed Adel 2021). Understanding the specific microbial shifts associated with these diseases offers new avenues for targeted diagnostic and therapeutic interventions.

1.2.7 The Future of Skin Health: Manipulating the Microbiome

The growing understanding of skin microbiome has opened up exciting possibilities for novel diagnostic and therapeutic strategies aimed at restoring and maintaining skin health.

1.2.7.1 Probiotics and Prebiotics for Skin

Similar to gut microbiome modulation, research is exploring the use of topical probiotics (live beneficial microbes, often *S. epidermidis* or *Lactobacillus* species) and prebiotics (nutrients that selectively support the growth and activity of beneficial microbes, such as oligosaccharides or certain plant extracts) to restore microbiome balance and improve skin health, particularly in conditions like AD (De Almeida et al. 2023; Gowda et al. 2024).

1.2.7.2 Postbiotics

These are beneficial compounds produced by microorganisms during fermentation, such as AMPs, enzymes, organic acids, and SCFAs. Applying these “dead microbes and their products” topically can exert beneficial effects on the skin, including anti-inflammatory, antioxidant, and barrier-

enhancing properties, without introducing live organisms (Prajapati et al. 2023).

1.2.7.3 Fecal Microbiota Transplantation for Skin Conditions

While primarily used for recurrent *Clostridioides difficile* infection, the concept of microbiome transplantation, specifically from healthy skin donors, for severe skin conditions, is a nascent area of research. Early studies exploring this for severe AD show some promise, though significant challenges remain regarding safety, efficacy, and standardization (Prajapati et al. 2023).

1.2.7.4 Targeted Antimicrobials

Instead of broad-spectrum antibiotics that indiscriminately kill both beneficial and pathogenic bacteria, future treatments may involve highly specific antimicrobial agents (e.g., bacteriocins, phage therapy) that target only specific pathogenic microbes while preserving the beneficial commensal flora (Myles et al. 2016).

1.2.7.5 Microbiome-Friendly Skincare

The skincare industry is increasingly developing products designed to support a healthy skin microbiome. These products avoid harsh chemicals, maintain skin pH, and incorporate ingredients that promote microbial balance, such as ceramides, prebiotics, and gentle cleansers (De Pessemier et al. 2021).

1.2.7.6 Personalized Dermatology

Future approaches may involve analyzing an individual's unique skin microbiome profile to tailor specific skincare routines, preventive strategies, and therapeutic interventions based on their microbial signature and predisposition to certain conditions (Markiewicz and Idowu 2018).

1.2.7.7 Early Life Interventions

Strategies aimed at promoting a healthy skin microbiome in early life, such as encouraging natural vaginal birth, breastfeeding, reduced antibiotic use, and exposure to diverse outdoor environments, may have long-term benefits for skin and immune health, potentially reducing the risk of developing allergic and inflammatory diseases (The Human Microbiome Project Consortium 2012; Nakatsuji and Gallo 2019).

1.2.8 A New Era of Skin Biology

Far from being passive bystanders or mere contaminants, these microorganisms are active participants in maintaining our protective barrier, educating our immune system, and defending against pathogens. As research continues to unravel the complexities of this fascinating ecosystem, enabled by advanced sequencing technologies and sophisticated computational analyses, we are poised for a new era of dermatology. This era will move beyond simply treating symptoms to actively nurturing and manipulating the skin microbiome for optimal health and well-being. The skin is not just a barrier; it is a vibrant, living community, and its health depends intrinsically on the delicate balance of its invisible residents. The future of dermatological care will increasingly involve strategies that acknowledge, respect, and leverage the power of our skin's tiniest allies.

1.3 The Oral Microbiome

The oral cavity represents one of the most intricate ecosystems in the human body, functioning as a gateway to both the respiratory and digestive systems. It contains over 700 bacterial species along with viruses, fungi, archaea, and protozoa; the oral microbiome is considered a dynamic and diverse community (Deo and Deshmukh, 2019). These microorganisms coexist in specific sites, such as the gingival crevice, tongue, hard and soft palates, and dental surfaces, each with unique environmental conditions, including oxygen availability, pH levels, and nutrient profiles (Table 1.1, Figure 1.3).

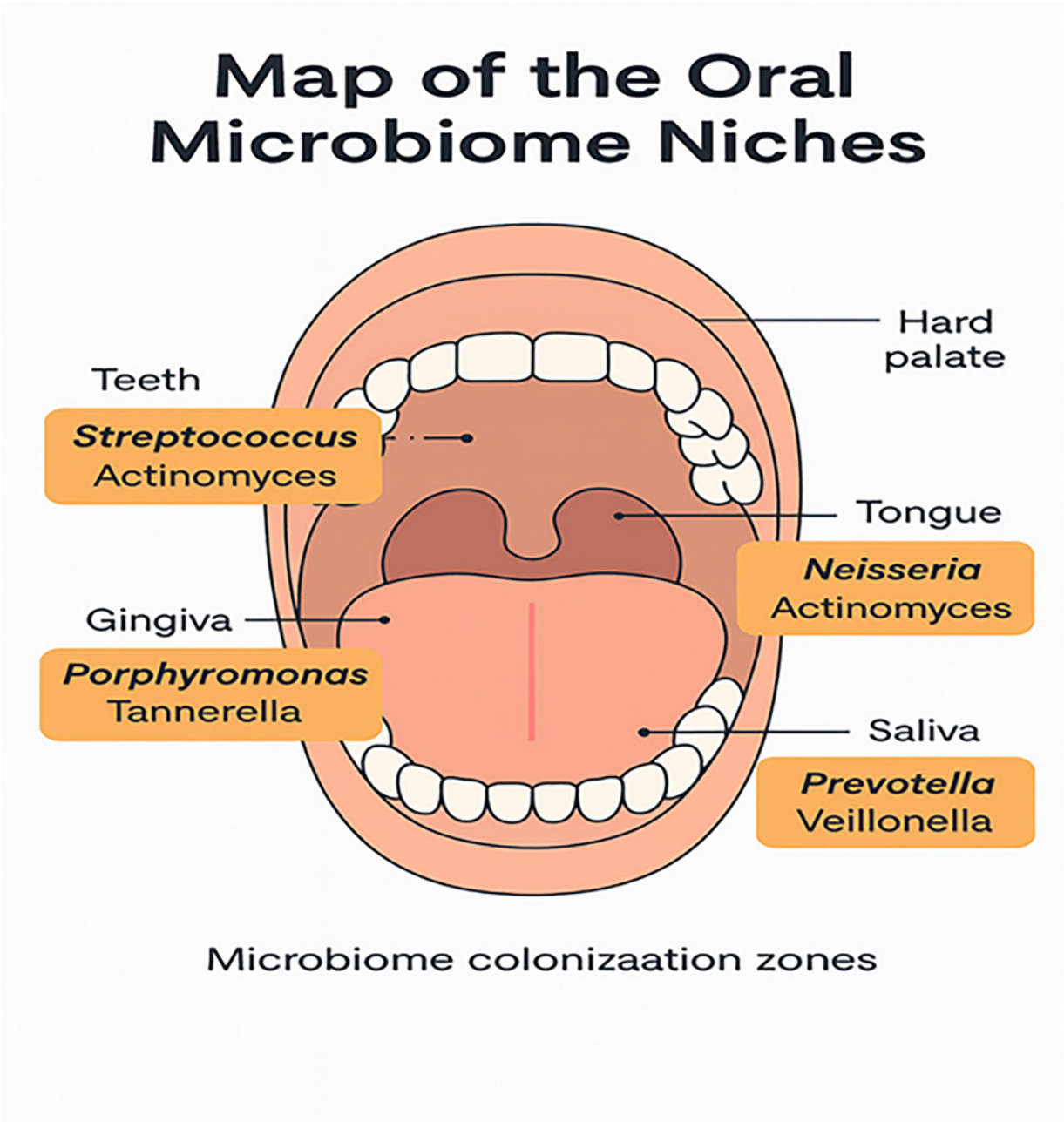

FIGURE 1.3 Schematic map of microbial colonization zones in the oral cavity. ⏎

TABLE 1.1

Key Genera in the Healthy Oral Microbiome by Site ⏎

Oral Site	Dominant Bacterial Genera	Notes

Oral Site	Dominant Bacterial Genera	Notes
Saliva	*Streptococcus, Prevotella, Veillonella*	Reflects a composite of oral microbiota from all environments
Supragingival plaque	*Streptococcus, Actinomyces, Capnocytophaga*	Early colonizers, contribute to biofilm formation
Subgingival crevice	*Porphyromonas, Treponema, Tannerella*	Anaerobic, linked to periodontal disease.
Tongue dorsum	*Neisseria, Rothia, Actinomyces*	Involved in nitrate metabolism and oral NO production
Buccal mucosa	*Streptococcus, Gemella*	Low diversity, mostly aerobic environment

The oral microbiome serves as a critical indicator and mediator of health. Besides its local role, it is intrinsically connected to systemic conditions through mechanisms like microbial translocation and immune modulation. The interaction between host factors, such as saliva and the immune system, and the microbial community ensures equilibrium, preventing overgrowth of potential pathogens (Rajasekaran et al. 2024). This section will provide an overview of the oral microbiome's development, roles, imbalances, and its potential as a therapeutic target.

1.3.1 Development and Influencing Factors

The development of the oral microbiome is a dynamic and lifelong process shaped by both intrinsic and extrinsic factors. From the earliest moments of life, microbial colonization begins through maternal transfer and feeding practices, laying the foundation for oral microbial communities. These communities continue to evolve across life stages, adapting to physiological changes, diet, and host development. Beyond natural progression, external exposures and systemic conditions exert powerful influences, modulating microbial balance and often tipping it toward dysbiosis. Together, these

processes highlight the complex interplay between biological development, lifestyle factors, and systemic health in shaping the oral microbiome.

1.3.1.1 Microbial Establishment

The colonization of the oral cavity begins at birth, with neonatal exposure to maternal microbiota through vaginal delivery, breastfeeding, and skin contact. For example, vaginally delivered infants commonly acquire *Lactobacillus* and *Prevotella* species, whereas cesarean delivery favors the presence of *Staphylococcus* and *Corynebacterium* (Dominguez-Bello et al. 2010). Early feeding practices have a significant impact on microbial diversity. Breast milk introduces immunoglobulins, oligosaccharides, and beneficial microbes, promoting the growth of commensals such as *Bifidobacterium* (Davis et al. 2022). In contrast, formula feeding alters the microbial course, with implications for later health (O'Sullivan et al. 2015).

1.3.1.2 Ecological Shifts across Life Stages

Teething introduces non-shedding surfaces that facilitate biofilm development, while dietary transitions during infancy and childhood, such as the introduction of solid foods, further expand the microbiome. Adolescence, marked by hormonal changes and shifts in dietary habits, leads to alterations in microbial composition (Teughels et al. 2006; Carson et al. 2023). Similarly, aging, tooth loss, and changes in salivary flow affect microbial communities in the elderly, often resulting in reduced diversity and increased susceptibility to oral infections (Schwartz et al. 2021).

1.3.1.3 External and Systemic Influences

Numerous external factors shape the oral microbiome. Diet, particularly the consumption of sugars and processed foods, fosters acidogenic and aciduric bacteria, promoting dental caries. Tobacco and alcohol disrupt microbial equilibrium, increasing the prevalence of anaerobic pathogens linked to periodontal disease (Senaratne et al. 2024). Systemic conditions such as diabetes mellitus alter immune responses and nutrient availability in the

oral cavity, worsening microbial imbalances (Negrini et al. 2021). Medications, including antibiotics and immunosuppressants, significantly disturb microbial communities, with long-term consequences for oral and systemic health (Kulis et al. 2025).

1.3.2 Functions in Health

The oral microbiome plays a pivotal role in maintaining oral and overall health. Saliva, enriched with antimicrobial proteins like lysozyme, lactoferrin, and immunoglobulins, interacts with the microbiome to limit pathogen colonization. Commensals such as *Streptococcus salivarius* produce bacteriocins that inhibit pathogenic species, exemplifying microbial mutualism (Vila et al. 2019).

1.3.2.1 Immune Modulation

Microbial interactions with epithelial cells and immune receptors, such as Toll-like receptors (TLRs), activate the immune system, promoting a balanced inflammatory response. For example, *Veillonella* and *Rothia* species have been shown to regulate cytokine production, preventing excessive inflammation while maintaining mucosal integrity (Constantino-Jonapa et al. 2025).

1.3.2.2 Metabolic Contributions

Oral bacteria facilitate nitrate metabolism, an essential pathway for systemic vascular health (Morou-Bermúdez et al. 2022). Nitrate-reducing species like *Neisseria* and *Actinomyces* convert dietary nitrates into nitrite, a precursor for nitric oxide (NO), which modulates blood pressure and has antimicrobial properties (Liu et al. 2023). Loss of these bacteria due to antiseptic overuse has been linked to hypertension and other systemic effects (Brookes et al. 2023).

1.3.3 Dysbiosis and Disease

Oral dysbiosis, defined as a disruption in the balance of the oral microbial community, has profound consequences for both local and systemic health. Within the oral cavity, shifts in microbial composition drive common pathologies such as dental caries and periodontitis, directly affecting oral tissues and host immunity. Beyond these localized effects, dysbiosis also establishes links with systemic diseases, as pathogenic species disseminate or exert indirect effects that contribute to chronic inflammation, metabolic dysfunction, and even tumorigenesis. Together, these findings underscore the dual role of oral dysbiosis as both a local driver of oral disease and a broader contributor to systemic pathology.

1.3.3.1 Local Manifestations

Oral dysbiosis is implicated in various dental and periodontal diseases. Dental caries arise from an ecological shift favoring acid-tolerant bacteria such as *Streptococcus mutans* and *Lactobacillus*, which produce lactic acid during carbohydrate fermentation. This acid demineralizes enamel, leading to cavity formation (Spatafora et al. 2024). Periodontitis, a chronic inflammatory condition, involves anaerobic species like *Porphyromonas gingivalis (P. gingivalis)* (How et al. 2016).These organisms degrade host tissues using proteolytic enzymes and evade immunity through virulence factors such as gingipains. Left untreated, periodontitis can result in tooth loss and contribute to systemic inflammation (Martínez-García and Hernández-Lemus 2021) (Figure 1.4).

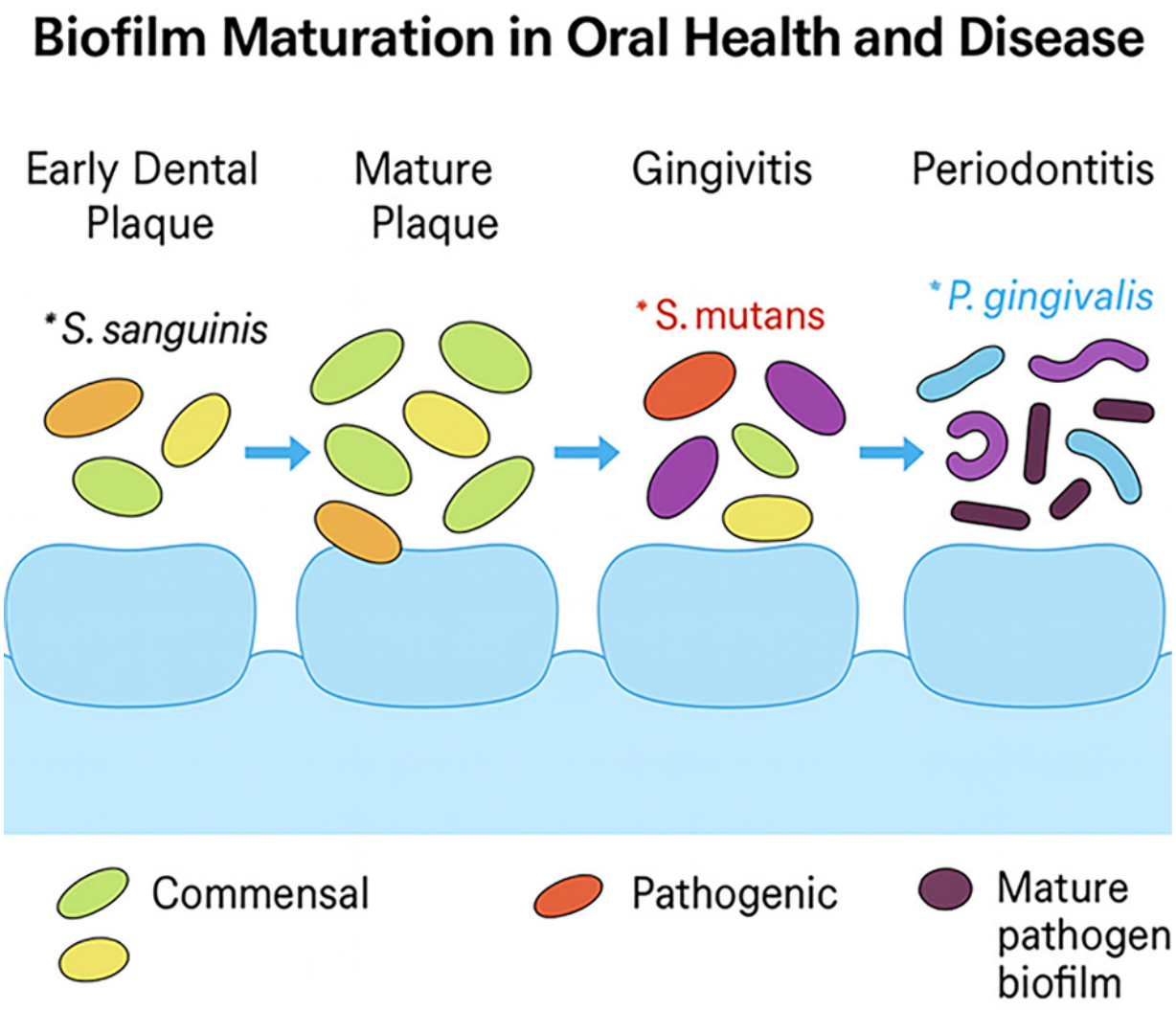

FIGURE 1.4 Flowchart showing progression from early commensal plaque to pathogenic biofilm leading to gingivitis and periodontitis. ⏎

1.3.3.2 Systemic Connections

Emerging evidence links oral dysbiosis to systemic diseases. *Fusobacterium nucleatum* has been associated with colorectal cancer due to its ability to adhere to epithelial cells and promote tumorigenesis (Rubinstein et al. 2013). Similarly, *P. gingivalis* has been detected in atherosclerotic plaques and is implicated in Alzheimer's disease through mechanisms involving amyloid deposition and neuroinflammation (Ryder 2020).

1.3.4 Microbiome–Host Interactions and Axes

The oral–gut axis demonstrates the bidirectional relationship between oral and gut microbiota. Daily swallowing of saliva introduces oral microbes to the gastrointestinal tract, potentially influencing gut microbial composition. For example, oral *Prevotella* species have been implicated in gut dysbiosis associated with systemic disease (Larsen 2017). The oral–brain axis represents another interesting area of research. Olfactory and trigeminal nerves provide potential routes for microbial migration, linking oral health to neurological disorders (Borrego-Ruiz and Borrego 2025a). Chronic oral inflammation may worsen systemic immune responses, contributing to conditions like systemic diseases (Martínez-García and Hernández-Lemus 2021).

1.3.5 Therapeutic and Preventive Strategies

As understanding of the oral microbiome deepens, attention has increasingly shifted toward strategies that not only treat dysbiosis but also promote long-term microbial balance. Conventional approaches are now complemented by microbiome-centered interventions, ranging from the use of probiotics and prebiotics to restore beneficial species, to novel therapies such as bacteriophages, quorum-sensing inhibitors, and microbial transplantation. Together, these therapeutic and preventive strategies reflect a growing focus on precision approaches that maintain oral health while minimizing disruption to commensal communities.

1.3.5.1 Probiotics and Prebiotics

Oral probiotics, such as *Lactobacillus reuteri* and *S. salivarius* K12, have demonstrated efficacy in reducing halitosis, preventing pharyngitis, and maintaining periodontal health. Prebiotics, including polyphenols and arginine, selectively promote beneficial bacteria, reducing the virulence of pathogens (Karbalaei et al. 2021).

1.3.5.2 Innovative Therapies

Phage therapy, targeting specific pathogens without disturbing commensals, is emerging as a promising approach for periodontal disease. Similarly, quorum-sensing inhibitors that disrupt microbial communication offer potential in preventing biofilm formation (Alum et al. 2025). Advances in microbiome repair, such as autologous microbial transplantation, are being explored for resistant cases of dysbiosis.

1.4 The Nasal Microbiome

The nasal cavity, a key component of the upper respiratory tract, serves as both a physical and immunological barrier between the external and the internal environments. It is lined by respiratory and olfactory epithelia and continuously exposed to inhaled air, particles, allergens, and microorganisms. This anatomical complexity supports a distinct and dynamic microbial community in the nasal microbiome (Table 1.2).

TABLE 1.2
Key Genera in the Healthy Nasal Microbiome ⏎

Nasal Region	Dominant Genera	Notes
Anterior nares	*Staphylococcus*, *Corynebacterium*	Primary site of *S. aureus* colonization
Nasopharynx	*Dolosigranulum*, *Moraxella*, *Haemophilus*	Influences immune development in early life
Olfactory epithelium	*Streptococcus*, *Corynebacterium*, *Propionibacterium*	Potential role in olfactory function and brain interaction

Comprising bacteria, viruses, fungi, and archaea, the nasal microbiome is essential for respiratory health and immune function. While traditionally most studies focused on the gut or oral microbiomes, recent advances in high-throughput sequencing have revealed that the nasal cavity harbors a rich and diverse microbial ecosystem that plays a critical role in maintaining mucosal homeostasis, preventing respiratory infections, and

potentially influencing systemic and neurological health through interconnected axes (Thangaleela et al. 2022).

1.4.1 Development and Influencing Factors

The nasal microbiome develops through a dynamic interplay of early-life exposures, environmental influences, and host-related factors. Colonization begins at birth, guided by delivery mode, feeding practices, and maternal transfer, and continues to evolve throughout infancy and adulthood. As shown in Figure 1.5, these microbial communities remain highly responsive to surrounding conditions, with environmental exposures, host physiology, and lifestyle shaping their long-term stability and function. Together, these influences underscore the complexity of nasal microbiome maturation and its role in health and disease.

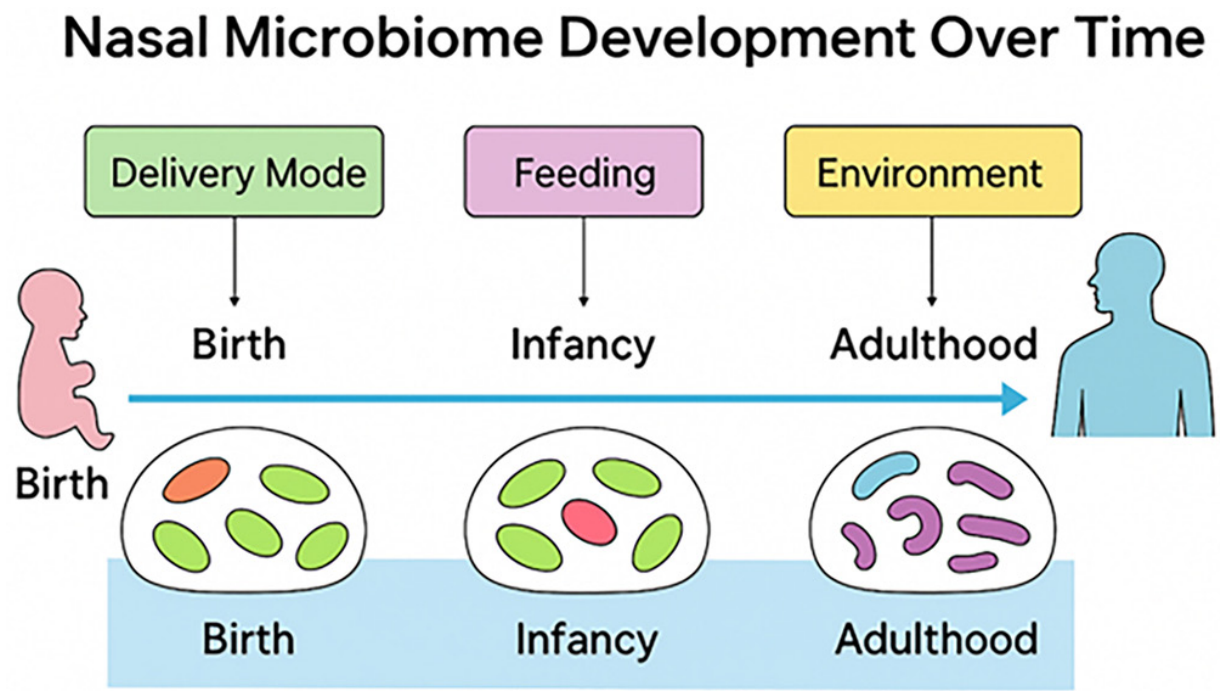

FIGURE 1.5 Nasal microbiome development over time. The nasal microbiome evolves from birth to adulthood. Early colonization is shaped by delivery mode, infancy by feeding, and adulthood by environmental exposure. The microbiota transitions from mainly commensal bacteria (e.g., *Corynebacterium*) to a stable community including pathogenic (e.g., *Staphylococcus aureus*) and opportunistic (e.g., *Moraxella catarrhalis*) species. ⏎

1.4.1.1 Early Life Colonization

Colonization of the nasal microbiome begins shortly after birth, influenced by mode of delivery, feeding practices, environmental exposures, and maternal microbiota. Vaginal delivery introduces *Lactobacillus* and other maternal vaginal flora, while cesarean section leads to skin-associated colonizers like *Staphylococcus* (Zhang et al. 2021a). Breastfeeding contributes to a favorable immune environment via maternal antibodies and anti-inflammatory agents, indirectly shaping nasal microbial communities (Camacho-Morales et al. 2021).

By the age of 2, the nasal microbiome begins to stabilize, although it remains more variable than the gut or oral microbiomes throughout life due

to environmental exposures and occasional infections. Infancy represents a sensitive period, as early microbial patterns are associated with future susceptibility to allergies, asthma, and respiratory diseases (Pattaroni et al. 2025).

1.4.1.2 Environmental and Host Factors

Environmental factors such as pollution, climate, urban lifestyle, and seasonal variation significantly influence nasal microbiota (Kim et al. 2014). For instance, exposure to indoor pollutants and smoking alters microbial composition, increasing colonization by pathogens like *Moraxella catarrhalis* (Bakhshaee et al. 2012). In rural or agricultural settings, higher microbial diversity in the air can enhance colonization with non-pathogenic commensals.

Antibiotic use, even systemic, has transient and sometimes permanent effects on nasal microbiota, particularly in children (Toivonen et al. 2021). Hormonal changes, allergic status, age, and the presence of nasal polyps or structural anomalies like a deviated septum could further contribute to inter-individual variation. Vaccination, especially with pneumococcal conjugate vaccines, has reshaped the ecology of nasal microbiota by altering carriage rates of *Streptococcus pneumonia* and allowing microenvironmental shift by non-vaccine serotypes or other organisms (Kielbik et al. 2022).

1.4.2 Functions in Health

The nasal microbiome performs several vital functions that support both local and systemic health. One of its primary roles is to provide a first line of defense against inhaled pathogens. Commensal microbes such as *S. epidermidis* occupy epithelial surfaces and limit pathogen colonization via nutrient competition, production of bacteriocins, and modulation of local immunity (Ortega-Peña et al. 2022).

1.4.2.1 Immune Priming and Regulation

Nasal commensals interact with the mucosal immune system through pattern recognition receptors such as TLRs on epithelial cells and immune cells (Kim et al. 2025). These interactions help maintain immune tolerance and prevent overactivation in response to harmless stimuli. They also stimulate the immune system to respond rapidly to invading pathogens, a function that is particularly important in children and immunocompromised individuals.

Mucus production, regulated partially by microbial signals, serves as a physical barrier and contains secretory IgA, AMPs (e.g., β-defensins, cathelicidins), and lysozyme (Kaiko and Stappenbeck 2014). These components work synergistically with the microbiota to maintain nasal homeostasis. Commensals like *Staphylococcus lugdunensis* produce antimicrobial compounds that inhibit *S. aureus*, a common nasal pathogen and reservoir for systemic infections (Rosenstein et al. 2024).

1.4.2.2 Support for Olfactory and Neurological Health

The olfactory epithelium, located in the upper nasal cavity, is beside the central nervous system via the olfactory bulb. While less studied, evidence suggests that the nasal microbiome may influence olfactory function and possibly impact neurological health through neuroimmune modulation and microbial metabolite signaling (Kumpitsch et al. 2024). Changes in the nasal microbiota have been observed in neurodegenerative diseases, suggesting an emerging nasal–brain axis.

1.4.3 Dysbiosis and Disease

Nasal microbiome dysbiosis is defined as a loss of microbial diversity, overgrowth of opportunistic microbes, or depletion of beneficial ones that has been implicated in several upper and lower respiratory tract diseases.

1.4.3.1 Chronic Rhinosinusitis and Nasal Polyps

Chronic rhinosinusitis (CRS), with or without nasal polyps, is characterized by persistent inflammation of the nasal and sinus mucosa (Stevens et al.

2016). Studies have shown reduced microbial diversity and a shift toward a pathogenic profile dominated by *S. aureus*, *P. aeruginosa*, and anaerobes. *S. aureus* produces enterotoxins that act as superantigens, triggering T-cell activation and sustaining chronic inflammation (Pinchuk et al. 2010).

Commensals like *Corynebacterium tuberculostearicum* and *Staphylococcus* are often depleted in CRS patients, suggesting their protective role. These bacteria may inhibit pathogen growth and modulate inflammatory signaling, offering potential for therapeutic intervention (De Boeck et al. 2019).

1.4.3.2 Allergic Rhinitis and Asthma

Nasal microbiota composition influences allergic sensitization and airway hyperreactivity. Allergic rhinitis is often associated with reduced *Dolosigranulum* and increased *Moraxella*, which may contribute to inflammation and mucosal barrier dysfunction. In children, early-life nasal colonization with Haemophilus and Streptococcus has been linked to increased asthma risk by age five, supporting the idea that nasal microbiota influences immune maturation and allergic outcomes (McCauley et al. 2024).

1.4.3.3 Viral Infections and COVID-19

The nasal cavity is a primary entry point for respiratory viruses, including influenza, rhinoviruses, and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Otter et al. 2022). Dysbiosis may impair mucosal defenses, increasing susceptibility and severity. Post-viral dysbiosis may contribute to prolonged symptoms and anosmia.

1.4.4 Microbiome–Host interactions and Axes

The nasal microbiome engages in multiple host microbe communication pathways that extend beyond the upper airway, shaping respiratory, neurological, and systemic immune processes. As shown in Figure 1.6, microbial metabolites and immune signaling connect the nasal cavity with

the lungs, the brain, and systemic immunity, forming the nasal lung, nasal brain, and nasal immune axes. These interconnected pathways highlight how alterations in nasal microbial communities can contribute not only to local airway health but also to broader physiological and pathological outcomes across multiple organ systems.

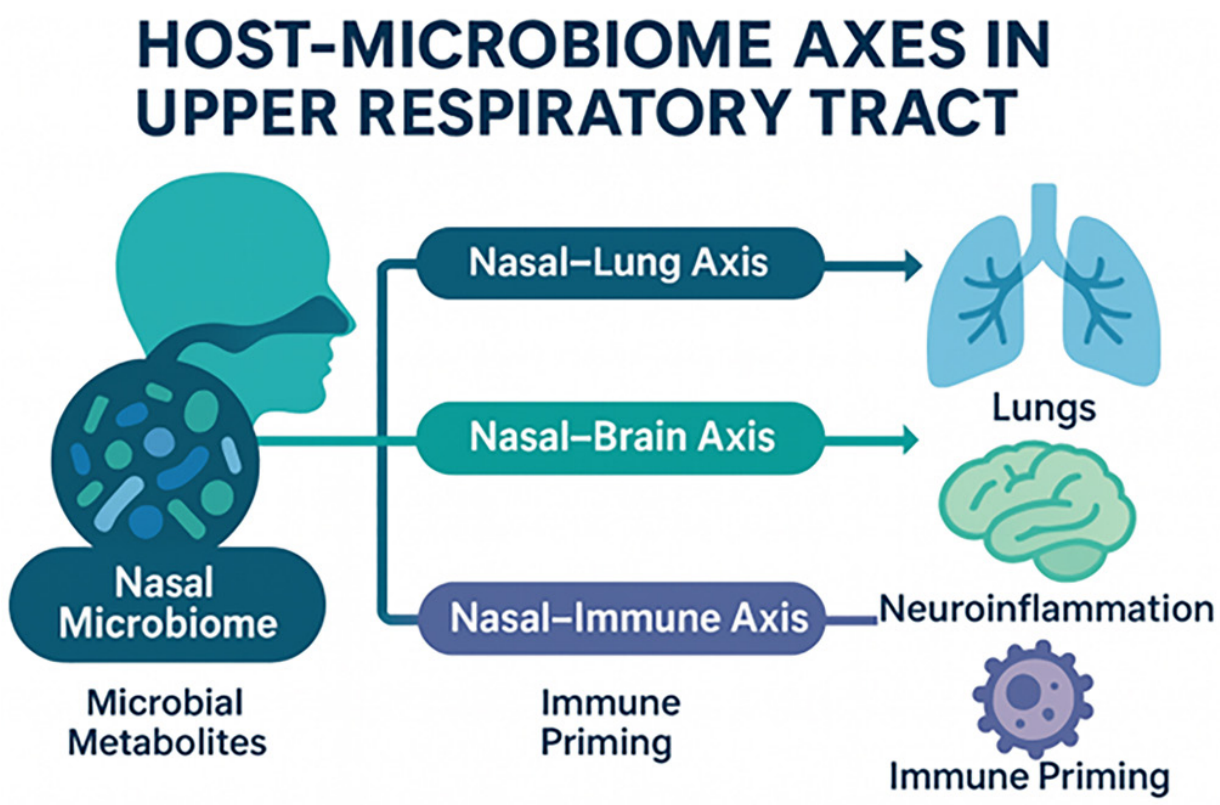

FIGURE 1.6 Diagram of the nasal–lung, nasal–brain, and nasal–immune axes illustrating microbiome influence across systems. ⏎

1.4.4.1 Nasal–Lung Axis

The nasal and lower respiratory microbiomes are anatomically and immunologically linked. Changes in the nasal microbiota can lead to and influence lower airway inflammation and infection. In diseases like chronic obstructive pulmonary disease (COPD) and cystic fibrosis, nasal microbial composition often predicts changes in the lung microbiota (Daniel E et al. 2018). Nasal colonization with pathogens such as *P. aeruginosa* is a known

risk factor for subsequent lung infections in vulnerable patients (Nickerson et al. 2024).

1.4.4.2 Nasal–Brain Axis

The anatomical closeness of the nasal cavity to the central nervous system raises the possibility of microbial or inflammatory signaling across the cribriform plate. Neurotropic viruses and microbial metabolites may reach the brain via the olfactory nerve, potentially contributing to neuroinflammatory conditions. Studies have reported altered nasal microbiota in individuals with Parkinson's disease, although causal relationships remain under investigation (Pal et al. 2021).

1.4.4.3 Systemic Immune Modulation

The nasal microbiome interacts with systemic immunity through mucosal immune responses and cytokine signaling. Nasal microbiota induced immune preparation that influence responses to vaccines and infections elsewhere in the body. This host systemic interaction makes the nasal cavity a promising target for immunomodulatory strategies (Gonçalves et al. 2022).

1.4.5 Therapeutic and Preventive Strategies

Rapid advances in microbiome science have paved the way for innovative therapeutic and preventive strategies aimed at restoring and maintaining nasal microbial balance. As research shifts from observational studies to translational applications, new approaches including probiotic interventions, microbiome-informed diagnostics, and targeted therapies are emerging as promising tools to combat dysbiosis and improve respiratory health. These strategies highlight the potential of precision medicine in leveraging the nasal microbiome for disease prevention and treatment.

1.4.5.1 Probiotics and LBTs

Although still in early development, nasal probiotic strategies show significant therapeutic potential. *Dolosigranulum* and *Corynebacterium* species are being explored as potential LBT agents due to their association with health and resistance to pathogens. Intranasal delivery of such probiotics could restore microbial balance in conditions like CRS or prevent post-viral complications (Brugger et al. 2020).

1.4.5.2 Microbiota-Informed Diagnostics

Nasal microbiome profiling using 16S rRNA sequencing or shotgun metagenomics can aid in disease diagnosis, estimation, and prediction (Matchado et al. 2024). Biomarker panels based on microbial signatures are under investigation for differentiating CRS subtypes or predicting treatment outcomes. Machine learning models trained on microbiome data are also being tested for early detection of respiratory conditions.

1.4.5.3 Targeted Therapies and Microbiome Restoration

Therapeutic strategies may include nasal sprays containing bacteriocins, AMPs, or quorum-sensing inhibitors that selectively inhibit pathogens without disrupting beneficial commensals (Newstead et al. 2020). Personalized microbiome modulation based on individual microbial profiles is an emerging concept. Antibiotic stewardship and environmental modifications such as air quality improvement and avoidance of overuse of nasal antiseptics are essential to preserve microbial diversity and prevent dysbiosis. Ongoing clinical trials will determine the efficacy and safety of these novel interventions.

1.5 Ear Microbiome

The human ear is divided into three sections: the external ear, which includes the ear canal or external auditory canal (EAC), the middle ear, and the inner ear (Figure 1.7) (Sundar et al. 2021). The EAC measures about 2.5–3.0 cm in length and consists of two segments: the distal cartilaginous

canal and the proximal bony canal. The outer cartilaginous third of the EAC is covered by thick, flexible skin and contains hair follicles as well as ceruminous apocrine and sebaceous glands, with around 1,000–2,000 ceruminous (modified apocrine glands) and sebaceous glands (Sjövall et al. 2021; Lee et al. 2022). Histologically, these components closely resemble those found in the outer skin (Lee et al. 2022). However, the skin of the inner bony two-thirds of the canal is continuous with the epithelium of the tympanic membrane and is characterized as being thin, immobile, and lacking hair follicles or glands. The EAC is naturally narrow, making it susceptible to blockage by earplugs or debris, which may have different characteristics compared to the external, open environment. Additionally, the ceruminous glands in the EAC produce a waxy substance known as cerumen, which consists of long-chain fatty acids, alcohols, squalene, and cholesterol (Lee et al. 2022).

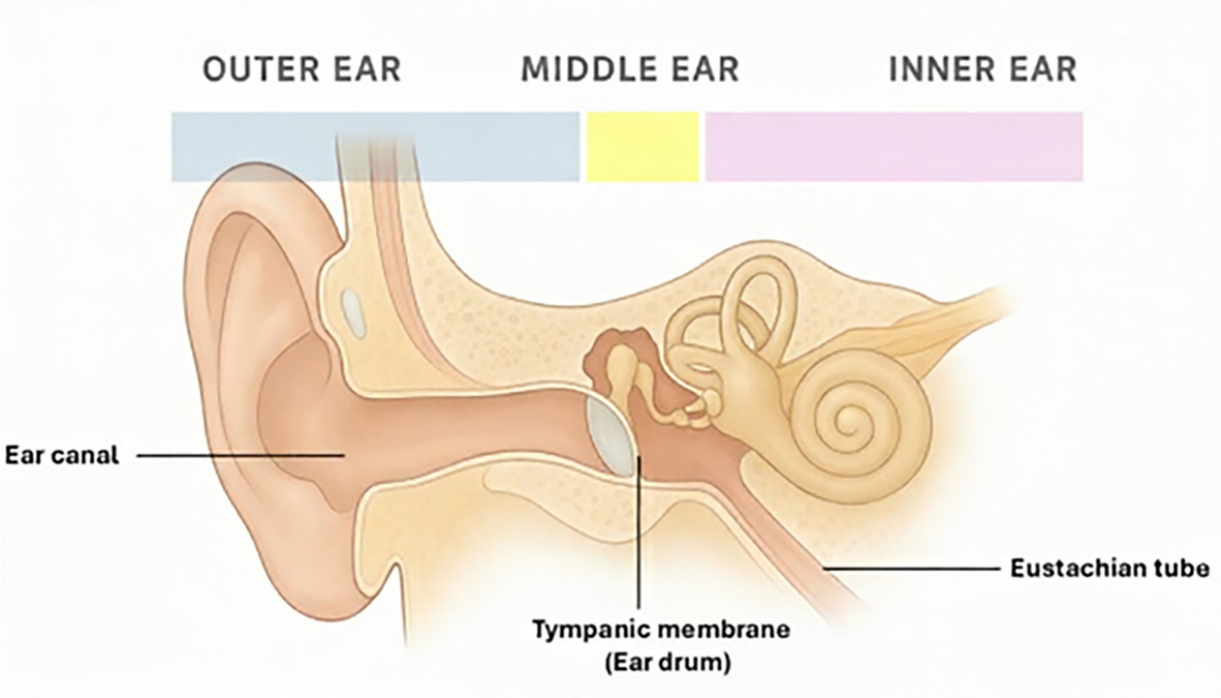

FIGURE 1.7 Anatomy of the human ear showing the outer ear, middle ear, and inner ear regions. ⏎

The EAC features a distinctive self-cleaning process, where the shed epithelium gradually moves outward to the membranous section and combines with glandular secretions to create cerumen. There are two main types of cerumen: (1) the wet variety, which is prevalent among Europeans and Africans, characterized by its brown, sticky texture and high lipid content, and (2) the dry variety, commonly found in East Asians, which is grayish and protein-rich. This waxy material serves a dual purpose: (1) it lowers the pH of the EAC, thereby maintaining an acidic environment that acts as an antimicrobial defense, and (2) it imparts water-repellent properties to the skin of the ear canal. Furthermore, cerumen aids in the removal of the sloughed epithelium, as well as in moisturizing, lubricating, and protecting the EAC (Sjövall et al. 2021; Lee et al. 2022).

The skin of the EAC is directly linked to cartilage or bone and is also connected to the eardrum. Functionally, the skin in the ear canal sheds tissue fragments that originate from the eardrum and nearby skin (Kim et al. 2022). The EAC is the most studied site of the ear microbiome. Culture-based studies assumed staphylococci, coryneforms, and streptococci as predominant commensals of the EAC (Stroman et al. 2001; Sjövall et al. 2021; Kim et al. 2022). Recently, a growing number of next-generation sequencing (NGS) culture-independent studies suggested *Alloiococcus otitis*, *Staphylococcus auricularis*, *Corynebacterium otitidis*, *Propionibacterium acnes*, and *Turicella otitidis* as predominant commensal microbiota of the healthy EAC (Sjövall et al. 2021; Kim et al. 2022). *Muribaculum, Rothia, Cutibacterium*, and *Pseudomonas* were also detected (Kim et al. 2022).

It was once thought that the healthy middle ear was free of any microorganisms. However, recent studies using molecular techniques have indicated the presence of a varied microbiome in the middle ear even when no disease is present (Westerberg et al. 2009; Fekete et al. 2024). Analyses using NGS 16S rRNA have identified *Firmicutes, Actinobacteria*, and *Proteobacteria* as the most prevalent phyla in a healthy middle ear (Minami et al. 2017; Fekete et al. 2024). In addition, several species that may be protective to the middle ear, such as lactic acid bacilli and

Dolorsigranulum, have been reported. These might play a role as a novel treatment modality in probiotic development (van den Broek et al. 2019; Nogues et al. 2020).

However, bacterial colonization of the middle ear is still debated (Jervis-Bardy et al. 2019; Fekete et al. 2024). Research on the middle ear microbiome often lacks support from culture-based methods and includes species that frequently contaminate low-biomass sequencing data (Jervis-Bardy et al. 2019). Many taxa identified in previous studies of the healthy middle ear microbiome were not found using parallel culture techniques, even though these species can be easily cultured with standard methods (Neeff et al. 2016; Minami et al. 2017; Jervis-Bardy et al. 2019). Additionally, many of the reported taxa belong to genera commonly linked to contamination of sequencing reagents and are very unlikely to exist in the human body, such as Bradyrhizobium (Jervis-Bardy et al. 2019). In 2019, Jervis-Bardy and colleagues employed a variety of methods, such as microscopy, diagnostic culture, dispersion analysis, quantitative polymerase chain reaction (PCR), and amplicon sequencing, to directly evaluate whether the bacterial taxa identified in the healthy middle ear ecosystem through molecular techniques were artifactual. Their findings indicated that bacterial quantification using qPCR revealed that the bacterial presence in the middle ear was not greater than that in negative controls (sterile irrigation buffer and DNA extracts from empty swabs). Additionally, Jervis-Bardy and colleagues discovered that the microbiota profiles in middle ear samples were predominantly composed of taxa previously identified as sequencing contaminants, like *Methylobacterium, Acinetobacter*, and *Pseudomonas*, or genera typically linked to extreme marine niche, such as *Marinilactibacillus, Psychrobacter*, and *Shewanella*, along with other genera that seemed biologically implausible in this context (Jervis-Bardy et al. 2019). The presence of *Ruminococcaceae* and *Pseudomonas* in negative controls further reinforced the likelihood that the bacterial taxa in middle ear samples were spurious. In concordance, Neeff and colleagues identified diverse bacterial taxa in the microbiome of healthy middle ears, including the genera *Staphylococcus*, *Propionibacterium*, *Streptococcus,*

Pseudomonas, Escherichia-Shigella, Novosphingobium, and *Burkholderia*. However, they failed to isolate any of these taxa by traditional culture techniques (Neeff et al. 2016). Similarly, Minami and coworkers used NGS to study mucosal samples of the middle ear from children and adults. They detected diverse bacterial taxa by sequencing, particularly members of the phylum Proteobacteria and the genus *Staphylococcus*. However, they were only able to isolate scant bacteria by traditional culture techniques (Minami et al. 2017). Jervis-Bardy and colleagues' (2019) conclusions cast substantial doubt on earlier NGS research identifying the microbiome of a healthy middle ear, as they found no significant difference in bacterial abundance of middle ear (ME) samples above those in healthy controls (Jervis-Bardy et al. 2019). Detected taxa included *Pseudomonas*, *Methylobacterium*, and *Acinetobacter*, which were among the commonly recognized sequencing contaminants. Besides, using linear regression of dominant ME taxa, they reported a negative correlation between bacterial load and relative abundance, which is consistent with contamination. They were unable to detect any bacteria in any middle ear sample by diagnostic culture or microscopy.

1.5.1 Most Common Ear Conditions Associated with Altered Microbiome

The most prevalent ear infections are otitis externa (OE), commonly known as "swimmer's ear," and otitis media (OM) (Figure 1.8). OE involves an infection and inflammation of the outer ear canal, which is often accompanied by pain, redness, and tenderness, sometimes with purulent discharge. OE can manifest as either an acute (AOE) or chronic (COE) condition. AOE is typically attributed to bacteria, whereas COE is more frequently caused by fungi (Osguthorpe and Nielsen 2006; Burton et al. 2022). OM is identified by inflammation in the middle ear, along with fluid buildup beneath the tympanic membrane, which is triggered or exacerbated by the proliferation of bacterial, fungal, and/or viral pathogens, resulting in infection, swelling, redness, and pain (Klein 2015; Burton et al. 2022). The

range of OM includes acute otitis media (AOM), recurrent acute otitis media (RAOM), otitis media with effusion (EOM), chronic otitis media with effusion (COME), and chronic suppurative otitis media (CSOM) (Schilder et al. 2016; Nogues et al. 2020). All these conditions are marked by middle ear effusion (MEE), which contains a diverse microbiome that has been extensively researched to gain a better understanding of its pathogenesis and etiology (Ngo et al. 2016; Nogues et al. 2020).

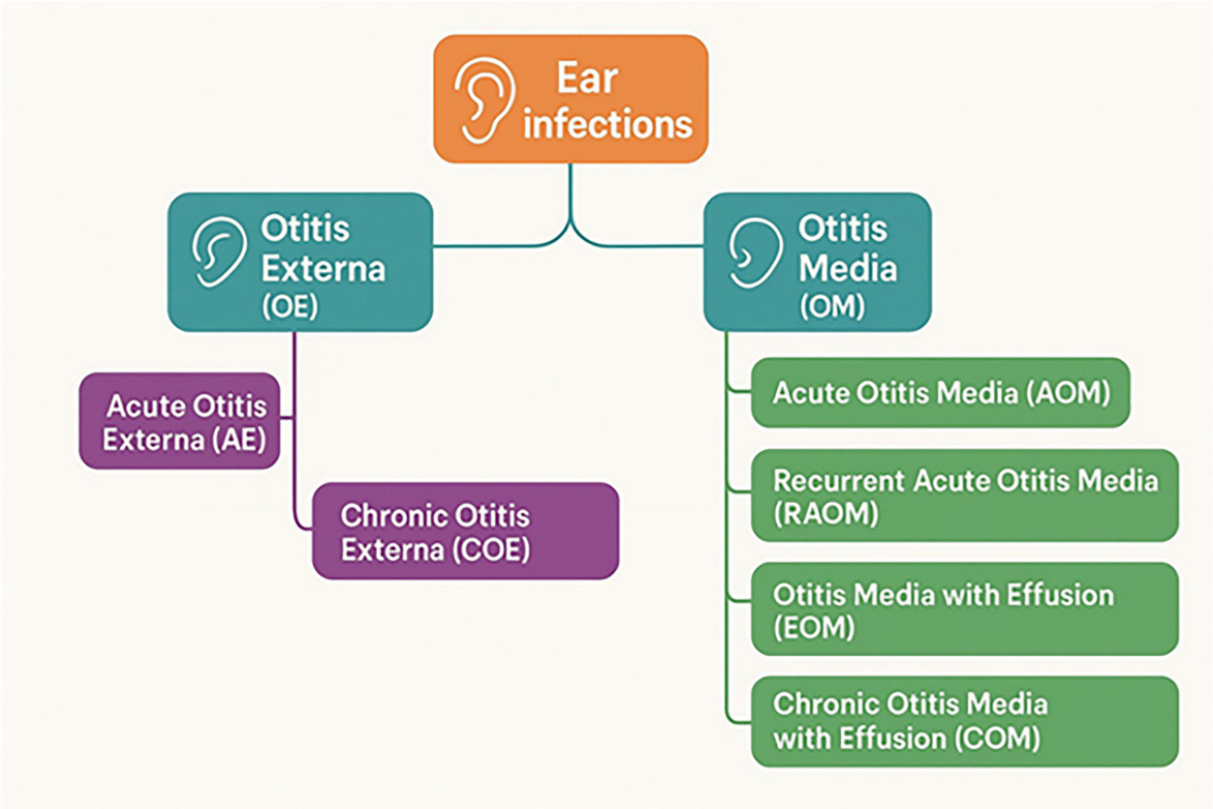

FIGURE 1.8 The most prevalent ear infections are OE, commonly known as "swimmer's ear," and OM. OE can manifest as either an AOE or COE condition. The range of OM includes AOM, RAOM, EOM, COME. ⏎

Although the tympanic membrane remains intact in OM, the discovery of microbes in the middle ear prompts questions about their source. It is generally believed that these infections stem from bacteria moving from the nasopharynx through the eustachian tube (Vergison et al. 2010; Fagö-Olsen et al. 2019; Jervis-Bardy et al. 2019). It has been suggested that bacteria

from the EAC may also play a role in the development of OM, entering the middle ear during the healing process of perforations in individuals who experience RAOM with perforation (Westerberg et al. 2009; Jervis-Bardy et al. 2019). However, the idea that bacteria might colonize the middle ear mucosa without inflammation or active disease is more debatable (Jervis-Bardy et al. 2019).

1.5.2 The OE Microbiome versus the OM Microbiome

OM and OE are often considered to arise from polymicrobial infections with biofilm formation, leading to antibiotic-resistant phenotypes. This may explain why antibiotic treatments sometimes offer limited benefits, as well as reduced effectiveness due to the rise of resistant microbes or the use of incorrect antimicrobials (Burton et al. 2022).

The OE microbiome is reported, with *P. aeruginosa*, *S. aureus*, and *Corynebacterium* as predominant members of the bacterial community (Burton et al. 2022; Kim et al. 2022). *Staphylococcus haemolyticus* and *Klebsiella* were also reported as primary bacterial pathogens in OE (Burton et al. 2022). On the other hand, *S. pneumonia*, *Haemophilus influenza*, and *M. catarrhalis* are historically the most isolated bacteria from the MEE of children with OM (Ruohola et al. 2006; Ngo et al. 2016; Nogues et al. 2020; Burton et al. 2022). *Streptococcus pyogenes* was also found to be commonly present in OM (Jervis-Bardy et al. 2019). NGS studies have confirmed these as primary otopathogens, in addition to *A. otitidis*, *S. auricularis*, and *T. otitidis*, which are being detected in OM at significant relative abundances (Sillanpää et al. 2017; Burton et al. 2022). However, the bacterium *A. otitidis*, which is found in the microbiome of both healthy and diseased ears, remains controversial as a pathogen. Further NGS analysis is needed to reveal how microbial species can be, at times, either pathogenic or benign (Burton et al. 2022).

An interesting study by Burton et al. in 2022 investigating both ear bacteriome and mycobiome, reported *C. acnes* as the most abundant species in healthy ear bacteriome, whereas *P. aeruginosa* dominated in OE samples,

followed by *C. acnes*, *S. aureus*, and *Corynebacterium jeikeium*. For the OM samples, *P. aeruginosa* was detected as the most prevalent species, followed by *S. aureus*, *C. jeikeium*, and *C. acnes*. A significant increase in bacterial biomass for both OE and OM samples was observed compared to the healthy samples, as revealed based on the 16S copy numbers. However, no significant difference was detected for the bacteriome across the study groups as revealed by alpha diversity. For the mycobiome, Burton et al. in 2022 reported *Malassezia* sp. as the most abundant fungal species in the healthy ear microbiome. The most dominant species were *Malassezia arunalokei*, followed by *M. restricta*, *Malasseziales* sp., and *M. globosa*. Taxa found in the healthy ear mycobiome were also present in otitis cases, though with lower abundances and frequencies. In addition, *Aspergillus sp*. were the most abundant fungal species among the OE samples, followed by *M. restricta*, *M. arunalokei*, *Candida albicans*, and *Malasseziales* sp. In the OM group, *M. restricta* was the most abundant species, followed *by M. arunalokei*, *Aspergillus* sp., and *C. parapsilosis*. There was a significant increase in the number of species detected in clinically affected samples of both OE and OM compared to the healthy samples. Interestingly, OM and OE groups had a significantly lower fungal biomass as measured by ITS copy numbers compared to the healthy group. Therefore, a healthy mycobiome was characterized by a lower fungal diversity and a higher fungal biomass compared to clinically affected samples. This is the opposite to what was found for the bacterial biomass (Burton et al. 2022).

The EOM microbiome was found to be dominated by six core bacterial genera: *Alloiococcus, Haemophilus, Streptococcus, Corynebacterium, Moraxella*, and *Sphingobium*, as revealed by a recent study by Fekete et al. (2024). *Alloiococcus* was the most predominant taxon, whereas *Haemophilus* was the most prevalent otopathogen, followed by *Streptococci* and *Moraxella*, respectively. It is noteworthy that *Alloiococcus, Haemophilus*, and *Pasteurella* were reported as predominant colonizers of the ear microbiome, if they were present.

Research on the COME microbiome is controversial. An investigation conducted by Kolbe et al. (2019) on the middle ear microbiome in children

with COME revealed the diverse and highly variable taxa comprising the MEE with no core microbiome. Based on the mean relative abundance, the most predominant genera were *Haemophilus*, *Turicella*, and *Moraxella*, although these genera were only present in approximately 50% of samples. However, the most prevalent genera were *Achromobacter* and *Pseudomonas*. Children with asthma or bronchiolitis showed a significantly higher abundance of *Staphylococcus, Haemophilus*, and *Moraxella*, whereas *Alloiococcus* and *Turicella* were significantly lower. However, earlier research reported Alloicoccus, Haemophilus, or *Moraxella* to predominantly colonize the middle ear microbiome as a single genus (Güvenç et al. 2010; Jervis-Bardy et al. 2015; Kolbe et al. 2019).

1.6 Gut Microbiome

The human gut microbiome is a highly diverse and dynamic microbial community located in the colon. It consists primarily of bacteria, but also archaea, fungi, viruses, and unicellular eukaryotes that exist predominantly in a mutualistic relationship with the host (Khalil et al. 2024). Far from being passive residents, these microbes, together with their collective genetic material (the metagenome) and metabolic products, are now regarded as a functional organ. The gut metagenome contains more than 3 million genes, approximately 150–200 times the number found in the human genome, highlighting its significant genetic and metabolic influence on host physiology (Qin et al. 2010). This microbial organ forms complex bidirectional communication pathways with other body systems, playing a crucial role in regulating diverse physiological processes vital to human health (Zeng et al. 2025).

1.6.1 Composition and Diversity of the Gut Microbiome

Microbial communities along the gastrointestinal tract exhibit distinct spatial organization, shaped by localized physicochemical gradients such as pH, oxygen tension, nutrient availability, osmolarity, and mechanical shear

forces. These gradients determine both longitudinal (mouth-to-colon) and radial (mucosal vs. luminal) microbial patterns. The oral cavity is among the most densely and taxonomically diverse microbial ecosystems in the human body. Humans swallow approximately 1–1.5 L of saliva daily, delivering millions of oral microbes into the GI tract. However, more than 99% of these microbes fail to survive transit through the acidic environment of the stomach and the digestive enzymes of the small intestine. Survival of ingested microbes depends critically on host factors such as gastric acidity, bile secretion, digestive enzymes, and gastrointestinal motility (Tropini et al. 2017; Schmidt et al. 2019; Barlow et al. 2021).

Within the gut (i.e., colon), the microbiome is dominated by obligate anaerobes, where the *Firmicutes* and *Bacteroidetes* are the dominant phyla. However, gut microbiome is highly personalized, as inter-individual variation in specific taxa and their abundances can be profound. Non-pathological factors shape this personalization, including genetics, early life events (mode of delivery, breastfeeding), and diet, as exemplified by the contrast between *Prevotella*-rich enterotypes in plant-based diets and Bacteroides-dominant profiles linked to diets high in animal fats and proteins. Additionally, lifestyle and environment have a significant impact on shaping a highly individualized gut ecosystem.

Antibiotic exposure profoundly disrupts gut microbiome composition, typically reducing microbial diversity and depleting beneficial taxa such as *Bifidobacterium* and butyrate-producing *Faecalibacterium prausnitzii*, while enabling overgrowth by opportunistic and potentially pathogenic members of *Proteobacteria* (e.g., *Enterobacteriaceae*). These perturbations can persist for weeks or months, with delayed or incomplete recovery even after therapy cessation. Functionally, antibiotic use reduces SCFA output, compromising mucosal integrity, metabolism, and host immune homeostasis. Host–microbiome interactions are further disturbed, with evidence for heightened mucosal immune activation, including elevated neutrophils and Th17/Th22 responses. In early life, such disturbances may have enduring consequences, including increased risk of metabolic and inflammatory disorders; for example, early antibiotic exposure correlates

with later obesity, insulin resistance, and even inflammatory bowel disease (IBD). While resilience mechanisms, including microbial redundancy and recolonization, may eventually restore equilibrium, recovery may be incomplete, prolonged, or even shift to alternative stable states (Manuzak et al. 2020; Nel Van Zyl et al. 2022; Wurm et al. 2024; Li et al. 2025; Cusumano et al. 2025).

1.6.2 Microbiome Development across the Lifespan

At birth, the newborn's gut begins a rapid colonization process shaped predominantly by mode of delivery and early environmental exposures. Infants delivered vaginally acquire microbes resembling their mother's vaginal and fecal microbiota, notably *Lactobacillus*, *Bifidobacterium*, and *Bacteroides*, whereas cesarean-delivered infants are more often colonized by maternal skin-associated taxa such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*. This initial microbial seeding profoundly influences early gut community assembly, which continues evolving through the first three years of life into a more adult-like configuration (Montoya-Williams et al. 2018; Suárez-Martínez et al. 2023).

During infancy, especially in exclusively breastfed infants, the gut microbiota is characterized by low overall diversity and a strong dominance of *Actinobacteria*, particularly *Bifidobacterium*, which thrive on human milk oligosaccharides (HMOs) that they uniquely ferment to produce beneficial metabolites such as acetate, lactate, and indole-3-lactic acid. Compared to breastfed infants, formula-fed infants exhibit higher diversity and relatively elevated abundances of *Streptococcus, Lachnospiraceae, Veillonella*, and *Clostridioides*. The introduction of solid foods triggers a phylogenetic shift, with increasing Bacteroidetes, reflecting adaptation to more complex dietary substrates (Lee et al. 2015; Ma et al. 2020).

In older adults, the gut microbiome undergoes significant compositional and functional remodeling, marked by both increases and declines in diversity depending on health status. Some studies, particularly among healthy centenarians, report higher alpha diversity and enrichment in taxa

like *Akkermansia*, contrasting with relatively lower levels in *Faecalibacterium, Bacteroidaceae*, and *Lachnospiraceae*. In frail elderly individuals, gut dysbiosis is characterized by reduced beneficial commensals (e.g., *Bifidobacterium*, SCFA producers), increased gut permeability, inflammation ("inflammaging"), and expansion of opportunistic taxa, hallmarks of unhealthy aging. Intriguingly, distinct microbial aging trajectories have been observed: healthy aging is associated with gradual loss of dominant core taxa (e.g., *Bacteroides*), increased compositional uniqueness, and lower mortality risk, suggesting that a personalized, evolving microbiome may support longevity (Saraswati and Sitaraman 2015; Badal et al. 2020; Ragonnaud and Biragyn 2021; Wilmanski et al. 2022; Zhang et al. 2025).

1.6.3 Functional Roles of the Gut Microbiome

The gut microbiome plays a pivotal role in digestion by fermenting indigestible dietary fibers into SCFAs, primarily acetate, propionate, and butyrate, which serve as essential energy substrates for colonocytes and modulate host metabolism. SCFAs contribute to maintaining intestinal epithelial integrity, regulating barrier function via induction of tight-junction proteins, and supporting overall gut health. Additionally, gut microbes synthesize essential vitamins such as vitamin K and certain B vitamins, which are absorbed and utilized by the host (Yao et al. 2022; Ney et al. 2023).

Gut-derived metabolites, especially SCFAs, have immune-modulatory functions: they enhance mucosal immunity by energizing epithelial cells, promoting mucus secretion, and facilitating barrier resilience. SCFAs also shape both innate and adaptive immune responses, promoting regulatory T cell differentiation, modulating macrophage and dendritic cell function, and regulating cytokine profiles, thus maintaining immune homeostasis and suppressing unwarranted inflammation (Yao et al. 2022; Akhtar et al. 2022; Ney et al. 2023).

The gut microbiome communicates bidirectionally with the central nervous system through the gut-brain axis (Figure 1.9), which includes neuronal (e.g., vagus nerve), immune, endocrine, and metabolic pathways. Microbial metabolites, such as SCFAs and neurotransmitter precursors, can influence central nervous system (CNS) function and modulate neuroinflammation, behavior, mood, and neurodevelopmental and neurodegenerative disease processes (Ma et al. 2019; Ullah et al. 2023).

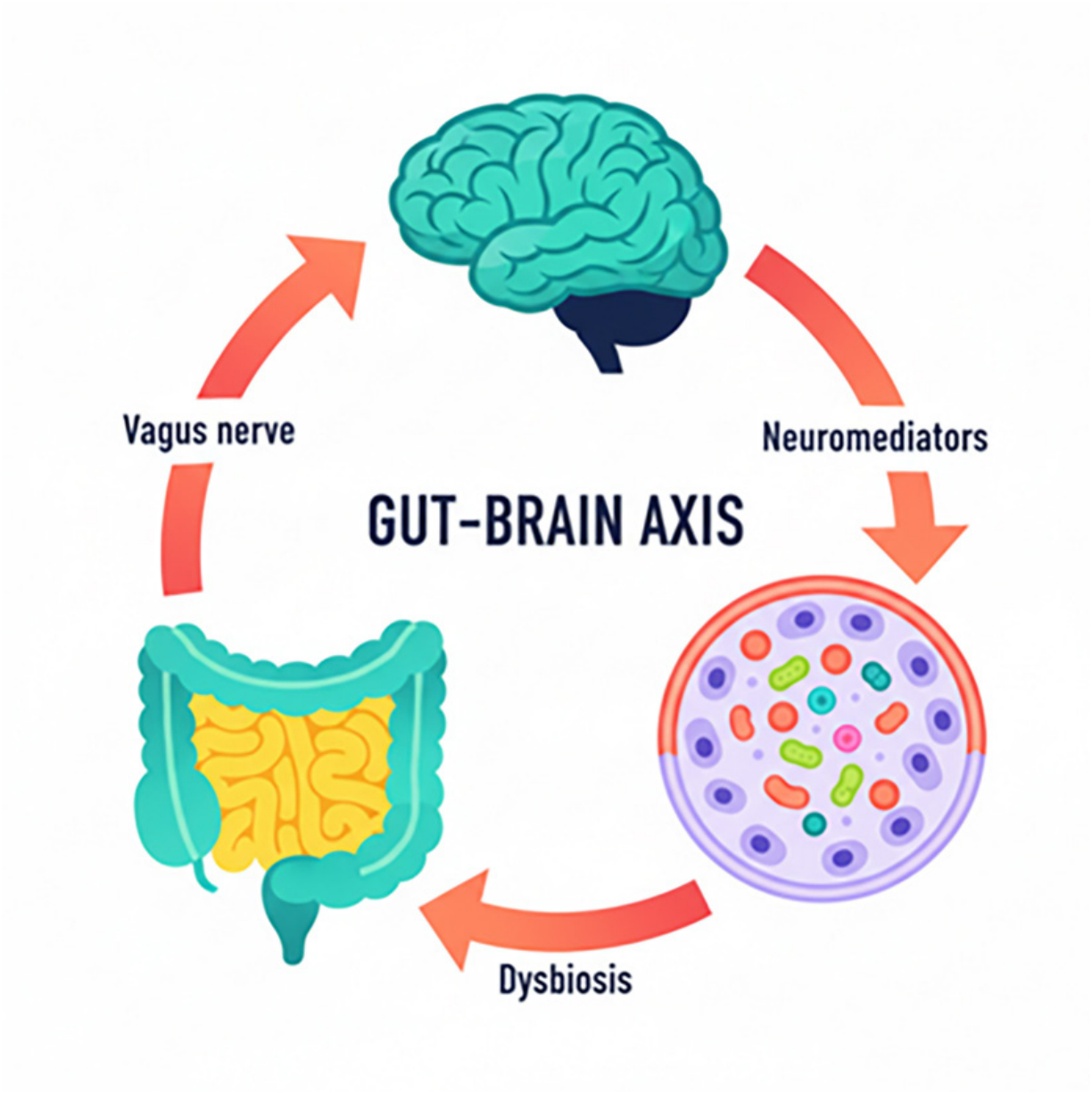

FIGURE 1.9 Schematic representation of the gut–brain axis. The diagram illustrates the bidirectional communication between the gut, brain, and intestinal microbiota. ⏎

Beyond SCFAs, the gut microbiome generates a spectrum of bioactive compounds with significant physiological and pathophysiological implications. These include:

- Gases (e.g., hydrogen, methane, hydrogen sulfide) which influence intestinal motility and redox balance.
- Trimethylamine N-oxide (TMAO) derived from microbial metabolism of dietary choline and carnitine—implicated in cardiovascular and metabolic risk.
- Polyamines (e.g., spermidine, putrescine), which regulate cell proliferation and gut epithelial homeostasis.
- Phenolic derivatives, produced from polyphenols by microbial catabolism, which modulate host oxidative stress responses and inflammatory pathways.

On the other hand, dysbiosis refers to a disruption in the structure or function of the gut microbiome encompassing reduced microbial diversity, alterations in taxonomic composition (e.g., overrepresentation of *Proteobacteria*), or functional imbalances deviating from a state of eubiosis. It can result from various factors, including antibiotic exposure, dietary shifts, stress, infection, medication use, and genetic predisposition. Dysbiosis contributes to compromised barrier function, immune dysregulation, and metabolic dysfunction, and is implicated in the pathogenesis of diseases such as IBD and metabolic disorders (Bidell et al. 2022; Shen et al. 2025).

1.6.4 Gut Microbiome-Associated Diseases

While dysbiosis is a common feature across many diseases, its specific microbial patterns and functional outcomes are disease-specific. This highlights the necessity for precision medicine approaches, where interventions are tailored to the unique microbial signatures of individual conditions. Additionally, microbiome profiling holds promise as a diagnostic tool for disease stratification and personalized therapies.

1.6.4.1 Inflammatory Bowel Disease

IBD comprises two main entities, Crohn's disease (CD) and ulcerative colitis (UC), which present as chronic, relapsing–remitting inflammation of the gastrointestinal tract. UC is limited to the colon with continuous mucosal inflammation beginning in the rectum, whereas CD can involve any segment from mouth to anus with typically segmental, transmural inflammation and a propensity for strictures and fistulas. Although etiology is multifactorial and not fully defined, converging evidence implicates genetic susceptibility, disruption of the epithelial barrier, dysregulated innate and adaptive immunity, and environmental exposures in disease pathogenesis and its global rise in incidence (Ungaro et al. 2017; Kaplan and Windsor 2021).

IBD is strongly associated with intestinal dysbiosis characterized by reduced alpha diversity, loss of beneficial *Firmicutes* (notably the butyrate-producer *F. prausnitzii*), and expansion of *Proteobacteria* (e.g., *Enterobacteriaceae*), alongside reproducible functional alterations (depleted SCFA pathways, perturbed bile-acid metabolism, and heightened oxidative stress signatures). Depletion of *F. prausnitzii* correlates with greater inflammatory activity and postoperative or clinical relapse, and this taxon exhibits anti-inflammatory properties in experimental systems. Beyond bacteria, virome alterations (expansion of *Caudovirales* bacteriophages) are disease-specific in CD and UC. Importantly, while microbes can precipitate and perpetuate mucosal immune activation, inflammation itself feeds back to intensify dysbiosis, so causality is bidirectional and context dependent (Sokol et al. 2008, 2009; Ni et al. 2017; Lloyd-Price et al. 2019).

1.6.4.2 Obesity and Metabolic Disorders

Multiple lines of evidence link obesity to dysbiosis. Classic gnotobiotic experiments showed that an "obese microbiota" from ob/ob mice or from obese human donors confers greater adiposity to germ-free recipients than a "lean microbiota," implicating community configuration as a causal determinant of host energy balance (Turnbaugh et al. 2006).

Mechanistically, obesity-associated microbiomes exhibit shifts in metabolic capacity (e.g., carbohydrate and bile-acid metabolism) and can

promote low-grade inflammation via increased gut permeability and endotoxin exposure ("metabolic endotoxemia"), which together impair insulin sensitivity and favor weight gain. In humans, reduced microbial gene richness and specific compositional signatures correlate with greater adiposity, dyslipidemia, and inflammatory phenotypes, supporting the clinical relevance of dysbiosis (Cani et al. 2007, 2008; Ridaura et al. 2013).

Dietary patterns are a primary, modifiable factor for influencing the gut microbiome. For example, a Mediterranean-style diet in people with overweight or obesity has been shown to consistently change the gut microbiome and metabolome, leading to improved cardiometabolic markers. Increasing the delivery of fermentable substrates (like inulin-propionate ester) to the colon can enhance the release of satiety hormones and help limit weight gain, as demonstrated in clinical trials. Similarly, structured exercise is linked to greater microbial diversity in athletes and can lead to beneficial shifts in the gut microbiota, although diet often plays a role in these effects (Clarke et al. 2014; Chambers et al. 2019; Meslier et al. 2020).

Medical therapies can also significantly alter the gut ecosystem. Bariatric surgery causes a long-lasting change in the gut microbiota. When this altered microbiota is transferred to germ-free animal models, it can reduce body fat (adiposity). Other promising microbiota-directed therapies include fecal microbiota transplantation (FMT) from a lean donor, which has been shown to temporarily improve insulin sensitivity in individuals with metabolic syndrome. Additionally, early human trials using a pasteurized formulation of the bacterium *Akkermansia muciniphila* have shown safety and improvements in both insulin sensitivity and blood lipid levels (Vrieze et al. 2012; Depommier et al. 2019).

1.6.4.3 Diabetes Mellitus

Again, an imbalance in the gut microbial community can trigger local and systemic inflammation and compromise the gut barrier. Such chronic inflammation is a common factor in the development and progression of various forms of diabetes, including type 1, type 2, and gestational diabetes.

A specific example provided is a single pathobiont from the family Muribaculaceae that, when enriched in the gut of mice, was sufficient to trigger local inflammation in the pancreas, leading to the destruction of insulin-producing β cells and the development of insulin-dependent diabetes. Bacteria can secrete extracellular vesicles (EVs) that contain metabolites and other molecules. Recent research suggests that these EVs can induce insulin resistance and impair glucose metabolism in skeletal muscle and adipose tissue, thereby contributing to the development of type 2 diabetes. The gut microbiome also influences various hormonal and immune pathways that are central to diabetes pathogenesis. The gut is home to enteroendocrine cells (EECs) that produce hormones like glucagon-like peptide-1 (GLP-1) and other incretins, which are key regulators of glucose metabolism. The microbiome can also affect the endocannabinoid (eCB) system, which regulates peripheral glucose and lipid metabolism in adipose tissue, the liver, and the pancreas. Furthermore, some gut microbes can affect the production of specific AMPs, which can either protect against or contribute to autoimmune destruction of pancreatic beta-cells (Byndloss et al. 2024; Garcia-Gutierrez et al. 2024).

1.6.4.4 Cancer

The relationship between gut dysbiosis and cancer is a complex two-way street, where the gut microbiome can both promote and inhibit tumor growth. This dynamic interaction involves several key mechanisms. First, the gut microbiome plays a crucial role in regulating the immune system. Dysbiosis, or an imbalance in these microbes, can lead to chronic inflammation, which creates a favorable environment for cancer development. Second, the microbiome produces metabolites that have a direct impact on cancer. For example, some bacteria like *F. nucleatum* secrete substances that can promote the proliferation of colorectal cancer cells. In contrast, beneficial bacteria produce SCFAs, which are known to have anti-tumor effects. Bacterial enzymes can also affect the metabolism of certain drugs, either activating inactive chemotherapy agents or altering hormone levels, which is particularly relevant in hormone-sensitive cancers.

On the other hand, the composition of the gut microbiota can significantly determine the success of cancer treatments, especially immunotherapies. A diverse gut microbiome is often correlated with a better response to immune checkpoint inhibitors. Specific beneficial species like *A. muciniphila* and *Bifidobacterium* have been shown to enhance the efficacy of these treatments by activating key immune pathways. Conversely, the presence of certain bacteria, such as *Actinomyces* and *Senegalimassilia*, is associated with a poor response to immunotherapy (Asseri et al. 2023; Sun et al. 2025).

1.6.4.5 Mental Health and Neurological Disorders

Recent research has shown a clear link between mental health and an imbalance in the gut microbiome. The gut and brain are in constant communication through the gut–brain axis, which involves neural, immune, and metabolic pathways. This connection allows gut microbes to influence the brain and behavior both directly (via the vagus nerve) and indirectly through the production of various chemicals, hormones, and metabolites. For example, the gut microbiome is responsible for producing a variety of important chemicals. It synthesizes or triggers the release of various neurotransmitters, including serotonin, melatonin, dopamine, and GABA, as well as their precursors and related compounds that can affect mood and anxiety. An imbalanced microbiome can disrupt these communication pathways, contributing to the development of conditions like depression and anxiety (Sonali et al. 2022).

The relationship between gut dysbiosis and Alzheimer's and Parkinson's diseases is a mechanistic one, primarily mediated by bacterial proteins and metabolites that impact the brain via the gut–brain axis. Gut dysbiosis, which is an imbalance in gut microbes, can lead to the production of bacterial proteins like curli and gingipain. Curli has been shown to "cross-seed" and accelerate the aggregation of the pathological proteins amyloid-beta (Aβ) and alpha-synuclein (α-syn), which are hallmarks of AD and PD, respectively. Both curli and gingipain can also trigger neuroinflammation and oxidative stress in the brain. The gut microbiome also produces

metabolites that are implicated in these diseases. For example, individuals with Alzheimer's and Parkinson's diseases often have reduced levels of beneficial SCFAs, which normally have neuroprotective, anti-inflammatory, and blood–brain barrier-strengthening effects (Denman et al. 2023).

1.6.4.6 Autoimmune Diseases

Across autoimmune diseases, alterations in gut microbial composition and function are repeatedly linked to aberrant immune activation and loss of tolerance. In rheumatoid arthritis (RA), the three mechanisms by which microbiota are thought to contribute to RA pathogenesis are inflammatory responses, molecular mimicry, and the loss of intestinal barrier integrity. An overabundance of certain bacteria, such as *Prevotella copri* and some *Lactobacillus* species, is associated with the induction of inflammatory responses, with murine studies showing that their presence can increase Th17 cells and related cytokines. Furthermore, *P. copri* may contribute through molecular mimicry, as its antigens are structurally similar to an RA autoantigen, potentially leading to the activation of T cells that then migrate to the joints. Lastly, the genus *Collinsella*, particularly the species *Collinsella aerofaciens*, may contribute to RA by increasing intestinal permeability. This breach in the gut barrier could allow bacterial antigens to enter the systemic circulation and trigger immune responses in distant areas, such as the joints. The study also notes a decrease in beneficial, butyrate-producing bacteria, like *Faecalibacterium*, which normally help maintain the intestinal barrier (Scher et al. 2013; Tsetseri et al. 2023).

In multiple sclerosis (MS), gut microbiome alterations are consistently reported, with depletion of SCFAs-producing bacteria and enrichment of pro-inflammatory taxa, which disrupt immune homeostasis along the gut–brain axis. These alterations promote reduced regulatory T-cell function, heightened peripheral inflammation, and compromised intestinal barrier integrity, ultimately contributing to neuroinflammation and MS progression. Classically, the increased abundance of *Akkermansia* in MS was thought as a detrimental factor. However, recently, this prevailing view of *Akkermansia* has shifted from being a potentially harmful bacterium in MS

to one that may be beneficial. The study by Cox et al. (2022) suggested that a higher abundance of *Akkermansia* in progressive MS patients was actually correlated with lower disability scores. This is supported by experiments where colonizing a mouse model of MS with *Akkermansia* helped to alleviate disease symptoms. The study's main point is that some bacteria, like *Akkermansia*, may not be directly causing the disease but could instead represent a positive, compensatory response by the host's body (Cox et al. 2021).

1.6.5 Clinical Applications and Therapeutics

Advances in microbiome research have catalyzed the development of a range of therapeutic strategies aimed at restoring microbial balance and improving disease outcomes. These include probiotics, prebiotics, postbiotics, FMT, synbiotics, and engineered microbial consortia. The field is transitioning from empirical treatments to mechanism-driven, precision-based interventions tailored to an individual's microbiome composition and functional profile. This evolution mirrors shifts seen in precision medicine, where diagnostic insights and personalized interventions are aligned for optimized outcomes. Artificial intelligence and multi-omics technologies are increasingly being applied to stratify patients, monitor therapeutic efficacy, and predict responses, laying the groundwork for microbiome-informed clinical decision-making.

1.6.5.1 Fecal Microbiota Transplantation

FMT involves the transfer of stool from a healthy donor to a recipient to restore microbial diversity and function. It has proven highly effective in treating recurrent *C. difficile* infection, with cure rates reaching approximately 90%, and is now being explored for broader applications, including IBD, metabolic disorders, neurodegenerative diseases such as Parkinson's and MS, and graft-versus-host disease. The therapeutic effects of FMT are thought to arise from multiple mechanisms: displacement of pathogenic microbes, reintroduction of beneficial taxa and their metabolites

such as SCFAs, modulation of immune signaling, and restoration of gut barrier function. FMT can be delivered via colonoscopy, enemas, or through oral capsules, enhancing accessibility and patient compliance. However, challenges persist regarding standardization, donor selection, and the risk of transmitting multidrug-resistant organisms. Future directions include the development of precision FMT, which employs high-resolution metagenomic and metabolomic profiling for donor-recipient matching and improved safety (Ianiro et al. 2018; Porcari et al. 2023).

1.6.5.2 Probiotics and Prebiotics

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. They contribute to gut health by enhancing immune responses, inhibiting pathogen colonization, and producing neuroactive and anti-inflammatory metabolites. Certain strains, such as *Lactobacillus* and *Bifidobacterium* species, have demonstrated potential in improving symptoms of anxiety, depression, and gastrointestinal disorders. However, probiotics face several challenges, including ensuring their viability and stability during production and storage, guaranteeing they reach the gut alive and colonize effectively, and addressing potential safety concerns. These challenges impact their efficacy and widespread use in food and therapeutic applications (Balta et al. 2021; Wolfe et al. 2023).

Prebiotics, on the other hand, are substrates selectively utilized by beneficial microbes to enhance their growth and activity. Compounds like inulin and fructooligosaccharides stimulate the production of SCFAs such as butyrate, which support intestinal barrier integrity and immune modulation. Although prebiotics are generally considered safe, their effectiveness depends on the presence of compatible microbial taxa in the host, and gastrointestinal side effects such as bloating are common. Regulatory inconsistencies and limited mechanistic understanding continue to hinder the standardization and clinical integration of both probiotics and prebiotics (Neyrinck et al. 2021).

1.6.5.3 Synbiotics

Synbiotics, which combine probiotics and prebiotics in a single formulation, aim to enhance microbial viability and functionality through synergistic interactions. These formulations have shown promise in clinical settings, particularly in improving gastrointestinal health and systemic inflammation. By delivering live beneficial bacteria along with their preferred substrates, synbiotics facilitate microbial engraftment and functional restoration. Clinical outcomes, however, remain variable and context-dependent, necessitating further research to refine formulations and define specific strain–substrate pairings for targeted therapeutic effects (Scorletti et al. 2020; Kanazawa et al. 2021).

1.6.5.4 Other Therapeutics

Beyond FMT, probiotics, prebiotics, and personalized nutrition, several other gut microbiome-based therapeutics are emerging:

- *Phage Therapy:* Utilizes bacteriophages (viruses that infect bacteria) for highly specific targeted bacterial depletion and microbiota remodeling (Li et al. 2022).
- *LBTs:* Are biological products containing live organisms intended for disease prevention or treatment, distinct from probiotics due to established clinical benefit for a specific disease. For example, *Clostridium scindens* has been inversely correlated with CDI establishment and can enhance the resistance to *C. difficile* in mice (Buffie et al. 2015).
- *Engineered Microbes:* Novel engineered microbes have been constructed and utilized in clinical trials for various non-cancer and cancer diseases, including IBD, diabetes, hyperammonemia, oral mucositis, HPV-positive cancers, and prostate cancer. For instance,
    - *L. reuteri* strain (PRB782) engineered to secrete high amounts of human interleukin-22 holds promise as a delivery platform for treating intestinal diseases (Ortiz-Velez et al. 2020).

- Engineered *Bacillus subtilis* producing butyrate has been shown to retard weight gain and fat accumulation in obesity mouse models (Bai et al. 2020).

1.6.6 Methods of Study: Recent Advances in Gut Microbiome Research

The study of the human gut microbiome has evolved rapidly due to major advances in sequencing technologies, bioinformatics, and integrated multi-omics approaches. These developments have shifted the field from descriptive taxonomy to mechanistic and predictive biology, enabling a deeper understanding of host-microbe interactions and their clinical relevance.

Modern microbiome research increasingly relies on multi-omics integration combining genomics, transcriptomics, proteomics, and metabolomics to capture microbial community structure, function, and dynamics. This systems-level approach allows for detailed exploration of microbial activity, host responses, and causal mechanisms in health and disease. The use of gnotobiotic models, synthetic microbial communities, and in vitro gut-mimicking platforms further enables hypothesis-driven mechanistic studies and translational research.

1.6.6.1 16S rRNA Gene Sequencing

16S rRNA gene amplicon sequencing remains a foundational, cost-effective approach to profiling gut microbial communities. It targets conserved and hypervariable regions of the 16S rRNA gene using broad-range primers, enabling taxonomic classification of bacteria in complex fecal or intestinal samples. Although limited to genus-level resolution in many cases, it is widely used to assess community structure (diversity metrics such as α- and β-diversity) and shifts associated with health or disease states. This method has guided insights into gut dysbiosis linked to obesity, IBD, and metabolic disorders, and serves as a routine starting point for broader multi-omic investigation (Jovel et al. 2016).

1.6.6.2 Metagenomics

Shotgun metagenomics overcomes the resolution constraints of amplicon sequencing by capturing all DNA within a sample, identifying not only bacterial taxa but also genomic content across bacteria, archaea, viruses, and fungi. This enables strain-level taxonomic assignment and inference of functional potential, such as metabolic pathways and antibiotic resistance. High-impact initiatives like the Human Microbiome Project and MetaHIT leveraged shotgun metagenomics to establish vast reference gene catalogs and reveal functional distinctions in healthy versus diseased populations (Qin et al. 2010; Jovel et al. 2016; Zhang et al. 2019).

1.6.6.3 Metatranscriptomics

Metatranscriptomics provides a dynamic view of gut microbiome functionality by sequencing community RNA to capture active gene expression. This approach reveals which metabolic pathways are transcriptionally active under specific conditions. It is crucial for understanding microbial contributions in situ. A recent study profiling the pediatric gut "secretome (secreted proteins)" via metatranscriptomics demonstrated that over one-quarter of expressed genes in the gut microbiome encode potentially secreted proteins, many of which differ in abundance between obese and healthy children, highlighting direct host–microbe communication via secreted factors (Zhang et al. 2019; Gallardo-Becerra et al. 2020).

1.6.6.4 Metabolomics

Metabolomics complements genomic and transcriptomic data by characterizing the chemical outputs of microbial activity. Approaches like mass spectrometry and NMR profiling of stool or serum link specific microbial metabolites (e.g., SCFAs) with host phenotypes and therapeutic responses. Meta-omics integration across sequencing and metabolite profiling has deepened insights into disease-associated metabolic signatures and microbiome-driven drug interactions (Zhang et al. 2019).

1.6.6.5 Gnotobiotic Models and Synthetic Microbial Communities (SynComs)

Gnotobiotic (germ-free or defined-flora) animal models, especially mice, enable controlled investigation of host–microbe causal relationships. Colonization with human-derived microbiota or simplified synthetic microbial communities (SynComs) allows for mechanistic dissection of microbial roles in host metabolism, immunity, and disease. Simplified communities assembled from known gut isolates are increasingly used to test hypotheses in reductionist yet well-defined systems. These approaches help connect specific microbial taxa or functions to host phenotypes under well-controlled environmental conditions (Bolsega et al. 2021).

1.6.6.6 Human-Relevant In Vitro Models

Advanced *in vitro* gut models now offer human-relevant platforms to study microbial dynamics under controlled conditions. Systems such as SHIME® (Simulator of the Human Intestinal Microbial Ecosystem) replicate successive digestive compartments, from stomach to colon, and enable longitudinal tracking of microbial composition and metabolite production. They are pivotal for screening dietary components, drugs, and probiotics, offering reproducible environments with reduced ethical and financial overhead compared to in vivo studies. Additionally, emerging microengineered models, including 3D electrospun scaffolds, organoids, and gut-on-a-chip platforms, provide physiologically relevant gut tissue–microbiome co-culture environments. These advanced systems support epithelial differentiation, peristalsis-like flow, and immune cell interactions, thus enhancing human relevance in mechanistic research (Biagini et al. 2020, 2023; Isenring et al. 2023; Zhu et al. 2024).

1.7 Vaginal Microbiome

The female reproductive tract (FRT) comprises both the upper reproductive tract (URT) and the lower reproductive tract (LRT). The former includes the

placenta, peritoneal fluid, ovaries, fallopian tubes, uterus, endometrium, and endocervix. The latter includes the vulva, vagina, and ectocervix (Lingasamy et al. 2023). Previous studies have reported on the sterility of the URT. However, this notion has been later revised, revealing the presence of microorganisms (Miles et al. 2017; Chen et al. 2017; Moreno and Simon, 2019; Schoenmakers et al. 2019; Quaranta et al. 2019; Rowe et al. 2020; Tomaiuolo et al. 2020; Feng and Liu, 2022; Lingasamy et al. 2023). Each part of the FRT, URT, and LRT has a specific microbiome composition (Figure 1.10). This composition is sensitive to several factors and may be altered in response to age, physiological state, pregnancy, lifestyle, and environmental elements (Lingasamy et al. 2023). It is noteworthy that generally higher microbial abundance is observed in the LRT, while microbial diversity increases from the outermost regions towards the innermost parts (Lingasamy et al. 2023). FRT microbiota plays a pivotal role in women's reproductive health. It represents approximately 9% of the total microbial burden of the body (Liptáková et al. 2022). Such microbes promote health by maintaining homeostasis as they secrete various biologically active compounds that positively interact with nervous, metabolic, endocrine, reproductive, and immune systems. Disruptions or changes in the microbial community ultimately lead to dysbiosis, which poses disease risks and has been suggested to have tremendous effects on immunity, hormones, metabolism, epigenetics, and gene expression (Wang et al. 2024). The FRT microbiome is crucial for maintaining reproductive well-being by encouraging fertility, preventing complicated pregnancies, and protecting against infections. However, alterations in the FRT microbiome are linked to reproductive disorders such as preterm birth, pregnancy loss, endometriosis, and infertility (Onderdonk et al. 2016; Donders et al. 2018; Lingasamy et al. 2023; Xiao et al. 2024).

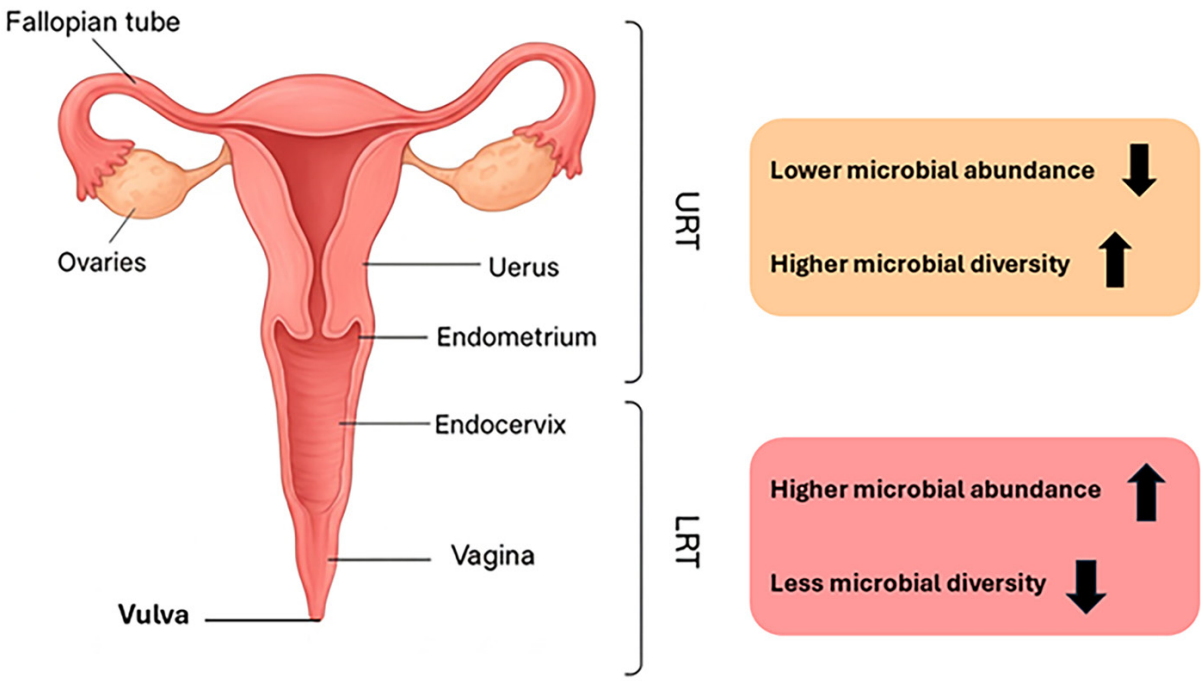

FIGURE 1.10 Anatomy of the FRT and microbiome distribution. The FRT comprises both the URT and the LRT. The former includes the placenta, peritoneal fluid, ovaries, fallopian tubes, uterus, endometrium, and endocervix. The latter includes the vulva, vagina, and ectocervix. Generally higher microbial abundance is observed in the LRT, while microbial diversity increases from the outermost regions towards the innermost parts. ⏎

1.7.1 The Vaginal Microbiome in Health and Disease

The vaginal microbiome consists of a diverse array of bacteria, yeast, viruses, and protozoa that are crucial for women's health. Disruptions in the normal balance of this microbiome can lead to vaginal diseases through interactions across different kingdoms, including those with the host's mucosa and immune system (Orton and Monaco, 2025). However, a healthy vaginal microbiome is dominated by *Lactobacillus* species. They play a crucial role in maintaining an acidic vagina primarily by producing lactic acid, which aids in neutralizing invading pathogens. A healthy vaginal epithelium is usually highly protective against *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Escherichia coli*, *Mycoplasma genitalium*, human

immunodeficiency virus (HIV), HPV, herpes simplex virus (HSV), and *Trichomonas vaginalis* (Valeriano et al. 2024). In women of childbearing age, estrogen stimulates the growth of vaginal epithelial cells, resulting in glycogen buildup, whereas progesterone causes the breakdown of these cells, aiding glycogen release (Ma et al. 2012; Borrego-Ruiz and Borrego, 2025b). Additionally, lactobacilli anaerobically metabolize glycogen to produce lactic acid, which reduces the vaginal pH to approximately 4.5. This acidic environment is hostile to microbial pathogens, preventing their colonization (Pekmezovic et al. 2019; Chee et al. 2020). Beyond acidification, lactobacilli bolster vaginal defense through various mechanisms, such as the generation of antimicrobial substances, including bacteriocins, hydrogen peroxide, biosurfactants, arginine deaminase, and co-aggregating molecules. These substances strengthen the epithelial barrier and trigger anti-inflammatory and immune responses in the body (Fuochi et al. 2019; Chee et al. 2020; Christensen et al. 2022; Miko and Barakonyi, 2023; Borrego-Ruiz and Borrego, 2025b). Moreover, cervical mucus shields the vaginal mucosa and improves its barrier function against microbial colonization and invasiveness. Vaginal secretions also contain various antimicrobial proteins, including lactoferrin, α- and β-defensins, lysozyme, calprotectin, elafin, cathelicidin, secretory leukocyte protease inhibitor, and other AMPs (Yarbrough et al. 2015; Vornhagen et al. 2018; Vagios and Mitchell, 2021). These secretions are also rich in immune mediators, such as immunoglobulins, chemokines, and cytokines (Gutzeit et al. 2025; Borrego-Ruiz and Borrego 2025b). *Lactobacillus* depletion or a decrease in its abundance results in dysbiosis and bacterial vaginosis (BV). This imbalance not only compromises the natural defenses of the vagina but also increases the likelihood of various health problems. Greater microbial diversity and dysbiosis in the vaginal ecosystem have been linked to a higher risk of sexually transmitted infections, pelvic inflammatory disease (PID), and other adverse effects on reproductive health (Qin et al. 2025).

Based on the *Lactobacillus*-dominant species, the vaginal microbiome is classified into five community state types (CSTs). CST I, II, III, and V are dominated by one distinct species of *Lactobacillus*, where CST-I is

dominated by *Lactobacillus crispatus*. CST-II is dominated by *Lactobacillus gasseri*. CST-III and CST-V were dominated by *Lactobacillus iners* and *jensenii jensenii*, respectively. Conversely, CST-IV is *Lactobacillus*-deficient, where diverse strict and facultative anaerobes, including *Gardnerella*, *Candidatus Lachnocurva vaginae* (formerly known as BVAB1), *Fannyhessea vaginae* (*Atopobium vaginae*), or *Prevotella* are common dominators (France et al. 2020; Djusse et al. 2025). Hallmarks of a healthy vagina include *L. crispatus, L. gasseri*, and *L. jensenii*, whereas *L. iners* is often reported as a transition species (Raimondi et al. 2021; Novak et al. 2022; Djusse et al. 2025). On the other hand, *Lactobacillus* depletion, as in CST-IV, is linked to BV, which is characterized by minimal lactic acid production, higher vaginal pH, increased concentrations of biogenic amines, and SCFAs. CST-IV is associated with a higher risk of adverse pregnancy outcomes and sexually transmitted diseases (Laghi et al. 2021; Occhipinti et al. 2024).

Nonetheless, because of the ever-changing nature of the human vaginal microbiome, not all women can be easily categorized into each CST (Lebeer et al. 2023; Valeriano et al. 2024). Moreover, the simultaneous presence of bacterial taxa commonly linked to BV and a healthy vaginal microbiome can exist in women without symptoms (Lebeer et al. 2023; Valeriano et al. 2024). Furthermore, some women, particularly those of Black or Hispanic descent, might naturally have a vaginal microbiome dominated by species other than *Lactobacillus* (France et al. 2020). As research continues to refine the definition of a healthy vaginal microbiome, more specific subtypes have been introduced based on the VAginaL community state typE Nearest CentroId clAssifier (VALENCIA) (France et al. 2020). Additionally, metagenomic community state types (mgCSTs) allow differentiation of the same species based on assembled metagenomes and functional diversity (Holm et al. 2023). Despite these findings, there is consensus that a healthy vaginal microbiome, which supports positive reproductive outcomes, is characterized by a low-diversity microbial community predominantly composed of different *Lactobacillus* species (Valeriano et al. 2024).

1.7.2 Factors Influencing the Composition of the Vaginal Microbiome

The composition of the vaginal microbiome can be influenced by several endogenous factors, such as hormonal fluctuations, pregnancy, menopause, the menstrual cycle, ethnicity, and host genetics, as well as several exogenous factors like diet, smoking, drugs, antibiotics, sexual habits, contraceptives, hygiene, and stress. All these factors can alter the vaginal microbiota (Djusse et al. 2025). Perturbation of the vaginal ecosystem by intrinsic or extrinsic factors alters the composition of the vaginal microbiome, shifting from eubiosis to dysbiosis, which is marked by *Lactobacillus* depletion, an increase in anaerobes, release of amino acids, an increase in vaginal pH, vaginal inflammation, abnormal immune response, and microbial dysbiosis-associated pathologies such as BV, sexually transmitted diseases, yeast infections, and other vaginal disturbances (Borrego-Ruiz and Borrego 2025b). Although dysbiosis refers to perturbed composition of the vaginal bacterial communities, the concept of what constitutes a healthy vaginal ecosystem is still controversial, as it is not stable but continuously fluctuates across a woman's lifespan (Borrego-Ruiz and Borrego 2025b).

1.7.3 Vaginal Microbiome across the Lifespan

1.7.3.1 Prepubescence to Menarche

In childhood, the vaginal microbiome exhibits a high level of diversity, primarily consisting of anaerobic bacteria, such as *Bacteroides*, *Prevotella*, *Actinomyces*, and *Fusobacterium* (Younes et al. 2018; Xiaoming et al. 2021; Lehtoranta et al. 2022). Additionally, it includes gram-positive cocci, such as *S. aureus* and *S. epidermidis*, and gram-negative bacilli, such as *Pseudomonas*, *Methylobacterium*, and *Escherichia* (Zhang et al. 2021b). The combination of these microorganisms and the absence of *Lactobacillus* results in a neutral vaginal pH during this stage of life (Zhang et al. 2021b; Xiaoming et al. 2021; Auriemma et al. 2021; Dubé-Zinatelli et al. 2024). As

the onset of menarche nears, rising estradiol levels lead to thickening of the vaginal lining and accumulation of glycogen, which is subsequently converted into maltose, maltotriose, and α-dextrins by α-amylase. These substances are then converted by *Lactobacillus* into lactic acid, resulting in an acidic environment (Hickey et al. 2015; Blaustein et al. 2016; Amabebe and Anumba 2018; Deka et al. 2021). Consequently, during puberty, the vaginal microbiome becomes increasingly dominated by *Lactobacillus*, resembling the bacterial composition found in women of reproductive age (Hickey et al. 2015; Curley and Forster, 2021). However, following menarche, the vaginal pH remains higher than the healthy range for reproductive-aged women, suggesting that the levels of lactobacilli do not reach adult concentrations until later in development (Hickey et al. 2015; Dubé-Zinatelli et al. 2024). These developmental changes collectively help prevent the proliferation of bacteria, such as *Gardnerella vaginalis*, and inhibit the growth of other pathogens associated with the initiation of sexual activity (Jespers et al. 2016; Bruins et al. 2021; Dubé-Zinatelli et al. 2024).

1.7.3.2 Reproductive Ages

In women of reproductive age, the vaginal microbiota is predominantly composed of *Lactobacillus* sp., which plays a vital role in sustaining vaginal health (Kaur et al. 2020; Chen et al. 2021; Dubé-Zinatelli et al. 2024). The presence of these bacteria is primarily influenced by hormonal fluctuations, with changes in bacterial composition in response to varying estradiol levels throughout the menstrual cycle (Hickey et al. 2015; Krog et al. 2022). During phases when estradiol is elevated, such as the follicular, preovulatory, and midluteal stages, glycogen builds up in the vaginal lining, encouraging the growth of lactobacilli and resulting in a higher abundance of lactobacilli (Brooks et al. 2017; Krog et al. 2022; Dubé-Zinatelli et al. 2024). In contrast, menstruation is characterized by low estradiol levels, reduced abundance of lactobacilli, and greater microbial diversity (Brooks et al. 2017; Krog et al. 2022; Dubé-Zinatelli et al. 2024). Notably, these changes are more evident in women who experience natural menstrual cycles than in those using combined oral contraceptive pills or

levonorgestrel intrauterine devices, indicating that synthetic hormones may disrupt the typical variations in the vaginal microbiota throughout the menstrual cycle (Krog et al. 2022; Dubé-Zinatelli et al. 2024).

1.7.3.3 Pregnancy

In a healthy pregnancy, the placenta produces estradiol and estriol, which result in an elevated abundance of *Lactobacillus* and a reduction in vaginal pH (Gupta et al. 2020; Nunn et al. 2021; Dubé-Zinatelli et al. 2024). This stable *Lactobacillus* population, maintained by an acidic environment throughout pregnancy, helps protect both the mother and fetus from infections (Shabayek et al. 2022; Baud et al. 2023). Notably, the vaginal bacterial composition of pregnant women is distinct from that of nonpregnant women, with pregnant women showing higher levels of *Bacteroidales* and *Clostridiales*, whereas nonpregnant women generally have more *Prevotella*, *Streptococcus*, and *Veillonellaceae* (Nunn et al. 2021; Shabayek et al. 2022; Baud et al. 2023; Dubé-Zinatelli et al. 2024). This highlights a prenatal shift, characterized by reduced bacterial diversity and increased *Lactobacillus* levels. However, after childbirth, estradiol levels drop significantly, leading to a decline in lactic acid-producing bacteria and a rise in other taxa, such as *G. vaginalis* and *Prevotella bivia*, making the vaginal microbiome more susceptible to pathogens. These changes can begin as early as labor onset and may last for up to a year postpartum (Nunn et al. 2021; Chopra et al. 2022; Li et al. 2024; Dubé-Zinatelli et al. 2024).

1.7.3.4 Menopause

Menopause is marked by a decrease in estrogen levels, which is linked to a reduction in *Lactobacillus* and glycogen levels and an increase in vaginal pH (Auriemma et al. 2021; Lehtoranta et al. 2022). At this stage, the vaginal ecosystem often exhibits high levels of anaerobic bacteria, such as *G. vaginalis*, *C. albicans*, and *Prevotella*, resulting in a vaginal microbiome similar to that of prepubescence (Hickey et al. 2015; Dubé-Zinatelli et al. 2024). Subsequently, these changes can initiate thinning of the vaginal

lining, causing symptoms such as dryness and discomfort in the vagina (Foschi et al. 2022; Tomczyk et al. 2023; Dubé-Zinatelli et al. 2024). Although this is a natural consequence of vaginal aging, estrogen treatments have been suggested to boost hormone production and glycogen levels in the vaginal niche to improve the vaginal mucosa (Shen et al. 2016; Tomczyk et al. 2023). However, the benefits of *Lactobacillus* therapeutics during menopause are debated, as studies on the effects of maintaining *Lactobacillus* dominance during this life stage show controversial results. Further research is needed to understand why continued estrogen production, but not *Lactobacillus*, seems to support vaginal health after menopause (Shen et al. 2016; Szymański et al. 2021; Tomczyk et al. 2023; Dubé-Zinatelli et al. 2024).

1.7.4 Microbial Conditions Associated with Dysbiosis of the Vaginal Microbiome

1.7.4.1 Bacterial Vaginosis

BV is an inflammatory condition that results from an imbalance in the vaginal microbiome. It is characterized by a marked reduction in *Lactobacillus* abundance and an increase in the relative abundance of anaerobic bacteria (Holdcroft et al. 2023; Borrego-Ruiz and Borrego 2025b). This occurs due to the significant proliferation of previously minor obligate or facultative anaerobes in the vaginal ecosystem, such as *G. vaginalis*, *Prevotella*, and *Mobiluncus* sp., along with bacterial vaginosis-associated bacteria (BVAB) (Muzny et al. 2019; Abou Chacra et al. 2022; Carter et al. 2023). BV is the most prevalent vaginal infection among women of reproductive age, affecting 25% of women worldwide (Holdcroft et al. 2023). Common symptoms of BV include fishy odor, excessive vaginal discharge, vaginal pH exceeding 4.5, and vaginal irritation (Bagnall and Rizzolo 2017). *Lactobacillus*-depleted microbiota during BV forms a biofilm on the vaginal epithelium, produces a cytotoxin that can lead to epithelial cell death, and generates proteolytic enzymes such as sialidase, fucosidase, and decarboxylases that convert amino acids and polyamines. If

not broken down, biogenic amines, including polyamines such as spermidine, agmatine, putrescine, cadaverine, tyramine, and trimethylamine, create a fishy odor due to increased vaginal pH (Nelson et al. 2015; Swidsinski et al. 2023; Mondal et al. 2023; Segui-Perez et al. 2024). Consequently, cytotoxicity from the interaction between organic acids and bacterial polyamines results in vaginal discharge, triggered by exfoliation of vaginal epithelial cells (Amegashie et al. 2017). Additionally, *G. vaginale* and other anaerobic bacteria coat the vaginal epithelial cells, leading to the formation of "clue cells," a hallmark of BV (Machado and Cerca 2015; Swidsinski et al. 2022). A BV-associated microbiome is closely similar to that of a CST-IV vaginal microbiome, which is particularly common in reproductive-aged African American women (Holdcroft et al. 2023). Notably, Black women are more likely to have *Lactobacillus*-depleted microbiomes than White women and are twice as likely to be diagnosed with BV (Fettweis et al. 2014; Saraf et al. 2021; Holdcroft et al. 2023). Asymptomatic BV is controversial, as women have a vaginal microbiome consistent with BV and receive treatment for it, despite not showing any symptoms (Holdcroft et al. 2023).

1.7.4.2 Aerobic Vaginitis

Aerobic vaginitis is associated with vaginal inflammation, acute pyelonephritis, bacteriuria, and lower urinary tract infections (Kaambo et al. 2018). This condition is characterized by an increased abundance of enteric aerobic bacteria such as group B Streptococcus (GBS) and *Streptococcus anginosus*, *S. aureus, S. epidermidis, E. coli*, and *Enterococcus faecalis* (Donders et al. 2017; Tao et al. 2019; Borrego-Ruiz and Borrego, 2025b). These aerobic bacteria are not related to BV and can cause inflammation of the vaginal mucosa by disrupting the dominance of lactobacilli, which leads to a higher turnover of the superficial epithelial cell layer and can result in small erosions, epidermal desquamation, ulceration, and atrophy of the vaginal epithelium (Fan et al. 2013; Oerlemans et al. 2020). Aerobic vaginitis occasionally leads to morbidity and is prevalent among pregnant and postmenopausal women, as they experience decreased estrogen levels

and local vaginal immunity (Borrego-Ruiz and Borrego, 2025b). Colonization of pregnant women with pathogenic aerobic vaginitis-associated bacteria, such as GBS, is considered a significant cause of severe neonatal infections, including pneumonia, sepsis, and meningitis. Besides GBS, *E. faecalis* has also been linked to puerperal sepsis, low birth weight, and preterm delivery, leading to serious maternal and neonatal morbidity and mortality (Krauss-Silva et al. 2014; Valeriano et al. 2024; Borrego-Ruiz and Borrego, 2025b).

1.7.4.3 Pelvic Inflammatory Disease

PID is an inflammatory syndrome of the upper FRT that can lead to serious, long-term reproductive issues, such as chronic pelvic pain, ectopic pregnancy, and tubal factor infertility (Hoenderboom et al. 2019; den Heijer et al. 2019). Due to the disruption of protective barriers and immune defense mechanisms in the context of inflammation, PID can be caused by several invading pathogens, including *C. trachomatis, N. gonorrhoeae*, and *M. genitalium* that ascend from the lower genital tract structures, such as the vagina or cervix, to infect the upper genital tract (Haggerty and Taylor 2011; Price et al. 2016; Tamarelle et al. 2019; Ravel et al. 2021; Xu and Gray-Owen 2021; Borrego-Ruiz and Borrego 2025b). Moreover, BV-associated bacteria have been implicated in the development of PID and in promoting the upward spread of sexually transmitted infections by increasing susceptibility to sexually transmitted microbes, contributing through synergistic interactions and hindering the clearance of invading pathogens. Hence, a vaginal microbiome dominated by lactobacilli may offer protection against PID, whereas a microbiome dominated by anaerobes such as *G. vaginale* may increase susceptibility (Taylor et al. 2013; Turpin et al. 2021; Xu and Gray-Owen, 2021; Borrego-Ruiz and Borrego, 2025b).

1.7.4.4 Vulvovaginal Candidiasis

Vulvovaginal candidiasis is one of the most common causes of vulvovaginal itching and discharge globally (Borrego-Ruiz and Borrego 2025b). This pathology is marked by inflammation of the vulval and vaginal lining, accompanied by yeast colonization, predominantly by *C. albicans*, which is responsible for over 90% of cases. However, non-albicans *Candida* species, such as *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*, have also been identified as causative agents (Willems et al. 2020; Borrego-Ruiz and Borrego 2025b). Candida is a commensal yeast found in the vagina of many women, typically migrating from the lower gastrointestinal tract. This colonization can remain asymptomatic for extended periods. However, overgrowth of *Candida* in the vaginal lining can lead to symptomatic infection (Borrego-Ruiz and Borrego 2025b). Symptoms linked to non-albicans *Candida* species are often mild and less severe than those associated with *C. albicans*. In certain cases, patients may also experience painful intercourse, increased vaginal discharge, painful urination, vaginal soreness, or swelling (Willems et al. 2020). While the majority of women encounter at least one episode of vulvovaginal candidiasis during their lifetime, some may experience recurrent infections (Nyirjesy et al. 2022; Borrego-Ruiz and Borrego 2025b).

Several factors are believed to contribute to the development of recurrent vulvovaginal candidiasis, such as sexual activity, oral contraceptives, or hormone replacement therapy, and the use of sponges or intrauterine devices (Borrego-Ruiz and Borrego 2025b). Genetic factors, such as polymorphisms in TLR2 and mannose-binding lectin 2, and a genetically determined hyperinflammatory response to *Candida* colonization and invasion, have been identified in women with recurrent vulvovaginal candidiasis, increasing their vulnerability to infection (Rosati et al. 2020). The role of host physiological factors in vulvovaginal candidiasis development remains unclear. Nonetheless, estrogen plays a significant role in determining susceptibility to the disease, where women in their reproductive years are at higher risk for the onset of the disease, whereas prepubertal girls and postmenopausal women are rarely affected (Cheng et al. 2006; Hong et al. 2014; Borrego-Ruiz and Borrego, 2025b).

1.7.4.5 Sexually Transmitted Infections

Vaginal microbiome dysbiosis and BV may increase the risk of sexually transmitted infections such as HIV, HPV, and *T. vaginalis* Infection (Velloza and Heffron 2017; Borrego-Ruiz and Borrego 2025b). This occurs through interactions with mucosal immunity in the female genital tract, which alters the protective epithelial barrier. Disruptions of the vaginal mucosal barrier by dysbiosis-associated bacteria such as *G. vaginale* and *Prevotella* result in the release of sialidase and causing host cell lysis, thereby increasing vulnerability to gynecological infections (France et al. 2022). Recent findings indicate that women with a non-*Lactobacilli*-dominant vaginal microbiome are at a greater risk of sexual transmission of HIV (Wang et al. 2023). BV is more prevalent and persistent in HIV-positive women, particularly in those with weakened immune systems (Borrego-Ruiz and Borrego 2025b). *L. crispatus* is linked to a reduced inflammatory state, clearance of BV-associated bacteria, and a lower risk of HIV. Conversely, *L. iners* is thought to offer less protection against HIV, possibly because of its role in a more intermediate transition to dysbiosis (Armstrong and Kaul 2021).

Similar to other sexually transmitted infections, vaginal dysbiosis or BV increases the risk of HPV infection and persistence. The sialidase genes of *Atopobium vaginae* and *G. vaginalis*, along with the dominance of *Snethia* species in the vaginal ecosystem, have been proposed as biomarkers for persistent HPV infection in women with BV (Lee et al. 2013; Di Paola et al. 2017; Borrego-Ruiz and Borrego 2025b). Notably, a vaginal microbiome dominated by *L. iners* or anaerobes may pose a risk for persistent HPV infection due to the reduced protective immunity associated with the loss of homeostasis caused by the depletion of commensal lactobacilli and disruption of the cervical epithelium's protective barrier by anaerobic microorganisms (Mei et al. 2022). These factors facilitate HPV entry into host cells, promoting the integration of HPV into host DNA and encouraging cell division, which supports viral replication (Cheng et al. 2020). In contrast, a vaginal microbiome dominated by *L. crispatus* offers

protection against HPV persistence and subsequent disease development, whereas a vaginal microbiome dominated by *Lactobacillus paragasseri* is associated with faster HPV clearance (Alimena et al. 2022; Mei et al. 2022). The vaginal microbiome significantly influences susceptibility to *T. vaginalis* infection, thereby increasing the risk of trichomoniasis in women with BV. Vaginal microbiome dysbiosis leads to a loss of lactobacilli and an elevated pH, creating conditions that are more favorable for *T. vaginalis* growth and colonization (Balkus et al. 2014; Margarita et al. 2020; Chiu et al. 2021). Furthermore, several anaerobic genera, such as *Prevotella* and *Sneathia*, may increase susceptibility to trichomoniasis by producing nicotinamide metabolites (Jarrett et al. 2019). Based on these interactions, interventions aimed at reducing the incidence of BV and promoting a balanced microbiota (eubiosis) could help decrease trichomoniasis rates (Borrego-Ruiz and Borrego 2025b).

1.8 Conclusion

The human microbiome constitutes a highly dynamic and interconnected ecosystem that is fundamental to host health. Distributed across diverse anatomical sites including the skin, oral cavity, nasal passages, auditory canal, gastrointestinal tract, and female reproductive system. These microbial consortia are far more than passive inhabitants. They participate directly in processes essential for host's health, including barrier protection, immune calibration, metabolic homeostasis, and defense against opportunistic pathogens.

Technological advances in sequencing and multi-omics have significantly expanded our ability to chart the composition and function of microbial communities, revealing their responsiveness to a broad spectrum of host-intrinsic factors (such as genetics, developmental stage, and hormonal milieu) and environmental influences (including diet, antibiotics, hygiene practices, and ecological exposures). Perturbations to this equilibrium (dysbiosis) are proven to be strongly associated with a wide

array of both localized and systemic disorders, encompassing dermatological, oral, metabolic, autoimmune, neurological, and reproductive conditions.

Importantly, the microbiome must be understood as a central integrator within the broader physiological network of the human body. Communication pathways such as the gut–brain, oral–gut, nasal–lung, and vaginal–immune axes exemplify the extensive reach of microbial signals across organs and systems. Through these axes, microbial communities exert influence that extends far beyond their immediate niches, shaping immunity, metabolism, and neurophysiology.

The recurrent identification of disease-associated microbial signatures underscores the microbiome's significance not merely as a marker of pathology but as a potential mechanistic driver and therapeutic lever. This realization has reframed the microbiome as a cornerstone of both preventive and precision medicine. Moving forward, strategies that restore eubiosis will be central to advancing human health, offering novel opportunities for diagnosis, intervention, and long-term well-being.

1.9 Future Directions

The next phase of microbiome research must extend beyond descriptive compositional studies toward mechanistic dissection of host–microbe interactions. Establishing causal relationships between specific taxa, their molecular outputs, such as metabolites, peptides, and EVs, and host physiological responses remains a central challenge. Achieving this will require the integration of longitudinal, multi-omics datasets encompassing metagenomics, metatranscriptomics, metabolomics, and host immunophenotyping, analyzed through advanced computational modeling and artificial intelligence. Such approaches promise to unravel the context-dependent roles of microbial communities in both health and disease. Complementary advances in physiologically relevant models will provide

the experimental rigor needed to validate mechanistic hypotheses in controlled environments.

On the translational front, the field is shifting from broad empirical approaches toward precision microbiome engineering. Rationally designed next-generation probiotics, engineered LBTs, bacteriophage-based antimicrobials, and tailored prebiotic or postbiotic interventions exemplify the promise of targeted strategies that align with an individual's microbial and metabolic profile.

Realizing these opportunities will depend on robust clinical validation, harmonized regulatory frameworks, and careful ethical oversight to ensure safety, efficacy, and equitable access. As the field matures, the microbiome will increasingly be positioned at the heart of preventive and precision medicine, opening new avenues for maintaining health, mitigating disease risk, and tailoring interventions at the level of the individual.

References

Abou CL, Fenollar F, Diop K (2022) Bacterial vaginosis: What do we currently know? *Front Cell Infect Microbiol* 11:672429. https://doi.org/10.3389/fcimb.2021.672429 ⏎

Aggarwal N, Kitano S, Puah GRY, *et al.* (2023) Microbiome and human health: current understanding, engineering, and enabling technologies. *Chem Rev* 123:31–72. https://doi.org/10.1021/acs.chemrev.2c00431 ⏎

Ahn J, Hayes RB (2021) Environmental influences on the human microbiome and implications for noncommunicable disease. *Annu Rev Public Health* 42:277–292. https://doi.org/10.1146/annurev-publhealth-012420-105020 ⏎

Akhtar M, Chen Y, Ma Z*, et al.* (2022) Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. *Anim Nutr* 8:350–360. https://doi.org/10.1016/j.aninu.2021.11.005 ⏎

Alimena S, Davis J, Fichorova RN, Feldman S (2022) The vaginal microbiome: A complex milieu affecting risk of human papillomavirus

persistence and cervical cancer. *Curr Probl Cancer* 46:100877. https://doi.org/10.1016/j.currproblcancer.2022.100877 ⏎

Alum EU, Gulumbe BH, Izah SC, *et al.* (2025) Natural product-based inhibitors of quorum sensing: A novel approach to combat antibiotic resistance. *Biochem Biophys Reports* 43:102111. https://doi.org/10.1016/j.bbrep.2025.102111 ⏎

Amabebe E, Anumba DOC (2018) Psychosocial stress, cortisol levels, and maintenance of vaginal health. *Front Endocrinol (Lausanne)* 9:568. https://doi.org/10.3389/fendo.2018.00568 ⏎

Amegashie CP, Gilbert NM, Peipert JF, *et al.* (2017) Relationship between nugent score and vaginal epithelial exfoliation. *PLoS One* 12:e0177797. https://doi.org/10.1371/journal.pone.0177797 ⏎

Armstrong E, Kaul R (2021) Beyond bacterial vaginosis: vaginal lactobacilli and HIV risk. *Microbiome* 9:239. https://doi.org/10.1186/s40168-021-01183-x ⏎

Asseri AH, Bakhsh T, Abuzahrah SS, *et al.* (2023) The gut dysbiosis-cancer axis: illuminating novel insights and implications for clinical practice. *Front Pharmacol* 14:1208044. https://doi.org/10.3389/fphar.2023.1208044 ⏎

Auriemma RS, Scairati R, del Vecchio G, *et al.* (2021) The vaginal microbiome: A long urogenital colonization throughout woman life. *Front Cell Infect Microbiol* 11:686167. https://doi.org/10.3389/fcimb.2021.686167 ⏎

Badal VD, Vaccariello ED, Murray ER, *et al.* (2020) The gut microbiome, aging, and longevity: A systematic review. *Nutrients* 12:3759. https://doi.org/10.3390/nu12123759 ⏎

Bagnall P, Rizzolo D (2017) Bacterial vaginosis. *JAAPA* 30:15–21. https://doi.org/10.1097/01.JAA.0000526770.60197.fa ⏎

Bai L, Gao M, Cheng X, *et al.* (2020) Engineered butyrate-producing bacteria prevents high fat diet-induced obesity in mice. *Microb Cell Fact* 19:94. https://doi.org/10.1186/s12934-020-01350-z ⏎

Bakhshaee M, Naderi HR, Ghazvini K, *et al.* (2012) Passive smoking and nasopharyngeal colonization by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in daycare children. *Eur Arch Oto-Rhino-Laryngology* 269:1127–1132. https://doi.org/10.1007/s00405-011-1811-8 ⏎

Balkus JE, Richardson BA, Rabe LK, *et al.* (2014) Bacterial vaginosis and the risk of *Trichomonas vaginalis* acquisition among HIV-1–negative women. *Sex Transm Dis* 41:123–128. https://doi.org/10.1097/OLQ.0000000000000075 ⏎

Balta I, Butucel E, Mohylyuk V, *et al.* (2021) Novel insights into the role of probiotics in respiratory infections, allergies, cancer, and neurological abnormalities. *Diseases* 9:60. https://doi.org/10.3390/diseases9030060 ⏎

Barlow JT, Leite G, Romano AE, *et al.* (2021) Quantitative sequencing clarifies the role of disruptor taxa, oral microbiota, and strict anaerobes in the human small-intestine microbiome. *Microbiome* 9:214. https://doi.org/10.1186/s40168-021-01162-2 ⏎

Baud A, Hillion K-H, Plainvert C, *et al.* (2023) Microbial diversity in the vaginal microbiota and its link to pregnancy outcomes. *Sci Rep* 13:9061. https://doi.org/10.1038/s41598-023-36126-z ⏎

Beardsley J, Kim HY, Dao A, *et al.* (2024) *Candida glabrata* (*Nakaseomyces glabrata*): A systematic review of clinical and microbiological data from 2011 to 2021 to inform the World Health Organization Fungal Priority Pathogens List. *Med Mycol* 62:myae041. https://doi.org/10.1093/mmy/myae041 ⏎

Biagini F, Calvigioni M, Lapomarda A, *et al.* (2020) A novel 3D in vitro model of the human gut microbiota. *Sci Rep* 10:21499. https://doi.org/10.1038/s41598-020-78591-w ⏎

Biagini F, Daddi C, Calvigioni M, *et al.* (2023) Designs and methodologies to recreate in vitro human gut microbiota models. *Bio-Design Manuf* 6:298–318. https://doi.org/10.1007/s42242-022-00210-6 ⏎

Bidell MR, Hobbs AL V., Lodise TP (2022) Gut microbiome health and dysbiosis: A clinical primer. *Pharmacother J Hum Pharmacol Drug Ther*

42:849–857. https://doi.org/10.1002/phar.2731 ⏎

Blaustein JD, Ismail N, Holder MK (2016) Review: Puberty as a time of remodeling the adult response to ovarian hormones. *J Steroid Biochem Mol Biol* 160:2–8. https://doi.org/10.1016/j.jsbmb.2015.05.007 ⏎

Bolsega S, Bleich A, Basic M (2021) Synthetic microbiomes on the rise—Application in deciphering the role of microbes in host health and disease. *Nutrients* 13:4173. https://doi.org/10.3390/nu13114173 ⏎

Borrego-Ruiz A, Borrego JJ (2024) Microbial dysbiosis in the skin microbiome and its psychological consequences. *Microorganisms* 12:1908. https://doi.org/10.3390/microorganisms12091908 ⏎

Borrego-Ruiz A, Borrego JJ (2025a) Human oral microbiome and its influence on mental health and brain disorders. *AIMS Microbiol* 11:242–294. https://doi.org/10.3934/microbiol.2025013 ⏎

Borrego-Ruiz A, Borrego JJ (2025b) Microbial pathogens linked to vaginal microbiome dysbiosis and therapeutic tools for their treatment. *Acta Microbiol Hell* 70:19. https://doi.org/10.3390/amh70020019 ⏎

Brookes Z, Teoh L, Cieplik F, Kumar P (2023) Mouthwash effects on the oral microbiome: Are they good, bad, or balanced? *Int Dent J* 73:S74–S81. https://doi.org/10.1016/j.identj.2023.08.010 ⏎

Brooks JP, Edwards DJ, Blithe DL, *et al.* (2017) Effects of combined oral contraceptives, depot medroxyprogesterone acetate and the levonorgestrel-releasing intrauterine system on the vaginal microbiome. *Contraception* 95:405–413. https://doi.org/10.1016/j.contraception.2016.11.006 ⏎

Brugger SD, Eslami SM, Pettigrew MM, *et al.* (2020) *Dolosigranulum pigrum* cooperation and competition in human nasal microbiota. *mSphere* 5:e00852-20. https://doi.org/10.1128/mSphere.00852-20 ⏎

Bruins MJ, dos Santos CO, Damoiseaux RAMJ, Ruijs GJHM (2021) Bacterial agents in vulvovaginitis and vaginal discharge: A 10-year retrospective study in the Netherlands. *Eur J Clin Microbiol Infect Dis* 40:2123–2128. https://doi.org/10.1007/s10096-021-04265-8 ⏎

Buch PJ, Chai Y, Goluch ED (2019) Treating polymicrobial infections in chronic diabetic wounds. *Clin Microbiol Rev* 32:e00091-18. https://doi.org/10.1128/CMR.00091-18 ⏎

Buffie CG, Bucci V, Stein RR, *et al.* (2015) Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 517:205–208. https://doi.org/10.1038/nature13828 ⏎

Burton M, Krumbeck JA, Wu G*, et al.* (2022) The adult microbiome of healthy and otitis patients: Definition of the core healthy and diseased ear microbiomes. *PLoS One* 17:e0262806. https://doi.org/10.1371/journal.pone.0262806 ⏎

Byndloss M, Devkota S, Duca F, *et al.* (2024) The gut microbiota and diabetes: Research, translation, and clinical applications—2023 Diabetes, Diabetes Care, and Diabetologia Expert Forum. *Diabetes Care* 47:1491–1508 https://doi.org/10.2337/dci24-0052 ⏎

Byrd AL, Belkaid Y, Segre JA (2018) The human skin microbiome. *Nat Rev Microbiol* 16:143–155. https://doi.org/10.1038/nrmicro.2017.157 ⏎

Camacho-Morales A, Caba M, García-Juárez M, *et al.* (2021) Breastfeeding contributes to physiological immune programming in the newborn. *Front Pediatr* 9:744104. https://doi.org/10.3389/fped.2021.744104 ⏎

Cani PD, Amar J, Iglesias MA*, et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56:1761–1772. https://doi.org/10.2337/db06-1491 ⏎

Cani PD, Bibiloni R, Knauf C, *et al*. (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. *Diabetes* 57:1470–1481. https://doi.org/10.2337/db07-1403 ⏎

Carson MD, Westwater C, Novince CM (2023) Adolescence and the microbiome. *Am J Pathol* 193:1900–1909. https://doi.org/10.1016/j.ajpath.2023.07.004 ⏎

Carter KA, Fischer MD, Petrova MI, Balkus JE (2023) Epidemiologic evidence on the role of *Lactobacillus iners* in sexually transmitted

infections and bacterial vaginosis: A series of systematic reviews and meta-analyses. *Sex Transm Dis* 50:224–235. https://doi.org/10.1097/OLQ.0000000000001744 ⏎

Celoria V, Rosset F, Pala V, *et al.* (2023) The skin microbiome and its role in psoriasis: A review. *Psoriasis Targets Ther* 13:71–78. https://doi.org/10.2147/PTT.S328439 ⏎

Chambers ES, Byrne CS, Morrison DJ, *et al.* (2019) Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: A randomised cross-over trial. *Gut* 68:1430–1438. https://doi.org/10.1136/gutjnl-2019-318424 ⏎

Chee WJY, Chew SY, Than LTL (2020) Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *Microb Cell Fact* 19:203. https://doi.org/10.1186/s12934-020-01464-4 ⏎

Chen C, Song X, Wei W, *et al.* (2017) The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat Commun* 8:875. https://doi.org/10.1038/s41467-017-00901-0 ⏎

Chen H, Zhao Q, Zhong Q, *et al.* (2022) Skin microbiome, metabolome and skin phenome, from the perspectives of skin as an ecosystem. *Phenomics* 2:363–382. https://doi.org/10.1007/s43657-022-00073-y ⏎

Chen X, Lu Y, Chen T, Li R (2021) The female vaginal microbiome in health and bacterial vaginosis. *Front Cell Infect Microbiol* 11:631972. https://doi.org/10.3389/fcimb.2021.631972 ⏎

Cheng G, Yeater KM, Hoyer LL (2006) Cellular and molecular biology of Candida albicans estrogen response. *Eukaryot Cell* 5:180–191. https://doi.org/10.1128/EC.5.1.180-191.2006 ⏎

Cheng L, Norenhag J, Hu YOO, *et al.* (2020) Vaginal microbiota and human papillomavirus infection among young Swedish women. *npj Biofilms Microbiomes* 6:39. https://doi.org/10.1038/s41522-020-00146-8 ⏎

Chiu S-F, Huang P-J, Cheng W-H, *et al.* (2021) Vaginal microbiota of the sexually transmitted infections caused by *Chlamydia trachomatis* and *Trichomonas vaginalis* in women with vaginitis in Taiwan. *Microorganisms* 9:1864. https://doi.org/10.3390/microorganisms9091864 ⏎

Chopra C, Bhushan I, Mehta M, *et al.* (2022) Vaginal microbiome: Considerations for reproductive health. *Future Microbiol* 17:1501–1513. https://doi.org/10.2217/fmb-2022-0112 ⏎

Christensen AO, Li G, Young CH, *et al.* (2022) Peptidylarginine deiminase enzymes and citrullinated proteins in female reproductive physiology and associated diseases. *Biol Reprod* 107:1395–1410. https://doi.org/10.1093/biolre/ioac173 ⏎

Clarke SF, Murphy EF, O'Sullivan O, *et al*. (2014) Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63:1913–1920. https://doi.org/10.1136/gutjnl-2013-306541 ⏎

Constantino-Jonapa LA, Aguilar-Villegas OR, Hernández-Ruiz P, *et al.* (2025) The link between inflammatory/SCFA profiles and oral/gut microbiome: An observational study in patients with ST-segment elevation myocardial infarction. *Curr Res Microb Sci* 9:100423. https://doi.org/10.1016/j.crmicr.2025.100423 ⏎

Cox LM, Calcagno N, Gauthier C, Madore C, Butovsky O, Weiner HL. (2022) The microbiota restrains neurodegenerative microglia in a model of amyotrophic lateral sclerosis. *Microbiome*;10:47. doi: 10.1186/s40168-022-01232-z.

Cox LM, Maghzi AH, Liu S, *et al.* (2021) Gut microbiome in progressive multiple sclerosis. *Ann Neurol* 89:1195–1211. https://doi.org/10.1002/ana.26084 ⏎

Curley T, Forster CS (2021) Recurrent UTIs in girls: What is the role of the microbiome? *Urology* 151:94–97. https://doi.org/10.1016/j.urology.2020.04.091 ⏎

Cusumano G, Flores GA, Venanzoni R, Angelini P (2025) The impact of antibiotic therapy on intestinal microbiota: dysbiosis, antibiotic

resistance, and restoration strategies. *Antibiotics* 14:371. https://doi.org/10.3390/antibiotics14040371 ⏎

D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T (2013) UV radiation and the skin. *Int J Mol Sci* 14:12222–12248. https://doi.org/10.3390/ijms140612222 ⏎

Daniel EF, Cameron TM, Warunya P, *et al.* (2019) A comparison between two pathophysiologically different yet microbiologically similar lung diseases: Cystic fibrosis and chronic obstructive pulmonary disease. *Metallomics* 11:1140–1153. 10.23937/2378-3516/1410098 ⏎

Davis EC, Castagna VP, Sela DA, *et al.* (2022) Gut microbiome and breast-feeding: Implications for early immune development. *J Allergy Clin Immunol* 150:523–534. https://doi.org/10.1016/j.jaci.2022.07.014 ⏎

De Almeida CV, Antiga E, Lulli M (2023) Oral and topical probiotics and postbiotics in skincare and dermatological therapy: A concise review. *Microorganisms* 11:1420. https://doi.org/10.3390/microorganisms11061420 ⏎

De Boeck I, Wittouck S, Martens K, *et al.* (2019) Anterior nares diversity and pathobionts represent sinus microbiome in chronic rhinosinusitis. *mSphere* 4:e00532-19. https://doi.org/10.1128/mSphere.00532-19 ⏎

De Pessemier B, Grine L, Debaere M, *et al.* (2021) Gut–skin axis: Current knowledge of the interrelationship between microbial dysbiosis and skin conditions. *Microorganisms* 9:353. https://doi.org/10.3390/microorganisms9020353 ⏎

Deka N, Hassan S, Seghal Kiran G, Selvin J (2021) Insights into the role of vaginal microbiome in women's health. *J Basic Microbiol* 61:1071–1084. https://doi.org/10.1002/jobm.202100421 ⏎

den Heijer CDJ, Hoebe CJPA, Driessen JHM, *et al.* (2019) *Chlamydia trachomatis* and the risk of pelvic inflammatory disease, ectopic pregnancy, and female infertility: A retrospective cohort study among primary care patients. *Clin Infect Dis* 69:1517–1525. https://doi.org/10.1093/cid/ciz429 ⏎

Denman CR, Park SM, Jo J (2023) Gut-brain axis: Gut dysbiosis and psychiatric disorders in Alzheimer's and Parkinson's disease. *Front Neurosci* 17:1268419. https://doi.org/10.3389/fnins.2023.1268419 ⏎

Deo P, Deshmukh R (2019) Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol* 23:122. https://doi.org/10.4103/jomfp.JOMFP_304_18 ⏎

Depommier C, Everard A, Druart C, *et al.* (2019) Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nat Med* 25:1096–1103. https://doi.org/10.1038/s41591-019-0495-2 ⏎

Di Cicco F, Evans RL, James AG, *et al.* (2023) Intrinsic and extrinsic factors affecting axillary odor variation. A comprehensive review. *Physiol Behav* 270:114307. https://doi.org/10.1016/j.physbeh.2023.114307 ⏎

Di Paola M, Sani C, Clemente AM, *et al.* (2017) Characterization of cervico-vaginal microbiota in women developing persistent high-risk human papillomavirus infection. *Sci Rep* 7:10200. https://doi.org/10.1038/s41598-017-09842-6 ⏎

Djusse ME, Prinelli F, Camboni T, *et al.* (2025) Dietary habits and vaginal environment: Can a beneficial impact be expected? *Front Cell Infect Microbiol* 15:1582283. https://doi.org/10.3389/fcimb.2025.1582283 ⏎

Dominguez-Bello MG, Costello EK, Contreras M, *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci* 107:11971–11975. https://doi.org/10.1073/pnas.1002601107 ⏎

Donders GGG, Bellen G, Grinceviciene S, *et al.* (2017) Aerobic vaginitis: No longer a stranger. *Res Microbiol* 168:845–858. https://doi.org/10.1016/j.resmic.2017.04.004 ⏎

Donders GGG, Bellen G, Ruban KS (2018) Abnormal vaginal microbioma is associated with severity of localized provoked vulvodynia. Role of aerobic vaginitis and Candida in the pathogenesis of vulvodynia. *Eur J*

Clin Microbiol Infect Dis 37:1679–1685. https://doi.org/10.1007/s10096-018-3299-2 ⏎

Dréno B, Araviiskaia E, Berardesca E, *et al.* (2016) Microbiome in healthy skin, update for dermatologists. *J Eur Acad Dermatology Venereol* 30:2038–2047. https://doi.org/10.1111/jdv.13965 ⏎

Dubé-Zinatelli E, Cappelletti L, Ismail N (2024) Vaginal microbiome: Environmental, biological, and racial influences on gynecological health across the lifespan. *Am J Reprod Immunol* 92:e70026. https://doi.org/10.1111/aji.70026 ⏎

El-Sokkary MMA. (2021) Microbial profiling of wound pathogens in isolates from an Egyptian hospital using a microarray chip. *J Appl Pharm Sci* 11:139–146.

Elias PM, Feingold KR (eds) (2005) *Skin Barrier*. CRC Press. ⏎

Ellis JR, Powell EJ, Tomasovic LM, *et al.* (2024) Changes in the skin microbiome following dermatological procedures: A scoping review. *Appl Microbiol* 4:972–985. https://doi.org/10.3390/applmicrobiol4020066 ⏎

Fagö-Olsen H, Dines LM, Sørensen CH, Jensen A (2019) The adenoids but not the palatine tonsils serve as a reservoir for bacteria associated with secretory otitis media in small children. *mSystems* 4:e00169-18. https://doi.org/10.1128/msystems.00169-18 ⏎

Fan A, Yue Y, Geng N, *et al.* (2013) Aerobic vaginitis and mixed infections: Comparison of clinical and laboratory findings. *Arch Gynecol Obstet* 287:329–335. https://doi.org/10.1007/s00404-012-2571-4 ⏎

Fekete S, Juhász J, Makra N, *et al.* (2024) Characterization of middle ear microbiome in otitis media with effusion in Hungarian children: *Alloiococcus otitidis* may potentially hamper the microbial diversity. *Heliyon* 10:e39380. https://doi.org/10.1016/j.heliyon.2024.e39380 ⏎

Feng T, Liu Y (2022) Microorganisms in the reproductive system and probiotic's regulatory effects on reproductive health. *Comput Struct Biotechnol J* 20:1541–1553. https://doi.org/10.1016/j.csbj.2022.03.017 ⏎

Fettweis JM, Brooks JP, Serrano MG, *et al.* (2014) Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology* 160:2272–2282. https://doi.org/10.1099/mic.0.081034-0 ⏎

Fitz-Gibbon S, Tomida S, Chiu B-H, *et al.* (2013) *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. *J Invest Dermatol* 133:2152–2160. https://doi.org/10.1038/jid.2013.21 ⏎

Foschi C, Alvisi S, Baldassarre M, *et al.* (2022) Vaginal metabolites in postmenopausal women with or without vulvo-vaginal atrophy at baseline and after ospemifeme and systemic hormone treatment. *Maturitas* 159:7–14. https://doi.org/10.1016/j.maturitas.2021.12.007 ⏎

France M, Alizadeh M, Brown S, *et al.* (2022) Towards a deeper understanding of the vaginal microbiota. *Nat Microbiol* 7:367–378. https://doi.org/10.1038/s41564-022-01083-2 ⏎

France MT, Ma B, Gajer P, *et al.* (2020) VALENCIA: A nearest centroid classification method for vaginal microbial communities based on composition. *Microbiome* 8:166. https://doi.org/10.1186/s40168-020-00934-6 ⏎

Fuochi V, Cardile V, Petronio Petronio G, Furneri PM (2019) Biological properties and production of bacteriocins-like-inhibitory substances by *Lactobacillus* sp. strains from human vagina. *J Appl Microbiol* 126:1541–1550. https://doi.org/10.1111/jam.14164 ⏎

Gallardo-Becerra L, Cornejo-Granados F, García-López R, *et al.* (2020) Metatranscriptomic analysis to define the Secrebiome, and 16S rRNA profiling of the gut microbiome in obesity and metabolic syndrome of Mexican children. *Microb Cell Fact* 19:61. https://doi.org/10.1186/s12934-020-01319-y ⏎

Garcia-Gutierrez E, O'Mahony AK, Dos Santos RS, *et al.* (2024) Gut microbial metabolic signatures in diabetes mellitus and potential preventive and therapeutic applications. *Gut Microbes* 16:2401654. https://doi.org/10.1080/19490976.2024.2401654 ⏎

Gilbert JA, Blaser MJ, Caporaso JG, *et al.* (2018) Current understanding of the human microbiome. *Nat Med* 24:392–400. https://doi.org/10.1038/nm.4517 ⏎

Gonçalves JIB, Borges TJ, de Souza APD (2022) Microbiota and the response to vaccines against respiratory virus. *Front Immunol* 13:889945. https://doi.org/10.3389/fimmu.2022.889945 ⏎

Gowda V, Sarkar R, Verma D, Das A (2024) Probiotics in dermatology: An evidence-based approach. *Indian Dermatol Online J* 15:571–583. https://doi.org/10.4103/idoj.idoj_614_23 ⏎

Grice EA, Kong HH, Conlan S, *et al.* (2009) Topographical and temporal diversity of the human skin microbiome. *Science (80-)* 324:1190–1192. https://doi.org/10.1126/science.1171700 ⏎

Grice EA, Segre JA (2011) The skin microbiome. *Nat Rev Microbiol* 9:244–253. https://doi.org/10.1038/nrmicro2537 ⏎

Gupta P, Singh MP, Goyal K (2020) Diversity of vaginal microbiome in pregnancy: Deciphering the obscurity. *Front Public Heal* 8:326. https://doi.org/10.3389/fpubh.2020.00326 ⏎

Gutzeit O, Gulati A, Izadifar Z, *et al.* (2025) Cervical mucus in linked human Cervix and Vagina Chips modulates vaginal dysbiosis. *npj Women's Heal* 3:5. https://doi.org/10.1038/s44294-025-00054-2 ⏎

Güvenç MG, Midilli K, Inci E, *et al.* (2010) Lack of *Chlamydophila pneumoniae* and predominance of *Alloiococcus otitidis* in middle ear fluids of children with otitis media with effusion. *Auris Nasus Larynx* 37:269–273. https://doi.org/10.1016/j.anl.2009.09.002 ⏎

Haahtela T, Holgate S, Pawankar R, *et al.* (2013) The biodiversity hypothesis and allergic disease: World Allergy Organization position statement. *World Allergy Organ J* 6:3. https://doi.org/10.1186/1939-4551-6-3 ⏎

Haggerty CL, Taylor BD (2011) Mycoplasma genitalium: An emerging cause of pelvic inflammatory disease. *Infect Dis Obstet Gynecol* 2011:1–9. https://doi.org/10.1155/2011/959816 ⏎

Hannigan GD, Meisel JS, Tyldsley AS, *et al.* (2015) The human skin double-stranded DNA virome: Topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. *MBio* 6:e01578-15. https://doi.org/10.1128/mBio.01578-15 ⏎

Hickey RJ, Zhou X, Settles ML, *et al.* (2015) Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *MBio* 6:e00097-15. https://doi.org/10.1128/mBio.00097-15 ⏎

Ho AW, Kupper TS (2019) T cells and the skin: From protective immunity to inflammatory skin disorders. *Nat Rev Immunol* 19:490–502. https://doi.org/10.1038/s41577-019-0162-3 ⏎

Hoenderboom BM, van Benthem BHB, van Bergen JEAM, *et al.* (2019) Relation between *Chlamydia trachomatis* infection and pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility in a Dutch cohort of women previously tested for *Chlamydia* in a chlamydia screening trial. *Sex Transm Infect 95:300*–306. https://doi.org/10.1136/sextrans-2018-053778 ⏎

Holdcroft AM, Ireland DJ, Payne MS (2023) The vaginal microbiome in health and disease—What role do common intimate hygiene practices play? *Microorganisms* 11:298. https://doi.org/10.3390/microorganisms11020298 ⏎

Holm JB, France MT, Gajer P, *et al.* (2023) Integrating compositional and functional content to describe vaginal microbiomes in health and disease. *Microbiome* 11:259. https://doi.org/10.1186/s40168-023-01692-x ⏎

Hong E, Dixit S, Fidel PL, *et al.* (2014) Vulvovaginal Candidiasis as a chronic disease. *J Low Genit Tract Dis* 18:31–38. https://doi.org/10.1097/LGT.0b013e318287aced ⏎

Hou K, Wu Z-X, Chen X-Y, *et al.* (2022) Microbiota in health and diseases. *Signal Transduct Target Ther* 7:135. https://doi.org/10.1038/s41392-022-00974-4 ⏎

How KY, Song KP, Chan KG (2016) Porphyromonas gingivalis: An overview of periodontopathic pathogen below the gum line. *Front*

Microbiol 7:53. https://doi.org/10.3389/fmicb.2016.00053 ⏎

Howard B, Bascom CC, Hu P, *et al.* (2022) Aging-associated changes in the adult human skin microbiome and the host factors that affect skin microbiome composition. *J Invest Dermatol* 142:1934–1946.e21. https://doi.org/10.1016/j.jid.2021.11.029 ⏎

Ianiro G, Maida M, Burisch J, *et al.* (2018) Efficacy of different faecal microbiota transplantation protocols for *Clostridium difficile* infection: A systematic review and meta-analysis. *United Eur Gastroenterol J* 6:1232–1244. https://doi.org/10.1177/2050640618780762 ⏎

Isenring J, Bircher L, Geirnaert A, Lacroix C (2023) In vitro human gut microbiota fermentation models: Opportunities, challenges, and pitfalls. *Microbiome Res Reports* 2:2. https://doi.org/10.20517/mrr.2022.15 ⏎

Iwase T, Uehara Y, Shinji H, *et al.* (2010) *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 465:346–349. https://doi.org/10.1038/nature09074 ⏎

Jarmuda S, O'Reilly N, Żaba R, *et al.* (2012) Potential role of Demodex mites and bacteria in the induction of rosacea. *J Med Microbiol* 61:1504–1510. https://doi.org/10.1099/jmm.0.048090-0 ⏎

Jarrett OD, Srinivasan S, Richardson BA, *et al.* (2019) Specific vaginal bacteria are associated with an increased risk of *Trichomonas vaginalis* acquisition in women. *J Infect Dis* 220:1503–1510. https://doi.org/10.1093/infdis/jiz354 ⏎

Jervis-Bardy J, Leong LEX, Papanicolas LE, *et al.* (2019) Examining the evidence for an adult healthy middle ear microbiome. *mSphere* 4:e00456-19. https://doi.org/10.1128/mSphere.00456-19 ⏎

Jervis-Bardy J, Rogers GB, Morris PS, *et al.* (2015) The microbiome of otitis media with effusion in Indigenous Australian children. *Int J Pediatr Otorhinolaryngol* 79:1548–1555. https://doi.org/10.1016/j.ijporl.2015.07.013 ⏎

Jespers V, Hardy L, Buyze J, *et al.* (2016) Association of sexual debut in adolescents with microbiota and inflammatory markers. *Obstet Gynecol* 128:22–31. https://doi.org/10.1097/AOG.0000000000001468 ⏎

Jovel J, Patterson J, Wang W, *et al.* (2016) Characterization of the gut microbiome using 16S or shotgun metagenomics. *Front Microbiol* 7:459. https://doi.org/10.3389/fmicb.2016.00459 ⏎

Kaambo E, Africa C, Chambuso R, Passmore J-AS (2018) Vaginal microbiomes associated with aerobic vaginitis and bacterial vaginosis. *Front Public Heal* 6:78. https://doi.org/10.3389/fpubh.2018.00078 ⏎

Kaiko GE, Stappenbeck TS (2014) Host–microbe interactions shaping the gastrointestinal environment. *Trends Immunol* 35:538–548. https://doi.org/10.1016/j.it.2014.08.002 ⏎

Kanazawa A, Aida M, Yoshida Y, *et al.* (2021) Effects of synbiotic supplementation on chronic inflammation and the gut microbiota in obese patients with Type 2 diabetes mellitus: A randomized controlled study. *Nutrients* 13:558. https://doi.org/10.3390/nu13020558 ⏎

Kaplan GG, Windsor JW (2021) The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 18:56–66. https://doi.org/10.1038/s41575-020-00360-x ⏎

Karbalaei M, Keikha M, Kobyliak NM, *et al.* (2021) Alleviation of halitosis by use of probiotics and their protective mechanisms in the oral cavity. *New Microbes New Infect* 42:100887. https://doi.org/10.1016/j.nmni.2021.100887 ⏎

Kaur H, Merchant M, Haque MM, Mande SS (2020) Crosstalk between female gonadal hormones and vaginal microbiota across various phases of women's gynecological lifecycle. *Front Microbiol* 11:551. https://doi.org/10.3389/fmicb.2020.00551 ⏎

Khalil M, Di Ciaula A, Mahdi L, *et al.* (2024) Unraveling the role of the human gut microbiome in health and diseases. *Microorganisms* 12:2333. https://doi.org/10.3390/microorganisms12112333 ⏎

Kielbik K, Pietras A, Jablonska J, *et al.* (2022) Impact of pneumococcal vaccination on nasopharyngeal carriage of *Streptococcus pneumoniae* and microbiota profiles in preschool children in South East Poland. *Vaccines* 10:791. https://doi.org/10.3390/vaccines10050791 ⏎

Kim B-J, Lee S-Y, Kim H-B, *et al.* (2014) Environmental changes, microbiota, and allergic diseases. *Allergy Asthma Immunol Res* 6:389. https://doi.org/10.4168/aair.2014.6.5.389 ⏎

Kim BE, Leung DYM (2018) Significance of skin barrier dysfunction in atopic dermatitis. *Allergy Asthma Immunol Res* 10:207. https://doi.org/10.4168/aair.2018.10.3.207 ⏎

Kim J, Kim BE, Ahn K, Leung DYM (2019) Interactions between atopic dermatitis and *Staphylococcus aureus* infection: Clinical implications. *Allergy Asthma Immunol Res* 11:593. https://doi.org/10.4168/aair.2019.11.5.593 ⏎

Kim S, Ndwandwe C, Devotta H, *et al.* (2025) Role of the microbiome in regulation of the immune system. *Allergol Int* 74:187–196. https://doi.org/10.1016/j.alit.2024.12.006 ⏎

Kim SK, Han SJ, Hong SJ, Hong SM (2022) Microbiome of acute otitis externa. *J Clin Med* 11:7074. https://doi.org/10.3390/jcm11237074 ⏎

Klein JO (2015) Otitis externa, otitis media, and mastoiditis. In: Mandell, Douglas, and *Bennett's Principles and Practice of Infectious Diseases*. Elsevier, pp 767–773.e1. ⏎

Kolbe AR, Castro-Nallar E, Preciado D, Pérez-Losada M (2019) Altered middle ear microbiome in children with chronic otitis media with effusion and respiratory illnesses. *Front Cell Infect Microbiol* 9:339. https://doi.org/10.3389/fcimb.2019.00339 ⏎

Kong HH, Oh J, Deming C, *et al.* (2012) Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 22:850–859. https://doi.org/10.1101/gr.131029.111 ⏎

Krauss-Silva L, Almada-Horta A, Alves MB, *et al.* (2014) Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: Prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. *BMC Pregnancy Childbirth* 14:107. https://doi.org/10.1186/1471-2393-14-107 ⏎

Krog MC, Hugerth LW, Fransson E, *et al.* (2022) The healthy female microbiome across body sites: Effect of hormonal contraceptives and the menstrual cycle. *Hum Reprod* 37:1525–1543. https://doi.org/10.1093/humrep/deac094 ⏎

Kulis E, Cvitkovic I, Pavlovic N, *et al.* (2025) A comprehensive review of antibiotic resistance in the oral microbiota: Mechanisms, drivers, and emerging therapeutic strategies. *Antibiotics* 14:828. https://doi.org/10.3390/antibiotics14080828 ⏎

Kumpitsch C, Fischmeister FPS, Lackner S, *et al.* (2024) Reduced olfactory performance is associated with changed microbial diversity, oralization, and accumulation of dead biomaterial in the nasal olfactory area. *Microbiol Spectr* 12:e0154923. https://doi.org/10.1128/spectrum.01549-23 ⏎

Laghi L, Zagonari S, Patuelli G, *et al.* (2021) Vaginal metabolic profiles during pregnancy: Changes between first and second trimester. *PLoS One* 16:e0249925. https://doi.org/10.1371/journal.pone.0249925 ⏎

Lambers H, Piessens S, Bloem A, *et al.* (2006) Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int J Cosmet Sci* 28:359–370. https://doi.org/10.1111/j.1467-2494.2006.00344.x ⏎

Landemaine L, Da Costa G, Fissier E, *et al.* (2023) *Staphylococcus epidermidis* isolates from atopic or healthy skin have opposite effect on skin cells: Potential implication of the AHR pathway modulation. *Front Immunol* 14:1098160. https://doi.org/10.3389/fimmu.2023.1098160 ⏎

Larsen JM (2017) The immune response to *Prevotella* bacteria in chronic inflammatory disease. *Immunology* 151:363–374. https://doi.org/10.1111/imm.12760 ⏎

Lebeer S, Ahannach S, Gehrmann T, *et al.* (2023) A citizen-science-enabled catalogue of the vaginal microbiome and associated factors. *Nat Microbiol* 8:2183–2195. https://doi.org/10.1038/s41564-023-01500-0 ⏎

Lee CH, Min M, Jin SS, Sivamani RK (2025) Skin microbiome shifts in various dermatological conditions. *J Clin Med* 14:6137.

https://doi.org/10.3390/jcm14176137 ⏎

Lee HJ, Jeong SE, Lee S, *et al.* (2018) Effects of cosmetics on the skin microbiome of facial cheeks with different hydration levels. *Microbiologyopen* 7:e00557. https://doi.org/10.1002/mbo3.557 ⏎

Lee JE, Lee S, Lee H, *et al.* (2013) Association of the vaginal microbiota with human papillomavirus infection in a Korean Twin cohort. *PLoS One* 8:e63514. https://doi.org/10.1371/journal.pone.0063514 ⏎

Lee JS, Lee SM, Son HS, *et al.* (2022) Analysis of the microbiome of the ear canal in normal individuals and patients with chronic otitis externa. *Ann Dermatol* 34:461. https://doi.org/10.5021/ad.22.153 ⏎

Lee SA, Lim JY, Kim B-S, *et al.* (2015) Comparison of the gut microbiota profile in breast-fed and formula-fed Korean infants using pyrosequencing. *Nutr Res Pract* 9:242. https://doi.org/10.4162/nrp.2015.9.3.242 ⏎

Lehtoranta L, Ala-Jaakkola R, Laitila A, Maukonen J (2022) Healthy vaginal microbiota and influence of probiotics across the female life span. *Front Microbiol* 13:819958. https://doi.org/10.3389/fmicb.2022.819958 ⏎

Leonard JM, Toro D Del (2023) Defining the microbiome components (bacteria, viruses, fungi) and microbiome geodiversity. *Surg Infect (Larchmt)* 24:208–212. https://doi.org/10.1089/sur.2023.014 ⏎

Leyden JJ, McGinley KJ, Hölzle E, *et al.* (1981) The microbiology of the human axilla and its relationship to axillary odor. *J Invest Dermatol* 77:413–416. https://doi.org/10.1111/1523-1747.ep12494624 ⏎

Li KT, Li F, Jaspan H, *et al.* (2024) Changes in the vaginal microbiome during pregnancy and the postpartum period in South African women: A longitudinal study. *Reprod Sci* 31:275–287. https://doi.org/10.1007/s43032-023-01351-4 ⏎

Li S, Liu J, Zhang X, *et al.* (2025) The potential impact of antibiotic exposure on the microbiome and human health. *Microorganisms* 13:602. https://doi.org/10.3390/microorganisms13030602 ⏎

Li Y, Zhu F, Li Y, *et al.* (2022) Bacteriophages allow selective depletion of gut bacteria to produce a targeted-bacterium-depleted mouse model. *Cell Rep Methods* 2:100324. https://doi.org/10.1016/j.crmeth.2022.100324 ⏎

Lingasamy P, Modhukur V, Mändar R, Salumets A (2023) Exploring immunome and microbiome interplay in reproductive health: Current knowledge, challenges, and novel diagnostic tools. *Semin Reprod Med* 41:172–189. https://doi.org/10.1055/s-0043-1778017 ⏎

Liptáková A, Čurová K, Záhumenský J, *et al.* (2022) Microbiota of female genital tract—functional overview of microbial flora from vagina to uterine tubes and placenta. *Physiol Res* 71:S21–S33. https://doi.org/10.33549/physiolres.934960 ⏎

Liu H, Huang Y, Huang M, *et al.* (2023) From nitrate to NO: Potential effects of nitrate-reducing bacteria on systemic health and disease. *Eur J Med Res* 28:425. https://doi.org/10.1186/s40001-023-01413-y ⏎

Lloyd-Price J, Arze C, Ananthakrishnan AN, *et al.* (2019) Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 569:655–662. https://doi.org/10.1038/s41586-019-1237-9 ⏎

Łuczaj W, Wroński A, Domingues P, *et al.* (2020) Lipidomic analysis reveals specific differences between fibroblast and keratinocyte ceramide profile of patients with psoriasis vulgaris. *Molecules* 25:630. https://doi.org/10.3390/molecules25030630 ⏎

Ma B, Forney LJ, Ravel J (2012) Vaginal microbiome: Rethinking health and disease. *Annu Rev Microbiol* 66:371–389. https://doi.org/10.1146/annurev-micro-092611-150157 ⏎

Ma J, Li Z, Zhang W, *et al.* (2020) Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: A study of 91 term infants. *Sci Rep* 10:15792. https://doi.org/10.1038/s41598-020-72635-x ⏎

Ma Q, Xing C, Long W, *et al.* (2019) Impact of microbiota on central nervous system and neurological diseases: The gut–brain axis. *J Neuroinflammation* 16:53. https://doi.org/10.1186/s12974-019-1434-3 ⏎

Ma Z, Zuo T, Frey N, Rangrez AY (2024) A systematic framework for understanding the microbiome in human health and disease: From basic principles to clinical translation. *Signal Transduct Target Ther* 9:237. https://doi.org/10.1038/s41392-024-01946-6 ⏎

Machado A, Cerca N (2015) Influence of biofilm formation by *Gardnerella vaginalis* and other anaerobes on bacterial vaginosis. *J Infect Dis* 212:1856–1861. https://doi.org/10.1093/infdis/jiv338 ⏎

Manuzak JA, Zevin AS, Cheu R, *et al.* (2020) Antibiotic-induced microbiome perturbations are associated with significant alterations to colonic mucosal immunity in *Rhesus macaques*. *Mucosal Immunol* 13:471–480. https://doi.org/10.1038/s41385-019-0238-1 ⏎

Margarita V, Fiori PL, Rappelli P (2020) Impact of symbiosis between *Trichomonas vaginalis* and *Mycoplasma hominis* on vaginal dysbiosis: A mini review. *Front Cell Infect Microbiol* 10:179. https://doi.org/10.3389/fcimb.2020.00179 ⏎

Markiewicz E, Idowu O (2018) Personalized skincare: From molecular basis to clinical and commercial applications. *Clin Cosmet Investig Dermatol* 11:161–171. https://doi.org/10.2147/CCID.S163799 ⏎

Martínez-García M, Hernández-Lemus E (2021) Periodontal inflammation and systemic diseases: An overview. *Front Physiol* 12:709438. https://doi.org/10.3389/fphys.2021.709438 ⏎

Matchado MS, Rühlemann M, Reitmeier S, *et al.* (2024) On the limits of 16S rRNA gene-based metagenome prediction and functional profiling. *Microb Genomics* 10:001203. https://doi.org/10.1099/mgen.0.001203 ⏎

McCauley KE, Durack J, Lynch K V., *et al.* (2024) Early-life nasal microbiota dynamics relate to longitudinal respiratory phenotypes in urban children. *J Allergy Clin Immunol* 153:1563–1573. https://doi.org/10.1016/j.jaci.2023.12.032 ⏎

McLaughlin J, Watterson S, Layton AM, *et al.* (2019) Propionibacterium acnes and acne vulgaris: New insights from the integration of population genetic, multi-omic, biochemical and host–microbe studies.

Microorganisms 7:128. https://doi.org/10.3390/microorganisms7050128 ⏎

Mei L, Wang T, Chen Y, *et al.* (2022) Dysbiosis of vaginal microbiota associated with persistent high-risk human papilloma virus infection. *J Transl Med* 20:12. https://doi.org/10.1186/s12967-021-03201-w ⏎

Meslier V, Laiola M, Roager HM, *et al.* (2020) Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* 69:1258–1268. https://doi.org/10.1136/gutjnl-2019-320438 ⏎

Miko E, Barakonyi A (2023) The role of hydrogen-peroxide (H_2O_2) produced by vaginal microbiota in female reproductive health. *Antioxidants* 12:1055. https://doi.org/10.3390/antiox12051055 ⏎

Miles SM, Hardy BL, Merrell DS (2017) Investigation of the microbiota of the reproductive tract in women undergoing a total hysterectomy and bilateral salpingo-oopherectomy. *Fertil Steril* 107:813–820.e1. https://doi.org/10.1016/j.fertnstert.2016.11.028 ⏎

Miller LS, Cho JS (2011) Immunity against *Staphylococcus aureus* cutaneous infections. *Nat Rev Immunol* 11:505–518. https://doi.org/10.1038/nri3010 ⏎

Minami SB, Mutai H, Suzuki T, *et al.* (2017) Microbiomes of the normal middle ear and ears with chronic otitis media. *Laryngoscope* 127:E371–E377. https://doi.org/10.1002/lary.26579 ⏎

Misic AM, Gardner SE, Grice EA (2014) The wound microbiome: Modern approaches to examining the role of microorganisms in impaired chronic wound healing. *Adv Wound Care* 3:502–510. https://doi.org/10.1089/wound.2012.0397 ⏎

Mladěnka P, Macáková K, Kujovská Krčmová L, *et al.* (2022) Vitamin K—sources, physiological role, kinetics, deficiency, detection, therapeutic use, and toxicity. *Nutr Rev* 80:677–698. https://doi.org/10.1093/nutrit/nuab061 ⏎

Mohamed Mohamed Adel E-S (2021) Microbial profiling of wound pathogens in isolates from an Egyptian hospital using a microarray chip. *J Appl Pharm Sci*. https://doi.org/10.7324/JAPS.2021.110819 ⏎

Moitinho-Silva L, Degenhardt F, Rodriguez E, *et al.* (2022) Host genetic factors related to innate immunity, environmental sensing and cellular functions are associated with human skin microbiota. *Nat Commun* 13:6204. https://doi.org/10.1038/s41467-022-33906-5 ⏎

Mondal AS, Sharma R, Trivedi N (2023) Bacterial vaginosis: A state of microbial dysbiosis. *Med Microecol* 16:100082. https://doi.org/10.1016/j.medmic.2023.100082 ⏎

Montoya-Williams D, Lemas DJ, Spiryda L, *et al.* (2018) The neonatal microbiome and its partial role in mediating the association between birth by cesarean section and adverse pediatric outcomes. *Neonatology* 114:103–111. https://doi.org/10.1159/000487102 ⏎

Moreno I, Simon C (2019) Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol* 18:40–50. https://doi.org/10.1002/rmb2.12249 ⏎

Morou-Bermúdez E, Torres-Colón JE, Bermúdez NS, *et al.* (2022) Pathways linking oral bacteria, nitric oxide metabolism, and health. *J Dent Res* 101:623–631. https://doi.org/10.1177/00220345211064571 ⏎

Mousa WK, Chehadeh F, Husband S (2022) Recent advances in understanding the structure and function of the human microbiome. *Front Microbiol* 13:825338. https://doi.org/10.3389/fmicb.2022.825338 ⏎

Muzny CA, Taylor CM, Swords WE, *et al.* (2019) An updated conceptual model on the pathogenesis of bacterial vaginosis. *J Infect Dis* 220:1399–1405. https://doi.org/10.1093/infdis/jiz342 ⏎

Myles IA, Williams KW, Reckhow JD, *et al.* (2016) Transplantation of human skin microbiota in models of atopic dermatitis. *JCI Insight* 1:e86955. https://doi.org/10.1172/jci.insight.86955 ⏎

Nakatsuji T, Gallo RL (2019) The role of the skin microbiome in atopic dermatitis. *Ann Allergy Asthma Immunol* 122:263–269.

https://doi.org/10.1016/j.anai.2018.12.003 ⏎

Neeff M, Biswas K, Hoggard M, *et al.* (2016) Molecular microbiological profile of chronic suppurative otitis media. *J Clin Microbiol* 54:2538–2546. https://doi.org/10.1128/JCM.01068-16 ⏎

Negrini T de C, Carlos IZ, Duque C, *et al.* (2021) Interplay among the oral microbiome, oral cavity conditions, the host immune response, diabetes mellitus, and its associated-risk factors—An overview. *Front Oral Heal* 2:697428. https://doi.org/10.3389/froh.2021.697428 ⏎

Nel Van Zyl K, Matukane SR, Hamman BL, *et al.* (2022) Effect of antibiotics on the human microbiome: A systematic review. *Int J Antimicrob Agents* 59:106502. https://doi.org/10.1016/j.ijantimicag.2021.106502 ⏎

Nelson TM, Borgogna J-LC, Brotman RM, *et al.* (2015) Vaginal biogenic amines: Biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? *Front Physiol* 6:253. https://doi.org/10.3389/fphys.2015.00253 ⏎

Newstead LL, Varjonen K, Nuttall T, Paterson GK (2020) Staphylococcal-produced bacteriocins and antimicrobial peptides: Their potential as alternative treatments for *Staphylococcus aureus* infections. *Antibiotics* 9:40. https://doi.org/10.3390/antibiotics9020040 ⏎

Ney L-M, Wipplinger M, Grossmann M, *et al.* (2023) Short chain fatty acids: Key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biol* 13:230014. https://doi.org/10.1098/rsob.230014 ⏎

Neyrinck AM, Rodriguez J, Zhang Z, *et al.* (2021) Prebiotic dietary fibre intervention improves fecal markers related to inflammation in obese patients: Results from the Food4Gut randomized placebo-controlled trial. *Eur J Nutr* 60:3159–3170. https://doi.org/10.1007/s00394-021-02484-5 ⏎

Ngo CC, Massa HM, Thornton RB, Cripps AW (2016) Predominant bacteria detected from the middle ear fluid of children experiencing otitis

media: A systematic review. *PLoS One* 11:e0150949. https://doi.org/10.1371/journal.pone.0150949 ⏎

Ni J, Wu GD, Albenberg L, Tomov VT (2017) Gut microbiota and IBD: Causation or correlation? *Nat Rev Gastroenterol Hepatol* 14:573–584. https://doi.org/10.1038/nrgastro.2017.88 ⏎

Nickerson R, Thornton CS, Johnston B, *et al.* (2024) Pseudomonas aeruginosa in chronic lung disease: Untangling the dysregulated host immune response. *Front Immunol* 15:1405376. https://doi.org/10.3389/fimmu.2024.1405376 ⏎

Nogues JC, Pérez-Losada M, Preciado D (2020) Review of otitis media microbiome studies: What do they tell us? *Laryngoscope Investig Otolaryngol* 5:936–940. https://doi.org/10.1002/lio2.460 ⏎

Nørreslet LB, Agner T, Clausen M-L (2020) The skin microbiome in inflammatory skin diseases. *Curr Dermatol Rep* 9:141–151. https://doi.org/10.1007/s13671-020-00297-z ⏎

Novak J, Ravel J, Ma B, *et al.* (2022) Characteristics associated with *Lactobacillus iners*-dominated vaginal microbiota. *Sex Transm Infect* 98:353–359. https://doi.org/10.1136/sextrans-2020-054824 ⏎

Nunn KL, Witkin SS, Schneider GM, *et al.* (2021) Changes in the vaginal microbiome during the pregnancy to postpartum transition. *Reprod Sci* 28:1996–2005. https://doi.org/10.1007/s43032-020-00438-6 ⏎

Nyirjesy P, Brookhart C, Lazenby G, *et al.* (2022) Vulvovaginal candidiasis: A review of the evidence for the 2021 Centers for Disease Control and Prevention of Sexually Transmitted Infections Treatment Guidelines. *Clin Infect Dis* 74:S162–S168. https://doi.org/10.1093/cid/ciab1057 ⏎

O'Sullivan A, Farver M, Smilowitz JT (2015) Correction to "The Influence of Early Infant-Feeding Practices on the Intestinal Microbiome and Body Composition in Infants." *Nutr Metab Insights* 8s1:87. https://doi.org/10.4137/NMI.S41125 ⏎

Occhipinti S, Incognito GG, Palumbo M (2024) The influence of the vaginal ecosystem on vaginitis, bacterial vaginosis, and sexually

transmitted diseases: An epidemiological study and literature review. *Arch Gynecol Obstet* 311:347–353. https://doi.org/10.1007/s00404-024-07626-8 ⏎

Oerlemans EFM, Wuyts S, Bellen G, *et al.* (2020) The dwindling microbiota of aerobic vaginitis, an inflammatory state enriched in pathobionts with limited TLR stimulation. *Diagnostics* 10:879. https://doi.org/10.3390/diagnostics10110879 ⏎

Ogunrinola GA, Oyewale JO, Oshamika OO, Olasehinde GI (2020) The human microbiome and its impacts on health. *Int J Microbiol* 2020:1–7. https://doi.org/10.1155/2020/8045646 ⏎

Onderdonk AB, Delaney ML, Fichorova RN (2016) The human microbiome during bacterial vaginosis. *Clin Microbiol Rev* 29:223–238. https://doi.org/10.1128/CMR.00075-15 ⏎

Ortega-Peña S, Rodríguez-Martínez S, Cancino-Diaz ME, Cancino-Diaz JC (2022) *Staphylococcus epidermidis* controls opportunistic pathogens in the nose, could it help to regulate SARS-CoV-2 (COVID-19) Infection? *Life* 12:341. https://doi.org/10.3390/life12030341 ⏎

Ortiz-Velez L, Goodwin A, Schaefer L, Britton RA (2020) Challenges and pitfalls in the engineering of human interleukin 22 (hIL-22) secreting *Lactobacillus reuteri*. *Front Bioeng Biotechnol* 8:543. https://doi.org/10.3389/fbioe.2020.00543 ⏎

Orton K l'Cess, Monaco CL (2025) The vaginal virome in women's health and disease. *Microorganisms* 13:431. https://doi.org/10.3390/microorganisms13020431 ⏎

Osguthorpe JD, Nielsen DR (2006) Otitis externa: Review and clinical update. *Am Fam Physician* 74:1510–1516 ⏎

Otter CJ, Fausto A, Tan LH, Khosla AS, Cohen NA, Weiss SR. (2023) Infection of primary nasal epithelial cells differentiates among lethal and seasonal human coronaviruses. *Proc Natl Acad Sci U S A*. 120:e2218083120. doi: 10.1073/pnas.2218083120. ⏎

Pal G, Ramirez V, Engen PA, *et al.* (2021) Deep nasal sinus cavity microbiota dysbiosis in Parkinson's disease. *npj Park Dis* 7:111.

https://doi.org/10.1038/s41531-021-00254-y ⏎

Pastar I, O'Neill K, Padula L, *et al.* (2021) Corrigendum: *Staphylococcus epidermidis* boosts innate immune response by activation of gamma delta T cells and induction of perforin-2 in human skin. *Front Immunol* 12:741437. https://doi.org/10.3389/fimmu.2021.741437 ⏎

Pattaroni C, Marsland BJ, Harris NL (2025) Early-life host–microbial interactions and asthma development: A lifelong impact? *Immunol Rev* 330:e70019. https://doi.org/10.1111/imr.70019 ⏎

Pekmezovic M, Mogavero S, Naglik JR, Hube B (2019) Host–pathogen interactions during female genital tract infections. *Trends Microbiol* 27:982–996. https://doi.org/10.1016/j.tim.2019.07.006 ⏎

Pinchuk I V., Beswick EJ, Reyes VE (2010) Staphylococcal enterotoxins. *Toxins (Basel)* 2:2177–2197. https://doi.org/10.3390/toxins2082177 ⏎

Podwojniak A, Tan IJ, Sauer J, *et al.* (2025) Acne and the cutaneous microbiome: A systematic review of mechanisms and implications for treatments. *J Eur Acad Dermatology Venereol* 39:793–805. https://doi.org/10.1111/jdv.20332 ⏎

Porcari S, Baunwall SMD, Occhionero AS, *et al.* (2023) Fecal microbiota transplantation for recurrent *C. difficile* infection in patients with inflammatory bowel disease: A systematic review and meta-analysis. *J Autoimmun* 141:103036. https://doi.org/10.1016/j.jaut.2023.103036 ⏎

Prajapati N, Patel J, Singh S, *et al.* (2023) Postbiotic production: Harnessing the power of microbial metabolites for health applications. *Front Microbiol* 14:1306192. https://doi.org/10.3389/fmicb.2023.1306192 ⏎

Price MJ, Ades AE, Welton NJ, *et al.* (2016) Proportion of pelvic inflammatory disease cases caused by *Chlamydia trachomatis*: Consistent picture from different methods. *J Infect Dis* 214:617–624. https://doi.org/10.1093/infdis/jiw178 ⏎

Qin J, Li R, Raes J, *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464:59–65. https://doi.org/10.1038/nature08821 ⏎

Qin L, Sun T, Li X, *et al.* (2025) Population-level analyses identify host and environmental variables influencing the vaginal microbiome. *Signal Transduct Target Ther* 10:64. https://doi.org/10.1038/s41392-025-02152-8 ⏎

Quaranta G, Sanguinetti M, Masucci L (2019) Fecal microbiota transplantation: A potential tool for treatment of human female reproductive tract diseases. *Front Immunol* 10:2653. https://doi.org/10.3389/fimmu.2019.02653 ⏎

Ragonnaud E, Biragyn A (2021) Gut microbiota as the key controllers of "healthy" aging of elderly people. *Immun Ageing* 18:2. https://doi.org/10.1186/s12979-020-00213-w ⏎

Raimondi S, Candeliere F, Amaretti A, *et al.* (2021) Vaginal and anal microbiome during *Chlamydia trachomatis* infections. *Pathogens* 10:1347. https://doi.org/10.3390/pathogens10101347 ⏎

Rajasekaran JJ, Krishnamurthy HK, Bosco J, *et al.* (2024) Oral microbiome: A review of its impact on oral and systemic health. *Microorganisms* 12:1797. https://doi.org/10.3390/microorganisms12091797 ⏎

Ramadan M, Solyman S, Taha M, Hanora A (2016) Preliminary characterization of human skin microbiome in healthy Egyptian individuals. *Cell Mol Biol* 62:21–27 ⏎

Ramadan M, Solyman S, Yones M, *et al.* (2019) Skin microbiome differences in atopic dermatitis and healthy controls in Egyptian children and adults, and association with serum immunoglobulin E. *OMICS* 23:247–260. https://doi.org/10.1089/omi.2019.0011 ⏎

Rather P, Hassan I (2014) Human demodex mite: The versatile mite of dermatological importance. *Indian J Dermatol* 59:60. https://doi.org/10.4103/0019-5154.123498 ⏎

Ravel J, Moreno I, Simón C (2021) Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. *Am J Obstet Gynecol* 224:251–257. https://doi.org/10.1016/j.ajog.2020.10.019 ⏎

Reynoso-García J, Miranda-Santiago AE, Meléndez-Vázquez NM, *et al.* (2022) A complete guide to human microbiomes: Body niches, transmission, development, dysbiosis, and restoration. *Front Syst Biol* 2:951403. https://doi.org/10.3389/fsysb.2022.951403 ⏎

Ridaura VK, Faith JJ, Rey FE, *et al.* (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341:1241214. https://doi.org/10.1126/science.1241214 ⏎

Rosati D, Bruno M, Jaeger M, *et al.* (2020) Recurrent vulvovaginal Candidiasis: An immunological perspective. *Microorganisms* 8:144. https://doi.org/10.3390/microorganisms8020144 ⏎

Rosenstein R, Torres Salazar BO, Sauer C, *et al.* (2024) The *Staphylococcus aureus*-antagonizing human nasal commensal *Staphylococcus lugdunensis* depends on siderophore piracy. *Microbiome* 12:213. https://doi.org/10.1186/s40168-024-01913-x ⏎

Rowe M, Veerus L, Trosvik P, *et al.* (2020) The reproductive microbiome: An emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. *Trends Ecol Evol* 35:220–234. https://doi.org/10.1016/j.tree.2019.11.004 ⏎

Rubinstein MR, Wang X, Liu W, *et al.* (2013) *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-Cadherin/β-Catenin signaling via its FadA Adhesin. *Cell Host Microbe* 14:195–206. https://doi.org/10.1016/j.chom.2013.07.012 ⏎

Ruohola A, Meurman O, Nikkari S, *et al.* (2006) Microbiology of acute otitis media in children with tympanostomy tubes: Prevalences of bacteria and viruses. *Clin Infect Dis* 43:1417–1422. https://doi.org/10.1086/509332 ⏎

Ryder MI (2020) Porphyromonas gingivalis and Alzheimer disease: Recent findings and potential therapies. *J Periodontol* 91:S45–S49. https://doi.org/10.1002/JPER.20-0104 ⏎

Saraf VS, Sheikh SA, Ahmad A, *et al.* (2021) Vaginal microbiome: Normalcy vs dysbiosis. *Arch Microbiol* 203:3793–3802. https://doi.org/10.1007/s00203-021-02414-3 ⏎

Saraswati S, Sitaraman R (2015) Aging and the human gut microbiota: From correlation to causality. *Front Microbiol* 5:764. https://doi.org/10.3389/fmicb.2014.00764 ⏎

Scharschmidt TC, Fischbach MA (2013) What lives on our skin: Ecology, genomics and therapeutic opportunities of the skin microbiome. *Drug Discov Today Dis Mech* 10:e83–e89. https://doi.org/10.1016/j.ddmec.2012.12.003 ⏎

Scher JU, Sczesnak A, Longman RS, *et al.* (2013) Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2:e01202. https://doi.org/10.7554/eLife.01202 ⏎

Schilder AGM, Chonmaitree T, Cripps AW, *et al.* (2016) Otitis media. *Nat Rev Dis Prim* 2:16063. https://doi.org/10.1038/nrdp.2016.63 ⏎

Schmidt TS, Hayward MR, Coelho LP, *et al.* (2019) Extensive transmission of microbes along the gastrointestinal tract. *Elife* 8:e42693. https://doi.org/10.7554/eLife.42693 ⏎

Schoenmakers S, Steegers-Theunissen R, Faas M (2019) The matter of the reproductive microbiome. *Obstet Med* 12:107–115. https://doi.org/10.1177/1753495X18775899 ⏎

Schwartz JL, Peña N, Kawar N, *et al.* (2021) Old age and other factors associated with salivary microbiome variation. *BMC Oral Health* 21:490. https://doi.org/10.1186/s12903-021-01828-1 ⏎

Scorletti E, Afolabi PR, Miles EA, *et al.* (2020) Synbiotics alter fecal microbiomes, but not liver fat or fibrosis, in a randomized trial of patients with nonalcoholic fatty liver disease. *Gastroenterology* 158:1597–1610.e7. https://doi.org/10.1053/j.gastro.2020.01.031 ⏎

Segui-Perez C, de Jongh R, Jonkergouw RLW, *et al.* (2024) Prevotella timonensis degrades the vaginal epithelial glycocalyx through high fucosidase and sialidase activities. *MBio* 15:e0069124. https://doi.org/10.1128/mbio.00691-24 ⏎

Senaratne NLM, Yung On C, Shetty NY, Gopinath D (2024) Effect of different forms of tobacco on the oral microbiome in healthy adults: A

systematic review. *Front Oral Heal* 5:1310334. https://doi.org/10.3389/froh.2024.1310334 ⏎

Shabayek S, Abdellah AM, Salah M, *et al.* (2022) Alterations of the vaginal microbiome in healthy pregnant women positive for group B *Streptococcus* colonization during the third trimester. *BMC Microbiol* 22:313. https://doi.org/10.1186/s12866-022-02730-8 ⏎

Shen J, Song N, Williams CJ, *et al.* (2016) Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. *Sci Rep* 6:24380. https://doi.org/10.1038/srep24380 ⏎

Shen Y, Fan N, Ma S, *et al.* (2025) Gut microbiota dysbiosis: Pathogenesis, diseases, prevention, and therapy. *MedComm* 6:e70168. https://doi.org/10.1002/mco2.70168 ⏎

Sillanpää S, Kramna L, Oikarinen S, *et al.* (2017) Next-generation sequencing Combined with specific PCR assays to determine the bacterial 16S rRNA gene profiles of middle ear fluid collected from children with acute otitis media. *mSphere* 2:e00006-17. https://doi.org/10.1128/mSphere.00006-17 ⏎

Sjövall A, Aho VTE, Hyyrynen T, *et al.* (2021) Microbiome of the healthy external auditory canal. *Otol Neurotol* 42:e609–e614. https://doi.org/10.1097/MAO.0000000000003031 ⏎

Sokol H, Pigneur B, Watterlot L, *et al.* (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci* 105:16731–16736. https://doi.org/10.1073/pnas.0804812105 ⏎

Sokol H, Seksik P, Furet JP, *et al.* (2009) Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 15:1183–1189. https://doi.org/10.1002/ibd.20903 ⏎

Sonali S, Ray B, Ahmed Tousif H, *et al.* (2022) Mechanistic insights into the link between gut dysbiosis and major depression: An extensive review. *Cells* 11:1362. https://doi.org/10.3390/cells11081362 ⏎

Spatafora G, Li Y, He X, *et al.* (2024) The evolving microbiome of dental caries. *Microorganisms* 12:121.

https://doi.org/10.3390/microorganisms12010121 ⏎

Stevens WW, Schleimer RP, Kern RC (2016) Chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol Pract* 4:565–572. https://doi.org/10.1016/j.jaip.2016.04.012 ⏎

Strachan DP (1989) Hay fever, hygiene, and household size. *BMJ* 299:1259–1260. https://doi.org/10.1136/bmj.299.6710.1259 ⏎

Stroman DW, Roland PS, Dohar J, Burt W (2001) Microbiology of normal external auditory canal. *Laryngoscope* 111:2054–2059. https://doi.org/10.1097/00005537-200111000-00035 ⏎

Suárez-Martínez C, Santaella-Pascual M, Yagüe-Guirao G, Martínez-Graciá C (2023) Infant gut microbiota colonization: Influence of prenatal and postnatal factors, focusing on diet. *Front Microbiol* 14:1236254. https://doi.org/10.3389/fmicb.2023.1236254 ⏎

Sun J, Song S, Liu J, *et al.* (2025) Gut microbiota as a new target for anticancer therapy: From mechanism to means of regulation. *npj Biofilms Microbiomes* 11:43. https://doi.org/10.1038/s41522-025-00678-x ⏎

Sundar PS, Chowdhury C, Kamarthi S (2021) Evaluation of human ear anatomy and functionality by axiomatic design. *Biomimetics* 6:31. https://doi.org/10.3390/biomimetics6020031 ⏎

Swidsinski A, Loening-Baucke V, Swidsinski S, *et al.* (2022) Clue cells and pseudo clue cells in different morphotypes of bacterial vaginosis. *Front Cell Infect Microbiol* 12:905739. https://doi.org/10.3389/fcimb.2022.905739 ⏎

Swidsinski S, Moll WM, Swidsinski A (2023) Bacterial vaginosis—vaginal polymicrobial biofilms and dysbiosis. *Dtsch Arztebl Int 120:*347–354. https://doi.org/10.3238/arztebl.m2023.0090 ⏎

Szymański JK, Słabuszewska-Jóźwiak A, Jakiel G (2021) Vaginal aging—What we know and what we do not know. *Int J Environ Res Public Health* 18:4935. https://doi.org/10.3390/ijerph18094935 ⏎

Tamarelle J, Thiébaut ACM, de Barbeyrac B, *et al.* (2019) The vaginal microbiota and its association with human papillomavirus, *Chlamydia*

trachomatis, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* infections: A systematic review and meta-analysis. *Clin Microbiol Infect* 25:35–47. https://doi.org/10.1016/j.cmi.2018.04.019 ⏎

Tao Z, Zhang L, Zhang Q, *et al.* (2019) The pathogenesis of Streptococcus anginosus in aerobic vaginitis. *Infect Drug Resist* 12:3745–3754. https://doi.org/10.2147/IDR.S227883 ⏎

Taylor BD, Darville T, Haggerty CL (2013) Does bacterial vaginosis cause pelvic inflammatory disease? *Sex Transm Dis* 40:117–122. https://doi.org/10.1097/OLQ.0b013e31827c5a5b ⏎

Teughels W, Van Assche N, Sliepen I, Quirynen M (2006) Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res* 17:68–81. https://doi.org/10.1111/j.1600-0501.2006.01353.x ⏎

Thangaleela S, Sivamaruthi BS, Kesika P, *et al.* (2022) Nasal microbiota, olfactory health, *Neurological Disorders and Aging—A review. Microorganisms* 10:1405. https://doi.org/10.3390/microorganisms10071405 ⏎

The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486:207–214. https://doi.org/10.1038/nature11234 ⏎

Toivonen L, Schuez-Havupalo L, Karppinen S, *et al.* (2021) Antibiotic treatments during infancy, changes in nasal microbiota, and asthma development: Population-based cohort study. *Clin Infect Dis* 72:1546–1554. https://doi.org/10.1093/cid/ciaa262 ⏎

Tomaiuolo R, Veneruso I, Cariati F, D'Argenio V (2020) Microbiota and human reproduction: The case of female infertility. *High-Throughput* 9:12. https://doi.org/10.3390/ht9020012 ⏎

Tomczyk K, Chmaj-Wierzchowska K, Wszołek K, Wilczak M (2023) New possibilities for hormonal vaginal treatment in menopausal women. *J Clin Med* 12:4740. https://doi.org/10.3390/jcm12144740 ⏎

Tropini C, Earle KA, Huang KC, Sonnenburg JL (2017) The gut microbiome: Connecting spatial organization to function. *Cell Host*

Microbe 21:433–442. https://doi.org/10.1016/j.chom.2017.03.010 ⏎

Tsetseri M-N, Silman AJ, Keene DJ, Dakin SG (2023) The role of the microbiome in rheumatoid arthritis: A review. *Rheumatol Adv Pract* 7:rkad034. https://doi.org/10.1093/rap/rkad034 ⏎

Turnbaugh PJ, Ley RE, Mahowald MA, *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–1031. https://doi.org/10.1038/nature05414 ⏎

Turpin R, Tuddenham S, He X, *et al.* (2021) Bacterial vaginosis and behavioral factors associated with incident pelvic inflammatory disease in the longitudinal study of vaginal flora. *J Infect Dis* 224:S137–S144. https://doi.org/10.1093/infdis/jiab103 ⏎

Ullah H, Arbab S, Tian Y, *et al.* (2023) The gut microbiota–brain axis in neurological disorder. *Front Neurosci* 17:1225875. https://doi.org/10.3389/fnins.2023.1225875 ⏎

Ungaro R, Mehandru S, Allen PB, *et al.* (2017) Ulcerative colitis. *Lancet* 389:1756–1770. https://doi.org/10.1016/S0140-6736(16)32126-2 ⏎

Vagios S, Mitchell CM (2021) Mutual preservation: A review of interactions between cervicovaginal mucus and microbiota. *Front Cell Infect Microbiol* 11:676114. https://doi.org/10.3389/fcimb.2021.676114 ⏎

Valeriano VD, Lahtinen E, Hwang I-C, *et al.* (2024) Vaginal dysbiosis and the potential of vaginal microbiome-directed therapeutics. *Front Microbiomes* 3:1363089. https://doi.org/10.3389/frmbi.2024.1363089 ⏎

van den Broek MFL, De Boeck I, Kiekens F, *et al.* (2019) Translating recent microbiome insights in otitis media into probiotic strategies. *Clin Microbiol Rev* 32:e00010-18. https://doi.org/10.1128/CMR.00010-18 ⏎

Velloza J, Heffron R (2017) The vaginal microbiome and its potential to impact efficacy of HIV pre-exposure prophylaxis for women. *Curr HIV/AIDS Rep* 14:153–160. https://doi.org/10.1007/s11904-017-0362-z ⏎

Vergison A, Dagan R, Arguedas A, *et al.* (2010) Otitis media and its consequences: Beyond the earache. *Lancet Infect Dis* 10:195–203.

https://doi.org/10.1016/S1473-3099(10)70012-8 ⏎

Vila T, Rizk AM, Sultan AS, Jabra-Rizk MA (2019) The power of saliva: Antimicrobial and beyond. *PLoS Pathog* 15:e1008058. https://doi.org/10.1371/journal.ppat.1008058 ⏎

Vornhagen J, Quach P, Santana-Ufret V, *et al.* (2018) Human cervical mucus plugs exhibit insufficiencies in antimicrobial activity towards Group B *Streptococcus*. *J Infect Dis* 217:1626–1636. https://doi.org/10.1093/infdis/jiy076 ⏎

Vrieze A, Van Nood E, Holleman F, *et al.* (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143:913–916.e7. https://doi.org/10.1053/j.gastro.2012.06.031 ⏎

Wang N, Chen L, Yi K, *et al.* (2024) The effects of microbiota on reproductive health: A review. *Crit Rev Food Sci Nutr* 64:1486–1507. https://doi.org/10.1080/10408398.2022.2117784 ⏎

Wang Y, Noël-Romas L, Perner M, *et al.* (2023) Non- *Lactobacillus*-dominant and polymicrobial vaginal microbiomes are more common in younger South African women and predictive of increased risk of human immunodeficiency virus acquisition. *Clin Infect Dis* 76:1372–1381. ⏎

Westerberg BD, Kozak FK, Thomas EE, *et al.* (2009) Is the healthy middle ear a normally sterile site? *Otol Neurotol* 30:174–177. ⏎

Willems HME, Ahmed SS, Liu J, *et al.* (2020) Vulvovaginal candidiasis: A current understanding and burning questions. *J Fungi* 6:27. https://doi.org/10.3390/jof6010027 ⏎

Wilmanski T, Gibbons SM, Price ND (2022) Healthy aging and the human gut microbiome: Why we cannot just turn back the clock. *Nat Aging* 2:869–871. https://doi.org/10.1038/s43587-022-00294-w ⏎

Wolfe W, Xiang Z, Yu X, *et al.* (2023) The challenge of applications of probiotics in gastrointestinal diseases. *Adv Gut Microbiome Res* 2023:1–10. https://doi.org/10.1155/2023/1984200 ⏎

Wu G, Zhao H, Li C, *et al.* (2015) Genus-wide comparative genomics of malassezia delineates its phylogeny, physiology, and niche adaptation on

human skin. *PLoS Genet* 11:e1005614. https://doi.org/10.1371/journal.pgen.1005614 ⏎

Wurm J, Curtis N, Zimmermann P (2024) The effect of antibiotics on the intestinal microbiota in children—A systematic review. *Front Allergy* 5:1458688. ⏎

Xiao L, Zuo Z, Zhao F (2024) Microbiome in female reproductive health: Implications for fertility and assisted reproductive technologies. *Genomics Proteomics Bioinformatics* 22:qzad005. ⏎

Xiaoming W, Jing L, Yuchen P, *et al.* (2021) Characteristics of the vaginal microbiomes in prepubertal girls with and without vulvovaginitis. *Eur J Clin Microbiol Infect Dis* 40:1253–1261. ⏎

Xu SX, Gray-Owen SD (2021) Gonococcal pelvic inflammatory disease: Placing mechanistic insights into the context of clinical and epidemiological observations. *J Infect Dis* 224:S56–S63. ⏎

Yao Y, Cai X, Fei W, *et al.* (2022) The role of short-chain fatty acids in immunity, inflammation and metabolism. *Crit Rev Food Sci Nutr* 62:1–12. https://doi.org/10.1080/10408398.2020.1854675 ⏎

Yarbrough VL, Winkle S, Herbst-Kralovetz MM (2015) Antimicrobial peptides in the female reproductive tract: A critical component of the mucosal immune barrier with physiological and clinical implications. *Hum Reprod Update* 21:353–377. https://doi.org/10.1093/humupd/dmu065 ⏎

Yerushalmi M, Elalouf O, Anderson M, Chandran V (2019) The skin microbiome in psoriatic disease: A systematic review and critical appraisal. *J Transl Autoimmun* 2:100009. https://doi.org/10.1016/j.jtauto.2019.100009 ⏎

Younes JA, Lievens E, Hummelen R, *et al.* (2018) Women and their microbes: The unexpected friendship. *Trends Microbiol* 26:16–32. https://doi.org/10.1016/j.tim.2017.07.008 ⏎

Zeng Q, Feng X, Hu Y, Su S (2025) The human gut microbiota is associated with host lifestyle: A comprehensive narrative review. *Front Microbiol* 16:1549160. https://doi.org/10.3389/fmicb.2025.1549160 ⏎

Zhang C, Li L, Jin B, *et al.* (2021a) The effects of delivery mode on the gut microbiota and health: State of art. *Front Microbiol* 12:724449. https://doi.org/10.3389/fmicb.2021.724449 ⏎

Zhang X, Li L, Butcher J, *et al.* (2019) Advancing functional and translational microbiome research using meta-omics approaches. *Microbiome* 7:154. https://doi.org/10.1186/s40168-019-0767-6 ⏎

Zhang Y, Liu T, Lin J, *et al.* (2021b) STROBE-sequencing analysis of the vaginal microecology of 4- to 6-year-old girls in Southwest China. *Medicine (Baltimore)* 100:e25362. ⏎

Zhang Z-J, Gao R, Lu Y-T, *et al.* (2025) Factors affecting dysbiosis of the gut microbiota in the elderly and the progress of interventions in traditional Chinese and Western medicine. *Front Cell Infect Microbiol* 15:1529347. ⏎

Zhu W, Zhang X, Wang D, *et al.* (2024) Simulator of the Human Intestinal Microbial Ecosystem (SHIME®): Current developments, applications, and future prospects. *Pharmaceuticals* 17:1639. ⏎

2

Human Microbiome: Dynamics and Host Interactions

Ashvin Dumadiya, Dhyey R. Kothari, Vishal L. Handa, B.R.M. Vyas, and Ramesh K. Kothari

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2.1 Human Microbiome

A microbiota can be defined as the community of microorganisms living in a specific environment, whereas a microbiome is the entire collection of all the genomic elements of a specific microbiota (Berg et al., 2020). The human microbiome is considered the collection of microbes, their genes, and products that colonize our body since birth and are transferred vertically (De Vos et al., 2022). While all body sites are colonized, the highest microbial numbers are found in the gut, which has been studied extensively (Lloyd-Price et al., 2017). The human body is estimated to harbor trillions of microbes that contribute to host physiology, disease, and behavior (Lozupone, 2012). Bacterial populations inhabit the entire length of the human gut from the oral cavity to the rectum. However, the density and composition of the resident microbial communities vary significantly

between anatomical regions and depend on transit rates, host secretions, substrate availability, environmental conditions, and integrity of the gut wall (Graf et al., 2015). These microbial communities are important for human physiology, immune system development, digestion and detoxification reactions, and synthesis of vitamins (Qin et al., 2010; Flint et al., 2012).

Human microbiome is extremely dynamic and can be influenced by a number of factors, such as age, diet, hormonal balance, travel, medicinal treatment, and diseases (D'Argenio and Salvatore, 2015). Gut-colonizing bacteria primarily belong to the phyla *Bacteroidetes, Actinobacteria, Proteobacteria, Firmicutes, Fusobacteria, Cyanobacteria*, and *Verrucomicrobia* (Qin et al., 2010). These microorganisms play essential roles in host physiology by facilitating the digestion of complex polysaccharides and proteins, synthesizing vitamins and bioactive peptides, and supporting immune function, overall health, and behavioral development (Sekirov et al., 2010; Cryan and O'Mahony, 2011). In addition, bacterial fermentation in the gut produces short-chain fatty acids such as butyrate, propionate, and acetate, which serve as the primary energy source for colonic epithelial cells and contribute to the maintenance of the mucosal barrier and intestinal defense mechanisms (Topping and Clifton, 2001). Here, we review the main and most recent findings on how human microbiomes, their activities, and their mediator molecules can contribute to health and disease. Approaches such as 16S rRNA sequencing, metagenomics, and metabolomics help researchers study its composition and function. The immune system interacts closely with the microbiome, using antibodies and antimicrobial proteins to shape microbial communities while microbes help regulate immune responses. Disruptions in these interactions can lead to dysbiosis, which is associated with various diseases, including metabolic disorders, inflammatory bowel disease, allergies, and neurological conditions.

2.2 Approaches to Study the Human Microbiome

The human microbiome can be examined using various techniques, such as 16S rRNA sequencing, next-generation sequencing, metatranscriptomics, and bioinformatic tools.

2.2.1 Next-Generation Sequencing

Traditionally, microbial studies relied on direct cultivation and isolation of microbes. According to Li et al. (2014), it is estimated that up to 99% of microbes are currently uncultivable. Over the past few years, rapid development of next-generation sequencing technologies (NGS) such as shotgun metagenomic sequencing, 16S rRNA gene sequencing, and RNA sequencing, which increase the throughput of bases sequenced, has had a major impact on the field of metagenomics. In fact, a specific microbiome can be qualitatively and quantitatively characterized in depth using NGS-based approaches without the selection bias and constraints associated with cultivation methods. These technologies are also being used in the Human Microbiome Project, to obtain a complete information of the microbes living in the human body and to define their functions (Qin et al., 2010). Although NGS-based strategies have greatly improved our understanding of metagenomics, they have limitations. In fact, some technical issues still need to be resolved, and NGS-based strategies depend on continuously updated databases, bioinformatic tools, and functional information. The combination of several analytic strategies, including traditional cultivation methods, to characterize the genomic and metabolic properties of specific bacteria will provide further insight into the role of the microbiota.

2.2.2 Shotgun Sequencing

Shotgun sequencing involves analyzing an entire microbial community. It starts with extracting genomic DNA directly from an environmental sample, which is then used to prepare an NGS library for high-throughput sequencing. Data analysis using specialized bioinformatics tools is necessary to assign the obtained reads to both the host and its microbial

components, as well as to perform genome assembly. A major advantage of this method is that it bypasses both cultivation and PCR steps, as the DNA is analyzed directly. It can also identify bacteria down to the species level. However, accurately assigning sequencing reads can be challenging due to limitations in current reference databases. Additionally, genome assembly may be flawed, especially in cases involving less abundant or closely related organism species. Function assignment may be difficult and could also be ambiguous (Morgan et al., 2015).

2.2.3 16S rRNA Sequencing

Targeted sequencing of specific genes enables one to study the microbiome in all its complexity easily and cost-effectively. All bacteria harbor the 16S rRNA gene, which is commonly used for phylogenetic analysis. The 16S rRNA gene has a peculiar structure, characterized by hypervariable regions separated by ultraconserved regions. Although 16S rRNA sequencing is easy to perform, fast, and relatively inexpensive, DNA extraction may be biased, and PCR methodology may confound the analysis and produce ambiguous results (Lee et al., 2012; Morgan et al., 2015). In addition, in this case, the assignment of reads depends on the accuracy of the reference databases used, and the procedure does not produce data on bacterial functions.

2.2.4 Metatranscriptomics

Metatranscriptomics offers a powerful window into the functional activity of the human microbiome by profiling all community RNA, but recent studies highlight persistent challenges in its application. For example, efficient rRNA depletion remains difficult, although a newly developed pan-human probe set using RNase H has significantly improved rRNA removal and reduced bias across diverse body sites (Tan et al., 2023). Moreover, extracting high-quality microbial RNA from human tissues with low microbial biomass remains a challenge, as demonstrated by Pereira-

Marques et al. (2024), who showed that even with optimized workflows, host RNA still predominates and deep sequencing is required. Longitudinal metatranscriptomic work in periodontitis further illustrates the complexity of distinguishing host–microbiome interactions in disease progression (Duran-Pinedo et al., 2025).

2.2.5 Bioinformatic Tools

Application of the abovementioned metagenomic strategies has increased the need for specific data analysis tools able to interpret the complex NGS data generated (Logares et al., 2012). Various tools for the analysis of 16S rRNA reads have been developed in recent years, such as taxonomic identification and diversity analysis (Meyer et al., 2008; Schloss et al., 2009). The accuracy of taxonomic identification depends not only on the specific tools used but also on the portion of the 16S rRNA sequence analyzed, PCR biases, and the availability of updated databases. In general, taxonomic assignment is from phylum to genus, while species identification is more difficult (Vetrovsky et al., 2013). In fact, the 16S rRNA sequencing approach gives a picture of the entire qualitative and quantitative composition of a microbiome, but does not provide information about a specific bacterial genome and/or functions. Recent studies using specially developed catheters confirmed these communities and revealed that the duodenal microbiota exhibited higher compositional dynamics correlating with the pH as compared with the jejunum, which is the intestinal compartment with the largest surface and is responsible for most sugar, protein, and lipid digestion and absorption (Seekatz et al., 2019).

2.3 Factors Affecting the Dynamics of Human Microbiome Composition

The dynamics of the human gut microbiome are largely shaped by numerous factors, such as host genotype, age, gender, diet, probiotics, and use of antibiotics.

2.3.1 Host Genotypes

Microbial genome-wide association studies (mGWAS), which combine human whole-genome sequencing with microbiome analysis (16S rRNA or metagenomics sequencing) from the same individual, are one of the main approaches to examine microbiome–host genome associations (Hall et al., 2017). Evidence for genetic influence on the human microbiome comes from observations that the gut microbial composition of related individuals is more similar than that of unrelated individuals (Dicksved et al., 2008; Turnbaugh et al., 2009), suggesting that host genetic background plays an important role in shaping intestinal microbial communities. Consistent with this, the relative abundances of several microbial taxa, particularly species within the phyla *Bacteroidetes, Firmicutes*, *Proteobacteria*, and *Actinobacteria*, have been linked to host genetic variation (Benson et al., 2010). However, the extent to which host genotype broadly affects microbiome composition, or influences only specific taxa, remains an area of active investigation and debate (Benga et al., 2024). Together, these findings support the presence of meaningful interactions between host genetics and the human gut microbiota.

2.3.2 Age

The composition of the human microbiota changes significantly throughout life, beginning at birth and continuing into adulthood. Infancy is a critical period for intestinal colonization, as the newborn gastrointestinal tract is almost sterile. Initial microbial acquisition occurs through exposure to the mother's vagina and feces, as well as the hospital environment (Figure 2.1). After delivery, the earliest colonizers of the infant gut primarily include *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Enterobacter*, *Ruminococcus*, and *Bacteroides* (Favier et al., 2002; Marques et al., 2010). The composition of the infant's intestine is influenced by several factors during the first 24 months of life (O'Toole and Claesson, 2010), including delivery mode, feeding method, and environmental factors like nutrition, medical

care, and antibiotics (Dominguez-Bello et al., 2010; Adlerberth and Wold, 2009). In adulthood, the gut microbiota becomes relatively stable and is largely dominated by species belonging to *the phyla Firmicutes and Bacteroidetes* (Figure 2.2). Although the adult microbiota can be perturbed by diet, antibiotics, or the introduction of new microbial species, it generally maintains stability due to the resilience of the established microbial community. However, if the disturbances overload the capacity that the microsystem can tolerate, shifts in gut microbiota occur and may induce dysbacteriosis, resulting in various diseases (Lozupone et al., 2012).

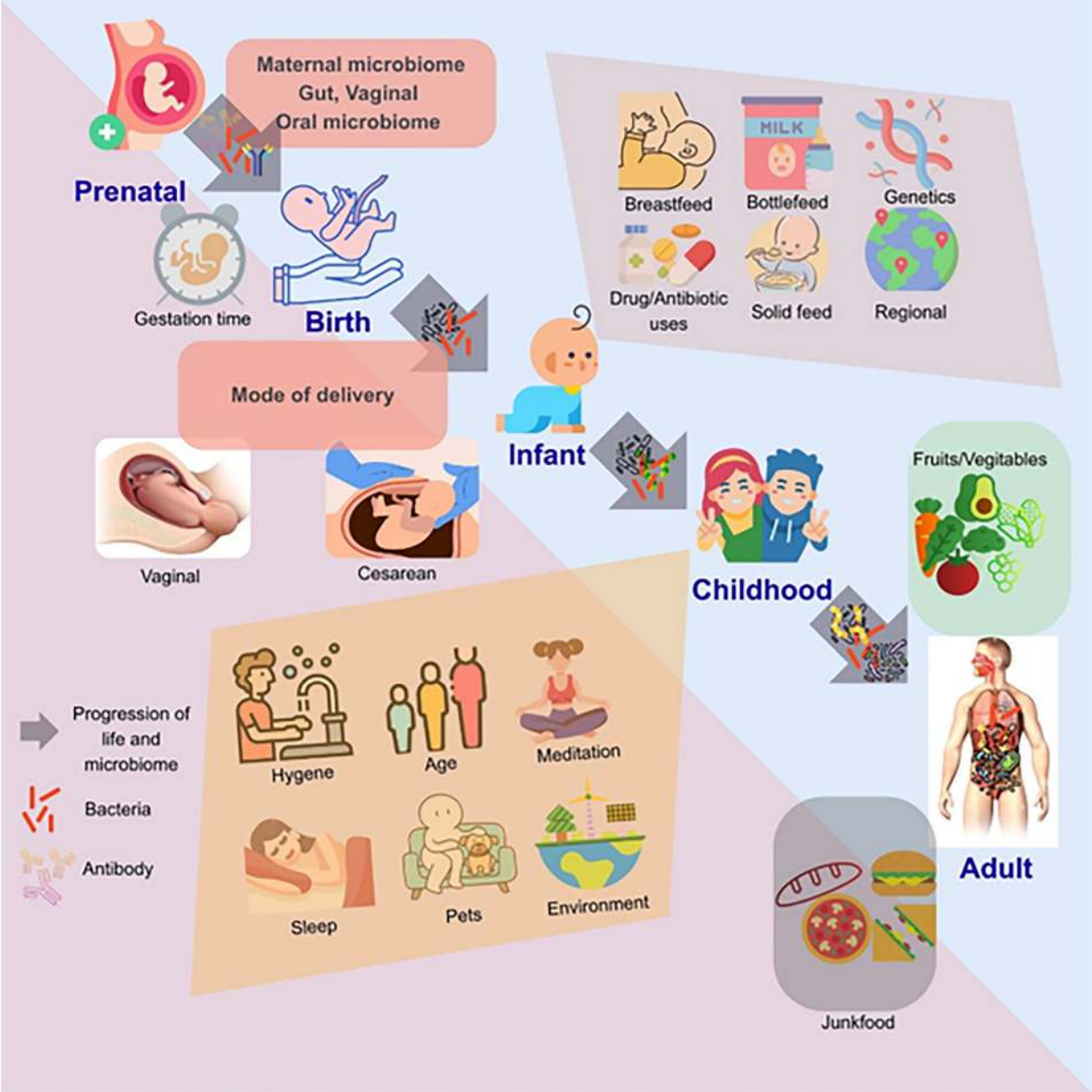

FIGURE 2.1 Factors shaping the human microbiome from prenatal stage to adulthood. The diagram illustrates how microbiome development begins with maternal gut, vaginal, and oral microbiomes during the prenatal period, followed by influences at birth through vaginal or cesarean delivery. In infancy, breastfeeding, formula feeding, antibiotics, solid foods, genetics, and geographic region modulate microbial composition. During childhood, diet, hygiene, sleep, pets, environment, and lifestyle practices further impact microbial diversity. In adulthood, healthy foods or junk-food diets

continue to shape gut microbiota. The graphic highlights the dynamic progression of microbiome structure across life stages. ⏎

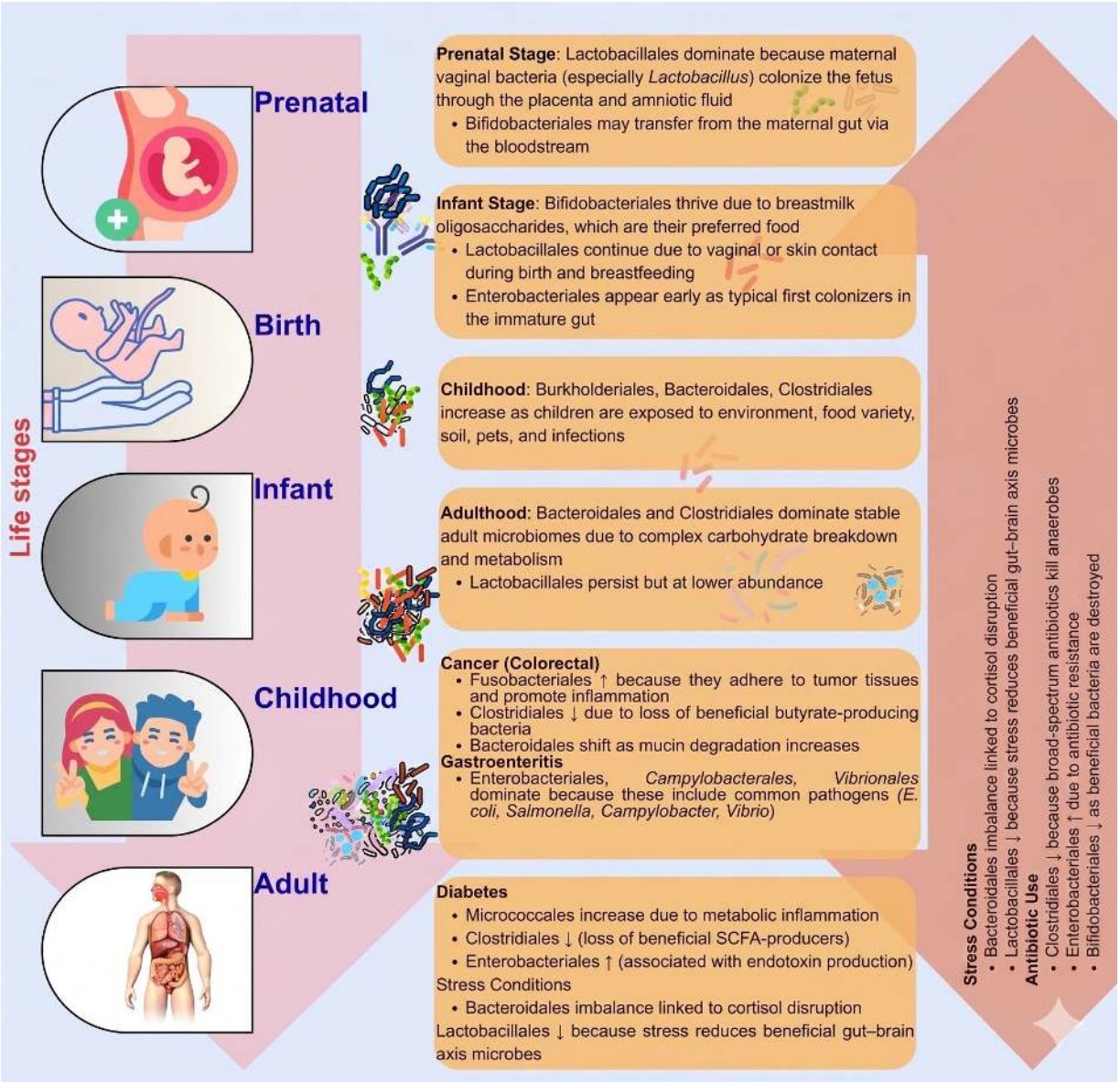

FIGURE 2.2 Bacterial orders across life stages and their alterations under various physiological, dietary, and disease conditions. The figure illustrates how the human gut microbiome evolves from prenatal development to adulthood. During the prenatal stage, *Lactobacillales* and *Bifidobacteriales* dominate due to maternal microbial (vertical) transfer. In infants, early colonizers such as *Bifidobacteriales*, *Lactobacillales*, and *Enterobacteriales* establish the immature gut. Childhood introduces increased environmental and dietary exposure, promoting *Burkholderiales*, *Bacteroidales*, and *Clostridiales*. Adulthood shows a stable community dominated by *Bacteroidales* and *Clostridiales*. The right panel highlights microbial shifts

driven by health conditions and external factors: tumor-associated rise of *Fusobacteriales* in cancer, pathogenic overgrowth (*Enterobacteriales*, *Campylobacterales*, *Vibrionales*) in gastroenteritis, dysbiosis in diabetes, stress-induced reductions in beneficial taxa, and antibiotic-driven depletion of microbial diversity. ⏎

The human microbiome changes significantly throughout the aging process. Age-related variations in the gut microbiota have been well documented in numerous studies. For example, the dominating roles of *Firmicutes* and *Bacteroidetes* remain constant across life stages, although the ratio of *Firmicutes* to *Bacteroidetes* changes to 0.4, 10.9, and 0.6 for newborns, adults, and elderly individuals, respectively (Mariat et al., 2009). In a study that examined the gut microbiota composition of adults (mean age, 35 years) and elderly people (mean age, 70 years), a higher prevalence of Enterobacteria was found (Mueller et al., 2006). Another study examining age-related variations in gut microbial composition in young individuals, elderly individuals (mean age, 70 years), and centenarians (>100 years) found that the former two groups' gut microbial compositions were very similar, whereas centenarians showed significant changes. *Eubacterium limosum* and its relatives were more than ten times increased in centenarians. The proportion of facultative anaerobes also increased. In contrast, centenarians showed a significant reduction in *Bifidobacteria*, *Faecalibacterium prauznitzii*, and related symbiotic species with documented anti-inflammatory properties (Biagi et al., 2010).

2.3.3 Gender

A healthy woman generally shows greater antigen-presenting activity and mitogenic responses, higher immunoglobulin levels, and enhanced antibody production than males, being more resistant to infectious diseases and much more likely to develop autoimmune disease, while healthy males are thought to have an immune system that maintains tolerance (Fish, 2008). Typically, steroid hormones exert opposite effects on the immune response: estrogens promote humoral immunity by enhancing B-cell survival, while

androgens and progesterone act to prevent or suppress autoimmunity (Ngo et al., 2014; Asaba et al., 2015). Recent research suggests a link with Toll-like receptor (TLR) function and estrogen and estrogen receptor α (ERα) and β (ERβ), suggesting a possible contribution of different microbial sensing and TLR activation to sex bias in autoimmune disease, likely linked to gut microbe-associated molecular patterns (MAMPs) recognition (Cunningham and Gilkeson, 2011). Host hormones can affect bacterial gene expression, virulence, and growth, with consequences for host physiology (Neuman et al., 2015). The sex hormone progesterone has been shown to promote the growth of oral *Bacteroides* species and *Prevotella intermedius*, whereas estriol and estradiol decrease bacterial virulence by inhibiting quorum-sensing signaling (Beury-Cirou et al., 2013).

2.3.4 Dietary Factors

Food components such as polysaccharides, proteins, fats, and vitamins consumed by the host can be absorbed and utilized by the gut microbiota. The microbial species have their preferential targets, and thus, diet–microbe interactions within the gut are believed to be crucial for host health, with links to reducing the risk of developing metabolic disorders, impact on blood lipids, and suppression of pathogens (Costabile et al., 2008). The microbial species in the gut can be more adept at degrading polysaccharides than the hosts themselves, resulting in higher production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. The study about the diet-related effects on the gut microbiota is of great significance for human health. For instance, the vegetarian diet and the animal-derived diet are distinctly different dietary habits, with the former characterized by low fat and high fiber, while the latter is the opposite. Several studies have found differences in gut flora composition between two dietary habits. The gut flora of vegetarians is dominated by *Clostridium coccoides, Clostridium ramosum*, and the absence of *Faecalibacterium prausnitzii* (Hayashi et al., 2002). High proportions of *F. prausnitzii* were found in the populations that mostly eat meat and fish (Mueller et al., 2006).

The establishment of the gut microbial community in early life is crucial for shaping the host's intestinal microbiota and immune system in later life (Olszak et al., 2012). Feeding type is one of the most significant factors that affect the newborn's gut microbiota composition (Figure 2.1). Breast-fed infants were largely colonized with *Bifidobacteria*. Human milk oligosaccharides are thought to act as growth factors for beneficial gut bacteria, as inhibitory receptors that bind different pathogens, and may induce the development of the early immune system (Kunz et al., 2000; Hemarajata and Versalovic, 2013). In contrast, bottle-fed infants harbor a more diverse microbiota, including Bacteroides, Enterobacteriaceae, and *Clostridium* (Martin and Walker, 2008; Martin & Davey-Smith., 2009).

2.3.5 Probiotics

Probiotics are live microbes that, when administered in adequate amounts, can confer a health benefit to the host (Sanders, 2008). The most common examples of probiotics are the genera *Lactobacillus* and *Bifidobacterium* (Parracho et al., 2007). Probiotics have been demonstrated to maintain the normal microbial community structure, improve the mucosal integrity, decrease gut permeability, and inhibit pathogen invasion in the human gut by increasing mucus secretion (Spinler et al., 2008; O'Shea et al., 2012). Introducing probiotics may change the expression of microbiome-encoded enzymes involved in the metabolism of plant polysaccharides (McNulty et al., 2011). Several studies have proved the ability of *Bifidobacteria* to improve the gut barrier function and reduce the intestinal endotoxin level (Griffiths et al., 2004). Probiotics can also influence the gut immune system and the sensitivity of immune cells and gut epithelia to microbes in the gut lumen (Thomas and Versalovic, 2010). Some studies also address the potential use of probiotics to prevent infections in immunocompromised patients and reduce the risk of necrotizing enterocolitis in infants (Martin and Walker 2008). Additionally, probiotics may function as a supplementary tool to reduce obesity and related disorders (Shen et al., 2013). A study on diet-induced obese mice has confirmed the antiobesity

effect of *Lactobacillus rhamnosus* PL60, a human-originated bacterium (Lee et al., 2006). *Lactobacillus paracasei* may reduce fat accumulation along with a high level of Angptl4, a circulating lipoprotein lipase inhibitor that regulates triglyceride deposition into adipocytes (Aronsson et al., 2010). A high level of intestinal lactobacilli can increase the risk of obesity and hyperglycemia in healthy individuals (Stsepetova et al., 2011), but the effects of the probiotics on body weight and obesity are thought to be strain-specific. For instance, *Lactobacillus acidophilus* and *Lactobacillus ingluviei* are linked with a weight-gain effect, whereas *Lactobacillus gasseri*, *Lactobacillus plantarum*, and *Lactobacillus casei/paracasei* showed an antiobesity effect (Million et al., 2012). Prebiotic supplements such as fructo-oligosaccharides and inulin can promote the growth of beneficial gut bacteria, particularly *Lactobacillus* and/or *Bifidobacterium* (Roberfroid et al., 2010; Cani et al., 2012).

2.3.6 Antibiotics

Antibiotics are used to target the harmful microbes. However, most antibiotics have broad-spectrum activity and may also kill the normal gut flora. The antibiotic treatment may alter the human microbiota, thereby disrupting its composition, reducing diversity and evenness of the community (Dethlefsen et al., 2008). Antibiotics have a negative effect (Figure 2.2) on the composition of microbiota, as they cause a decrease in obligate anaerobes, such as *Bifidobacteria* and *Bacteroides* (Martin and Walker, 2008; Reinhardt et al., 2009). Reduced bacterial diversity of the intestinal flora precedes asthma (Abrahamsson et al., 2013), allergic sensitization, allergic rhinitis, and peripheral blood eosinophilia (Bisgaard et al., 2011). However, the effect on gut microbiota varies with the type of antibiotic (Penders et al., 2006). Decreasing the overuse of antibiotics and increasing the use of probiotics and prebiotics are effective in preventing several diseases, such as necrotizing enterocolitis and atopic dermatitis, as well as in improving short- and long-term health (Wall et al., 2009). Excessive antibiotic use has been linked to both acute and chronic health

problems, such as diarrhea, asthma, and inflammatory bowel diseases (IBDs) (Beaugerie and Petit, 2004; Floistrup et al., 2006; Guarner et al., 2006). A study on the gut flora of infants treated with ampicillin and gentamicin indicated that the gut microbiota changes significantly with higher proportions of *Proteobacteria* and lower proportions of *Actinobacteria* as well as genus *Lactobacillus* than the untreated counterparts, and recovery was still not complete after 2 months, which suggest that the antibiotic disruption in early life can exert significant impact on the development of the infant gut microbiota (Fouhy et al., 2012).

2.4 Gut Microbiome

Most (but not all) gut microbes are either harmless or beneficial to the host. The gut microbiota protects against enteropathogens, extracts nutrients and energy from our diets, and contributes to normal immune function (Candela et al., 2008). Dysbiosis and disruption of the gut microbiota have been associated with obesity, malnutrition, IBDs, neurological disorders, and cancer (Turnbaugh et al., 2008; Dicksved et al., 2008). Furthermore, the gut microbiota is believed to be involved in appetite control, energy balance, obesity, diabetes, allergies, immune function, behavioral perturbations, cardiovascular disease, and cancers, including stomach cancer. Most of these diseases are becoming more common as the role of infectious diseases decreases; it is therefore crucial to address the variety of potential contributions of the microbiota to these medical problems, especially because some of them progress to a point where they become irreversible. Therefore, a shift in emphasis from individual pathogens to an ecological approach taking into account the community as a whole is necessary to understand how the gut microbiota affects health and disease.

The presence of microbiota in the gut is affected by many factors, such as the acidity of the stomach, the production of bile acids, digestive enzymes, and antimicrobial proteins in the intestine. Various other variables also affect the microbial colonization, such as chemical parameters like pH,

level of oxygen, redox potential, and biological production of mucus, bile, and antibodies. The colonic microbiome is dominated mainly by anaerobic bacteria, including thousands of genes, distributed among the major phyla of *Firmicutes* (predominantly *Ruminococcaceae* and *Lachnospiraceae*), *Proteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* (Li et al., 2014).

2.4.1 Gut Microbiome and Diet

While the microbiome of a healthy individual is relatively stable, gut microbial composition can certainly be influenced by host lifestyle and dietary choices. The diet might be expected to have a strong influence on the gut microbiota and to be able to modify the impact of the microbiota upon health, with either beneficial or deleterious consequences. Therefore, it is important to determine whether the intestinal microbiota is essentially static within an individual or influenced by their diet.

Research indicates that intentional dietary modification, such as adding non-digestible carbohydrates, can alter the composition of the gut microbiota; however, not all bacterial groups are sensitive. Changes occur mainly to groups that have specialized nutritional niches, whereas bacteria that are more nutritionally versatile or that depend largely on host-derived substrates may be less affected (Sonnenburg et al., 2005). Studies suggest that habitual diet had a minor influence on the overall microbiota profiles, although a more recent study has reported an association between intake of fat, protein, and carbohydrate and the frequency of *Bacteroides* and *Prevotella* dominated microbiotas (Wu et al., 2011).

An increase in dietary fiber intake has been shown to enhance the gut transit time and modulate gut microbial community, resulting in increased populations of total bacteria and increased production of fermentation metabolites such as SCFAs. For example, a randomized controlled trial in healthy adults demonstrated that fiber supplementation significantly increased SCFA-producing genera (e.g., *Bifidobacterium*, *Anaerostipes*, *Faecalibacterium*). Moreover, dietary fiber fermentation in the colon has been linked with a decrease in luminal pH, especially in the proximal colon,

due to the accumulation of SCFAs (Flint & Louis, 2024). This drop in pH can influence microbial ecology by favoring acid-tolerant, butyrate-producing bacteria and suppressing proteolytic fermentation pathways (Deehan et al., 2024; Kumar et al., 2025). The dietary regulation exerts influences on microbial metabolism and host immune functions through several pathways including selective bacterial fermentation of nutrients, lower intestinal barrier function, disruptions of intestinal function by inducing immune responses, and overexpression of genes associated with disorders (Kau et al., 2011; Shulzhenko et al., 2011). The majority of the gut microbes play a crucial role in the host's metabolism by improving energy harvest from foods, for instance, they can degrade the polysaccharide that the host cannot digest. Additionally, they can improve intestinal permeability, mucosal immunity, and modulate the host-derived compounds, thus having a profound effect on human life (Hooper et al., 2002; Ley et al., 2005).

Carbohydrates can be digestible or non-digestible for humans. Digestible carbohydrates are enzymatically degraded in the small intestine such as starch, glucose, sucrose, fructose, and lactose. Individuals fed with high digestible carbohydrates had increased relative abundance of *Bifidobacteria* and reduced *Bacteroides* (Eid et al., 2014). In contrast, non-digestible carbohydrates such as fiber and resistant starch are not enzymatically degraded in the small intestine; instead, they move to the large intestine and undergo fermentation by resident microbes. Dietary fiber is a good source of "microbiota accessible carbohydrates," and can be utilized by microbes to provide the host with energy and a carbon source (Lozupone et al., 2012; Sonnenburg et al., 2014). Several studies suggest that a diet rich in non-digestible carbohydrates mainly increases intestinal bifidobacteria and lactic acid bacteria. For example, non-digestible carbohydrate diets that are rich in wheat bran and whole grain are linked to increase *Bifidobacteria* and *Lactobacilli* in the gut (Costabile et al., 2008; Carvalho-Wells et al., 2010). Other non-digestible carbohydrates, such as resistant starch and whole grain barley, appear to increase the abundance of *Ruminococcus* and *Roseburia* (Keim and Martin 2014; Leitch et al., 2007). Furthermore,

fructooligosaccharides, polydextrose, and arabinooligosaccharides-based prebiotics have been shown to reduce *Clostridium* and *Enterococcus* species (Liu et al., 2014; Kedia et al., 2009)

Studies conducted on mice have shown that high-fat diet significantly reduces *Lactobacillus intestinalis* and increases species producing propionate and acetate, such as *Clostridiales, Bacteroides*, and *Enterobacteriales*. Furthermore, the abundance of *L. intestinalis* is negatively correlated with mice body weight and fat mass (Lecomte et al., 2015). In mice fed with a high-fat diet, microbial alterations have also been shown to control metabolic endotoxemia-induced inflammation (Cani et al., 2008). Studies on mice have also compared the differential effects of various lipids on intestinal microflora. A comparison of pig-derived and fish oil-derived lipids revealed that *Bacteroides* and *Bilophila* were increased in pig-fed mice, while *Actinobacteria*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, and *Verrucomicrobia* were increased in fish-oil-fed mice.

A study demonstrated lower counts of *Bifidobacterium adolescentis* and increased counts of *Bacteroides* and *Clostridia* in a population consuming a high beef diet as compared to a population consuming a meatless diet. Furthermore, several microbial genera promoted by the intake of red meat have also been linked to an increase in the levels of trimethylamine-N-oxide, a compound that increases the risk of cardiovascular disease (De Filippis et al., 2016).

2.4.2 Microbiota–Gut–Brain Axis

Gut microbiota homeostasis affect not only the gastrointestinal tract environment but also distant organs, such as brain (Sommer and Backhed, 2013). Early colonization of the gut microbiome is vital for brain function and behavior because its absence impairs the blood–brain barrier, alters synaptic plasticity, and social behavior deficits (Sampson and Mazmanian, 2015; Chu et al., 2019). Communication between the gut microbiome and the central nervous system (CNS) is mainly facilitated through microbial-

derived intermediates, with the best described examples including SCFAs, tryptophan metabolites and secondary bile acids (Tolhurst et al., 2012). Best characterized example of the interaction between the microbiota and CNS involves microglial development. Microbial-derived SCFAs have been shown to have a crucial role in fostering microglial maturity and proper functioning (Erny et al., 2015). Bidirectional communication between gut and brain suggests that gut microbiota plays a crucial role in controlling the host's metabolic, circulatory, and immunological systems (Cryan et al., 2019). Germ-free mice show immature microglial phenotype leading to impaired immune responses (Erny et al., 2015). Furthermore, the gut microbiota may also influence CNS via vagus nerve by transmitting gut microbiome signals to the brain and vice versa in case of both health and disease (Spielman et al., 2018). Dysregulation of the hypothalamic–pituitary–adrenal axis is linked to depressive episodes (Barden, 2004), and it has been suggested that the gut microbiota plays a role in both the programming of the hypothalamic–pituitary–adrenal axis early in life and stress response over the lifespan (Figure 2.2). Microbiota may also influence CNS function through neuronal activation of stress circuits. Studies using oral administration of *Campylobacter jejuni* and *Citrobacter rodentium* show that bacteria living in the gastrointestinal tract can activate stress circuits through activation of vagal pathways (Goehler, et al., 2008; Lyte et al., 2006). γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the CNS, and dysfunctions in GABA signaling are associated with anxiety and depression. Interestingly, *Lactobacillus* and *Bifidobacterium* residing in the gut can metabolize glutamate to produce GABA (Barrett et al., 2012). The gut microbiota also regulates a vital neurotransmitter such as serotonin by altering levels of precursors; for instance, *Bifidobacterium infantis* has been shown to increase plasma tryptophan level and thus influence central 5-hydroxytryptamine receptors transmission (Desbonnet et al., 2010). Recent studies have shown that the stroke risk and outcome are significantly affected by the gut microbiota composition (Benakis et al., 2016). Experimental evidences showed that the bacterial diversity in stool is reduced in mice with a severe stroke, whereas

the microbiome of mice subjected to a mild stroke was identical to controls. The overall changes were observed as a reduction of the *Firmicutes* and overgrowth of *Bacteroidetes*, which represents the two major bacterial phyla in the gut.

Analysis of fecal samples collected from patients of Alzheimer's disease has revealed a reduced diversity and increased abundance in *Bacteroidetes*, and this was reproducible in animal models (Vogt et al., 2017). Furthermore, the composition of microbiome in Parkinson's disease patients is characterized by increased abundances of *Enterobacteriaceae, Bifidobacteriaceae, Akkermansia, Lactobacillaceae*, and drastic reduction of *Prevotellaceae* from the phylum *Bacteroidetes* (Barichella et al., 2019).

2.5 Oral Microbiome

The oral microbiota is the second most intricate microbial community after the gut, comprising a diverse and dynamic array of microbes that inhabit the oral cavity and interact with host genetics, diet, immune system, and many other factors (Wade, 2013). The oral cavity is a highly complex habitat that supports a vast population of microbes, making it one of the most densely colonized sites in the human body. Moreover, the oral cavity contains multiple distinct niches, each fostering complex and heterogeneous microbial communities. The bacterial microbiome is the predominant component, with species consisting mainly of obligate aerobes like *Rothia* and *Neisseria*, facultative aerobes such as *Streptococcus and Actinomyces*, and obligate anaerobes including members of *Firmicutes*, *Bacteroidetes*, and *Spirochaetes* (Dewhirst et al., 2010).

Even though the core microbial composition in the oral cavities is roughly similar, the specific species present may vary depending on the host's genetics, diet and nutrition, hormonal factors, alcohol consumption, and antibiotic exposure. When this shift led to harmful microbial load, the imbalances, termed as dysbiosis, can trigger various changes in both oral and overall health through several pathophysiological mechanisms

(Appanna, 2018; Zaura, 2014). Dysbiosis has been linked to involved in the development of various oral diseases such as dental caries, gingivitis, periodontitis, oral mucosal diseases, and/or systemic diseases (Gao et al., 2018; Appanna, 2018). Furthermore, the interaction between the oral microbiome and the host is dynamic and is shaped by many aspects of lifestyle, such as diet, smoking, stress, and overall health status, that may alter the composition and functions, ultimately disrupting the delicate balance of this ecosystem (Willis and Gabaldon, 2020; Martinon, 2021). Within the oral cavity, distinct microenvironments, such as the tongue, gingival sulcus, tonsils, hard palate, and soft palate, provide a rich environment in which microorganisms can flourish (Dewhirst et al., 2010). Disruption of the host–microbial interaction may occur because of significant changes in the oral environment or lifestyle of an individual that favor the colonization of disease-associated microbiota (Lamont et al., 2018).

Smoking is a significant factor that affects oral microbiota composition (Al Bataineh et al., 2020), as it enhances biofilm formation that results in greater epithelial adherence by certain pathogens, for instance, *Streptococcus mutans*, thereby increasing susceptibility for dental caries in the smokers (Tomar et al., 2019). Recent research showed that smokers tend to have higher levels of the genera *Atopobium*, *Campylobacter*, and *Prevotella* and depletion of certain phyla including *Proteobacteria* (Wu et al., 2016). Toxic components in cigarettes can induce the loss of beneficial oral species, pathogen colonization, and, eventually, disease, directly or indirectly through immunosuppression, oxygen deprivation, or biofilm formation (Nociti et al., 2015). Toxic substances in cigarettes may lead to the loss of beneficial oral species and colonization of pathogenic microbes, eventually contributing to disease, either directly or indirectly through immunosuppression, oxygen deprivation, or biofilm formation (Nociti et al., 2015). In smokers, microbes such as *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Fusobacterium nucleatum*, and *Fusobacterium naviforme* were observed with a high taxonomic diversity and richness, which was closely matched with a disease-associated

microbiota in clinically healthy individuals. Kumar et al. (2011) reported the differences in the formation of marginal and subgingival biofilms between smokers and non-smokers. In smokers, early biofilms showed higher taxonomic diversity, reduced niche saturation, and comparatively unstable initial colonization. Moreover, periodontal pathogens from the genera *Fusobacterium*, *Synergistes, Cardiobacterium*, and Selenomonas, as well as respiratory pathogens such as *Pseudomonas* and *Haemophilus*, were detected in the early biofilms of smokers (Kumar et al., 2011).

Dietary intake also influences oral microbes. For instance, frequent sugar intake increases acid production, which dissolves tooth structure and increases the risk of dental caries via the fermentation of dietary carbohydrates by oral bacteria (Gupta et al., 2013). Dietary sugars and specific oral microbiota, including *Tannerella forsythia*, *Streptococcus sobrinus*, and *Eikenella corrodens*, can have a feedforward loop in patients with dental caries (Inquimbert et al., 2019; Lamont et al., 2018). Subsequent acid production by repeated intake of high levels of carbohydrates led to sustained reductions in pH, along with the low buffering capacity of saliva. In turn, this can change the oral microbiota composition and upregulate aciduric species (Takahashi and Nyvad, 2011). Bacterial species such as *S. mutans* and *Lactobacilli*, which are considered to be caries-associated microbiota, also produce acid (Gross et al., 2010). Antimicrobial use has also been suggested as an important factor affecting the composition of oral microbiota. Amoxicillin treatment reduced species richness and diversity (Lazarevic et al., 2013).

Oral microbiota also contributes to disease. Dental caries is characterized by the demineralization of susceptible dental hard tissues by acidic bacterial by-products from sugar metabolism that leads to cavitation and a chronic continuous process from sub-clinical decay to dentinal involvement (Featherstone, 2004). Dental caries results from an ecological imbalance toward acidogenic and aciduric bacterial shifts and environmental acidification within the dental biofilm. *S. mutans* are the initial colonizers of the supragingival biofilm, which produce water-insoluble glucans that promote bacterial adhesion and only comprise <1%

of the total community (Gross et al., 2012). In addition, *mutans streptococci*, especially *S. mutans* and *S. sobrinus*, were first identified as prominent pathogens because of their extensive acidogenic and aciduric properties (Simon, 2007). Apart from *mutans streptococci*, other acidogenic species, including *Lactobacillus, Bifidobacterium*, and *Scardovia wiggsiae*, showed a strong association with caries (Badet and Thebaud, 2008; Mantzourani et al., 2009). It was also revealed that a complex community associated with caries included other prominent species such as *Atopobium, Prevotella, Corynebacterium, Veillonella*, and *Capnocytophaga* (Simon-Soro et al., 2013). These species produce weak organic acids after carbohydrate fermentation and decrease the local pH to demineralize the tooth tissues.

The oral microbiome plays a critical role in maintaining mucosal health, and growing evidence links its dysbiosis to the development of several cancers. Altered microbial communities can promote chronic inflammation, genotoxicity, and immune modulation, all of which are key mechanisms in tumor initiation and progression (Elinav et al., 2019). For example, *F. nucleatum*, a common oral anaerobic bacterium, has been associated with colorectal cancer due to its ability to alter epithelial signaling and suppress antitumor immune responses (Bullman et al., 2017). Similarly, periodontal pathogens such as *Porphyromonas gingivalis* have been implicated in oral squamous cell carcinoma through mechanisms involving epithelial cell invasion, inhibition of apoptosis, and activation of inflammatory pathways (Gao et al., 2016). Understanding these microbial mechanisms may improve early detection and targeted cancer prevention strategies.

Neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, have also been reported to be associated with the oral microbiome. The most complete study in this regard is the association between *P. gingivalis* and Alzheimer's disease (Dominy et al., 2019; Mihaila et al., 2019). *P. gingivalis* infection in mice resulted in brain colonization, and gingipain proteases produced by *P. gingivalis* have neurotoxic characteristics. It has also been shown that typical oral species

of the phylum Spirochaetes, including multiple species of the genus *Treponema*, often comprise amyloid plaques (Miklossy, 2016).

2.6 Skin Microbiome

The human skin serves as the primary interface with the external environment. One of its most critical features is its complex and dynamic microbiome. Over the past two decades, advances in metagenomic sequencing had helped to understand the composition and dynamics of the skin microbiota, revealing a system essential for immune development, pathogen defense, and overall skin homeostasis (Grice and Segre, 2011). The skin microbiome differs significantly across individuals and skin sites. Its distribution is largely influenced by local biophysical conditions such as moisture, sebum levels, pH, and temperature (Byrd et al., 2018). In healthy individuals, normal skin flora is dominated primarily by members of the genera *Propionibacterium*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* occupying specific skin regions, while fungi such as *Malassezia* predominate in lipid-rich areas (Findley et al., 2013; Grice and Segre, 2011). Moisture in the skin promotes the growth of *Corynebacterium, Staphylococcus*, and *Micrococcus* species (Byrd et al., 2018). Sebaceous regions support lipophilic organisms, most notably *Propionibacterium acnes*, which thrives in sebum-rich follicles. *Staphylococcus epidermidis* and *Malassezia* species are also abundant (Dreno et al., 2016). Host-produced lipids, antimicrobial peptides, and sweat composition also selectively support specific microbes, demonstrating a dynamic interplay between skin physiology and microbial ecology. Intrinsic factors such as host genetics, age, immune function, and local environment influence the shape of the microbial community. For example, infants initially host a less diverse microbiome that matures with age and shows increased stability by adulthood (Capone et al., 2011).

Skin microbiome plays a vital role in the inhibition of pathogen colonization (Cogen et al., 2008). Some species also produce bacteriocins,

lipases, and proteases, which inhibit the growth of harmful organisms. For instance, *S. epidermidis* produces antimicrobial peptides such as phenol-soluble modulins that suppress *Staphylococcus aureus* colonization (Cogen et al., 2010). *P. acnes* produces SCFAs that contribute to maintaining acidic skin pH, limiting pathogen survival (Beylot et al., 2014). Skin microbiome also plays a crucial role in the development and modulation of cutaneous immunity. Skin microbes stimulate pattern recognition receptors (PRRs), which prime the skin's immune system for rapid responses to pathogens while maintaining tolerance to resident microbes (Kobayashi et al., 2015). *S. epidermidis*, in particular, enhances T-cell-mediated immune responses and promotes tissue repair after injury (Naik et al., 2012). Similarly, *P. acnes* participates in lipid metabolism by hydrolyzing triglycerides in sebum into free fatty acid compounds that lower skin pH and create an inhospitable environment for many pathogens (Gribbon et al., 1993). Skin bacteria also metabolize components of sweat, sebum, and epidermal lipids to produce molecules such as fatty acids and lactic acid that maintain an acidic skin surface (Sanford and Gallo, 2013). These interactions highlight the microbiome as not merely a passive resident but an active participant in skin health.

However, the skin microbiome is not static; it is sensitive to a wide array of extrinsic factors including hygiene habits, environmental conditions, diet, climate, pollutants, antibiotics, and importantly, the widespread use of skin-care products, all of which may alter microbial composition and function (Chen et al., 2018).

Modern skin-care products significantly affect the microbial ecosystem. Cleansers containing surfactants remove dirt and oils but can also disrupt lipid layers and reduce microbial diversity. Harsh surfactants such as sodium lauryl sulfate can damage the barrier, alter pH, and promote shifts toward less beneficial microbes (Ananthapadmanabhan et al., 2012). Moisturizers may increase microbial diversity by restoring hydration and lipid balance. Products containing ceramides support barrier integrity, indirectly favoring the growth of commensals like *S. epidermidis* (Draelos, 2018). Alcohol-based sanitizers, chlorhexidine, and benzoyl peroxide

rapidly reduce microbial load but may also eliminate protective commensals. Benzoyl peroxide lowers both pathogenic and beneficial bacterial counts on acne-prone skin, with population recovery typically occurring over several weeks (O'Neill and Gallo, 2018).

Some species of skin bacteria may also cause diseases. Atopic dermatitis is one of the most extensively studied microbiome-associated skin diseases. Patients with atopic dermatitis typically experience reduced microbial diversity and overgrowth of *S. aureus* (Kong et al., 2012). *S. aureus* worsens inflammation by producing superantigens and proteases that damage the barrier. Conversely, *S. epidermidis* and *S. hominis* produce antimicrobial peptides that inhibit *S. aureus* and appear beneficial in restoring balance (Nakatsuji et al., 2017). *P. acnes* is a dominant organism in sebaceous areas and is associated with skin acne (Fitz-Gibbon et al., 2013). Dysbiosis may involve decreased diversity and an increase in strains that trigger inflammation and sebum oxidation. Psoriasis is an immune-mediated disease where microbial shifts may play a sustaining role. Studies showed increased abundance of *Streptococcus* and reduction in *Propionibacterium* in psoriatic skin (Tett et al., 2017). Skin-care products and antibiotics used in acne treatment both influence the microbiome, sometimes with paradoxical effects. For example, long-term antibiotic therapy can disrupt commensal populations, encourage resistant organisms, and alter host immunity.

2.7 Vaginal Microbiome

The vaginal microbiome is a complex and dynamic microbial ecosystem that plays a crucial role in maintaining women's reproductive health. Vaginal microbial composition is influenced by numerous biological and behavioral factors, such as hormonal fluctuations, sexual activity, antibiotic exposure, hygiene practices, and host genetics. In a healthy woman, the vaginal microbiome is typically dominated by species of *Lactobacillus*, most commonly *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii* (Ravel et

al., 2011). These species produce lactic acid that maintains a low vaginal pH (3.5–4.5) that protects against pathogenic invasion and is considered a hallmark of healthy vaginal ecology (Miller et al., 2016). The commensal bacteria help maintain vaginal homeostasis through the production of lactic acid and other antimicrobial substances, which prevent colonization and expansion of pathogenic species. The composition of vaginal microbiome has been shown to influence susceptibility to infection, reproductive health, pregnancy, and overall gynecological well-being (Brotman, 2011). Disruption in the composition of vaginal microbiome is closely associated with bacterial vaginosis, sexually transmitted infections (STIs), infertility, and adverse obstetric outcomes such as preterm birth (Lehtoranta et al., 2022).

The vaginal microbiome is a vital determinant of female reproductive health. *Lactobacillus*-dominated communities promote homeostasis, prevent pathogen overgrowth, and support fertility. The mechanisms underlying the protective roles of the vaginal microbiome center on lactic acid production, immune modulation, and competitive exclusion of pathogens. Lactic acid, produced by most vaginal lactobacilli, maintains acidic pH and exerts direct antimicrobial effects, enhances epithelial barrier function, and modulates innate immune responses (Tachedjian et al., 2017). Some lactobacilli species produce bacteriocins that inhibit pathogens such as *Gardnerella* and *Escherichia coli*. Among *Lactobacillus* species, *L. crispatus* is considered particularly protective because it produces high levels of D-lactic acid and possesses strong antimicrobial potential (Witkin et al., 2013). The vaginal microbiome also protects against urinary tract infections (UTIs) by preventing uropathogens such as *E. coli* from colonizing the vaginal vestibule. Reduced *Lactobacillus* abundance is associated with recurrent UTI risk, while re-establishment of *L. crispatus* has been shown to reduce UTI recurrence in clinical trials (Stapleton et al., 2011).

Vaginal dysbiosis is commonly associated with bacterial vaginosis, which is the most common vaginal condition in women of reproductive age. Bacterial vaginosis is marked by the depletion of protective lactobacilli and

an overabundance of anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella*, *Megasphaera*, and *Sneathia* (Fredricks et al., 2005; Onderdonk et al., 2016). These bacteria can produce amines responsible for bacterial vaginosis's characteristic odor, elevate vaginal pH, and form biofilms, particularly those initiated by *Gardnerella* that adhere to epithelial cells of vagina (Swidsinski et al., 2005). Bacterial vaginosis significantly increases the risk of several STIs, including human immunodeficiency virus (HIV), chlamydia, and gonorrhea (Atashili et al., 2008). Another common vaginal infection is candidiasis, typically caused by *Candida albicans*, which often arises when *Lactobacillus* populations are disrupted by antibiotic exposure, hormonal fluctuations, or immune changes. The vaginal microbiome also exerts a substantial influence on susceptibility to STIs. For example, women with low-lactobacillus microbial communities face a significantly higher risk of acquiring HIV (Atashili et al., 2008). Vaginal lactic acid, particularly the D-isomer produced by *L. crispatus*, can inactivate HIV virions and reduce target cell activation (Tachedjian et al., 2017). Similarly, dysbiotic microbiota are associated with persistent human papillomavirus (HPV) infection and increased risk of cervical dysplasia, likely due to impaired antiviral immune responses and increased epithelial permeability (Curty et al., 2019).

Studies suggest that a *Lactobacillus*-dominated vaginal microbiome correlates with higher implantation, clinical pregnancy, and live birth rates, whereas overgrowth of anaerobic bacteria such as *Gardnerella* or *Atopobium* has been linked to implantation failure and early pregnancy loss (Moreno et al., 2016). The endometrial microbiome, although present in low abundance, may also affect embryo implantation through immune modulation, though its exact clinical role remains under investigation. During pregnancy, hormonal changes typically promote increased *Lactobacillus* dominance and stability in the vaginal microbiome. This protective state helps reduce the risk of infections (MacIntyre et al., 2015). Estrogen plays a significant role by promoting glycogen deposition in the vaginal epithelium, which provides substrate for *Lactobacillus* growth; thus, *lactobacillus*-dominant communities are more prevalent before

menstruation, during pregnancy, and in hormone-treated postmenopausal women (Miller et al., 2016). Understanding the vaginal microbiome in healthy and diseased states, as well as its relationship to reproductive physiology, is therefore crucial.

2.8 Microbiome and Immune System

Immune response can be innate or adaptive. Innate immunity constitutes the first line of defense and is nonspecific; this includes elements of mucus membranes, the skin, chemical responses, and phagocytosis. Adaptive immunity, in contrast, is activated by antigens derived from invading cells or pathogens and involves a set of specialized cells that recognize such and neutralize such antigens (Thaiss et al., 2016).

Gut bacteria ferment some food components and produce SCFA, which induce a rapid increase in the production of neutrophils, eosinophils, and basophils (Mathews et al., 2014). Among gut bacteria, *Bacteroides fragilis* and some *Clostridia* species have been reported to cause an anti-inflammatory response, while some segmented filamentous bacteria have been found to cause inflammatory cytokine production (Madhogaria et al., 2022). Due to disturbance in intestinal barrier bacteria from intestinal lumen reaching the tissue, which triggers many inflammatory responses, one of them is the induction of PRRs, which are linked to cancer progression; these receptors include mainly membrane-bound TLRs and Nod-like receptors (NLRs) (Madhogaria et al., 2022).

Bacteria also provoke the immune response to combat pathogenic infections. For instance, flagellin, a structural component of bacterial flagella in the intestinal mucosa, is detected by germline-encoded receptors (TLR5 and NLRC4) of the innate immune system (Vijay-Kumar and Gewirtz, 2009). When administered intraperitoneally, flagellin confers resistance to rotavirus infection in neonatal mice as assessed by reduction in virus shedding, shortening the period of diarrhea, and even cures previously infected mice. The protective role of flagellin was eliminated in the absence

of both TLR5 and NLRC4. Further research has demonstrated that flagellin-triggered antiviral response required both the production of IL-22 by innate lymphoid cells (ILCs) and IL-18 by dendritic cells. Prophylactic administration of IL-22 and IL-18 conferred protection against rotavirus or eradicated virus shedding in Rag1–/– mice chronically infected with RV (Zhang et al., 2014).

Th17 cells are specialized in fighting infections at mucosal surfaces, while regulatory T (reg) cells suppress inflammatory responses mediated by Th17 cells to prevent damage from autoimmunity and chronic inflammation (Littman and Rudensky, 2010). Th17 cells have been shown to mediate defensive responses against a variety of diseases. For example, Th17 cells in the lamina propria are strongly induced by rodent segmented filamentous bacteria (SFB), and these cells contain TCRs that specifically recognize SFB antigens (Yang et al., 2014). Th17 cell differentiation is also induced by other infections such as the bacteria *C. rodentium* and the fungus *C. albicans* (Hernandez-Santos and Gaffen, 2012; Mangan et al., 2006). Th17 cells do not have antimicrobial activity themselves, but mount an integrated response that includes activation of neutrophils, epithelial cells, and IgA-producing plasma cells (Weaver et al., 2013). Atarashi et al. (2015) demonstrated that at least 20 bacterial strains from genera *Bifidobacterium*, *Ruminococcus*, *Clostridium*, and *Bacteroides* from the human gut can induce Th17 cells. It is known that several viruses, such as HIV, hepatitis C virus (HCV), and simian immunodeficiency virus (SIV), persistently stimulate TCRs, which causes Th1 cells to differentiate into T follicular helper cells (Tfh) (Lindqvist et al., 2012; Petrovas et al., 2012; Feng et al., 2012). Tfh are a special type of CD4+ T cells that express the B cell follicle homing receptor CXCR5. Virus persistence typically results from the depletion of Th1 cells and the subsequent degradation of CD8+ T cells because Th1 cells are necessary to initiate an antiviral response mediated by CD8+ effector T cells.

2.9 Microbiome in Health and Diseases

The gut microbiome appears to play a significant role in health and disease and has been linked to a number of clinical problems, including inflammatory bowel disease, irritable bowel syndrome, frailty of the elderly, colorectal cancer, and gut-derived infections. Although the gut microbiome remains almost stable in the healthy human body, its qualitative and quantitative alterations result in functional modifications and have been reported in a number of human diseases (Kostic, 2014; Schnabl, 2014). Many large studies investigating the gut microbiome and its relevance have been performed in specific gastrointestinal disorders, such as intestinal bowel diseases, celiac disease, irritable bowel syndrome, chronic liver diseases, prototypic inflammatory disorders of the intestine, are associated with deviating gut microbiome composition and indeed facultative anaerobes outgrow have been reported, especially in the context of active inflammation and metabolite disturbances including bile acids, SCFAs, and acylcarnitine pathways (Lloyd- Price et al,. 2019; Tilg et al., 2016). Chronic liver diseases, such as liver cirrhosis, are characterized by profound microbial aberrations (Trebicka et al., 2021).

In general, microbial richness is usually considered an indicator of a healthy status: reduced bacterial diversity has been related to obesity and immune-related and inflammatory diseases (Cox, 2014; Clarke et al., 2014). Appropriate microbiome composition is required for a normal physiological function (Sekirov, 2010; Flint et al., 2012). Qualitative alterations of the core microbiome may lead to the development of diseases, as it has been mostly studied in IBD, such as Crohn's disease and ulcerative colitis (Elson and Cong, 2012).

Studies revealed that the composition and role of the intestinal microbiome in various diseased states have been linked with IBD, atopic dermatitis, autoimmune arthritis, type 2 diabetes, obesity, and atherosclerosis. For example, IBD patients often have less bacterial diversity and lower numbers of *Bacteroides* and *Firmicutes*, which together may contribute to decreased levels of microbial-derived butyrate. Butyrate and other SCFAs are thought to have anti-inflammatory effect in the gut (Lucas Lopez et al., 2017).

Lactobacilli species were found to be less in number during the active phase of inflammatory bowel disease; however, some species, such as *Lactobacillus salivarus*, *Lactobacillus manihotivorans*, and *Pediococcus acidilactici*, were found during the remission phase and not during active inflammation. Higher *E. coli* loads have been linked to the pathophysiology and development of ulcerative colitis, according to studies. Also, there were fewer *Roseburia hominis* and *F. prausnitzii* in fecal samples of ulcerative colitis patients (Madhogaria et al., 2022). Patients with Crohn's disease have more *Enterobacteriaceae* compared to healthy individuals. In addition, there is an increase in *Ruminococcus gnavus* and a decrease in *F. prausnitzii, Dialister invisus*, and *B. adolescentis*. Pediatric Crohn's disease patients had higher level of *Enterobacteriaceae*, *Pasteurellaceae*, *Fusobacteriaceae*, *Veillonellaceae* bacteria and lower level of *Bacteroidales*, *Erysipelotrichales*, and *Clostridiales* bacteria (Dahlhamer, 2016).

Diabetes (type 1 and type 2 diabetes) are metabolic disorders and are strongly associated with gut microbiota. Studies revealed that children with type 1 diabetes have lower levels of *Lactobacillus*, Bifidobacterium, *Eubacterium rectale*, and *Blautiacoccoides* and higher levels of *Clostridium*, *Veillonella*, and *Bacteroides* (Figure 2.2). Furthermore, a positive correlation was found between *Clostridium* and plasma glucose level, while a negative correlation was found between plasma glucose level and *Bifidobacterium*, *Lactobacillus* spp., *Firmicutes*, and *Bacteroidetes*. *F. prausnitzii* was also reduced, while *B. fragilis* was present in lower amounts. Additionally, type 2 diabetes patients had higher abundances of *Bifidobacterium* and lower abundances of the *Lactobacillus* species (Ahmad et al., 2019). The gut flora of patients with type 2 diabetes has been characterized with diabetes-associated markers, showing enriched membrane transport of sugars and branched-chain amino acids, xenobiotic metabolism, and sulfate reduction along with decreased bacterial chemotaxis and metabolism of cofactors and vitamins (Qin, 2012). Type 2 diabetes has also been characterized by an impaired gut microbiome in Asian and European populations (Karlsson et al., 2013). In type 2 diabetes,

microbial variations were strongly correlated with the presence of insulin resistance, and several studies implied that the gut microbiome affects glucose regulation (Wu et al., 2020).

Rheumatoid arthritis is a systemic autoimmune inflammatory disease that results in joint damage. Patients with rheumatoid arthritis show expansion of enteric *Prevotella copri*, and autoantigens in these patients exhibit a high degree of homology to *Prevotella*-associated peptides (Scher et al., 2013; Pianta et al., 2017). In HIV patients, pathogenic species like *C. albicans* and *Pseudomonas aeruginosa* were shown to be more prevalent, while *Bifidobacterium* and lactobacilli decreased significantly (Mutlu et al., 2009). A recent study also demonstrated that gut bacteria can produce significant amounts of amyloid and lipopolysaccharides, which are key players in the pathophysiology of Alzheimer's disease (Pistollato et al., 2016).

2.10 Human Microbiome and Cancer

The microbiome has an impact on cancer as well. Persistent T-cell activation by co-stimulatory proteins is linked with expression of co-inhibitory immune checkpoint inhibitors, Immune checkpoint inhibitors (ICIs) (e.g., cytotoxic T-lymphocyte associated protein 4, CTLA4, and programmed cell death 1, PD1), which prevent autoimmune reactions (Wherry and Kurachi, 2015; Gubin et al., 2014). Malignant cells adapt such mechanisms by expressing immune checkpoint homologs, and monoclonal antibodies that block such proteins hold great potential for treating various cancers (Escudier, 2012; Creelan, 2014; Tsai & Daud, 2015; Zhao et al., 2016). The gut microbiome has been demonstrated to modulate the clinical response to ICI, for instance, *Bacteroides* was linked to be associated with efficacy of anti-CTLA4 inhibition in melanoma in both mice and humans (Sivan et al., 2015; Vetizou, 2015). Moreover, *Bifidobacterium* species could enhance the efficacy of anti-PDL1 immunotherapy in mice. Surprisingly, *Bifidobacterium* alone improved tumor control to the same

extent as PDL1-specific checkpoint blocking, and combination treatment nearly abolished tumor outgrowth (Sivan et al., 2015). Furthermore, one of the primary immune-related adverse effects that restricts treatment, especially with anti-CTLA4 drugs, is colitis and other gastrointestinal problems, which have been linked to certain microbial activity. Examining how the gut microbiota and the anticancer immune response interact can help anticipate, prevent, and better manage side effects while also increasing the effectiveness of immunotherapies.

Research reveals that *F. nucleatum* suppresses the host immune response and promotes cell division. Butyrate is an important nutrient for a healthy colon. Genera that produce butyrate were almost absent in colorectal cancer patient's stool. *Prevotella* species were completely absent from several cancer samples that were examined. On the other hand, stool samples from patients with colorectal cancer contained substantial amounts of bacteria such as *Acidaminobacter*, *Phascolarctobacterium*, and *Citrobacter* (Madhogaria et al., 2022). *Bifidobacterium longum* showed strong antitumor activity in the treatment of colon cancer (Hsieh et al., 2018). Colorectal cancer, the most common malignancy in the lower gut, correlated with a disturbed gut microbiome and implicated certain bacteria such as *F. nucleatum, E. coli*, or *B. fragilis* (Figure 2.2), some of which are derived from the oral microbiome (Tilg et al., 2018). *Helicobacter pylori* is associated with a major risk of stomach cancer in humans. Furthermore, an elevated population of bacterial species such as *Fusobacterium* and *Clostridium* was observed in stomach cancer patients. Bacterial strains such as *F. nucleatum* and *C. colicanis* are used as markers for the diagnosis of stomach cancer. *Bacteroides massiliensis* has a significant impact on prostate cancer (Snyder et al., 2015). In case of cancer due to disturbance in intestinal barrier, bacteria from intestinal lumen enter the tissue, which triggers many inflammatory responses, for instance, they induce PRRs, e.g., membrane-bound TLRs and NLRs, which are linked to cancer progression.

2.11 Effect of Drugs on Microbiome

The human microbiome constitutes a complex ecosystem that can mediate the interaction of the human host with their environment. The interaction between the microbes and commonly used non-antibiotic drugs is complex and bidirectional. Drugs can affect the composition of the microbiome, but, vice versa, the microbiome can also influence an individual's response to a drug by enzymatically transforming the structure of drug and altering its bioavailability, bioactivity, or toxicity (Weersma et al., 2020). The gut microbiome can indirectly impact an individual's response to immunotherapy in cancer treatment by affecting the host's general immune status (Zitvogel et al., 2017).

The gut microbiota plays a crucial role in several processes, such as protection against overgrowth of pathogenic microbes, development of the host immune response, synthesis and metabolism of several compounds, such as SCFAs, and in the neurologic signaling (Lynch et al., 2016; Nogal et al., 2021). The use of drugs, such as proton pump inhibitors, corticosteroids, and statins, may alter the composition of gut microbiota (Minalyan et al., 2017; Caparros-Martin et al., 2017; Lange et al., 2016).

Proton pump inhibitors are the most commonly used drugs worldwide and are used to treat disorders such as peptic ulcers, dyspepsia, and gastroesophageal reflux. Proton pump inhibitors, such as pantoprazole and omeprazole, have grown in popularity in recent decades due to their effectiveness and safety profile (Othman et al., 2016). While there is clear evidence-based indication for the use of proton pump inhibitors, it has been suggested that up to 70% of proton pump inhibitor prescriptions may be unnecessary (Forgacs and Loganayagam, 2008). The large population-based study showed that proton pump inhibitors were the drugs most associated to a decreased diversity and taxonomical changes in the gut microbiome (Bonder et al., 2016). According to Imhann et al. (2016), the relative abundance of up to 20% of bacterial taxa changed (either decreased or increased) in proton pump inhibitor users compared to non-users. Overall, the taxonomic alterations in proton pump inhibitor users show an increase in the families *Enterobacteriaceae*, *Enterococcaceae*, and *Lactobacillaceae* and a decrease in *Ruminococcaceae* and *Bifidobacteriaceae*, and oral

bacteria are increases in the species *Rothia dentocariosa* and *Rothia mucilaginosa* (Imhann et al., 2016). A higher dosage appears to be associated with larger microbial changes (Vich Vila et al., 2020). While the efficacy and safety profiles of proton pump inhibitors are still very favorable when suggested with prescriptions, the medical community should rethink about widespread and chronic use.

Statins are often prescribed drugs used to prevent cardiovascular events. Statin inhibits the hydroxyl-methyl-glutaryl-coenzyme A reductase in the liver and causes a decrease in the level of low-density lipoprotein cholesterol (LDL-C), hence, frequently prescribed to prevent cardiovascular diseases. Statins were originally discovered for their antimicrobial properties and can cause gastrointestinal side effects, suggesting that statins may also affect the composition of gut microbiome (Thompson et al., 2016). Studies in the large population showed that statin users had significantly higher abundance in *Streptococcus vestibularis, S. parasanguinis, Clostridium bolteae, Ruminococcus bacterium, Ruminococcus torques*, and *Coprobacillus*. In contrast, a lower abundance in *C. comes, D. formicigenerans, D. longicatena, Eubacterium ramulus*, and *Ruminococcus lactaris* (Zhernakova et al., 2016). In comparison to controls, statin medication was positively correlated with *Burkholderiaceae*, *Enterobacteriaceae*, *Enterococcaceae, Propionibacteriaceae*, and *Actinomycetaceae* in idiopathic Parkinson's disease patients and healthy controls (Bedarf et al., 2017).

Metformin is a blood glucose-lowering drug used in the treatment of type 2 diabetes. Metformin inhibits gluconeogenesis in liver, and studies suggest that some of its beneficial effects are mediated by the gut microbiota (Wu et al., 2017; Sun et al., 2018). Studies in healthy volunteers showed the use of metformin resulted in a change in more than 80 species compared with a control group receiving placebo. Notably, when metformin-treated patients with type 2 diabetes were compared to untreated patients, metformin treatment significantly increased *E. coli* and lowered *Intestinibacter* abundance. Subsequently, the investigators observed lower blood glucose level in metformin-treated volunteers, suggesting a direct

effect of the gut microbiota on blood glucose level. This effect is thought to be mediated by metformin's effect on SCFA, for example, butyrate-producing bacteria and the abundance of *Akkermansia muciniphila* (Forslund et al., 2015; Shin et al., 2014).

Levodopa, a drug used for the treatment of Parkinson's disease, is a fascinating example of microbial impact on drug efficacy. Levodopa must be absorbed in the small intestine after oral treatment to pass through the blood–brain barrier and reach the brain, where it is converted to therapeutically active dopamine by the human enzyme aromatic amino acid decarboxylase. The bioavailability of levodopa to the brain is a key factor for drug efficacy. Recent studies have demonstrated that levodopa may be metabolized by the microbial decarboxylases found in gut microbial species. Tyrosine decarboxylases (tyrDCs), which are mostly driven by *Enterococcus faecalis*, have been found to be involved in novel bacterial L-dopa metabolism (Maini Rekdal et al., 2019).

Furthermore, research has demonstrated that other commonly used medications like statins, laxatives, opioids, and antidepressants can contribute to some of the variations in the composition of the gut microbiome (Falony et al., 2016; Jackson et al., 2018; Imhann et al., 2017).

2.12 Conclusion

The human microbiome, composed of trillions of microorganisms, and plays a crucial and dynamic role in maintaining human health throughout life. Its composition is shaped by numerous intrinsic and extrinsic factors including host genetics, age, gender, diet, probiotics, antibiotic exposure, and various environmental influences. Modern sequencing technologies such as NGS, shotgun metagenomics, 16S rRNA sequencing, and metatranscriptomics, together with sophisticated bioinformatic tools, have transformed our understanding of this complex ecosystem and enabled detailed characterization of microbial diversity, function, and interactions with the host.

The gut microbiota, the largest and most metabolically active microbial community in the body, profoundly affects digestion, nutrient metabolism, immune regulation, and protection against pathogens. Disruptions to this ecosystem due to diet, disease, or medications can lead to dysbiosis, which has been implicated in metabolic disorders, IBD, autoimmune conditions, neurological disorders, and cancer. Microbial metabolites such as SCFAs, tryptophan derivatives, and bile acid products mediate many of these interactions and influence both local gut physiology and systemic processes, including the gut–brain axis. Growing evidence highlights the bidirectional relationship between drugs and the microbiome: while medications such as antibiotics, proton pump inhibitors, statins, metformin, and levodopa alter microbial composition, microbial communities can likewise modify drug metabolism, bioavailability, and therapeutic efficacy. This underscores the need to consider the microbiome in precision medicine and drug development.

Overall, maintaining a balanced microbiome is critical for health, and disturbances in microbial ecosystems can contribute to a wide spectrum of diseases. Understanding the mechanisms that shape microbiome dynamics through advanced sequencing technologies, functional assays, and integrative analyses holds great promise for developing microbiome-informed diagnostics, personalized nutrition strategies, and improved clinical interventions for complex human diseases.

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Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

References

Adlerberth, I., & Wold, A. E. (2009). Establishment of the gut microbiota in Western infants. *Acta Paediatrica*, *98*(2), 229–238. https://doi.org/10.1111/j.1651-2227.2008.01060.x ⏎

Ahmad, A. F., Dwivedi, G., O'Gara, F., Caparros-Martin, J., & Ward, N. C. (2019). The gut microbiome and cardiovascular disease: current knowledge and clinical potential. *American Journal of Physiology—Heart and Circulatory Physiology*, *317*(5), H923–H938. https://doi.org/10.1152/ajpheart.00376.2019 ⏎

Al Bataineh, M. T., Dash, N. R., Elkhazendar, M., Alnusairat, D. A. M. H., Darwish, I. M. I., Al-Hajjaj, M. S., & Hamid, Q. (2020). Revealing oral microbiota composition and functionality associated with heavy cigarette smoking. *Journal of Translational Medicine*, *18*(1), 421. https://doi.org/10.1186/s12967-020-02579-3 ⏎

Ananthapadmanabhan, K. P., Moore, D. J., Subramanyan, K., Misra, M., & Meyer, F. (2012). Cleansing without compromise: The impact of cleansers on the skin barrier and the technology of mild cleansing. *Dermatologic Therapy*, *25*(3), 244–253. https://doi.org/10.1111/j.1529-8019.2012.01499.x ⏎

Appanna, V. D. (2018). Dysbiosis, probiotics, and prebiotics: In diseases and health. In *Human microbes—The power within: Health, healing and beyond* (pp. 81–122). Singapore: Springer. https://doi.org/10.1007/978-981-10-7684-8_3 ⏎

Aronsson, L., Huang, Y., Parini, P., Korach-André, M., Håkansson, J., Gustafsson, J. Å., … & Rafter, J. (2010). Decreased fat storage by *Lactobacillus paracasei* is associated with increased levels of

angiopoietin-like 4 protein (ANGPTL4). *PloS One*, *5*(9), e13087. https://doi.org/10.1371/journal.pone.0013087 ⏎

Asaba, J., Bandyopadhyay, M., Kindy, M., & Dasgupta, S. (2015). Estrogen receptor signal in regulation of B cell activation during diverse immune responses. *International Journal of Biochemistry & Cell Biology*, *68*, 42–47. https://doi.org/10.1016/j.biocel.2015.08.012 ⏎

Atarashi, K., Tanoue, T., Ando, M., Kamada, N., Nagano, Y., Narushima, S., … & Honda, K. (2015). Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell*, *163*(2), 367–380. https://doi.org/10.1016/j.cell.2015.08.058 ⏎

Atashili, J., Poole, C., Ndumbe, P. M., Adimora, A. A., & Smith, J. S. (2008). Bacterial vaginosis and HIV acquisition: A meta-analysis of published studies. *AIDS*, *22*(12), 1493–1501. https://doi.org/10.1097/QAD.0b013e3283021a37 ⏎

Badet, C., & Thebaud, N. B. (2008). Ecology of lactobacilli in the oral cavity: A review of literature. *Open Microbiology Journal*, *2*, 38–48. https://doi.org/10.2174/1874285800802010038 ⏎

Barden, N. (2004). Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. *Journal of Psychiatry and Neuroscience*, *29*(3), 185–193. ⏎

Barichella, M., Severgnini, M., Cilia, R., Cassani, E., Bolliri, C., Caronni, S., … & Pezzoli, G. (2019). Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. *Movement Disorders*, *34*(3), 396–405. https://doi.org/10.1002/mds.27581 ⏎

Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., & Stanton, C. (2012). γ-Aminobutyric acid production by culturable bacteria from the human intestine. *Journal of Applied Microbiology*, *113*(2), 411–417. https://doi.org/10.1111/j.1365-2672.2012.05344.x ⏎

Beaugerie, L., & Petit, J. C. (2004). Antibiotic-associated diarrhoea. *Best Practice & Research Clinical Gastroenterology*, *18*(2), 337–352. https://doi.org/10.1016/j.bpg.2003.10.002 ⏎

Bedarf, J. R., Hildebrand, F., Coelho, L. P., Sunagawa, S., Bahram, M., Goeser, F., … & Wüllner, U. (2017). Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. *Genome Medicine*, *9*(1), 39. https://doi.org/10.1186/s13073-017-0428-y ⏎

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., … & Anrather, J. (2016). Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nature Medicine*, *22*(5), 516–523. https://doi.org/10.1038/nm.4068 ⏎

Benga, L., Rehm, A., Gougoula, C., Westhoff, P., Wachtmeister, T., Benten, W. P. M., … & Janssen, S. (2024). The host genotype actively shapes its microbiome across generations in laboratory mice. *Microbiome*, *12*(1), 256. https://doi.org/10.1186/s40168-024-01954-2 ⏎

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., … & Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences*, *107*(44), 18933–18938. https://doi.org/10.1073/pnas.1007028107 ⏎

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., … & Schloter, M. (2020). Microbiome definition re-visited: Old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0 ⏎

Beury-Cirou, A., Tannieres, M., Minard, C., Soulere, L., Rasamiravaka, T., Dodd, R. H., … & Faure, D. (2013). At a supra-physiological concentration, human sexual hormones act as quorum-sensing inhibitors. *PLoS One*, *8*(12), e83564. https://doi.org/10.1371/journal.pone.0083564 ⏎

Beylot, C., Auffret, N., Poli, F., Claudel, J. P., Leccia, M.-T., Del Giudice, P., & Dreno, B. (2014). Propionibacterium acnes: An update on its role in the pathogenesis of acne. *Journal of the European Academy of Dermatology and Venereology*, 28(3), 271–278. https://doi.org/10.1111/jdv.12224 ⏎

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., … & De Vos, W. (2010). Through ageing, and beyond: Gut microbiota and inflammatory status in seniors and centenarians. *PLoS One*, *5*(5), e10667. https://doi.org/10.1371/journal.pone.0010667 ⏎

Bisgaard, H., Li, N., Bonnelykke, K., Chawes, B. L. K., Skov, T., Paludan-Müller, G., … & Krogfelt, K. A. (2011). Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *Journal of Allergy and Clinical Immunology*, *128*(3), 646–652. https://doi.org/10.1016/j.jaci.2011.04.060 ⏎

Bonder, M. J., Tigchelaar, E. F., Cai, X., Trynka, G., Cenit, M. C., Hrdlickova, B., … & Zhernakova, A. (2016). The influence of a short-term gluten-free diet on the human gut microbiome. *Genome Medicine*, *8*(1), 45. https://doi.org/10.1186/s13073-016-0295-y ⏎

Brotman, R. M. (2011). Vaginal microbiome and sexually transmitted infections: An epidemiologic perspective. *Journal of Clinical Investigation*, *121*(12), 4610–4617. https://doi.org/10.1172/JCI57149 ⏎

Bullman, S., Pedamallu, C. S., Sicinska, E., *et al*. (2017). Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science*, *358*(6369), 1443–1448. https://doi.org/10.1126/science.aal5240 ⏎

Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*, 143–155. https://doi.org/10.1038/nrmicro.2017.157 ⏎

Candela, M., *et al*. (2008). Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production. *International Journal of Food Microbiology*, *125*(3), 286–292. https://doi.org/10.1016/j.ijfoodmicro.2008.04.012 ⏎

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced

obesity and diabetes in mice. *Diabetes*, *57*(6), 1470–1481. https://doi.org/10.2337/db07-1403 ⏎

Caparros-Martin, J. A., Lareu, R. R., Ramsay, J. P., Peplies, J., Reen, F. J., Headlam, H. A., … & O'Gara, F. (2017). Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. *Microbiome*, *5*(1), 95. https://doi.org/10.1186/s40168-017-0312-4 ⏎

Capone, K. A., *et al*. (2011). Diversity of the human skin microbiome early in life. *Journal of Investigative Dermatology*, *131*(10), 2026–2032. https://doi.org/10.1038/jid.2011.168 ⏎

Carvalho-Wells, A. L., Helmolz, K., Nodet, C., Molzer, C., Leonard, C., McKevith, B., … & Tuohy, K. M. (2010). Determination of the in vivo prebiotic potential of a maize-based whole grain breakfast cereal: a human feeding study. *British Journal of Nutrition*, *104*(9), 1353–1356. https://doi.org/10.1017/S0007114510002084 ⏎

Chu, C., Murdock, M. H., Jing, D., Won, T. H., Chung, H., Kressel, A. M., … & Artis, D. (2019). The microbiota regulate neuronal function and fear extinction learning. *Nature*, *574*(7779), 543–548. https://doi.org/10.1038/s41586-019-1644-y ⏎

Cogen, A. L., *et al*. (2010). *Staphylococcus epidermidis* antimicrobial peptides targeting *Staphylococcus aureus*. *Journal of Investigative Dermatology*, *130*(3), 641–648. https://doi.org/10.1038/jid.2009.287 ⏎

Cogen, A. L., Nizet, V., & Gallo, R. L. (2008). Skin microbiota: A source of disease or defence? *British Journal of Dermatology*, *158*(3), 442–455. https://doi.org/10.1111/j.1365-2133.2008.08437.x ⏎

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., … & Tuohy, K. M. (2008). Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: A double-blind, placebo-controlled, crossover study. *British Journal of Nutrition*, *99*(1), 110–120. https://doi.org/10.1017/S0007114507793923 ⏎

Cox, A. J., West, N. P., & Cripps, A. W. (2015). Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinology, 3(3), 207*–215. http://dx.doi.org/10.1016/S2213-8587(14)70134-2 ⏎

Creelan, B. C. (2014). Update on immune checkpoint inhibitors in lung cancer. *Cancer Control*, *21*(1), 80–89. https://doi.org/10.1177/107327481402100112 ⏎

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: From bowel to behavior. *Neurogastroenterology & Motility*, *23*(3), 187–192. https://doi.org/10.1111/j.1365-2982.2010.01664.x ⏎

Cryan, J. F., O'Riordan, K. J., Cowan, C. S., Sandhu, K. V., Bastiaanssen, T. F., Boehme, M., … & Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews, 99, 1877*–2013. https://doi.org/10.1152/physrev.00018.2018 ⏎

Cunningham, M., & Gilkeson, G. (2011). Estrogen receptors in immunity and autoimmunity. *Clinical Reviews in Allergy & Immunology*, *40*(1), 66–73. https://doi.org/10.1007/s12016-010-8203-5 ⏎

Curty, G., *et al*. (2019). The female genital tract microbiome in HPV infection: Implications for cervical cancer development. *Microbial Pathogenesis*, *132*, 164–172. https://doi.org/10.1016/j.micpath.2019.04.016 ⏎

D'Argenio, V., & Salvatore, F. (2015). The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta*, *451*, 97–102. https://doi.org/10.1016/j.cca.2015.01.003 ⏎

Dahlhamer, J. M. (2016). Prevalence of inflammatory bowel disease among adults aged≥ 18 years-United States, 2015. *MMWR Morbidity and Mortality Weekly Report*, *65*(42), 1166–1169. http://dx.doi.org/10.15585/mmwr.mm6542a3 ⏎

De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I. B., La Storia, A., Laghi, L., … & Ercolini, D. (2016). High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut*, *65*(11), 1812–1821. https://doi.org/10.1136/gutjnl-2015-309957 ⏎

Deehan, E. C., *et al*. (2024). Effects of dietary fibre on metabolic health and obesity. *Nature*, *771*(7764), 331–337. 10.1038/s41586-019-1311-3 ⏎

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.07.005 ⏎

Dethlefsen, L., Huse, S., Sogin, M. L., & Relman, D. A. (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology*, *6*(11), e280. https://doi.org/10.1371/journal.pbio.0060280 ⏎

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., … Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*(19), 5002–5017. https://doi.org/10.1128/jb.00542-10 ⏎

Dicksved, J., Halfvarson, J., Rosenquist, M., Järnerot, G., Tysk, C., Apajalahti, J., … & Jansson, J. K. (2008). Molecular analysis of the gut microbiota of identical twins with Crohn's disease. *ISME Journal*, *2*(7), 716–727. https://doi.org/10.1038/ismej.2008.37 ⏎

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences*, *107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107 ⏎

Dominy, S. S., Lynch, C., Ermini, F., Benedyk, M., Marczyk, A., Konradi, A., … Potempa, J. (2019). *Porphyromonas gingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Science Advances*, *5*(1), eaau3333. https://doi.org/10.1126/sciadv.aau3333 ⏎

Draelos, Z. D. (2018). The science behind skin care: Moisturizers. *Journal of Cosmetic Dermatology*, *17*(2), 138–144. https://doi.org/10.1111/jocd.12505 ⏎

Dreno, B., *et al*. (2016). *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and acne vulgaris: A brief update. *Journal of the European*

Academy of Dermatology and Venereology, *30*(12), 1978–1983. https://doi.org/10.1111/jdv.13965 ⏎

Duran-Pinedo, A., Solbiati, J. O., Teles, F., Yanping, Z., & Frias-Lopez, J. (2025). Longitudinal host–microbiome dynamics of metatranscription identify hallmarks of progression in periodontitis. *Microbiome*, *13*, 119. https://doi.org/10.1186/s40168-025-02014-8 ⏎

Eid, N., Enani, S., Walton, G., Corona, G., Costabile, A., Gibson, G., … & Spencer, J. P. (2014). The impact of date palm fruits and their component polyphenols, on gut microbial ecology, bacterial metabolites and colon cancer cell proliferation. *Journal of Nutritional Science*, *3*, e46. https://doi.org/10.1017/jns.2014.16 ⏎

Elinav, E., Garrett, W. S., Trinchieri, G., & Wargo, J. (2019). The cancer microbiome. *Nature Reviews Cancer*, 19(7), 371–376. https://doi.org/10.1038/s41568-019-0155-3 ⏎

Erny, D., Hrabe de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … & Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, *18*(7), 965–977. https://doi.org/10.1038/nn.4030 ⏎

Escudier, B. (2012). Emerging immunotherapies for renal cell carcinoma. *Annals of Oncology*, *23*, viii35–viii40. https://doi.org/10.1093/annonc/mds261 ⏎

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., … & Raes, J. (2016). Population-level analysis of gut microbiome variation. *Science*, *352*(6285), 560–564. https://doi.org/10.1126/science.aad3503 ⏎

Favier, C. F., Vaughan, E. E., De Vos, W. M., & Akkermans, A. D. (2002). Molecular monitoring of succession of bacterial communities in human neonates. *Applied and Environmental Microbiology*, *68*(1), 219–226. https://doi.org/10.1128/AEM.68.1.219-226.2002 ⏎

Featherstone, J. D. B. (2004). The continuum of dental caries: Evidence for a dynamic disease process. *Journal of Dental Research*, *83*(1-suppl), 39–42. https://doi.org/10.1177/154405910408301s08 ⏎

Feng, J., Hu, X., Guo, H., Sun, X., Wang, J., Xu, L., & Jiang, Y. (2012). Patients with chronic hepatitis C express a high percentage of CD4-CXCR5- T follicular helper cells. *Journal of Gastroenterology*, *47*(9), 1048–1056. https://doi.org/10.1007/s00535-012-0568-1 ⏎

Fish, E. N. (2008). The X-files in immunity: Sex-based differences predispose immune responses. *Nature Reviews Immunology*, *8*(9), 737–744. https://doi.org/10.1038/nri2394 ⏎

Fitz-Gibbon, S., *et al*. (2013). *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. *Journal of Investigative Dermatology*, *133*(9), 2152–2160. https://doi.org/10.1038/jid.2013.21 ⏎

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, *3*, 289–306. https://doi.org/10.4161/gmic.19897 ⏎

Flint, H. J., & Louis, P. (2024). Why does increased microbial fermentation in the human colon shift toward butyrate? *AIMS Microbiology*, *10*(2), 311–319. https://doi.org/10.3934/microbiol.2024016 ⏎

Floistrup, H., Swartz, J., Bergstrom, A., Alm, J. S., Scheynius, A., van Hage, M., … & Parsifal Study Group. (2006). Allergic disease and sensitization in Steiner school children. *Journal of Allergy and Clinical Immunology*, *117*(1), 59–66. https://doi.org/10.1016/j.jaci.2005.09.039 ⏎

Forgacs, I., & Loganayagam, A. (2008). Overprescribing proton pump inhibitors. *BMJ*, *336*(7634), 2–3. https://doi.org/10.1136/bmj.39406.449456.BE ⏎

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., … & Pedersen, O. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, *528*(7581), 262–266. https://doi.org/10.1038/nature15766 ⏎

Fouhy, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., … & Cotter, P. D. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin.

Antimicrobial Agents and Chemotherapy, *56*(11), 5811–5820. https://doi.org/10.1128/aac.00789-12 ⏎

Fredricks, D. N., Fiedler, T. L., & Marrazzo, J. M. (2005). Molecular identification of bacteria associated with bacterial vaginosis. *New England Journal of Medicine*, *353*(18), 1899–1911. https://doi.org/10.1056/NEJMoa043802 ⏎

Gao, L., Xu, T., Huang, G., Jiang, S., Gu, Y., & Chen, F. (2018). Oral microbiomes: More and more importance in oral cavity and whole body. *Protein & Cell*, *9*(5), 488–500. https://doi.org/10.1007/s13238-018-0548-1 ⏎

Gao, S., Li, S., Ma, Z., Liang, S., Shan, T., *et al*. (2016). Presence of *Porphyromonas gingivalis* in esophageal cancer and its association with the clinicopathological characteristics and survival. *Infectious Agents and Cancer*, *11*(3), 1–10. https://doi.org/10.1186/s13027-016-0049-x ⏎

Goehler, L. E., Park, S. M., Opitz, N., Lyte, M., & Gaykema, R. P. (2008). *Campylobacter jejuni* infection increases anxiety-like behavior in the holeboard: Possible anatomical substrates for viscerosensory modulation of exploratory behavior. *Brain, Behavior, and Immunity*, *22*(3), 354–366. https://doi.org/10.1016/j.bbi.2007.08.009 ⏎

Graf, D., Di Cagno, R., Fak, F., Flint, H. J., Nyman, M., Saarela, M., & Watzl, B. (2015). Contribution of diet to the composition of the human gut microbiota. *Microbial Ecology in Health and Disease*, *26*(1), 26164. https://doi.org/10.3402/mehd.v26.26164 ⏎

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*, 244–253. https://doi.org/10.1038/nrmicro2537 ⏎

Griffiths, E. A., Duffy, L. C., Schanbacher, F. L., Qiao, H., Dryja, D., Leavens, A., … & Ogra, P. L. (2004). In vivo effects of *bifidobacteria* and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Digestive Diseases and Sciences*, *49*(4), 579–589. https://doi.org/10.1023/B:DDAS.0000026302.92898.ae ⏎

Gross, E. L., Beall, C. J., Kutsch, S. R., Firestone, N. D., Leys, E. J., & Griffen, A. L. (2012). Beyond *Streptococcus mutans*: Dental caries onset

linked to multiple species by 16S rRNA community analysis. *PLoS One*, *7*(10), e47722. https://doi.org/10.1371/journal.pone.0047722 ⏎

Gross, E. L., Leys, E. J., Gasparovich, S. R., Firestone, N. D., Schwartzbaum, J. A., Janies, D. A., … Griffen, A. L. (2010). Bacterial 16S sequence analysis of severe caries in young permanent teeth. *Journal of Clinical Microbiology*, *48*(11), 4121–4128. https://doi.org/10.1128/JCM.01232-10 ⏎

Guarner, F., Bourdet-Sicard, R., Brandtzaeg, P., Gill, H. S., McGuirk, P., Van Eden, W., … & Rook, G. A. (2006). Mechanisms of disease: The hygiene hypothesis revisited. *Nature Clinical Practice Gastroenterology & Hepatology*, *3*(5), 275–284. https://doi.org/10.1038/ncpgasthep0471 ⏎

Gubin, M. M., Zhang, X., Schuster, H., Caron, E., Ward, J. P., Noguchi, T., … & Schreiber, R. D. (2014). Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*, *515*(7528), 577–581. https://doi.org/10.1038/nature13988 ⏎

Gupta, P., Gupta, N., Pawar, A. P., Birajdar, S. S., Natt, A. S., & Singh, H. P. (2013). Role of sugar and sugar substitutes in dental caries: A review. *International Scholarly Research Notices*, *2013*(1), 519421. https://doi.org/10.1155/2013/519421 ⏎

Hall, A. B., Tolonen, A. C., & Xavier, R. J. (2017). Human genetic variation and the gut microbiome in disease. *Nature Reviews Genetics*, *18*(11), 690–699. https://doi.org/10.1038/nrg.2017.63 ⏎

Hayashi, H., Sakamoto, M., & Benno, Y. (2002). Fecal microbial diversity in a strict vegetarian as determined by molecular analysis and cultivation. *Microbiology and Immunology*, *46*(12), 819–831. https://doi.org/10.1111/j.1348-0421.2002.tb02769.x ⏎

Hernandez-Santos, N., & Gaffen, S. L. (2012). Th17 cells in immunity to *Candida albicans*. *Cell Host & Microbe*, *11*(5), 425–435. https://doi.org/10.1016/j.chom.2012.04.008 ⏎

Hooper, L. V., Midtvedt, T., & Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine.

Annual Review of Nutrition, *22*(1), 283–307. https://doi.org/10.1146/annurev.nutr.22.011602.092259 ⏎

Hsieh, Y. Y., Tung, S. Y., Pan, H. Y., Yen, C. W., Xu, H. W., Lin, Y. J., … & Li, C. (2018). Increased abundance of *Clostridium* and *Fusobacterium* in gastric microbiota of patients with gastric cancer in Taiwan. *Scientific Reports*, *8*(1), 158. https://doi.org/10.1038/s41598-017-18596-0 ⏎

Imhann, F., Bonder, M. J., Vila, A. V., Fu, J., Mujagic, Z., Vork, L., … & Zhernakova, A. (2016). Proton pump inhibitors affect the gut microbiome. *Gut*, *65*(5), 740–748. https://doi.org/10.1136/gutjnl-2015-310376 ⏎

Imhann, F., Vich Vila, A., Bonder, M. J., Lopez Manosalva, A. G., Koonen, D. P., Fu, J., … & Weersma, R. K. (2017). The influence of proton pump inhibitors and other commonly used medication on the gut microbiota. *Gut Microbes*, *8*(4), 351–358. https://doi.org/10.1080/19490976.2017.1284732 ⏎

Inquimbert, C., Bourgeois, D., Bravo, M., Viennot, S., Tramini, P., Llodra, J. C., … Carrouel, F. (2019). The oral bacterial microbiome of interdental surfaces in adolescents according to carious risk. *Microorganisms*, *7*(9), 319. https://doi.org/10.3390/microorganisms7090319 ⏎

Jackson, M. A., Verdi, S., Maxan, M. E., Shin, C. M., Zierer, J., Bowyer, R. C., … & Steves, C. J. (2018). Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nature Communications*, *9*(1), 2655. https://doi.org/10.1038/s41467-018-05184-7 ⏎

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., Nielsen, J., & Bäckhed, F. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*, *498*(7452), 99–103. https://doi.org/10.1038/nature12198 ⏎

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, *474*(7351), 327–336. https://doi.org/10.1038/nature10213 ⏎

Kayıran, M. A., Karadag, A. S., Al-Khuzaei, S., Chen, W., & Dreno, B. (2020). Antibiotic resistance in acne: Mechanisms, complications and management. *American Journal of Clinical Dermatology*, 21(6), 813–819. https://doi.org/10.1007/s40257-020-00556-6

Kedia, G., Vázquez, J. A., Charalampopoulos, D., & Pandiella, S. S. (2009). In vitro fermentation of oat bran obtained by debranning with a mixed culture of human fecal bacteria. *Current Microbiology*, *58*(4), 338–342. https://doi.org/10.1007/s00284-008-9335-1 ⏎

Keim, N. L., & Martin, R. J. (2014). Dietary whole grain–microbiota interactions: Insights into mechanisms for human health. *Advances in Nutrition*, *5*(5), 556–557. https://doi.org/10.3945/an.114.006536 ⏎

Kobayashi, T., *et al*. (2015). Dysbiosis and skin immunity. *Journal of Clinical Investigation*, *125*(5), 1984–1993. https://doi.org/10.1172/JCI78090 ⏎

Kong, H. H., *et al*. (2012). Temporal shifts in the skin microbiome associated with atopic dermatitis flares and treatment. *Genome Research*, *22*(5), 850–859. https://doi.org/10.1101/gr.131029.111 ⏎

Kostic, A. D., Xavier, R. J., & Gevers, D. (2014). The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*, *146*, 1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009 ⏎

Kumar, A., *et al*. (2025). Impact of diet on microbial ecology in the gut: A comprehensive review. *Frontiers in Microbiology*, *16*, Article 1675065. https://doi.org/10.3389/fmicb.2025.1675065 ⏎

Kumar, P. S., Matthews, C. R., Joshi, V., de Jager, M., & Aspiras, M. (2011). Tobacco smoking affects bacterial acquisition and colonization in oral biofilms. *Infection and Immunity*, *79*(11), 4730–4738. https://doi.org/10.1128/iai.05371-11 ⏎

Lamont, R. J., Koo, H., & Hajishengallis, G. (2018). The oral microbiota: Dynamic communities and host interactions. *Nature Reviews Microbiology*, *16*(12), 745–759. https://doi.org/10.1038/s41579-018-0089-x ⏎

Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of antibiotics on gut microbiota. *Digestive Diseases*, *34*(3), 260–268. https://doi.org/10.1159/000443360 ⏎

Lazarevic, V., Manzano, S., Gaïa, N., Girard, M., Whiteson, K., Hibbs, J., … Schrenzel, J. (2013). Effects of amoxicillin treatment on the salivary microbiota in children with acute otitis media. *Clinical Microbiology and Infection*, *19*(8), e335–e342. https://doi.org/10.1111/1469-0691.12213 ⏎

Lecomte, V., Kaakoush, N. O., Maloney, C. A., Raipuria, M., Huinao, K. D., Mitchell, H. M., & Morris, M. J. (2015). Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. *PloS One*, *10*(5), e0126931. https://doi.org/10.1371/journal.pone.0126931 ⏎

Lee, C. K., Herbold, C. W., Polson, S. W., Wommack, K. E., Williamson, S. J., McDonald, I. R., & Cary, S. C. (2012). Groundtruthing next-gen sequencing for microbial ecology—Biases and errors in community structure estimates from PCR amplicon pyrosequencing. *PLoS ONE*, *7*(9), e44224. https://doi.org/10.1371/journal.pone.0044224 ⏎

Lee, H. Y., Park, J. H., Seok, S. H., Baek, M. W., Kim, D. J., Lee, K. E., … & Park, J. H. (2006). Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1761*(7), 736–744. https://doi.org/10.1016/j.bbalip.2006.05.007 ⏎

Lehtoranta, L., *et al*. (2022). Vaginal microbiota and risk of preterm birth: A systematic review and meta-analysis. *American Journal of Obstetrics and Gynecology*, *226*(2), 272.e1–272.e13. https://doi.org/10.1016/j.ajog.2021.10.020 ⏎

Leitch, E. C. M., Walker, A. W., Duncan, S. H., Holtrop, G., & Flint, H. J. (2007). Selective colonization of insoluble substrates by human faecal bacteria. *Environmental Microbiology*, *9*(3), 667–679. https://doi.org/10.1111/j.1462-2920.2006.01186.x ⏎

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences*, *102*(31), 11070–11075. https://doi.org/10.1073/pnas.0504978102 ⏎

Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, *5*, 258. https://doi.org/10.3389/fmicb.2014.00258 ⏎

Lindqvist, M., van Lunzen, J., Soghoian, D. Z., Kuhl, B. D., Ranasinghe, S., Kranias, G., … & Streeck, H. (2012). Expansion of HIV-specific T follicular helper cells in chronic HIV infection. *Journal of Clinical Investigation*, *122*(9), 3271–3280. https://doi.org/10.1172/JCI64314 ⏎

Littman, D. R., & Rudensky, A. Y. (2010). Th17 and regulatory T cells in mediating and restraining inflammation. *Cell*, *140*(6), 845–858. https://doi.org/10.1016/j.cell.2010.02.021 ⏎

Liu, Z., Lin, X., Huang, G., Zhang, W., Rao, P., & Ni, L. (2014). Prebiotic effects of almonds and almond skins on intestinal microbiota in healthy adult humans. *Anaerobe*, *26*, 1–6. https://doi.org/10.1016/j.anaerobe.2013.11.007 ⏎

Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., Andrews, E., Ajami, N. J., Bonham, K. S., Brislawn, C. J., Casero, D., Courtney, H., Gonzalez, A., Graeber, T. G., Hall, A. B., Lake, K., Landers, C. J., Mallick, H., Plichta, D. R., Prasad, M., Rahnavard, G., Sauk, J., Shungin, D., Vázquez-Baeza, Y., White, R. A., & Huttenhower, C. (2019). Multi-Omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*, *569*(7758), 655–662. https://doi.org/10.1038/s41586-019-1237-9 ⏎

Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A. B., … & Huttenhower, C. (2017). Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*, *550*(7674), 61–66. https://doi.org/10.1038/nature23889 ⏎

Logares, R., Haverkamp, T. H. A., Kumar, S., Lanzén, A., Nederbragt, A. J., Quince, C., & Kauserud, H. (2012). Environmental microbiology through the lens of high-throughput DNA sequencing: Synopsis of current platforms and bioinformatics approaches. *Journal of Microbiological Methods*, *91*(1), 106–113. https://doi.org/10.1016/j.mimet.2012.07.017 ⏎

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220–230. https://doi.org/10.1038/nature11550 ⏎

Lynch, S. V., & Pedersen, O. (2016). The human intestinal microbiome in health and disease. *New England Journal of Medicine*, *375*(24), 2369–2379. 10.1056/NEJMra1600266 ⏎

MacIntyre, D. A., Chandiramani, M., Lee, Y. S., *et al*. (2015). The vaginal microbiome during pregnancy and the postpartum period in a European population. *Scientific Reports*, *5*, 8988. https://doi.org/10.1038/srep08988 ⏎

Madhogaria, B., Bhowmik, P., & Kundu, A. (2022). Correlation between human gut microbiome and diseases. *Infectious Medicine*, *1*(3), 180–191. https://doi.org/10.1016/j.imj.2022.08.004 ⏎

Maini Rekdal, V., Bess, E. N., Bisanz, J. E., Turnbaugh, P. J., & Balskus, E. P. (2019). Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science*, *364*(6445), eaau6323. doi: 10.1126/science.aau6323 ⏎

Mangan, P. R., Harrington, L. E., O'Quinn, D. B., Helms, W. S., Bullard, D. C., Elson, C. O., … & Weaver, C. T. (2006). Transforming growth factor-β induces development of the TH17 lineage. *Nature*, *441*(7090), 231–234. https://doi.org/10.1038/nature04754 ⏎

Mantzourani, M., Fenlon, M., & Beighton, D. (2009). Association between Bifidobacteriaceae and the clinical severity of root caries lesions. *Oral Microbiology and Immunology*, *24*(1), 32–37. doi: 10.1111/j.1399-302X.2008.00470.x ⏎

Mariat, D., Firmesse, O., Levenez, F., Guimaraes, V. D., Sokol, H., Dore, J., … & Furet, J. (2009). The *Firmicutes*/*Bacteroidetes* ratio of the human microbiota changes with age. *BMC Microbiology*, *9*(1), 123. https://doi.org/10.1186/1471-2180-9-123 ⏎

Marques, T. M., Wall, R., Ross, R. P., Fitzgerald, G. F., Ryan, C. A., & Stanton, C. (2010). Programming infant gut microbiota: Influence of dietary and environmental factors. *Current Opinion in Biotechnology*, *21*(2), 149–156. https://doi.org/10.1016/j.copbio.2010.03.020 ⏎

Martin, C. R., & Walker, W. A. (2008). Probiotics: Role in pathophysiology and prevention in necrotizing enterocolitis. *Seminars in Perinatology*, *32*(2), 127–137. https://doi.org/10.1053/j.semperi.2008.01.006 ⏎

Martinon, P., Fraticelli, L., Giboreau, A., Dussart, C., Bourgeois, D., & Carrouel, F. (2021). Nutrition as a key modifiable factor for periodontitis and main chronic diseases. *Journal of Clinical Medicine*, *10*(2), 197. https://doi.org/10.3390/jcm10020197 ⏎

Mathews, S., Henderson, S., & Reinhold, D. (2014). Uptake and accumulation of antimicrobials, triclocarban and triclosan, by food crops in a hydroponic system. *Environmental Science and Pollution Research*, *21*(9), 6025–6033. https://doi.org/10.1007/s11356-013-2474-3 ⏎

McNulty, N. P., Yatsunenko, T., Hsiao, A., Faith, J. J., Muegge, B. D., Goodman, A. L., … & Gordon, J. I. (2011). The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Science Translational Medicine*, *3*(106), 106. https://doi.org/10.1126/scitranslmed.3002701 ⏎

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., & Edwards, R. A. (2008). The metagenomics RAST server—A public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics*, *9*, 386. https://doi.org/10.1186/1471-2105-9-386 ⏎

Mihaila, D., Donegan, J., Barns, S., LaRocca, D., Du, Q., Zheng, D., … Middleton, F. A. (2019). The oral microbiome of early stage Parkinson's

disease and its relationship with functional measures of motor and non-motor function. *PLoS One*, *14*(6), e0218252. https://doi.org/10.1371/journal.pone.0218252 ⏎

Miklossy, J. (2016). Bacterial amyloid and DNA are important constituents of senile plaques: Further evidence of the spirochetal and biofilm nature of senile plaques. *Journal of Alzheimer's Disease*, *53*(4), 1459–1473. https://doi.org/10.3233/JAD-160451 ⏎

Miller, E. A., Beasley, D. E., Dunn, R. R., & Archie, E. A. (2016). Lactobacilli dominance and vaginal pH: Why are they important? *Trends in Microbiology*, *24*(11), 757–769. https://doi.org/10.1016/j.tim.2016.07.007 ⏎

Minalyan, A., Gabrielyan, L., Scott, D., Jacobs, J., & Pisegna, J. R. (2017). The gastric and intestinal microbiome: Role of proton pump inhibitors. *Current Gastroenterology Reports*, *19*(8), 42. https://doi.org/10.1007/s11894-017-0577-6 ⏎

Moreno, I., *et al*. (2016). Evidence that the endometrial microbiota has an effect on implantation success. *American Journal of Obstetrics and Gynecology*, *215*(6), 684–703. https://doi.org/10.1016/j.ajog.2016.09.075 ⏎

Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T., … & Blaut, M. (2006). Differences in fecal microbiota in different European study populations in relation to age, gender, and country: A cross-sectional study. *Applied and Environmental Microbiology*, *72*(2), 1027–1033. https://doi.org/10.1128/AEM.72.2.1027-1033.2006 ⏎

Mutlu, E., Keshavarzian, A., Engen, P., Forsyth, C. B., Sikaroodi, M., & Gillevet, P. (2009). Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcoholism: Clinical and Experimental Research*, *33*(10), 1836–1846. https://doi.org/10.1111/j.1530-0277.2009.01022.x ⏎

Naik, S., *et al*. (2012). Commensal–host immune interactions at the skin barrier. *Nature Immunology*, *13*(5), 475–483. https://doi.org/10.1038/ni.2260 ⏎

Nakatsuji, T., *et al*. (2017). Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus*. *Science Translational Medicine*, *9*(378), eaah4680. https://doi.org/10.1126/scitranslmed.aah4680 ⏎

Neuman, H., Debelius, J. W., Knight, R., & Koren, O. (2015). Microbial endocrinology: The interplay between the microbiota and the endocrine system. *FEMS Microbiology Reviews*, *39*(4), 509–521. https://doi.org/10.1093/femsre/fuu010 ⏎

Ngo, S. T., Steyn, F. J., & McCombe, P. A. (2014). Gender differences in autoimmune disease. *Frontiers in Neuroendocrinology*, *35*(3), 347–369. https://doi.org/10.1016/j.yfrne.2014.04.004 ⏎

Nociti Jr, F. H., Casati, M. Z., & Duarte, P. M. (2015). Current perspective of the impact of smoking on the progression and treatment of periodontitis. *Periodontology 2000*, *67*(1), 187–210. https://doi.org/10.1111/prd.12063 ⏎

Nogal, A., Valdes, A. M., & Menni, C. (2021). The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes*, *13*(1), 1897212. https://doi.org/10.1080/19490976.2021.1897212 ⏎

O'Neill, A. M., & Gallo, R. L. (2018). Host–microbiome interactions and recent progress into understanding the biology of acne. *Journal of Investigative Dermatology*, *138*(5), 1022–1027. https://doi.org/10.1016/j.jid.2017.10.017 ⏎

Olszak, T., An, D., Zeissig, S., Vera, M. P., Richter, J., Franke, A., … & Blumberg, R. S. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*, *336*(6080), 489–493. https://doi.org/10.1126/science.1219328 ⏎

Onderdonk, A. B., Delaney, M. L., & Fichorova, R. N. (2016). The human microbiome during bacterial vaginosis. *Clinical Microbiology Reviews*, *29*(2), 223–238. https://doi.org/10.1128/CMR.00075-15 ⏎

O'Shea, E. F., Cotter, P. D., Stanton, C., Ross, R. P., & Hill, C. (2012). Production of bioactive substances by intestinal bacteria as a basis for

explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. *International Journal of Food Microbiology*, *152*(3), 189–205. https://doi.org/10.1016/j.ijfoodmicro.2011.05.025 ⏎

Othman, F., Card, T. R., & Crooks, C. J. (2016). Proton pump inhibitor prescribing patterns in the UK: A primary care database study. *Pharmacoepidemiology and Drug Safety*, *25*(9), 1079–1087. https://doi.org/10.1002/pds.4043 ⏎

O'Toole, P. W., & Claesson, M. J. (2010). Gut microbiota: Changes throughout the lifespan from infancy to elderly. *International Dairy Journal*, *20*(4), 281–291. https://doi.org/10.1016/j.idairyj.2009.11.010 ⏎

Parracho, H., McCartney, A. L., & Gibson, G. R. (2007). Probiotics and prebiotics in infant nutrition. *Proceedings of the Nutrition Society*, *66*(3), 405–411. https://doi.org/10.1017/S0029665107005678 ⏎

Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., … & Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*, *118*(2), 511–521. https://doi.org/10.1542/peds.2005-2824 ⏎

Pereira-Marques, J., Ferreira, R. M., & Figueiredo, C. (2024). A metatranscriptomics strategy for efficient characterization of the microbiome in human tissues with low microbial biomass. *Gut Microbes*, *16*(1), 2323235. https://doi.org/10.1080/19490976.2024.2323235 ⏎

Petrovas, C., Yamamoto, T., Gerner, M. Y., Boswell, K. L., Wloka, K., Smith, E. C., … & Koup, R. A. (2012). CD4 T follicular helper cell dynamics during SIV infection. *Journal of Clinical Investigation*, *122*(9), 3281–3294. https://doi.org/10.1172/JCI63039 ⏎

Pistollato, F., Sumalla Cano, S., Elio, I., Masias Vergara, M., Giampieri, F., & Battino, M. (2016). Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. *Nutrition Reviews*, *74*(10), 624–634. https://doi.org/10.1093/nutrit/nuw023 ⏎

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … & Wang, J. (2010). A human gut microbial gene catalogue established by

metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821 ⏎

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., … & Wang, J. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, *490*(7418), 55–60. https://doi.org/10.1038/nature11450 ⏎

Ravel, J., Gajer, P., Abdo, Z., *et al*. (2011). Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences*, *108*(Suppl 1), 4680–4687. https://doi.org/10.1073/pnas.1002611107 ⏎

Reinhardt, C., Reigstad, C. S., & Backhed, F. (2009). Intestinal microbiota during infancy and its implications for obesity. *Journal of Pediatric Gastroenterology and Nutrition*, *48*(3), 249–256. https://doi.org/10.1097/MPG.0b013e318183187c ⏎

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., … & Meheust, A. (2010). Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition*, *104*(S2), S1–S63. https://doi.org/10.1017/S0007114510003363 ⏎

Sampson, T. R., & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell Host & Microbe*, *17*(5), 565–576. https://doi.org/10.1016/j.chom.2015.04.011 ⏎

Sanford, J. A., & Gallo, R. L. (2013). Functions of the skin microbiota in health and disease. *Seminars in Immunology*, *25*(5), 370–377. https://doi.org/10.1016/j.smim.2013.09.005 ⏎

Schloss, P. D., Westcott, S. L., & Ryabin, T. (2009). Introducing mothur: open-source, platform independent, community supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*, 7537–7541. https://doi.org/10.1128/AEM.01541-09 ⏎

Schnabl, B., & Brenner, D. A. (2014). Interactions between the intestinal microbiome and liver diseases. *Gastroenterology*, *146*, 1513–1524. https://doi.org/10.1053/j.gastro.2014.01.020 ⏎

Seekatz, A. M., Schnizlein, M. K., & Koenigsknecht, M. J. (2019). Spatial and temporal analysis of the stomach and small- intestinal microbiota in fasted healthy humans. *mSphere*, *4*, e00126–19. https://doi.org/10.1128/mSphere.00126-19 ⏎

Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*, 859–904. https://doi.org/10.1152/physrev.00045.2009 ⏎

Shen, J., Obin, M. S., & Zhao, L. (2013). The gut microbiota, obesity and insulin resistance. *Molecular Aspects of Medicine*, *34*(1), 39–58. https://doi.org/10.1016/j.mam.2012.11.001 ⏎

Shin, N. R., Lee, J. C., Lee, H. Y., Kim, M. S., Whon, T. W., Lee, M. S., & Bae, J. W. (2014). An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut*, *63*(5), 727–735. https://doi.org/10.1136/gutjnl-2012-303839 ⏎

Shulzhenko, N., Morgun, A., Hsiao, W., Battle, M., Yao, M., Gavrilova, O., … & Matzinger, P. (2011). Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nature Medicine*, *17*(12), 1585–1593. https://doi.org/10.1038/nm.2505 ⏎

Simon, L. (2007). The role of *Streptococcus mutans* and oral ecology in the formation of dental caries. *Lethbridge, Undergraduate Research Journal*. 2007. *2*(2). 1-6. https://hdl.handle.net/10133/1196 ⏎

Simon-Soro, A., Tomás, I., Cabrera-Rubio, R., Catalan, M. D., Nyvad, B., & Mira, A. (2013). Microbial geography of the oral cavity. *Journal of Dental Research*, *92*(7), 616–621. https://doi.org/10.1177/0022034513488119 ⏎

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., … & Gajewski, T. F. (2015). Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy. *Science*, *350*(6264), 1084–1089. https://doi.org/10.1126/science.aac4255 ⏎

Snyder, A., Pamer, E., & Wolchok, J. (2015). Could microbial therapy boost cancer immunotherapy? *Science*, *350*(6264), 1031–1032. https://doi.org/10.1126/science.aad7706 ⏎

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), 227–238. https://doi.org/10.1038/nrmicro2974 ⏎

Sonnenburg, E. D., & Sonnenburg, J. L. (2014). Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metabolism*, *20*(5), 779–786. http://dx.doi.org/10.1016/j.cmet.2014.07.003 ⏎

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., … & Gordon, J. I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, *307*(5717), 1955–1959. https://doi.org/10.1126/science.1109051 ⏎

Spielman, L. J., Gibson, D. L., & Klegeris, A. (2018). Unhealthy gut, unhealthy brain: The role of the intestinal microbiota in neurodegenerative diseases. *Neurochemistry International*, *120*, 149–163. https://doi.org/10.1016/j.neuint.2018.08.005 ⏎

Spinler, J. K., Taweechotipatr, M., Rognerud, C. L., Ou, C. N., Tumwasorn, S., & Versalovic, J. (2008). Human-derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. *Anaerobe*, *14*(3), 166–171. https://doi.org/10.1016/j.anaerobe.2008.02.001 ⏎

Stapleton, A. E., *et al*. (2011). Randomized controlled trial of *Lactobacillus crispatus* for prevention of recurrent UTI. *Clinical Infectious Diseases*, *52*(10), 1212–1217. https://doi.org/10.1093/cid/cir183 ⏎

Sun, L., Xie, C., Wang, G., Wu, Y., Wu, Q., Wang, X., … & Jiang, C. (2018). Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nature Medicine*, *24*(12), 1919–1929. https://doi.org/10.1038/s41591-018-0222-4 ⏎

Tachedjian, G., O'Hanlon, D. E., & Ravel, J. (2017). The role of lactic acid in vaginal health and disease. *Nature Reviews Microbiology*, *15*(10),

647–660. https://doi.org/10.1038/nrmicro.2017.55 ⏎

Takahashi, N., & Nyvad, B. (2011). The role of bacteria in the caries process: Ecological perspectives. *Journal of Dental Research*, *90*(3), 294–303. https://doi.org/10.1177/0022034510379602 ⏎

Tan, A., Murugapiran, S., Mikalauskas, A., Koble, J., Kennedy, D., Hyde, F., … Gohl, D. M. (2023). Rational probe design for efficient rRNA depletion and improved metatranscriptomic analysis of human microbiomes. *BMC Microbiology*, *23*, 299. https://doi.org/10.1186/s12866-023-03037-y ⏎

Tett, A., Pasolli, E., Farina, S. et al. (2017). Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. *npj Biofilms Microbiomes 3*(14). https://doi.org/10.1038/s41522-017-0022-5 ⏎

Thaiss, C. A., Zmora, N., Levy, M., & Elinav, E. (2016). The microbiome and innate immunity. *Nature*, *535*(7610), 65–74. https://doi.org/10.1038/nature18847 ⏎

Thomas, C. M., & Versalovic, J. (2010). Probiotics-host communication: modulation of signaling pathways in the intestine. *Gut Microbes*, *1*(3), 148–163. https://doi.org/10.4161/gmic.1.3.11712 ⏎

Thompson, P. D., Panza, G., Zaleski, A., & Taylor, B. (2016). Statin-associated side effects. *Journal of the American College of Cardiology*, *67*(20), 2395–2410. https://doi.org/10.1016/j.jacc.2016.02.071 ⏎

Tilg, H., Cani, P. D., & Mayer, E. A. (2016). Gut microbiome and liver diseases. *Gut*, *65*, 2035–2044. https://doi.org/10.1136/gutjnl-2016-312729 ⏎

Tilg, H., Adolph, T. E., Gerner, R. R., & Moschen, A. R. (2018). The intestinal microbiota in colorectal cancer. *Cancer Cell*, *33*(6), 954–964. https://doi.org/10.1016/j.ccell.2018.03.004 ⏎

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., … & Gribble, F. M. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein–coupled

receptor FFAR2. *Diabetes*, *61*(2), 364–371. https://doi.org/10.2337/db11-1019 ⏎

Tomar, S. L., Hecht, S. S., Jaspers, I., Gregory, R. L., & Stepanov, I. (2019). Oral health effects of combusted and smokeless tobacco products. *Advances in Dental Research*, *30*(1), 4–10. https://doi.org/10.1177/0022034519872480 ⏎

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81(3), 1031*–1064. https://doi.org/10.1152/physrev.2001.81.3.1031 ⏎

Trebicka, J., Macnaughtan, J., Schnabl, B., Shawcross, D. L., & Bajaj, J. S. (2021). The microbiota in cirrhosis and its role in hepatic decompensation. *Journal of Hepatology*, *75*(Suppl. 1), S67–S81. https://doi.org/10.1016/j.jhep.2020.11.013 ⏎

Tsai, K. K., & Daud, A. I. (2015). Nivolumab plus ipilimumab in the treatment of advanced melanoma. *Journal of Hematology & Oncology*, *8*(1), 123. https://doi.org/10.1186/s13045-015-0219-0 ⏎

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host & Microbe*, *3*(4), 213–223. https://doi.org/10.1016/j.chom.2008.02.015 ⏎

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., … & Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540 ⏎

Vetizou, M., Pitt, J. M., Daillere, R., Lepage, P., Waldschmitt, N., Flament, C., … & Zitvogel, L. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, *350*(6264), 1079–1084. https://doi.org/10.1126/science.aad1329 ⏎

Vetrovsky, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community

analyses. *PLoS One*, *8*, e57923. https://doi.org/10.1371/journal.pone.0057923 ⏎

Vich Vila, A., Collij, V., Sanna, S., Sinha, T., Imhann, F., Bourgonje, A. R., … & Weersma, R. K. (2020). Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nature Communications*, *11*(1), 362. https://doi.org/10.1038/s41467-019-14177-z ⏎

Vijay-Kumar, M., & Gewirtz, A. T. (2009). Flagellin: Key target of mucosal innate immunity. *Mucosal Immunology*, *2*(3), 197–205. https://doi.org/10.1038/mi.2009.9 ⏎

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., … & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*, *7*(1), 13537. https://doi.org/10.1038/s41598-017-13601-y ⏎

Wall, R., Ross, R. P., Ryan, C. A., Hussey, S., Murphy, B., Fitzgerald, G. F., & Stanton, C. (2009). Role of gut microbiota in early infant development. *Clinical Medicine Pediatrics*, *3*, CMPed-S2008. https://doi.org/10.4137/CMPed.S2008 ⏎

Weaver, C. T., Elson, C. O., Fouser, L. A., & Kolls, J. K. (2013). The Th17 pathway and inflammatory diseases of the intestines, lungs, and skin. *Annual Review of Pathology: Mechanisms of Disease*, *8*(1), 477–512. https://doi.org/10.1146/annurev-pathol-011110-130318 ⏎

Weersma, R. K., Zhernakova, A., & Fu, J. (2020). Interaction between drugs and the gut microbiome. *Gut*, *69*(8), 1510–1519. https://doi.org/10.1136/gutjnl-2019-320204 ⏎

Wherry, E. J., & Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nature Reviews Immunology*, *15*(8), 486–499. https://doi.org/10.1038/nri3862 ⏎

Willis, J. R., & Gabaldon, T. (2020). The human oral microbiome in health and disease: From sequences to ecosystems. *Microorganisms*, *8*(2), 308. https://doi.org/10.3390/microorganisms8020308 ⏎

Witkin, S. S., Linhares, I. M., & Giraldo, P. (2013). Bacterial interactions with the female genital tract. *American Journal of Obstetrics and Gynecology*, *208*(4), 288–294. https://doi.org/10.1016/j.ajog.2012.12.023 ⏎

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., … & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*(6052), 105–108. https://doi.org/10.1126/science.1208344 ⏎

Wu, J., Peters, B. A., Dominianni, C., Zhang, Y., Pei, Z., Yang, L., … Ahn, J. (2016). Cigarette smoking and the oral microbiome in a large study of American adults. *ISME Journal*, *10*(10), 2435–2446. https://doi.org/10.1038/ismej.2016.37 ⏎

Yang, Y., Torchinsky, M. B., Gobert, M., Xiong, H., Xu, M., Linehan, J. L., … & Littman, D. R. (2014). Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. *Nature*, *510*(7503), 152–156. https://doi.org/10.1038/nature13279 ⏎

Zaura, E., Nicu, E. A., Krom, B. P., & Keijser, B. J. (2014). Acquiring and maintaining a normal oral microbiome: Current perspective. *Frontiers in Cellular and Infection Microbiology*, *4*, 85. https://doi.org/10.3389/fcimb.2014.00085 ⏎

Zhang, B., Chassaing, B., Shi, Z., Uchiyama, R., Zhang, Z., Denning, T. L., … & Gewirtz, A. T. (2014). Prevention and cure of rotavirus infection via TLR5/NLRC4–mediated production of IL-22 and IL-18. *Science*, *346*(6211), 861–865. https://doi.org/10.1126/science.1256999 ⏎

Zhang, Y., Wang, X., Li, H., & Ni, C. (2019). The oral microbiome and human cancer. *Journal of Oral Microbiology*, *11*(1), 1654784. https://doi.org/10.1080/20002297.2019.1654784

Zhao, L. H., Liu, X., Yan, H. X., Li, W. Y., Zeng, X., Yang, Y., … & Wang, H. Y. (2016). Genomic and oncogenic preference of HBV integration in hepatocellular carcinoma. *Nature Communications*, *7*(1), 12992. https://doi.org/10.1038/ncomms12992 ⏎

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T., … & Fu, J. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, *352*(6285), 565–569. https://doi.org/10.1126/science.aad3369 ⏎

Zitvogel, L., Daillère, R., Roberti, M. P., Routy, B., & Kroemer, G. (2017). Anticancer effects of the microbiome and its products. *Nature Reviews Microbiology*, *15*(8), 465–478. https://doi.org/10.1038/nrmicro.2017.44 ⏎

Section II

The Microbiome of the Digestive System

3

Model Systems for Studying the Human Gut Microbiome

Jyoti Batgire, Niteeka Chandel, Shreya Peddakolmi, and Vikrant M. Bhor

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3.1 Introduction

The human gut microbiome represents one of the most intricate biological partnerships, operating at the intersection of ecology, physiology, and evolution. Rather than functioning as a passive assemblage of microorganisms, the gut microbiome constitutes a dynamic and adaptive system that continuously exchanges metabolic, immunological, and molecular signals with its host. These interactions influence fundamental aspects of human biology, including nutrient processing, immune development, neuroendocrine communication, and systemic metabolism. Understanding the gut microbiome, therefore, requires approaches that extend beyond cataloguing microbial taxa to encompass functional capacity, ecological interactions, and consequences for host health and disease. Because of this complexity, no single experimental or analytical framework can fully capture the behavior of the gut microbiome in its native context. Instead, insight has emerged from the combined use of complementary model systems, including *in vitro*, *in vivo*, and computational approaches, each of which provides access to distinct but interdependent dimensions of host–microbiome biology. Together, these approaches enable researchers to move between controlled mechanistic interrogation, physiological relevance, and system-level analysis.

In vitro models offer highly controlled experimental environments in which specific variables can be isolated and manipulated with precision. By enabling defined microbial communities, substrates, and selected host components to be studied in isolation, these systems allow detailed examination of metabolic pathways, microbial interactions, and host–microbe signaling mechanisms. Such reductionist approaches are particularly valuable for hypothesis generation, mechanistic dissection, and systematic screening. However, their explanatory power is achieved through deliberate simplification, and they cannot fully recapitulate the spatial organization, immune complexity, or environmental variability of the living host.

In vivo studies, in contrast, preserve the integrated physiological and environmental context in which the gut microbiome naturally operates. Animal models and human cohort studies capture emergent properties of host–microbiome interactions that arise only within intact biological systems, including immune maturation, neuroendocrine signaling, and whole-body metabolic regulation. These models are therefore essential for assessing biological relevance and testing causal relationships. Their limitations lie in reduced experimental control and mechanistic resolution, which can complicate the interpretation of complex, multivariate outcomes.

Computational approaches provide a complementary perspective by integrating large-scale sequencing, metabolomic, and clinical datasets into predictive and inferential models. These methods enable pattern discovery, functional inference, and hypothesis generation across spatial and temporal scales that are inaccessible to experimental systems alone. Computational analyses can reveal latent structure within microbial communities and generate testable predictions about host–microbiome interactions, but their conclusions remain provisional unless grounded in empirical validation.

When employed in isolation, each of these approaches offers only a partial view of gut microbiome biology. When integrated, however, they form a coherent and iterative investigative framework. Mechanistic insights from *in vitro* systems inform *in vivo* experimentation, observations from living hosts refine computational models, and computational predictions guide targeted experimental design. This integrative strategy is essential for translating descriptive observations into a mechanistic understanding. More broadly, the gut microbiome challenges traditional distinctions between the host and its environment by illustrating how host genetics, diet, lifestyle, and microbial ecology jointly shape biological outcomes. Health emerges from a dynamic equilibrium within this system, whereas disease more often reflects disruptions in community structure or function rather than the presence or absence of individual microbial species. Studying the gut microbiome, therefore, demands not only methodological diversity but also conceptual integration, recognizing that biological meaning arises from interactions

across scales rather than from isolated components. This chapter presents *in vitro*, *in vivo*, and computational approaches as interconnected tools within a unified framework for understanding how humans and their microbial partners coexist, adapt, and, under certain conditions, function out of balance.

3.2 *In Vitro* Experimental Systems for Studying the Human Gut Microbiome

In vivo studies, including human trials and animal models, remain essential for applied insight, whereas *in vitro* systems provide controlled and mechanistically possible environments to dissect community behavior, host responses, and substrate–microbe interactions. These *in vitro* gut models vary widely in complexity from simple anaerobic batch cultures to advanced multi-level bioreactors and microfluidic "gut-on-chip" devices. Their design showcases relationships between physiological environment, experimental control, cost, and throughput. Depending on the intended use, these can be categorized as models for rapid screening of substrates and drugs, long-term and region-specific gut ecosystem simulation, host–microbe interaction assessment, and specialized co-culture and immune-integrated systems.

3.3 Rapid *In Vitro* Screening Models for Gut Microbiome–Substrate and Drug Interactions

Rapid *in vitro* screening platforms are often used in the early stages of microbiome research. Their main purpose is to evaluate how dietary substrates, prebiotics, pharmaceuticals, xenobiotics, or natural compounds interact with the gut microbiota before moving to complex host systems (Figure 3.1; Table 3.1).

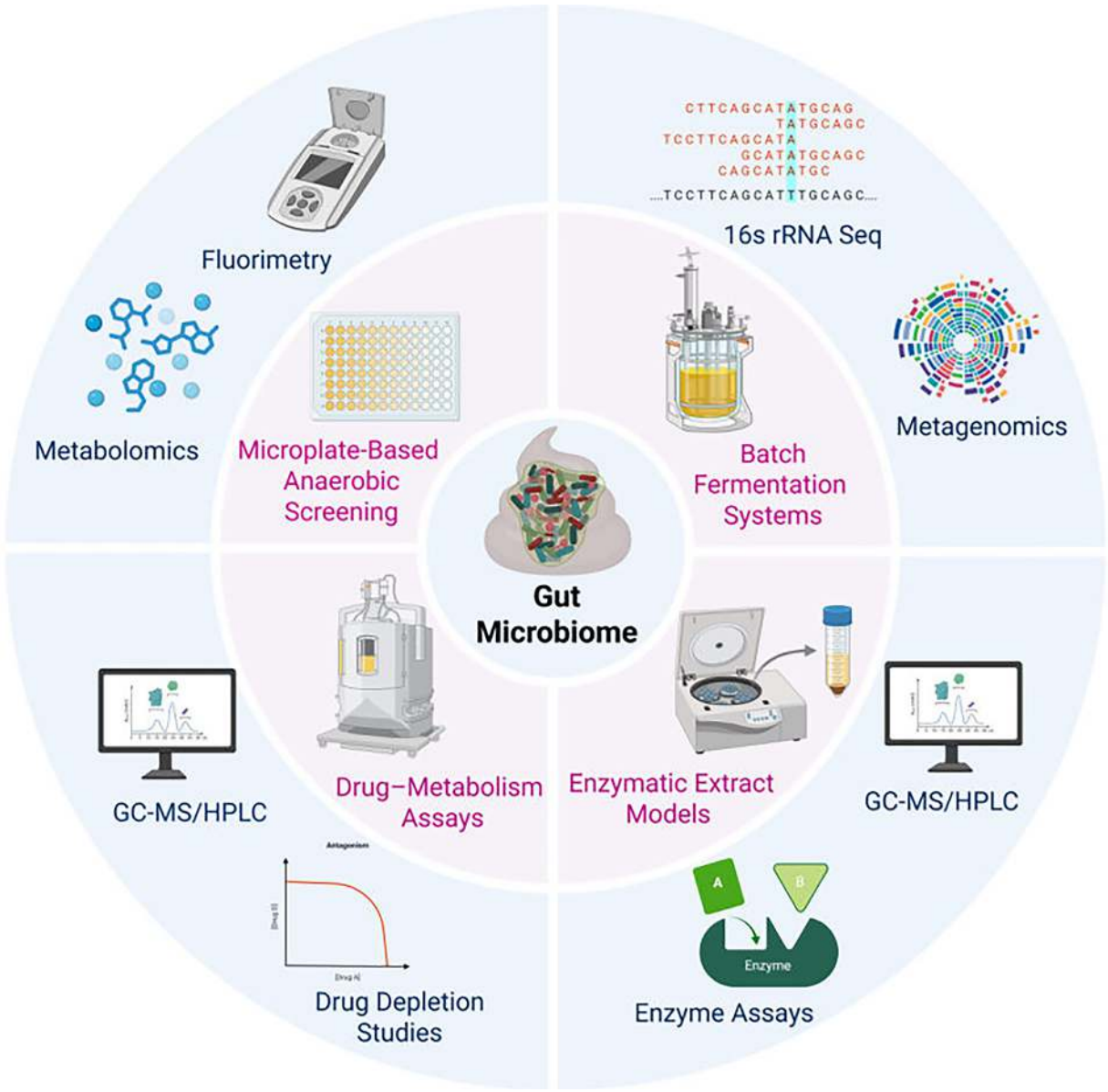

FIGURE 3.1 Rapid *in vitro* screening models for gut microbiome–substrate and drug. High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS). ⏎

3.3.1 Batch Fecal Fermentation Systems

In this approach, freshly collected human stool is homogenized under anaerobic conditions to preserve obligate anaerobes, followed by inoculation into growth media supplemented with the test substrate (Macfarlane & Gibson, 1991). This fermentation occurs in sealed reactors or serum bottles, which maintain strict anaerobiosis. After inoculation, these cultures are incubated for 24–72 hours, during which microbial

metabolism and compositional changes take place. The environment becomes progressively acidic and nutrient-depleted due to a lack of waste removal, similar to early colonic fermentation. It begins with ethical donor recruitment and stool collection into anaerobic transport jars. Samples are transferred to an anaerobic chamber and diluted in phosphate-buffered or carbonate-buffered media that are enriched with yeast extract, bile salts, peptone, and hemin that support anaerobic growth. After thorough homogenization and filtration, this inoculum is then added to pre-equilibrated basal medium. Test substrates are introduced at physiologically similar concentrations. Incubation occurs in temperature-controlled shaking incubators (37°C) that ensure uniform distribution of nutrients. Sampling is carried out at different time points to capture dynamic changes. Analytical endpoints include microbial composition (16S rRNA gene sequencing and metagenomics), metabolite profiling (short-chain fatty acids (SCFAs) via gas chromatography, lactate, and ammonia), gas production, and pH decline. This platform is suitable for rapid hypothesis screening, prebiotic profiling, and ranking dietary compounds based on their fermentability. However, due to its closed-system nature, metabolic waste accumulation and pH drop can inhibit slow-growing organisms and rare taxa, resulting in community drift. Thus, even though batch fermentations provide important early insight, findings should typically correlate with continuous fermentation systems or *in vivo* models.

3.3.2 Microplate-Based Anaerobic Screening

Principles of batch fermentations can be adapted into a microscale variant through fecal or microbiological cultures using microplates, in which several hundred test conditions can be simultaneously tested (Falony et al., 2016). In pharmaceutical research on the microbiome interactions of drugs, testing the metabolism of gut bacteria or the subsequent toxicity of drugs toward bacteria may require exploring several chemical libraries in this manner (Zimmermann et al., 2019). Anaerobic chambers containing gas-producing packets are used as containers for microplates. Fecal material or specific species of gut bacteria are added per well after homogenization in growth medium, and specific chemicals are also introduced. End-point metabolomic analyses, fluorometric assays of enzymatic or metabolic activity, and optical density-based measurements of growth kinetics are commonly used analytical readouts, as growth in microtiter plates may be influenced by depletion of medium components and by oxygen diffusion into the wells (Macfarlane & Gibson, 1991).

3.3.3 Drug Metabolism Assays Using Gut Microbiota

Drug-microbiome metabolism tests aim to determine whether a pharmaceutical or xenobiotic is metabolized by bacteria in the gastrointestinal tract to form active, inactive, or toxic metabolites (Sousa et al., 2008). Several prescription drugs undergo first-pass microbial metabolism in the colon and liver due to microbial reductases and hydrolases. Fecal slurry culture or specific strains identified to biocatalytically metabolize a drug (Eggerthella lenta metabolizing digoxin) (Haiser et al., 2013) are often used as biological samples. Anaerobic bioreactor flasks or serum bottles containing a physiological concentration of a drug are spiked with an inoculum. To stimulate bioreactor microbial utilization of a pharmaceutical as a substrate, the culture media are often low in nutrients or carbohydrates. To prevent settling, bioreactors are sealed and flushed with a defined mixture of anaerobic gases ($N_2/CO_2/H_2$). Incubation is done under gentle agitation and a temperature of 37°C. Sampling is done across time points, and aliquots are immediately placed on ice to halt metabolism. Analytical workflows rely heavily on liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) or high-performance liquid chromatography (HPLC) to quantify parent drug and degradative metabolites. Pairing metabolomics with microbial profiling (16S rRNA/metagenomics) reveals microbial taxa linked with specific conversion pathways. The generation of toxic or off-target metabolites, individual differences in drug response based on differences in microbiome, and loss of drug effect due to microbial activity can be easily modelled using this approach. However, to translate metabolism results to systemic pharmacokinetics, further analysis in more complex models would be required, as raw feces do not have host variables such as intestinal enzymes and mucosa absorption.

3.3.4 Fecal Enzymatic Extract Models

By focusing on the enzyme pool, fecal enzyme extracts provide an *in vitro*, cell-free technique for studying the metabolism of gut microbiota. The techniques involve assays, which examine isolated biochemical reactions for gut microbiota towards specific substrates, including drugs, food substances, bile acids, and environmental chemicals. The anaerobic fecal samples are prepared by dilution in phosphate buffers with added reducing agents. Homogenization follows. Differential centrifugation techniques are employed on the homogenized mixture for extracting the microbial cells. The enzyme-enriched supernatant can be filtered through a 0.22 µm filter if sterility is desired. To ensure equal concentration from different subjects, assays are measured for protein content (e.g., by using the Bradford technique). The sterile microbial enzyme supernatant is incubated with specific substances in anaerobic microtubes at 37°C for 1–24 hours. This model shows its inherent enzyme functionality as it does not rely upon

growth conditions, primarily for β-glucuronidase (responsible for reversing many drugs that undergo liver conjugation) (Wallace et al., 2010), azoreductases, nitroreductases, and sulfatases. HPLC, LC-MS, or specialized fluorometric enzyme activity assays are employed for analysis. Enzyme-specific inhibitor dose–response, inter-dataset variability regarding enzyme-specific risk, and rapid mechanistic analysis of detoxification processes could all be enabled for this cell-free system. Microbe-cross talk, reduced metabolism as a result of the presence of only soluble enzymes, and the lack of dynamic feedback regulation represent drawbacks for its use. The strongest use case for this model is as a mechanistic adjunct for culture-based microbial analysis.

TABLE 3.1
Comparison of Rapid *In Vitro* Screening Models Used to Study Gut Microbiome–Substrate and Drug Interactions

Aspect	Batch Fecal Fermentation	Microplate Anaerobic Screening	Drug–Metabolism Assay	Fecal Enzymatic Extract
Main purpose	Study microbial response to dietary substrates.	High-throughput testing of multiple substrates or drugs.	Examine microbial biotransformation of pharmaceuticals/xenobiotics.	Assess enzyme-specific reactions in cell-free fecal extracts.
Focus	Microbiome composition and fermentation output.	Rapid screening of growth or activity patterns.	Fate of compound and metabolite formation.	Direct enzymatic activity without microbial growth.
Setup	Anaerobic batch reactors with fecal inoculum (24–72 h).	Anaerobic multi-well plates (micro-scale).	Anaerobic fecal slurry or isolates with drug substrate.	Sterile enzyme-rich supernatant incubated with substrate.
Endpoints	16S profiling, SCFAs, gas, pH.	OD/fluorescence, metabolic readouts.	LC-MS metabolite mapping, drug depletion.	Enzyme activity, product quantification.
Throughput	Moderate	Very high	Low–moderate	Moderate
Advantages	Simple, physiologically relevant.	Fast, cost-efficient, parallelizable.	Mechanistic insight into drug metabolism.	Rapid, specific, mechanistic clarity.

Aspect	Batch Fecal Fermentation	Microplate Anaerobic Screening	Drug–Metabolism Assay	Fecal Enzymatic Extract
Limitations	Closed system, pH drift.	Limited realism, oxygen sensitivity.	Lacks host absorption and systemic context.	No growth regulation, enzyme instability.

3.4 Long-Term and Region-Specific Gut Ecosystem Simulation

Substrates, dietary compounds, or drugs of interest showing promising microbial interaction in short-term batch fermentations will be further studied using continuous or semi-continuous *in vitro* models. Compared to the batch models, these systems more accurately simulate the long-term stability, nutrient flow, and geographic variability of the human colon. They can model the microbial ecology steady state *in vivo* by continuously adding fresh media and removing the spent material to examine long-term effects, resilience, and kinetics of metabolites (Table 3.2).

3.4.1 Chemostat-Based Continuous Fermentation Systems

The basic continuous culture model used to support microbial growth in steady-state conditions is the chemostat (Herbert et al., 1956). It consists of an agitated bioreactor. The bioreactor maintains a steady volume and dilution rate through the simultaneous removal of an equal volume of culture and the addition of a steady infusion of fresh nutritional media. The chemostats have been modified to support anaerobic conditions. They are equipped with the use of pH and redox electrodes, temperatures of 37°C, magnetically stirred reactors, and continuous gas sparging with N_2 gas or CO_2. The basal medium, consisting of proteins, carbohydrates, bile salts, vitamins, and trace metals, is a simulant of the intestinal chyme. The reactor is allowed three to five retention times of the test substance prior to inoculation with the addition of the test substance. The retention time, typically between 20 and 40 hours, representing the time it takes for the substance to pass through the human intestine, is determined through the flow rate (Macfarlane & Macfarlane, 2007). These models enable sampling for drug degradation, metabolites, and microbe populations over extended periods. Repeatability is a notable attribute associated with the chemostat, which provides quantitative information regarding drug metabolism parameters, as all factors, such as pH, flow, and dilution rate, can be controlled. However, chemostats do not have the gradients found in

the colon, which vary from the proximal part to the distal part, as a chemostat represents a single homogeneous compartment. It must also be operated aseptically and anaerobically, which may impose selective pressure on communities, leading to reduced diversity (Figure 3.2).

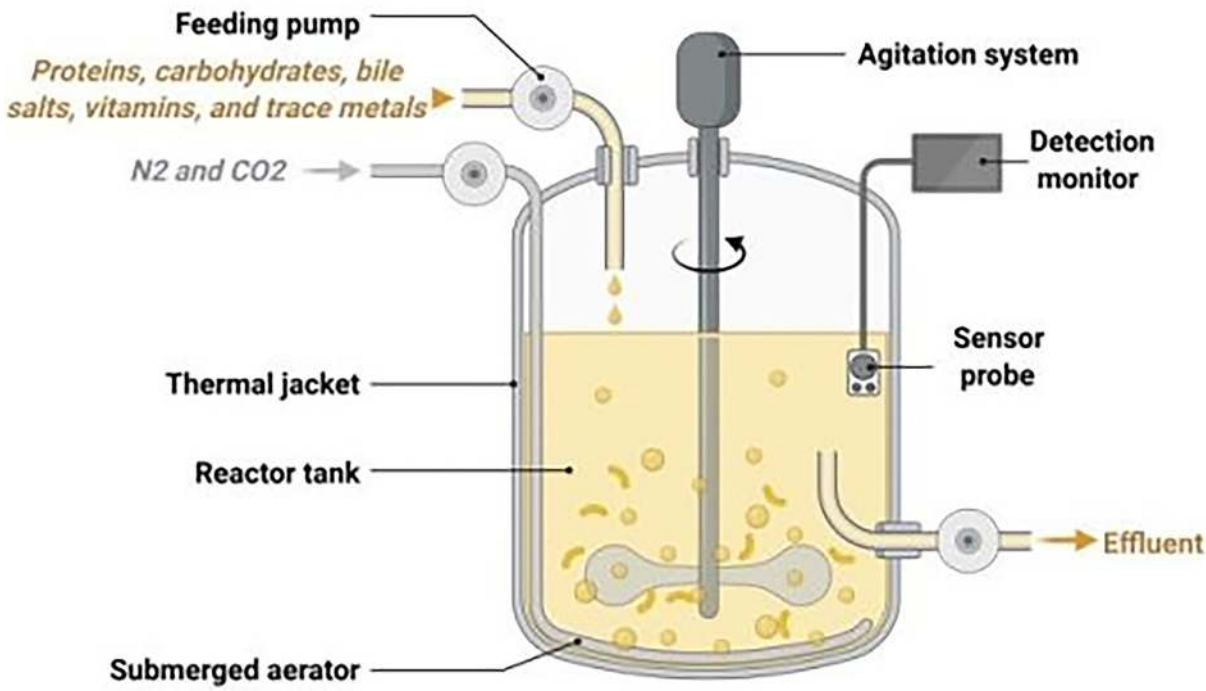

FIGURE 3.2 Chemostat-based continuous fermentation systems. ⏎

3.4.2 SHIME – Simulator of the Human Intestinal Microbial Ecosystem

The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) model, developed at Ghent University, Belgium, simulates the sequential compartments of the human gastrointestinal tract by extending the chemostat concept into a series of interconnected reactors (Molly et al., 1993). The system enables investigation of microbial colonization and metabolic activity under region-specific physicochemical gradients. The SHIME model typically consists of five or more interconnected reactors representing the stomach, small intestine, and the three regions of the human colon: the ascending, transverse, and descending colon. Digested material is sequentially transferred between compartments using peristaltic pumps operating at defined flow rates. Anaerobic conditions are maintained by continuous flushing with anaerobic gas, while pH and temperature are independently controlled in each compartment. Once a stable microbial community is established following an initial colonization period of approximately 2–3 weeks using a fecal inoculum, test compounds (e.g., probiotics,

pharmaceuticals, or nanoparticles) can be introduced into specific compartments. Administration via the gastric compartment is commonly used to simulate oral intake, whereas direct addition to colonic compartments may be applied when targeting distal intestinal regions (Van den Abbeele et al., 2010). In addition, the SHIME model can be coupled with simulated mucus layers or cultured epithelial cell systems to investigate microbial adhesion and metabolite absorption (Figure 3.3).

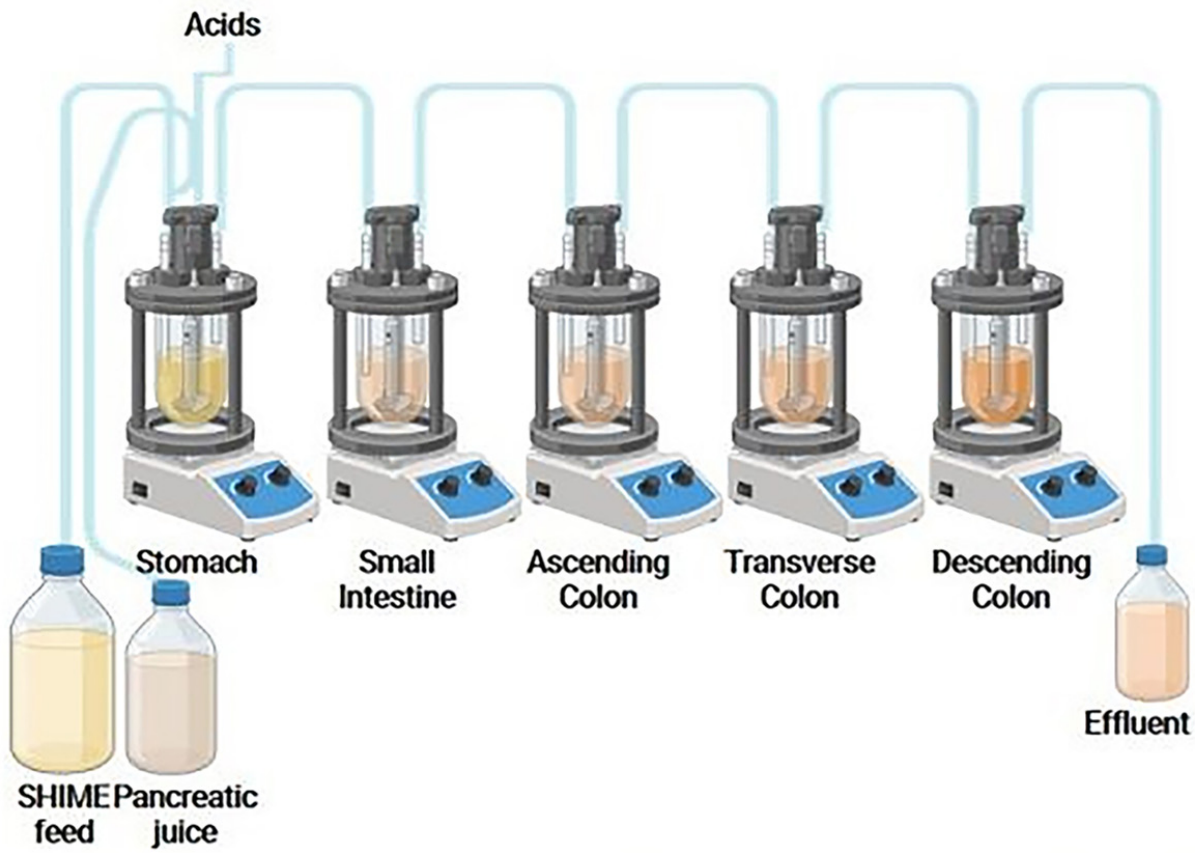

FIGURE 3.3 SHIME – Simulator of the Human Intestinal Microbial Ecosystem. ⏎

3.4.3 TIM-2 – TNO Intestinal Model (Colon Compartment)

The TIM-2 system, developed by the Netherlands Organisation for Applied Scientific Research, or TNO (Minekus et al., 1995), is a highly dynamic, computer-controlled model of the human large intestine. Unlike conventional chemostats, the pH control, continuous dialysis, and peristaltic mingling in TIM-2 mimic the physiologic conditions of the proximal colon. Via the semi-permeable dialysis membrane that removes microbial by-products (such as SCFAs) at rates approaching *in vivo* values, TIM-2 sustains the luminal environment in a constant manner, excluding the risk of acid

inhibition and S-equilibrium. Furthermore, peristaltic contractions of the flexible wall sectors driven by pneumatic pressure successfully mimic the mixing process of the contents in TIM-2.

The colon compartment, which is filled with the complex nutritional medium, is inoculated with fecal matter from healthy donors. The test chemicals are administered after stabilization, and microbial response or degradation of the compound is monitored for a period of 2–4 weeks. Sampling ports allow for the withdrawal of the dialysate and luminal fractions for compositional and metabolic analysis. TIM-2 is often applied for assessing microbial stability in controlled conditions, prebiotic efficacy, and the release of pharmaceuticals and their metabolism (Van Nuenen et al., 2003). It bridges the gap between static *in vitro* and *in vivo* systems by sustaining microbial balance in the human colon. However, TIM-2 is costly, technically complex to operate, and characterized by low throughput. Consequently, its use is largely restricted to advanced research facilities, as operation of the specialized equipment requires substantial technical training.

TABLE 3.2
Characteristics of Long-Term and Region-Specific *In Vitro* Gut Ecosystem Simulation Models

Feature	Chemostat	SHIME	TIM-2
Complexity	Low–moderate	High	Very high
Colon region simulation	Single, uniform environment	Multi-regional (ascending, transverse, descending)	Proximal colon with absorption/dialysis
Steady-state duration	Weeks	Months	Weeks
Flow and control	Continuous inflow/outflow	Sequential peristaltic transfer	Computer-controlled peristaltic and dialysis system
Anaerobiosis maintenance	Gas flushing	Gas flushing per compartment	Dynamic control
Analytical focus	Kinetic studies, steady-state fermentation	Regional metabolism, long-term adaptation	Dynamic absorption, realistic microbial stability
Advantages	Simple, reproducible	Physiologically comprehensive	Closest mimic of human colonic physiology
Limitations	No regional specificity	Complex, costly	Very complex, low throughput

3.5 Host–Microbe Interaction Models

Although batch and continuous fermentation models replicate key aspects of microbial community organization and metabolism, they lack host-related features such as mucus secretion, immune signaling, epithelial barrier function, and nutrient absorption. To address these limitations, host–microbe interaction models have been developed that enable controlled exposure of human gut microbiota, or their metabolites, to human intestinal epithelial cells or suitable surrogate systems. These models are particularly valuable for investigating microbial contributions to inflammation, drug and metabolite uptake or biotransformation, interactions at the mucus interface, and host cellular responses to microbial products, including SCFAs and lipopolysaccharides (LPSs) (Table 3.3).

3.5.1 Transwell-Based Co-Culture Systems

Speaking of *in vitro* methods, the oldest and easiest model system available for the analysis of host–microbiota interactions is the transwell system (Artursson & Karlsson, 1991). This involves the use of polarized epithelial cell lines of enterocytic origin (e.g., Caco2, HT29, T84) plated on a semipermeable membrane insert separating the basolateral (serosal) and apical (luminal) compartments. With this model system, the separated compartments for microorganisms and host cells can be maintained while allowing the transit of drugs, paracrine factors, or microbial products. In order to establish the tight junctions, cells can be seeded on transwell membranes coated with collagen or fibronectin and allowed to differentiate for 2–3 weeks. Transepithelial electric resistance (TEER) analysis can validate the integrity of the cell layers (Srinivasan et al., 2015). Cell proximity experiment, using bacterial products or conditioned medium, or microbial components (LPS, SCFAs, and others), represents some of the approaches by which the microbiota encounter can be achieved. Through the simulation of the systemic circulation, the basolateral medium enables the measurement of drug permeability, cytokine secretion such as TNF-α and IL-8, and ingested compounds. The above models can be applied to the integrity of the host barrier challenged with microorganisms, immune response of the epithelia to probiotics/microorganisms, studies of the signaling of SCFAs/LPS, and drug permeability and absorption studies (Caco-2 permeability studies) (Figure 3.4).

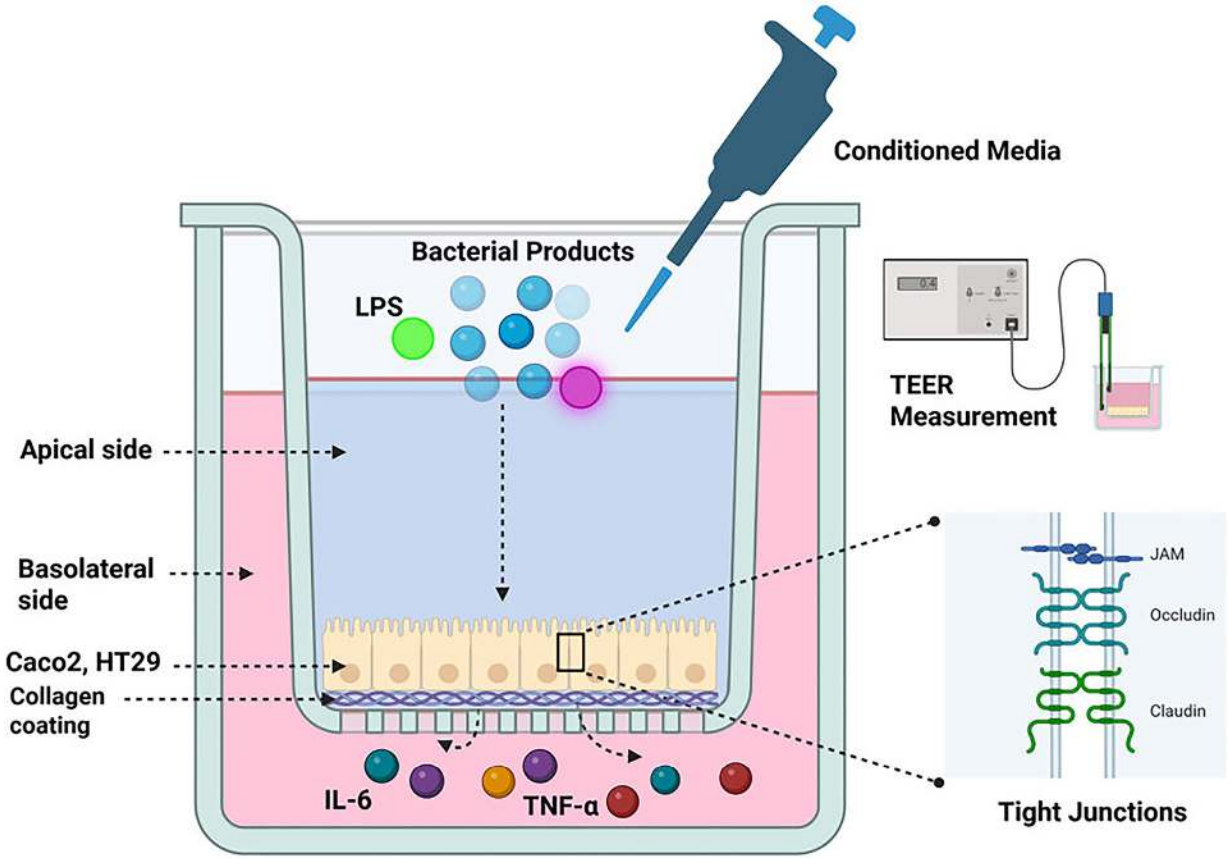

FIGURE 3.4 Transwell-based co-culture system. ⏎

3.5.2 Intestinal Organoids and Enteroids

Organoids, also referred to as intestinal enteroids or colonoids, are three-dimensional (3D) epithelial tissue models derived either from induced pluripotent stem cells (iPSCs) or from adult intestinal stem cells under defined *in vitro* conditions (Sato et al., 2009). These structures self-organize into crypt–villus–like architectures that recapitulate key physiological functions of the intestine, including mucus secretion and differentiation into multiple epithelial cell types, such as enterocytes, goblet cells, and Paneth cells. Organoids represent a valuable platform for modeling the host–microbiota communication interface in a physiologically relevant, tissue-like context. For their growth, a defined growth factor–supplemented medium containing Wnt3a, R-spondin, epidermal growth factor (EGF), and Noggin is typically used in combination with extracellular matrices such as Matrigel or collagen domes (Clevers, 2016). Several experimental approaches are employed to study host–microbe interactions using organoids, including microinjection of live microbes or bacterial components into the lumen; exposure of 2D organoid-derived monolayers to microbial metabolites or

anaerobic bacteria; and treatment with conditioned media to assess host gene expression and metabolic responses. Organoids are applied in modeling inherited enteropathies, investigating drug–host–microbe interactions, evaluating host responses to microbiota-derived metabolites, and studying epithelial reactions to commensal or pathogenic microorganisms. In addition, patient-derived organoids enable applications in personalized medicine. Organoids provide an almost physiological epithelial model while preserving cellular diversity and self-renewal capacity. However, maintaining strict anaerobic conditions remains challenging, and the closed-lumen architecture limits direct bacterial infection (Figure 3.5).

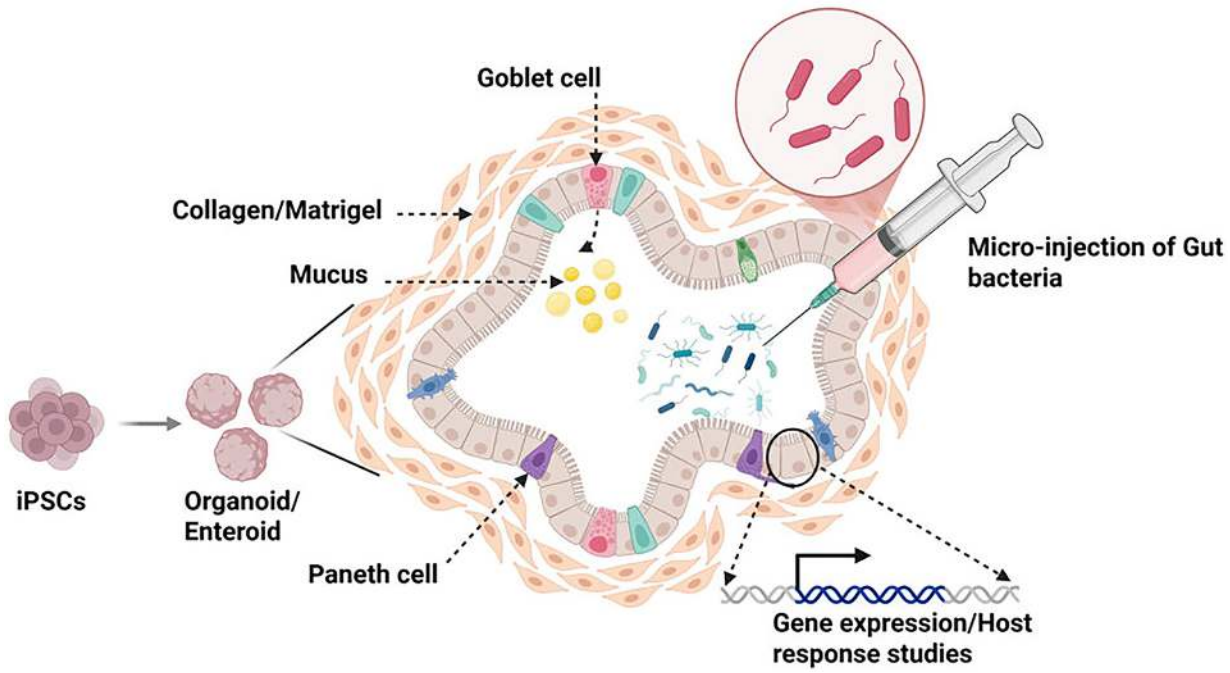

FIGURE 3.5 Intestinal organoid. ⏎

3.5.3 Gut-on-a-Chip Microfluidic Models

The Gut-on-a-Chip technology faithfully recreates the dynamic hemodynamics and biochemistry of the gut through the integration of microfluidics, biomaterials, and biomimetic materials in tissue engineering applications (Kim & Ingber, 2013). These micro-chips, which were first developed at the Wyss Institute at Harvard University, consist of the lower channels perfused with microbial or vascular cells, micro-channels lined with the intestinal epithelial layer, and the porous separation layer. The simulation of luminal shear stress, peristalsis, cyclic stretching, and continuous flow generates the required hemodynamics. Microbiological culture media, or the media for the microbe in the gut, are perfused through the higher (apical) micro-channel. The micro-channel is

perfused by endothelial cells or oxygen-infused microbe-bearing media. The flexible porous layer is cyclically expanded by the action of micro-pneumatic actuators, simulating peristalsis. Simultaneous co-culture of the flexible porous layer with the anaerobic bacteria is achieved by the exact control of the oxygen gradient in the layer, an innovation in microbiome science studies (Jalili-Firoozinezhad et al., 2019). It can also be employed to evaluate inflammatory bowel diseases, infection, and barriers; examine drug absorption, metabolism, and toxicity; analyze the reaction of the host to commensal and pathogenic microorganisms, including *Lactobacillus rhamnosus* and *E. coli*; and live–host microbial interaction analysis utilizing live cell imaging.

Unlike static models, Gut-on-a-Chip devices support dynamic host–microbe co-culturing in physiological flow, oxygenation, and mechanical stimulation conditions. This encourages the survival of microbial as well as epithelial cells in the system. The major drawbacks are the low throughput capacity, the complexity, and the cost involved in the preparation of the device. In addition to this, the immunological and neurological parts are absent in the multi-organ on-chip devices that are being developed (Figure 3.6).

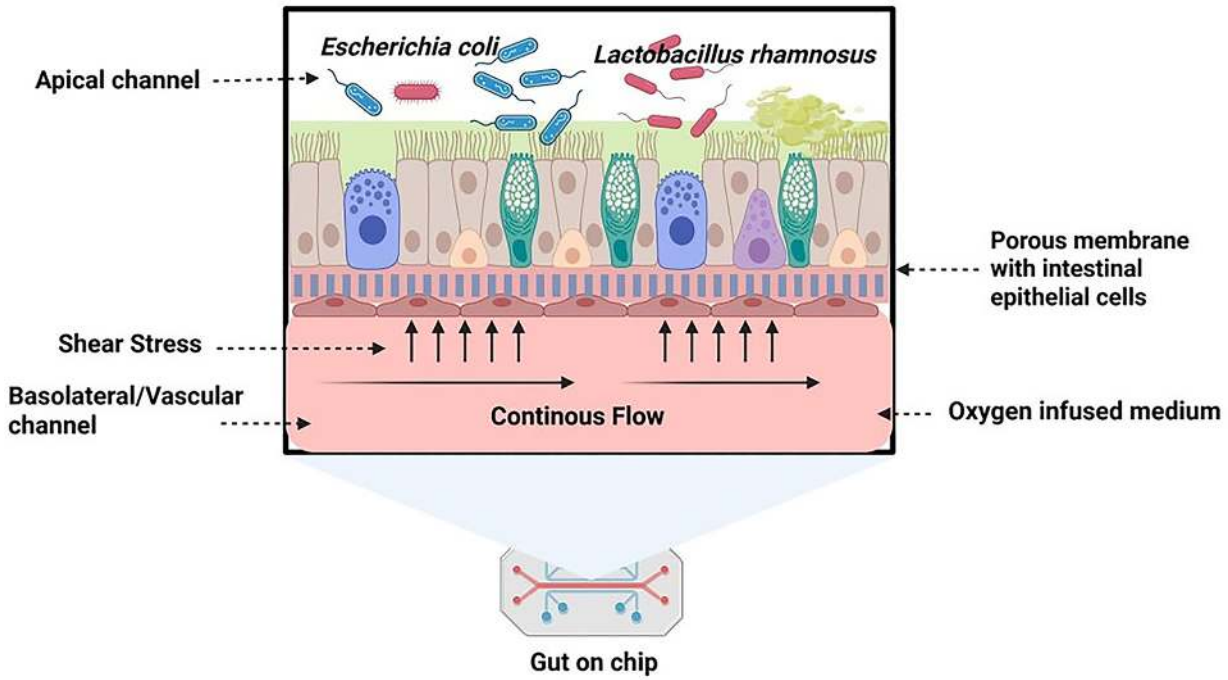

FIGURE 3.6 Gut-on-a-chip microfluidic models. ⏎

3.5.4 HuMiX – Human Microbial Cross-Talk Model

Among the most advanced and integrative *in vitro* platforms for host–microbiota co-culture is the Human–Microbial Cross-Talk (HuMiX) system (Wampach et al., 2017). Developed by Wampach and colleagues, HuMiX is a modular, three-chamber microfluidic device that enables the co-culture of human epithelial cells and microbial communities under physiologically relevant conditions, while maintaining spatial separation between aerobic and anaerobic environments. The HuMiX device consists of three vertically stacked compartments: a perfusion chamber that simulates microvascular flow, a bacterial chamber designed to sustain anaerobic microbial communities, and a human epithelial chamber typically seeded with Caco-2 or HT29-MTX cells. Semi-permeable membranes separate the chambers, allowing the exchange of nutrients, metabolites, and signaling molecules while preventing direct contact between host cells and microorganisms and preserving distinct oxygen gradients. This configuration enables detailed investigation of host–microbe molecular interactions, including the concurrent metabolism of drugs or xenobiotics by both microbial and host cells, as well as analyses of host transcriptional responses, immune signaling, and multi-omics readouts such as transcriptomics, metabolomics, and proteomics under controlled conditions. A key advantage of the HuMiX system is its ability to support prolonged co-culture of obligate anaerobic microorganisms alongside oxygen-requiring human epithelial cells, a capability that remains challenging in many other *in vitro* models. In addition, the modular design allows parallelization and adaptation to different host cell types or microbial communities, enabling a degree of experimental personalization. However, the widespread adoption of HuMiX is limited by the need for specialized microfabrication, precise flow control, and advanced calibration, restricting its use primarily to highly equipped research laboratories (Figure 3.7).

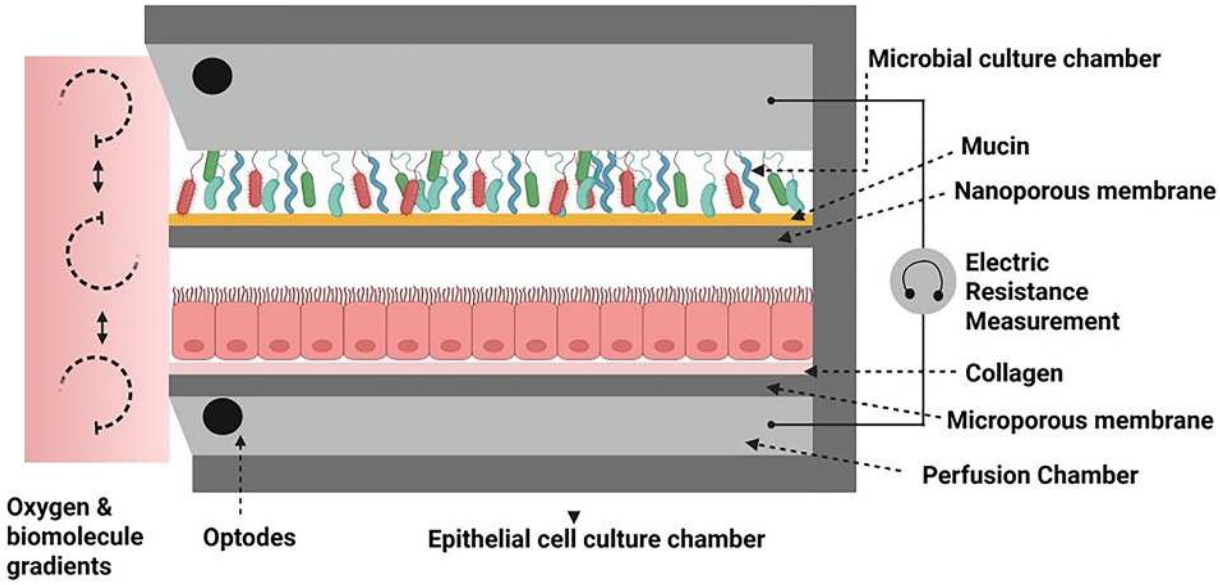

FIGURE 3.7 HuMiX – Human Microbial Cross-Talk Model. ⏎

TABLE 3.3
Overview of *In Vitro* Host–Microbe Interaction Models for Studying Intestinal Physiology and Immune Responses ⏎

Feature	Transwell	Organoid/Enteroid	Gut-on-a-Chip	HuMiX
Structure	2D monolayer on membrane	3D epithelial tissue	Microfluidic channels with flow	Modular tri-chamber microfluidic
Host cell type	Immortalized cell lines	Primary or stem-cell derived	Epithelial + endothelial (optional)	Epithelial + microbial + perfusion
Microbial interaction	Limited, short-term	Luminal injection or monolayer exposure	Dynamic co-culture	Long-term anaerobe–epithelial interaction
Physiological features	Static barrier	3D tissue, mucus	Flow, shear, peristalsis	Flow, anaerobic–aerobic gradient
Applications	Permeability, toxicity, cytokine assays	Host genetics, infection, drug response	Disease modeling, drug absorption	Integrated host–microbe metabolism
Advantages	Simple, reproducible	High biological relevance	Dynamic and imaging-friendly	Closest to *in vivo* physiology

Feature	Transwell	Organoid/Enteroid	Gut-on-a-Chip	HuMiX
Limitations	No flow, poor anaerobic tolerance	Closed lumen, no immune cells	Expensive, low throughput	Technically complex, costly

3.6 Specialized Co-Culture and Immune Integrated Systems

One of the key accomplishments in gut microbial studies has been the development of epithelial/immune/microbial co-culture models that set out to model the complex cellular environment that exists within the mucosa of the intestines (Leonard et al., 2010). Indeed, conventional models of *in vitro* analysis often study the microbial flora in isolation without considering the important interactions that exist within the complex interplay of immunological signaling pathways and mucus secretion that occur alongside epithelial cells. In an effort to address this deficiency, highly complex co-culture models have been developed that combine immunological components such as macrophages and/or dendritic cells with differentiated epithelial cells that line the intestines and mucus-producing goblet cells into a single system for study (Bermudez-Brito et al., 2012). Caco-2 or HT-29 MTX cell lines cultured on semi-permeable transwell insert systems, in which a polarized monolayer with well-defined apical and basolateral domains is established, have commonly been employed in the construction of such models (Bernet et al., 1994). The epithelium barrier may also be capable of producing mucus and antimicrobial peptides, besides serving as a physical barrier. If a lamina propria environment in which immune surveillance is naturally conducted is required, then immune cells such as THP-1-derived macrophages or monocyte-derived dendritic cells can be seeded in the basolateral compartment (Leonard et al., 2010). When strict anaerobes cannot be supported, microbial stimuli are added in the form of sterile microbial metabolites and supernatants, as well as live bacteria, after the co-culture reaches equilibrium. The balance of these two sides is preserved with respect to the concentration of the oxygen gradient, utilizing gas-permeable membranes or hypoxia chambers (Schulte et al., 2015).

Although the analysis of cytokine and chemokine signatures in the basolateral medium reflects immune activation, measurements of TEER as well as immunofluorescent staining of tight junction proteins such as ZO-1 and claudin-1 are used to analyze barrier function. At the same time, gene expression studies analyze the regulation of genes coding for mucin genes (such as MUC2), pattern-recognition receptors (such as TLR2 and TLR4), as well as inflammatory pathways (such as NF-κB) (Peterson & Artis, 2014). This enables the analysis of direct effects of

probiotics/microbial metabolites on inflammation, immunological tone, and epithelial differentiation.

3.7 Challenges and Limitations in the Use of *In Vitro* Animal Models for Studying the Human Gut Microbiome

In vitro models of the human gut microbiome have inherent limitations because host–microbe ecology is not accurately replicated *in vivo*. The biological complexity of *in vitro* models is limited, and they frequently omit critical host components essential for immunological surveillance, neuroendocrine signaling, vascular transport, and metabolism. These models also exhibit community drift and selective over-representation of fast-growing species compared with slow-growing, low-abundance, or spatially dependent taxa. Even in sophisticated microfluidic devices, it remains challenging to replicate the dynamic physicochemical gradients of the gut, including variations in nutrient availability, oxygen tension, mucus turnover, and peristaltic forces. Furthermore, donor-to-donor variability in fecal samples introduces reproducibility issues, complicating cross-study comparisons and standardization. Therefore, careful integration of computational and *in vitro* approaches is critical, as physiological observations do not always directly correspond to the mechanistic insights obtained from reductionist systems. Consequently, while *in vitro* models are indispensable for controlled hypothesis testing, their findings must be interpreted in context and validated using complementary experimental frameworks.

3.8 *In Vivo* Experimental Model Systems for Studying the Gut Microbiome

A deeper understanding of the gut microbiota is possible through *in vitro* and computational models by reproducing aspects of the intestinal environment and applying advanced algorithms, statistical methods, and integrated multi-omics approaches. However, these models cannot fully capture the complexity of host physiology or its dynamic interactions with the gut microbial ecosystem. Therefore, *in vivo* models are important to microbiome research to fully understand the functional roles of the gut microbiome in health and illness. *In vivo* models serve as a bridge between associative observations and causal relationships, enabling investigation of the complex interplay among host genetics, environmental factors, and microbial communities within a living system (Aluthge et al., 2020). *In vivo* models of the human

gut microbiome are the main topic of this section, and their application in studying microbiome function across diverse physiological and pathological conditions.

3.9 Rodents as a Classical *In Vivo* Model for Studying the Gut Microbiome

Rodent models, mainly mice, have been widely used for several decades for *in vivo* studies due to the flexibility of understanding the physiology, immunology, and genetic factors associated with a particular research setting (Bezirtzoglou et al., 2025). Mice models share similarities in the genetic makeup, along with the physiological and anatomical features of humans, including gut and gut-associated microbiota, making them one of the extensively utilized and studied models for gut microbiome research (Bibiloni, 2012). There are models such as germ-free (GF) and gnotobiotic mice models, specific pathogen-free and human-flora-associated models. Depending upon the research objective of the gut microbiome studies, researchers can explore the models to study the host–microbiome interactions (Berde et al., 2021).

3.9.1 GF Mice

GF mice, as the name suggests, are raised in sterile conditions from birth. This controlled environment ensures that there is a complete absence of microorganisms. They have a largely preserved colonic secretomotor function due to the absence of microorganisms, as they trigger secretion, which leads to reduced stimulation of the mucosal immune system (Lomasney et al., 2014). GF mice show decreased production of intestinal IgA, unless they are repopulated with bacteria (Hapfelmeier et al., 2010). The absence of microbial stimulation also leads to a thinner mucus layer, fewer and smaller Paneth cells, etc. (Herath et al., 2020). This allows researchers to explore the role of the human microbiome in different conditions, as the immune and phenotypic changes can be easily studied due to the absence of the existing microbiome (Huang et al., 2025; Sheikh et al., 2024). Despite their advantages, GF models present several limitations. They are expensive and require specialized facilities for their development and maintenance. Furthermore, these models may not fully recapitulate conventional or human physiology due to their underdeveloped immune, metabolic, and neural systems (Kennedy et al., 2018; Thomson et al., 2022).

3.9.2 Gnotobiotic Mice

Colonization with defined microbial consortia. This involves inoculating GF animals with either specific individual microbial species or a complex mixture of known microorganisms, enabling precise investigations into the functional contributions of individual microbes or defined communities to host physiology and disease (Kennedy et al., 2018). The colonization of a gnotobiotic model can be of different forms, ranging from low complexity (GF, mono colonization, and minimal microbial consortia) to more complex, defined microbial consortia and complex, undefined microbiota samples from murine or human origin (Jans & Vereecke, 2024). This allows researchers to investigate the role of the particular bacteria of interest in different conditions by introducing them into the GF mice and studying the immune functions, alteration in behavior, physiology, as well as the secretory functions, which are minimal in GF mice (Huang et al., 2025; Sheikh et al., 2024).

3.9.3 Antibiotic-Treated Mice

To overcome the limitations of the GF model, an alternative model is to treat the conventional mice with a broad-spectrum antibiotic to deplete the existing microbiome of any genotype or condition of the mice. It is inexpensive and does not require any special treatment. The limitation is that the whole bacterial population is not depleted, and some bacteria are still there. Antibiotic treatment can give rise to an overgrowth of bacteria or select for resistant bacteria (Kennedy et al., 2018).

3.9.4 Rats

Rats are extensively used in gut microbiome studies due to their large size and longer life span than mice. Some studies have shown that rats show greater similarity in microbiome composition with humans than mice, and after a single human microbiome transplant, they can capture more human microbial species than mice (Lleal et al., 2019). Human microbiota transplant can be performed in either GF or conventional rats. Conventional rats like mice undergo antibiotic or polyethylene glycol (PEG) treatment to deplete existing microbiota, followed by microbiota transplant. Rats as a model for studying human-related gut microbiota have been used for various diseases (Gheorghe et al., 2021; Hu et al., 2022). For instance, studies evaluating fecal microbiota transplantation in colitis and type 2 diabetes mellitus have utilized rat models to observe improvements in clinical manifestations and alterations in microbiome community structure (Lleal et al., 2019; Zhang et al., 2020).

3.9.5 Humanized Microbiota-Associated Mouse Models

Humanized microbiota-associated (HMA) mouse models can be generated through fecal microbiota transplantation (FMT) from human donors into mice. This approach can be applied across a range of mouse genotypes, including conventional, GF, and genetically modified models. In GF mice, FMT can be performed without prior treatment, whereas conventional or wild-type mice require depletion of the resident microbiota, typically achieved using PEG treatment or broad-spectrum antibiotics. Donor fecal material must be processed under anaerobic conditions prior to transplantation to preserve obligate anaerobic bacteria (Huang et al., 2025).

The primary advantage of HMA models is their ability to partially recapitulate the complex microbial communities of the human gut under both healthy and disease-associated conditions (Arrieta et al., 2016). These models also enable mechanistic investigation of host immune responses influenced by human-derived microbiota. However, a major limitation of HMA mouse models is that microbial engraftment is often incomplete and may not fully reflect the donor's original microbiota composition. This constraint arises from fundamental anatomical and physiological differences between mice and humans, including variations in diet, immune system function, and gut morphology (Wrzosek et al., 2018).

3.10 Alternative and Emerging Non-Rodent Models

3.10.1 Pigs

The exploration of non-rodent models is being driven by the need for therapeutically appropriate models of the human gut microbiome. Pigs are considered a better alternative as they are already in use for biomedical research and due to their overlapping similarity to humans in terms of anatomy, gut morphology, microbiota composition, and immune system development. Moreover, pigs are omnivores and have a comparable developmental period to humans during infancy, which makes them a valuable model for studying early life gut microbial colonization and its impact on host health (Pang et al., 2007; Zhang et al., 2013). Human flora-associated models developed in GF pigs from adults as well as children have shown the establishment and persistence of human-derived microbial communities (Zhang et al., 2013).

3.10.2 Zebrafish

Classical models, such as rodents, have limitations such as ethical issues and high cost, and have therefore shifted the focus towards non-mammalian models such as fish. Zebrafish (*Danio rerio*) offer distinct advantages for microbiome research, including

optical transparency during larval stages, high fecundity, and external development, which facilitate direct observation of host–microbe interactions and experimental manipulation (Douglas, 2019). The method of generating and colonizing gnotobiotic zebrafish is well established. A GF zebrafish model involves meticulously sterilizing embryos to eliminate all microorganisms. This process typically includes obtaining embryos, performing aseptic derivation with chemical sterilization agents like PVP-I or bleach, and then rearing them in sterile environments such as gnotobiotic isolators or culture flasks (Pham et al., 2008). Daily maintenance ensures sterility and high survival rates, making these models invaluable for studying host–microbe interactions (Pham et al., 2008). Researchers have tried introducing the human microbiome from a fecal sample in GF zebrafish, which has further resulted in successful engraftment of human microbial communities, which further warrants the use of this model system to study host–microbe interactions, particularly given the lower cost and faster reproduction cycles compared to mammalian models (Lu et al., 2021; Arias-Jayo et al., 2017). The limitation of this model is its evolutionary distance from humans, which may limit the direct translatability of some findings, despite shared genetic homology and similar gut structures (Zhong et al., 2022) (Toh et al., 2013).

3.10.3 Drosophila

Drosophila, as a model organism, is used due to its short life cycle and high fecundity, which results in the production of numerous genetically identical offspring in a short duration, as compared to typical rodent models, and typically, 75% human disease-causing genes are conserved in Drosophila. This genetic homology, along with its ease of handling in the lab, makes Drosophila an attractive model for studying human diseases and host-microbiome interactions (Douglas, 2018). Studies on transferring the human gut microbiome to the Drosophila gut have employed methodologies similar to human-to-mouse FMT. This involves initial antibiotic-mediated depletion of the existing microbiome in Drosophila larvae and adult flies. The human fecal suspension is added to the Drosophila food as a culture medium, and this treatment is introduced for a span of 30 days. It was observed that after 30 days, around 80–87.5% of the Operational Taxonomic Units (OTUs) from donor faeces were adopted by the recipient Drosophila (Ji et al., 2022). Studies in which Drosophila were inoculated with specific probiotic strains to examine the role of individual microbes and their protective effects against pathogenic strains have shown increased post-infection viability, comparable to that of control groups (Gavrilova et al., 2023).

3.10.4 *Caenorhabditis elegans*

C. elegans exhibits a high degree of genetic conservation with humans, particularly in core cellular and signaling pathways. Owing to its simple anatomy, transparent body, short life cycle, and well-characterized genetics, *C. elegans* serves as a powerful model for investigating the mechanistic basis of host–microbiome interactions under highly controlled conditions. As a bacterivorous nematode, *C. elegans* readily consumes microbial diets, facilitating experimental manipulation of its gut-associated microbial environment.

Human fecal samples can be diluted and cultured *in vitro*, after which defined bacterial communities are fed to GF or microbiota-depleted *C. elegans* to assess host-specific physiological and behavioral responses to microbial exposure (Kumar et al., 2019; Møller et al., 2023). While *C. elegans* does not naturally harbor a complex human-like gut microbiome, this approach enables functional screening of microbial effects on conserved host pathways.

3.10.5 Honeybees

Honeybees (*Apis mellifera*) live in highly organized social communities and exhibit complex social and cognitive behaviors. Gut microbiome analyses have revealed that honeybees possess a distinct and relatively simple core gut microbiota. Honeybees are a useful model for investigating microbiota–brain and microbiota–behavior interactions because this microbiome is crucial to host nutrition, immunological function, and cognitive processes (Engel et al., 2012; Zeng et al., 2024). Recent studies investigating the role of the gut microbiome in neurodevelopmental and neurodegenerative disorders have employed microbiota transplantation approaches in microbiota-depleted honeybees. For example, gut microbiota from children with autism spectrum disorder or from typically developing controls have been introduced into microbiota-free bees, followed by assessment of learning and memory through behavioral assays to evaluate the impact of autism-associated microbial communities (Li et al., 2023). By introducing fecal microbiota from Parkinson's disease patients into microbiota-free honeybees, the role of the gut microbiome in the disease has been investigated, resulting in measurable motor impairments and altered behavior in the host (Zeng et al., 2024). An overview of the *in vivo* models used to study gut microbiome and different routes to perform fecal microbiota transplantation is shown in Figure 3.8.

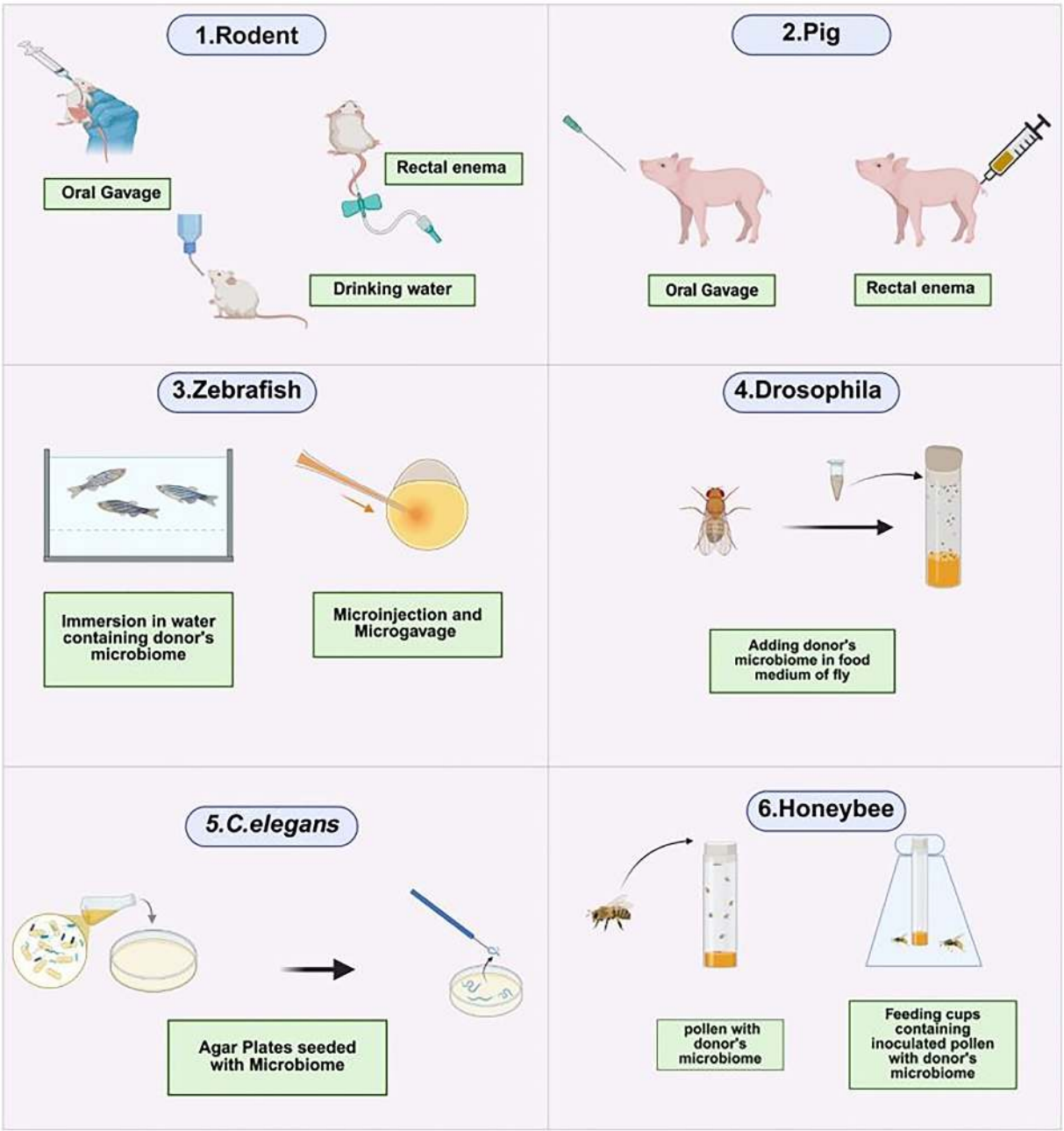

FIGURE 3.8 Routes of FMT delivery across different *in vivo* model organisms. ⏎

3.11 Challenges and Limitations in the Use of *In Vivo* Animal Models for Studying the Human Gut Microbiome

Despite the high relevance and advancements in microbiome research utilizing diverse animal models, several challenges and limitations persist when it comes to translating findings to human health outcomes (Berde et al., 2021). A primary concern is the

difference in the physiological makeup and gut architecture between model organisms and humans, which can significantly influence microbial community establishment, metabolic functions, and host–microbe interaction. In addition to this, the difference in the genetic makeup and immune responses among species further complicates the direct extrapolation of research findings to human physiology (Basic et al., 2022). Fecal microbiota transplant from the human to animal models, while valuable for studying host-microbiome interactions, often faces challenges in successful engraftment (Figure 3.9). With the aim of addressing these challenges necessitates the development of more sophisticated humanized animal models and innovative experimental designs that better mimic the complex human gut environment and immune system (Iyer, 2016).

Model Organisms	Rodents	Pig	Zebrafish	Drosophila	C.elegans	Honeybee
Key Features	• Rodents are genetically 90- 95% similar to humans • Different strains like Knockouts, transgenic and humanized are widely used for gut microbiome studies	• Pigs have gut microbiome closer to humans as compared to rodents • Repeated gut and blood sampling can be performed. • Useful for nutrition, metabolic disease, and immune studies	• Zebrafish germ free larvae can be easily generated and they are transparent which allows for direct visualization	• Drosophila has a well characterized genome and genetic tools make it easy to generate models for neurological disorders • Useful for Gut-brain axis, behavior, metabolism studies	• C.elegans have a transparent body which allows monitoring • of microbial colonization in live nematode • Host-microbiome interaction can be studied at molecular and genetic level	• Simple and stable gut microbiota • Honey bees possess similar symptoms to obesity, T2D, NAFLD, neurodegenerative disorders and inflammatory diseases in human. • Used in studies of nutrition, social interactions, and stress
Pros	• Well established FMT protocols • They can be generated into GF,Gnotobiotic, HMA models etc • They have similar gut microbiome as humans	• They have similar gastrointestinal anatomy and physiology as humans • GF, Gnotobiotic and HMA models are possible • They share similar gut microbiome as humans	• Rapid generation and low cost Real time monitoring of gut microbiome due to optical transparency at larval stage GF, Gnotobiotic and HMA models can be maintained	• Short life cycle and high fecundity rates • 75% of the disease causing genes in humans are conserved	• Show high genetic conservation with humans Can be colonized with single or human microbiota • It has a transparent body , direct observation of microbiome interaction is possible using fluorescent reporters	• Low Cost, high generation time and simple to handle Highly social in nature and hence useful for studying ecological and behavioural aspect
Cons	• Expensive, require specialized facilities and high maintenance	• Longer generation time • Expensive and difficult to maintain • Ethical Issues	• Evolutionary far from humans Gut anatomy and physiology not similar to humans	• Low diversity Microbiota • Gut anatomy and physiology not similar to humans	• Very simple gut anatomy as compared to humans limiting extrapolation of results	• Physiology is different from humans • Gut anatomy is different which makes it difficult to extrapolate to humans

FIGURE 3.9 Comparative account of *in vivo* models for FMT and gut microbiome research. ⏎

3.12 Computational Modeling for Human Microbiome Studies

The gut microbiome functions as a dynamic network. Within this network, microbes engage in interspecies interactions through chemical signaling such as quorum sensing. Additionally, exchange of metabolites and genetic material via horizontal gene transfer also facilitates this communication. These processes result in a robust and adaptive microbial community skilled enough in responding to environmental perturbations such as diet, antibiotics, or infection. Simultaneously, host epithelial and immune cells respond to microbial cues, maintaining a balanced state of mutualism (Heinken et al., 2021a). Any divergence in this mutualism leads to numerous pathophysiological conditions such as inflammatory bowel disease, obesity, and metabolic disorders (Hyun et al., 2021). Understanding these community interactions posed a challenge before the development of high-end computers. The complexity and high-dimensionality of the microbiome have been revealed by the development of computers and various omics data types, including metagenomics, meta-transcriptomics, and metabolomics (Dakal et al., 2025), leading to the exponential growth of microbiome research in various healthy and diseased conditions (Figure 3.10) (Segata et al, 2013).

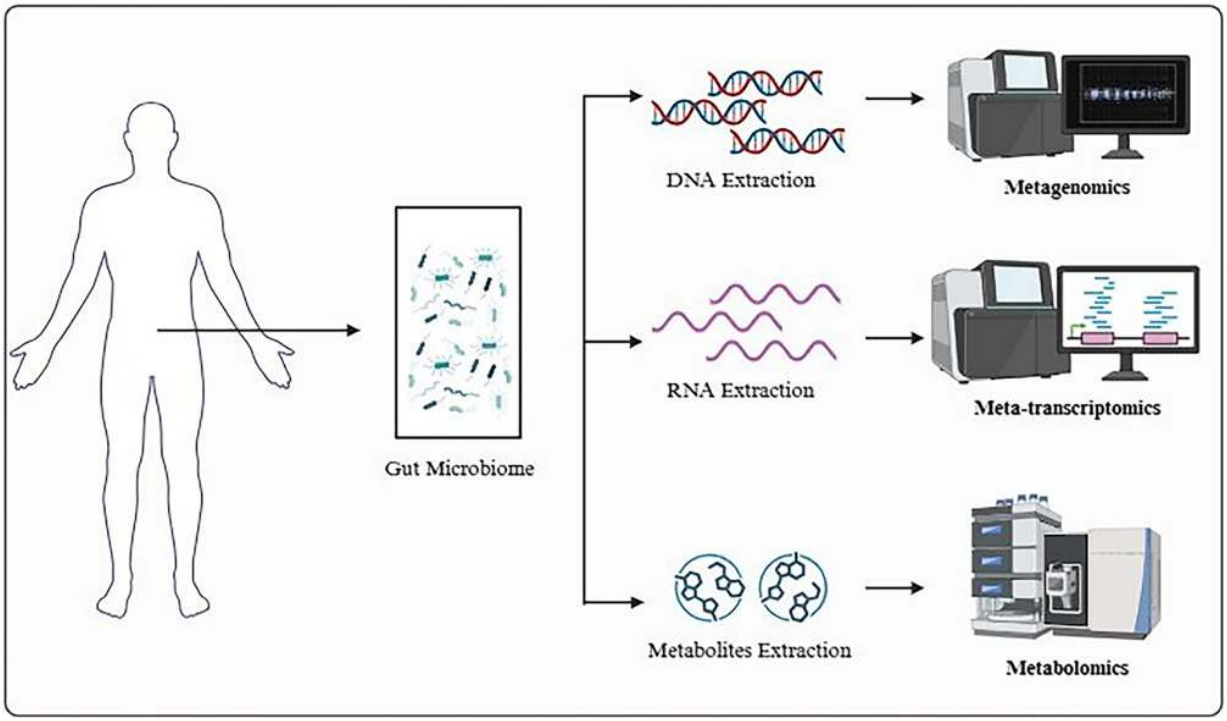

FIGURE 3.10 Stool samples can be systematically analyzed using integrated metagenomic, transcriptomic, and metabolomic approaches to achieve a comprehensive characterization of the microbiome. ⏎

The data generated through high-throughput technologies is submitted to various databases, which can be accessed to develop computational models. Researchers use the

available and developed multi-omics data for computational methods that range from traditional statistical approaches to unconventional machine learning and deep learning techniques that enable pattern recognition and predictive modeling (Marcos-Zambrano et al., 2021).

3.13 Different Types of Computational Models

Using the available and generated data, diverse computational modeling approaches can be developed to extract deeper and mechanistic insights into microbiome networks. These models enable the integration of multi-omics datasets, capture complex microbial interactions, and elucidate community structure–function relationships. Network-based models can be employed to infer co-occurrence, co-exclusion, and interaction dynamics among microbial taxa, thereby identifying keystone species and community hubs (Figure 3.11).

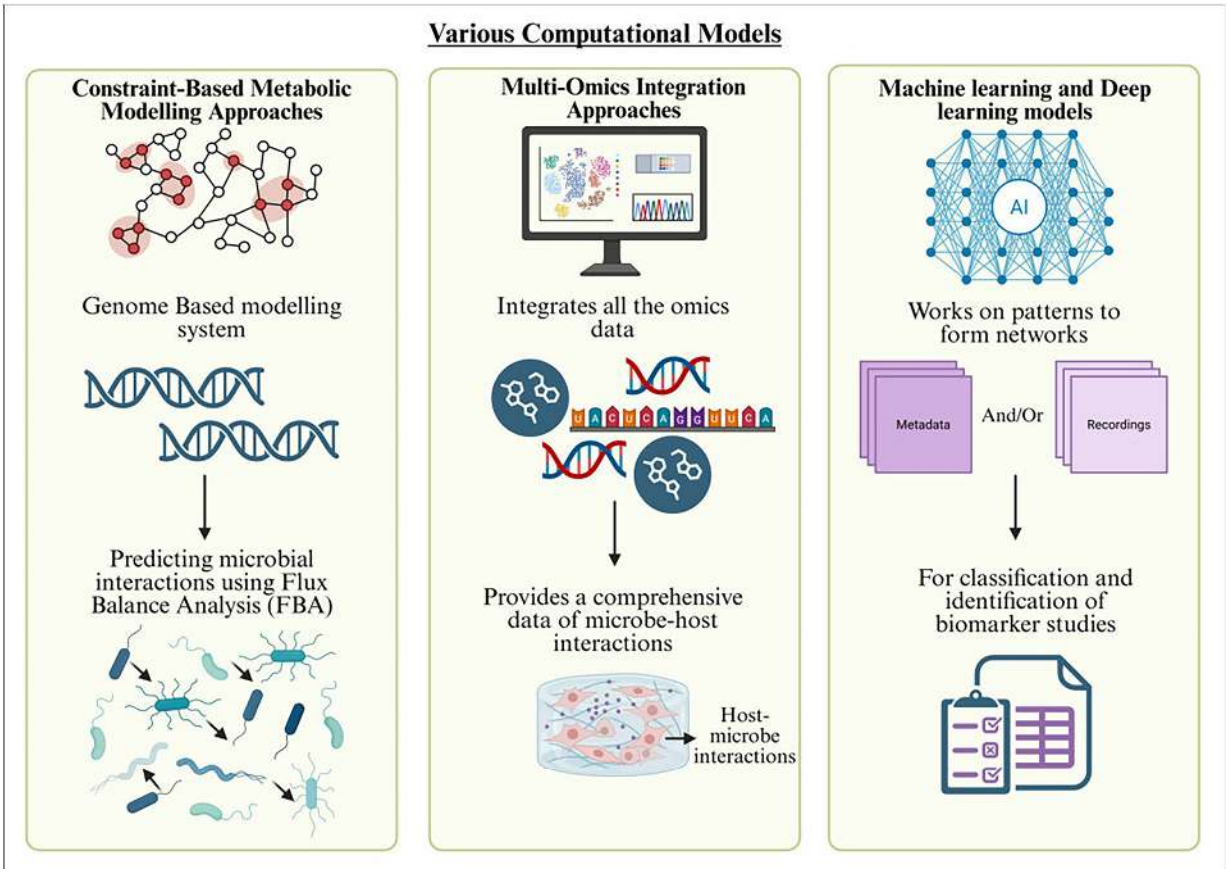

FIGURE 3.11 Using the available and generated data, different types of computations models can be developed to get deeper insights into the microbiome network. ⏎

3.13.1 Constraint-Based Metabolic Modeling Approaches

Constraint-based analysis, particularly genome-scale metabolic models (GEMs), emerges as a powerful mechanistic tool for predicting microbial metabolism and community interactions by taking into account all the known biochemical reactions present within an organism (Colarusso et al., 2021). These knowledge-based computational models use flux balance analysis (FBA), a technique that takes metabolic fluxes in a system by optimizing a function, typically growth rate, subject to steady-state assumptions and constraints imposed by the metabolic reactions present in the system (Raman et al., 2009). More thorough investigation of microbiome metabolism has been made possible by the growing availability of curated GEMs for thousands of human-associated microbial organisms. Community-scale metabolic modeling techniques designed especially for the human microbiome, like MICOM and mgPipe, have emerged to predict microbial community metabolic output from microbial abundance (Baldini et al., 2019; Deiner et al., 2020). These models enable the progress and *in silico* testing of biological hypotheses concerning the metabolism of microbial communities, offering insights into the relationship between ecosystem-level phenotypes in human host health and metabolites generated from these microbes (Table 3.4).

Constraint-based models on the microbiome scale, encompassing hundreds of organisms, may now be constructed and examined due to the significant advancements in the field (Heinkin et al., 2021). These multiscale, strain, and molecule-resolved models have complemented experimental approaches in various fields for novel biological knowledge through mechanistic prediction by metabolic models. Recent developments in dynamic flux balance analysis (dFBA) have further enhanced parameter fitting and data integration capabilities, with recurrent optimization algorithms achieving 98% and 96% accuracy when replicating *in vitro* and clinical bacterial abundance distributions, respectively (Gomez et al., 2018). This integration of data allows researchers to observe the evolution of metabolites and provides a deeper understanding of metabolic interactions.

TABLE 3.4

Tools for Constraint-Based Metabolic Models ⏎

Modeling Tool/Method	Core Algorithm	Primary Applications	Computational Requirements	Microbiome Study Contexts

Modeling Tool/Method	Core Algorithm	Primary Applications	Computational Requirements	Microbiome Study Contexts
MICOM (Deiner et al., 2020)	Community-scale flux balance analysis with co-operative trade off optimization	Human gut microbiome metabolism prediction, metabolite exchange analysis	Moderate to high; requires genome-scale metabolic networks for community members	Human health, disease associations, dietary interventions
mgPipe (F Baldini et al., 2019)	Pipeline integrating abundance data with flux balance analysis	Mechanistic prediction of community metabolic output from abundance profiles	Moderate; Python-based implementation with standard computational resources	Human microbiome observational studies, clinical cohorts
dFBA (Dynamic Flux Balance Analysis) (J. Gomez et al., 2018)	Time-resolved flux optimization with dynamic parameter estimation	Temporal modelling of metabolite evolution and community dynamics	High; requires iterative optimization and time-course data	Clinical microbiome tracking, *in vitro* community experiments, drug response studies

3.13.2 Multi-Omics Integration Approaches

Multi-omics integration methods combine microbiome data with host genomics, transcriptomics, and metabolomics to provide comprehensive insights into microbiome–host interactions, though statistical and computational methods for integrating these diverse data types are difficult (Chetty & Blekhman., 2024). Five main integration strategies have been systematically compared for microbiome research: Concatenation (a single input matrix combining features from all omics layers), Averaged Stacking (averaging predictions from individual omics models), Weighted Non-Negative Least Squares (NNLS, optimally weighting individual model predictions with non-negative constraints), Lasso Stacking (using L1-regularized regression to combine predictions), and Partial Least Squares (PLS, projecting data onto latent components that maximize covariance between omics layers and outcomes) (Kang et al., 2022). Among these approaches, Random Forest combined with NNLS integration achieved the best overall performance across a variety of datasets for continuous outcomes, while for binary classification tasks, Random Forest maintained consistent performance regardless of integration strategy (Kang et al., 2022). The integration of

multi-omics data enables researchers to identify molecular subtypes, predict biomarkers, and regulatory circuits, though challenges of data heterogeneity, scalability, and interpretability persist (Chetty & Blekhman, 2024) (Table 3.5).

Systems biology approaches integrate metabolic modelling with multi-omics data to enable mechanistic predictions of metabolic fluxes and community functions, moving beyond taxonomic composition to identify microbial activities and their roles in host health (Heinken & Thiele, 2015). In order to find biomarkers and possible therapeutic targets in precision medicine applications, computational frameworks for predicting metabolite profiles provide indirect insights into microbial metabolic outputs (Wishart et al., 2016). The integration of biological domain knowledge with functional gene activity reveals complex metabolic interactions within microbial communities and their contributions to host metabolism, with genome-scale metabolic models serving as frameworks for multi-omics data integration (Tarzi, 2025). Recent advances demonstrate that integrating population-based metabolomics with constraint-based microbiome community modelling can identify biomarkers reflective of protective diet–microbiome–host interactions, such as urinary methanol as a marker of microbial pectin degradation linked to lower all-cause mortality (Klier et al., 2025).

TABLE 3.5

Approaches for Integration of Omics Data to Build a Multi-Omics Model ⏎

Integration Strategy	Methodological Approach	Computational Requirements	Advantages	Limitations	Suitable Applications
Concatenation (M Kang et al., 2022)	Combines all omics features into single input matrix for unified modelling	Moderate; scales with total feature count across all omics layers	Simple implementation; enables joint feature selection across modalities	High dimensionality can lead to overfitting; equal weighting may not reflect biological importance	Exploratory analyzes with balanced omics data; compatible with feature selection methods

Integration Strategy	Methodological Approach	Computational Requirements	Advantages	Limitations	Suitable Applications
Averaged Stacking (M Kang et al., 2022)	Builds separate models for each omics layer and averages predictions	Low to moderate; parallelizable across individual omics models	Simple ensemble approach; robust to poor performance of single modality	Equal weighting assumes all omics contribute equally; may underperform if one modality is highly informative	Scenarios where all omics layers have similar predictive power; initial baseline comparisons
Weighted NNLS (M Kang et al., 2022)	Non-negative least squares optimization to weight individual omics model predictions	Moderate; requires iterative optimization with non-negativity constraints	Optimal weighting based on predictive performance; highest accuracy for continuous outcomes; interpretable weights	Requires sufficient validation data for weight estimation; computationally more intensive than averaging	Continuous outcome prediction; biomarker discovery requiring clear contribution quantification from each omics layer
Lasso Stacking (M Kang et al., 2022)	L1-regularized regression combines predictions with sparse model selection	Moderate; regularization path computation required	Automatic feature selection through sparsity; can identify most relevant omics layers	May exclude informative modalities if correlation exists; sensitive to regularization parameter choice	High-dimensional settings where dimension reduction across omics is needed; sparse multi-omics signatures

Integration Strategy	Methodological Approach	Computational Requirements	Advantages	Limitations	Suitable Applications
Partial Least Squares (PLS) (M Kang et al, 2022)	Projects omics data onto latent components maximizing covariance with outcomes	Moderate to high; requires singular value decomposition and cross-validation for component selection	Handles multicollinearity well; reduces dimensionality while preserving covariance structure	Interpretability of latent components can be challenging; assumes linear relationships	Highly correlated omics data; mechanistic studies requiring pathway-level interpretation

3.13.3 Machine Learning and Deep Learning Models

Machine learning approaches, including Random Forest, Support Vector Machines, and Elastic Net, have become widely used for microbiome data analysis, particularly for classification tasks and biomarker discovery (Marcus-Zambrano et al., 2021). These conventional machine learning methods address important challenges in microbiome research, including compositional constraints and taxonomic hierarchy, enabling researchers to identify disease-associated microbial signatures and predict clinical outcomes. Deep learning techniques have demonstrated remarkable capacities in managing high-dimensional, noisy, and complex microbiome data, providing exceptional accuracy in understanding the makeup of microbial communities and their influence on health and illness (Przymus et al., 2025). Deep learning-based metagenomics techniques enable novel pathogen detection, patient stratification, and mechanistic understanding of host–microbiome dynamics. Neural network architectures, such as convolutional neural networks and recurrent neural networks, have been successfully applied to microbial classification, functional prediction, and sequence analysis (Zhang et al., 2023). Across a variety of microbiome datasets, Random Forest plus NNLS integration has shown the best overall performance, especially for continuous outcomes. While single-omics models incorporating metabolomics data frequently outperform integrative techniques, Random Forest consistently performs well for binary classification tasks regardless of integration strategy. Compared to linear approaches, tree-based models show more consistency in feature selection across various dimensionalities and integration strategies (Palmer et al., 2025) (Table 3.6). These developments are comparable to the use of conventional machine learning in gut microbiome composition-based autism spectrum disorder

prediction, where random forest algorithms, support vector machines, and artificial neural networks achieved classification accuracies as high as 90% with sensitivities reaching 96.97% (Kasahun et al., 2024).

TABLE 3.6
Different Approaches in Machine Learning to Build Models for Microbiome

Algorithm	Data Type	Primary Study	Performance	Computational Requirement	Interpretability
Random Forest (Marcus-Zambrano et al., 2021)	Taxonomic, metabolomics, multi-omics	Classification, biomarker discovery, feature selection	High accuracy (90%+) for binary outcomes; consistent across integration strategies	Moderate; parallelizable with multiple trees	High; feature importance scores readily available
Support Vector Machines (Marcus-Zambrano et al., 2021)	High-dimensional taxonomic data	Binary classification, disease prediction	Strong performance with proper kernel selection; sensitivity up to 96.97%	Moderate to high; computationally intensive with large datasets	Moderate; kernel functions can obscure interpretability
Elastic Net (Marcus-Zambrano et al., 2021)	Sparse compositional data	Feature selection, regularized regression	Good performance with correlated features; handles sparsity well	Low to moderate; efficient linear optimization	High; coefficient-based feature importance
Convolutional Neural Networks (Zhang et al., 2023)	Metagenomic sequences, functional gene prediction	Sequence classification, pattern recognition	High accuracy for sequence-based tasks; excels at spatial feature extraction	High; GPU acceleration required for large datasets	Low; black-box nature limits direct interpretation

Algorithm	Data Type	Primary Study	Performance	Computational Requirement	Interpretability
Recurrent Neural Networks (Zhang et al., 2023)	Temporal microbiome data, longitudinal studies	Time-series analysis, dynamic community modeling	Captures temporal dependencies; effective for sequential data	High; memory-intensive with long sequences	Low; complex hidden state representations

3.14 Challenges and Future Directions in Computational Modelling for Microbiome Studies

Despite the rapid advancement of computational methods for microbiome analysis, several challenges continue to obstruct the translation of research findings into clinical applications. Current challenges include improving model interpretability, cross-dataset generalization, and integrating multi-omics data effectively (Nguyen et al., 2023). Sample sizes, uneven label distribution, varying experimental design, and lack of access to metadata contribute to reproducibility issues and limit applications into clinical practice (Cunha-Oliveira et al., 2024). Batch effects arising from technical and biological sources pose significant challenges, requiring computational methods to remove unwanted variation while preserving true biological signals (Leek et al., 2010). The lack of proper standardization complicates the assessment, preventing objective comparison of model performance across microbiome contexts and further making it difficult to determine which methods provide robust and generalizable results.

Future developments should focus on creating standardized methodologies and improved transparency guidelines to facilitate the shift from observational studies to causal inference and intervention (Harper et al., 2019). Promising paths for moving beyond descriptive profiling to predictive and prescriptive analytics include integrating AI into data-based models and integrative multi-omics frameworks (Ali, 2023). The development of standardized datasets with diverse population representation and comprehensive clinical metadata will facilitate the identification of robust, generalizable approaches for microbiome-based precision medicine. Emerging areas include the development of microbiome-targeted therapies, personalized treatment strategies, and the establishment of global database repositories with comprehensive host metadata enabling more accurate AI-based disease prediction models.

References

Ali, H. (2023). Artificial intelligence in multi-omics data integration: Advancing precision medicine, biomarker discovery, and genomic-driven disease interventions. *International Journal of Science and Research Archive*, *8*(1), 1012–1030. ⏎

Aluthge, N. D., Tom, W. A., Bartenslager, A. C., Burkey, T. E., Miller, P. S., Heath, K. D., Kreikemeier-Bower, C., Kittana, H., Schmaltz, R. J., Ramer-Tait, A. E., & Fernando, S. C. (2020). Differential longitudinal establishment of human fecal bacterial communities in germ-free porcine and murine models. *Communications Biology*, *3*(1), 760. https://doi.org/10.1038/s42003-020-01477-0 ⏎

Arias-Jayo, N., Alonso-Saez, L., Ramirez-Garcia, A., & Pardo, M. A. (2017). Zebrafish axenic larvae colonization with human intestinal microbiota. *Zebrafish*, *15*(2), 96–106. https://doi.org/10.1089/zeb.2017.1460 ⏎

Arrieta, M., Walter, J., & Finlay, B. B. (2016). Human microbiota-associated mice: A model with challenges. *Cell Host & Microbe*, *19*(5), 575–578. https://doi.org/10.1016/j.chom.2016.04.014 ⏎

Artursson, P., & Karlsson, J. (1991). Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochemical and Biophysical Research Communications*, *175*(3), 880–885. ⏎

Baldini, F. (2019). Developing individual-based gut microbiome metabolic models for the investigation of Parkinson's disease-associated intestinal microbial communities (Doctoral dissertation, University of Luxembourg). ORBilu. https://orbilu.uni.lu/handle/10993/41182 [orbilu.uni.lu]. ⏎

Basic, M., Dardevet, D., Abuja, P. M., Bolsega, S., Bornes, S., Caesar, R., Calabrese, F. M., Collino, M., De Angelis, M., Gérard, P., Gueimonde, M., Leulier, F., Untersmayr, E., Van Rymenant, E., De Vos, P., & Savary-Auzeloux, I. (2022). Approaches to discern if microbiome associations reflect causation in metabolic and immune disorders. *Gut Microbes*, *14*(1), 2107386. https://doi.org/10.1080/19490976.2022.2107386 ⏎

Berde, C. V., Sagar, P. S., Kunal, V. K., Suyoj, A. J., & Vikrant, B. B. (2021). Insight into the animal models for microbiome studies. In Bramhachari, P.V. (eds) *Microbiome in Human Health and Disease* Springer, Singapore. 259–273. https://doi.org/10.1007/978-981-16-3156-6_13 ⏎

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, *61*(2), 160–174. ⏎

Bernet, M. F., Brassart, D., Neeser, J. R., & Servin, A. L. (1994). *Lactobacillus acidophilus* LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut*, *35*(4), 483–489. ⏎

Bezirtzoglou, E., Plaza-Diaz, J., Song, J., Xie, G., & Stavropoulou, E. (2025). Editorial: Rodent model organisms: Therapeutic treatments and drugs interaction with the gut microbiome. *Frontiers in Microbiology*, *16*, 1581166. https://doi.org/10.3389/fmicb.2025.1581166 ⏎

Bibiloni, R. (2012). Rodent models to study the relationships between mammals and their bacterial inhabitants. *Gut Microbes*, *3*(6), 536–543. https://doi.org/10.4161/gmic.21905 ⏎

Blasco Aramburu, T. (2024). Reconstruction and analysis of gut microbiota metabolic networks in the context of personalized nutrition (Doctoral dissertation, Universidad de Navarra). Dialnet. https://dialnet.unirioja.es/servlet/tesis?codigo=327757 [dialnet.unirioja.es].

Clevers, H. (2016). Modeling development and disease with organoids. *Cell*, *165*(7), 1586–1597. ⏎

Colarusso, A. V., Goodchild-Michelman, I., Rayle, M., & Zomorrodi, A. R. (2021). Computational modeling of metabolism in microbial communities on a genome-scale. *Current Opinion in Systems Biology*, *26*, 46–57. ⏎

Cunha-Oliveira T, Ioannidis JPA, Oliveira PJ. Best practices for data management and sharing in experimental biomedical research. *Physiol Rev*. 2024 Jul 1;104(3):1387–1408. doi: 10.1152/physrev.00043.2023.

Dakal, T. C., Xu, C., & Kumar, A. (2025). Advanced computational tools, artificial intelligence and machine-learning approaches in gut microbiota and biomarker identification. *Frontiers in Medical Technology*, *6*, 1434799. ⏎

Diener, C., Gibbons, S. M., & Resendis-Antonio, O. (2020). MICOM: Metagenome-scale modeling to infer metabolic interactions in the gut microbiota. *MSystems*, *5*(1), 10–1128. ⏎

Douglas, A. E. (2018). The Drosophila model for microbiome research. *Lab Animal*, *47*(6), 157–164. https://doi.org/10.1038/s41684-018-0065-0 ⏎

Douglas, A. E. (2019). Simple animal models for microbiome research. *Nature Reviews Microbiology*, *17*(12), 764–775. https://doi.org/10.1038/s41579-019-0242-1 ⏎

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., et al. (2016). Population-level analysis of gut microbiome variation. *Science*, *352*(6285), 560–564. ⏎

Gavrilova, E., Kostenko, V., Zadorina, I., Khusnutdinova, D., Yarullina, D., Ezhkova, A., Bogachev, M., Kayumov, A., & Nikitina, E. (2023). Repression of

Staphylococcus aureus and *Escherichia coli* by *Lactiplantibacillus plantarum* Strain AG10 in *Drosophila melanogaster in vivo* model. *Microorganisms*, *11*(5), 1297. https://doi.org/10.3390/microorganisms11051297 ⏎

Gheorghe, C. E., Ritz, N. L., Martin, J. A., Wardill, H. R., Cryan, J. F., & Clarke, G. (2021). Investigating causality with fecal microbiota transplantation in rodents: Applications, recommendations and pitfalls. *Gut Microbes*, *13*(1), Article 1941711. https://doi.org/10.1080/19490976.2021.1941711 ⏎

Gomez, J. A. (2018). *Simulation, sensitivity analysis, and optimization of bioprocesses using dynamic flux balance analysis* (Doctoral dissertation, Massachusetts Institute of Technology). ⏎

Haiser, H. J., Gootenberg, D. B., Chatman, K., Sirasani, G., Balskus, E. P., & Turnbaugh, P. J. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science*, *341*(6143), 295–298. ⏎

Hapfelmeier, S., Lawson, M. a. E., Slack, E., Kirundi, J. K., Stoel, M., Heikenwalder, M., Cahenzli, J., Velykoredko, Y., Balmer, M. L., Endt, K., Geuking, M. B., Curtiss, R., McCoy, K. D., & Macpherson, A. J. (2010). Reversible microbial colonization of germ-free mice reveals the dynamics of IGA immune responses. *Science*, *328*(5986), 1705–1709. https://doi.org/10.1126/science.1188454 ⏎

Harper, S. (2019). A future for observational epidemiology: Clarity, credibility, transparency. *American Journal of Epidemiology*, *188*(5), 840–845. ⏎

Heinken, A., & Thiele, I. (2015). Systems biology of host–microbe metabolomics. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *7*(4), 195–219. ⏎

Heinken, A., Basile, A., & Thiele, I. (2021a). Advances in constraint-based modelling of microbial communities. *Current Opinion in Systems Biology*, *27*, 100346. ⏎

Heinken, A., Hertel, J., & Thiele, I. (2021b). Metabolic modelling reveals broad changes in gut microbial metabolism in inflammatory bowel disease patients with dysbiosis. *NPJ Systems Biology and Applications*, *7*(1), 19.

Herath, M., Hosie, S., Bornstein, J. C., Franks, A. E., & Hill-Yardin, E. L. (2020). The role of the gastrointestinal mucus system in intestinal homeostasis: Implications for neurological disorders. *Frontiers in Cellular and Infection Microbiology*, *10*, 248. https://doi.org/10.3389/fcimb.2020.00248 ⏎

Herbert, D., Elsworth, R., & Telling, R. C. (1956). The continuous culture of bacteria; a theoretical and experimental study. *Journal of General Microbiology*, *14*(3), 601–622. ⏎

Huang, X., Yu, Y., Tian, N., Huang, J., Zhang, X., & Yu, R. (2025). Human microbiota-associated animal models: A review. *Frontiers in Cellular and Infection Microbiology*, *15*, 1644187. https://doi.org/10.3389/fcimb.2025.1644187 ⏎

Hyun, C. K. (2021). Molecular and pathophysiological links between metabolic disorders and inflammatory bowel diseases. *International Journal of Molecular Sciences*, *22*(17), 9139. ⏎

Iyer, N. (2016). Methods in microbiome research. *Lab Animal*, *45*(9), 323–326. https://doi.org/10.1038/laban.1093 ⏎

Jalili-Firoozinezhad, S., Gazzaniga, F. S., Calamari, E. L., Camacho, D. M., Fadel, C.W., Bein, A., *et al.* (2019). A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nature Biomedical Engineering*, *3*(7), 520–531. ⏎

Jans, M., & Vereecke, L. (2024). A guide to germ-free and gnotobiotic mouse technology to study health and disease. *FEBS Journal*, *292*(6), 1228–1251. https://doi.org/10.1111/febs.17124 ⏎

Ji, D., Sun, H., Yang, W., Gao, M., & Xu, H. (2022). Transfer of human microbiome to Drosophila gut model. *Microorganisms*, *10*(3), 553. https://doi.org/10.3390/microorganisms10030553 ⏎

Kang, M., Ko, E., & Mersha, T. B. (2022). A roadmap for multi-omics data integration using deep learning. *Briefings in Bioinformatics*, *23*(1), bbab454. ⏎

Kasahun, M., & Legesse, A. (2024). Machine learning for urban land use/cover mapping: Comparison of artificial neural network, random forest and support vector machine, a case study of Dilla town. *Heliyon*, *10*(20), e39146. ⏎

Kennedy, E. A., King, K. Y., & Baldridge, M. T. (2018). Mouse microbiota models: Comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. *Frontiers in Physiology*, *9*, 1534. https://doi.org/10.3389/fphys.2018.01534 ⏎

Kim, H. J., & Ingber, D. E. (2013). Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integrative Biology*, *5*(9), 1130–1140. ⏎

Klier, K., Mehrjerd, A., Fässler, D., Franck, M., Weihs, A., Budde, K., … & Hertel, J. (2025). Integrating population-based metabolomics with computational microbiome modelling identifies methanol as a urinary biomarker for protective diet–microbiome–host interactions. *Food & Function*, *16*(18), 7067–7081. ⏎

Kumar, A., Baruah, A., Tomioka, M., Iino, Y., Kalita, M. C., & Khan, M. (2019). *Caenorhabditis elegans*: A model to understand host–microbe interactions. *Cellular and Molecular Life Sciences*, *77*(7), 1229–1249. https://doi.org/10.1007/s00018-019-03319-7 ⏎

Leek, J. T., Scharpf, R. B., Bravo, H. C., Simcha, D., Langmead, B., Johnson, W. E., … & Irizarry, R. A. (2010). Tackling the widespread and critical impact of batch effects in high-throughput data. *Nature Reviews Genetics*, *11*(10), 733–739. ⏎

Leonard, F., Collnot, E. M., & Lehr, C. M. (2010). A three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa *in vitro*. *Advanced Drug Delivery Reviews*, *62*(1), 64–82. ⏎

Lleal, M., Sarrabayrouse, G., Willamil, J., Santiago, A., Pozuelo, M., & Manichanh, C. (2019). A single faecal microbiota transplantation modulates the microbiome and improves clinical manifestations in a rat model of colitis. *EBioMedicine*, *48*, 630–641. https://doi.org/10.1016/j.ebiom.2019.10.002

Lomasney, K. W. (2014). *The impact of host–microbe interactions on murine colonic secretomotor function*. (PhD Thesis, University College Cork). ⏎

Lu, H., Li, P., Huang, X., Wang, C. H., Li, M., & Xu, Z. Z. (2021). Zebrafish model for human gut microbiome-related studies: Advantages and limitations. *Medicine in Microecology*, *8*, 100042. https://doi.org/10.1016/j.medmic.2021.100042 ⏎

Macfarlane, G. T., & Gibson, G. R. (1991). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. *Gastroenterology*, *100*(6), 1613–1621. ⏎

Macfarlane, G. T., & Macfarlane, S. (2007). Models for intestinal fermentation: Association between food components, delivery systems, bioavailability and functional interactions in the gut. *Current Opinion in Biotechnology*, *18*(2), 156–162. ⏎

Marcos-Zambrano, L. J., Karaduzovic-Hadziabdic, K., Loncar Turukalo, T., Przymus, P., Trajkovik, V., Aasmets, O., … & Truu, J. (2021). Applications of machine learning in human microbiome studies: A review on feature selection, biomarker identification, disease prediction and treatment. *Frontiers in Microbiology*, *12*, 634511. ⏎

Minekus, M., Marteau, P., Havenaar, R., & Huis in't Veld, J. H. J. (1995). A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Alternatives to Laboratory Animals*, *23*(2), 197–209. ⏎

Møller, K. V., Wesseltoft, J. B., Malazarte, R., Kousgaard, S. J., Nielsen, H. L., Yashiro, E., & Olsen, A. (2023). Usage of cultured human fecal microbiota for colonization of *Caenorhabditis elegans* to study host–microbe interaction. *Applied Microbiology*, *3*(4), 1130–1143. https://doi.org/10.3390/applmicrobiol3040078 ⏎

Molly, K., Vande Woestyne, M., & Verstraete, W. (1993). Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Applied Microbiology and Biotechnology*, *39*(2), 254–258. ⏎

Nguyen, P. C., Choi, J. B., Udaykumar, H. S., & Baek, S. (2023). Challenges and opportunities for machine learning in multiscale computational modeling. *Journal of Computing and Information Science in Engineering*, *23*(6), 060808. ⏎

Palmer, S. N., Mishra, A., Gan, S., Liu, D., Koh, A. Y., & Zhan, X. (2025). Identifying optimal machine learning approaches for human gut microbiome (Shotgun Metagenomics) and metabolomics integration with stable feature selection. *bioRxiv*. [Preprint]. 2025 Sep 23:2025.06.21.660858. doi: 10.1101/2025.06.21.660858. ⏎

Pang, X., Hua, X., Yang, Q., Ding, D., Che, C., Cui, L., Jia, W., Bucheli, P., & Zhao, L. (2007). Inter-species transplantation of gut microbiota from human to pigs. *ISME Journal*, *1*(2), 156–162. https://doi.org/10.1038/ismej.2007.23 ⏎

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, *14*(3), 141–153. ⏎

Pham, L. N., Kanther, M., Semova, I., & Rawls, J. F. (2008). Methods for generating and colonizing gnotobiotic zebrafish. *Nature Protocols*, *3*(12), 1862–1875. https://doi.org/10.1038/nprot.2008.186 ⏎

Przymus, P., Rykaczewski, K., Martín-Segura, A., Truu, J., Carrillo De Santa Pau, E., Kolev, M., … & Nechyporenko, A. (2025). Deep learning in microbiome analysis: A comprehensive review of neural network models. *Frontiers in Microbiology*, *15*, 1516667. ⏎

Raman, K., & Chandra, N. (2009). Flux balance analysis of biological systems: Applications and challenges. *Briefings in Bioinformatics*, *10*(4), 435–449. ⏎

Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., *et al.* (2009). Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche. *Nature*, *459*(7244), 262–265. ⏎

Schulte, L. N., Eulalio, A., Mollenkopf, H. J., Reinhardt, R., & Vogel, J. (2015). Analysis of hypoxia-induced noncoding RNAs in intestinal epithelial cells. *Cellular and Molecular Gastroenterology and Hepatology*, *1*(4), 397–408. ⏎

Segata, N., Boernigen, D., Tickle, T. L., Morgan, X. C., Garrett, W. S., & Huttenhower, C. (2013). Computational meta'omics for microbial community studies. *Molecular Systems Biology*, *9*(1), 666. ⏎

Sheikh, I. A., Bianchi-Smak, J., Laubitz, D., Schiro, G., Midura-Kiela, M. T., Besselsen, D. G., Vedantam, G., Jarmakiewicz, S., Filip, R., Ghishan, F. K., Gao, N., & Kiela, P. R. (2024). Transplant of microbiota from Crohn's disease patients to germ-free mice results in colitis. *Gut Microbes*, *16*(1), 2333483. https://doi.org/10.1080/19490976.2024.2333483 ⏎

Sousa, T., Paterson, R., Moore, V., Carlsson, A., Abrahamsson, B., & Basit, A. W. (2008). The gastrointestinal microbiota as a site for the biotransformation of drugs. *Biochemical Pharmacology*, *76*(10), 1353–1361. ⏎

Srinivasan, B., Kolli, A. R., Esch, M. B., Abaci, H. E., Shuler, M. L., & Hickman, J. J. (2015). TEER measurement techniques for *in vitro* barrier model systems. *Journal of*

Laboratory Automation, *20*(2), 107–126. ⏎

Tarzi, C. (2025). Towards genome-scale metabolic modeling of microbial communities and multi-omics data integration to predict human diseases states. Ph.D. Thesis, Teesside University. https://research.tees.ac.uk/ws/portalfiles/portal/94771740/PhD_Thesis_Tarzi_Chaimaa_Teesside_University.pdf

Thomson, C. A., Morgan, S. C., Ohland, C., & McCoy, K. D. (2022). From germ-free to wild: Modulating microbiome complexity to understand mucosal immunology. *Mucosal Immunology*, *15*(6), 1085–1094. https://doi.org/10.1038/s41385-022-00562-3 ⏎

Toh, M. C., Goodyear, M., Daigneault, M., Allen-Vercoe, E., & Van Raay, T. J. (2013). Colonizing the embryonic zebrafish gut with anaerobic bacteria derived from the human gastrointestinal tract. *Zebrafish*, *10*(2), 194–198. https://doi.org/10.1089/zeb.2012.0814 ⏎

Van den Abbeele, P., Roos, S., Eeckhaut, V., MacKenzie, D. A., Derde, M., Verstraete, W., *et al.* (2010). Microbial community development in a dynamic gut model is reproducible, compartment-specific and selective upon inoculation from fecal samples. *Applied and Environmental Microbiology*, *76*(15), 5237–5246. ⏎

Van Nuenen, M. H., Meyer, P. D., & Venema, K. (2003). The effect of antibiotics on the intestinal microbiota using the TIM-2 *in vitro* model. *Anaerobe*, *9*(4), 173–184. ⏎

Wallace, B. D., Wang, H., Lane, K. T., Scott, J. E., Orans, J., Koo, J. S., *et al.* (2010). Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science*, *330*(6005), 831–835. ⏎

Wampach, L., Heintz-Buschart, A., Fritz, J. V., Ramiro-Garcia, J., Habier, J., Herold, M., *et al.* (2017). Of bugs and brains: The microbiome–gut–brain axis *in vitro*. *Nature Communications*, *8*, 14889. ⏎

Wishart, D. S. (2016). Emerging applications of metabolomics in drug discovery and precision medicine. *Nature Reviews Drug Discovery*, *15*(7), 473–484. ⏎

Wrzosek, L., Ciocan, D., Borentain, P., Spatz, M., Puchois, V., Hugot, C., Ferrere, G., Mayeur, C., Perlemuter, G., & Cassard, A. (2018). Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. *Scientific Reports*, *8*(1), 6854. https://doi.org/10.1038/s41598-018-25300-3 ⏎

Zeng, J., Li, Y., Yan, J., Chang, R., Xu, M., Zhou, G., Meng, J., Liu, D., Mao, Z., & Yang, Y. (2024). Gut microbiota from patients with Parkinson's disease causes motor deficits in honeybees. *Frontiers in Microbiology*, *15*, 1418857. https://doi.org/10.3389/fmicb.2024.1418857 ⏎

Zhang, J., Li, C., Yin, Y., Zhang, J., & Grzegorzek, M. (2023). Applications of artificial neural networks in microorganism image analysis: A comprehensive review from conventional multilayer perceptron to popular convolutional neural network and potential visual transformer. *Artificial Intelligence Review*, *56*(2), 1013–1070. ⏎

Zhang, P., Li, L., Han, X., Li, Q., Zhang, X., Liu, J. J., & Wang, Y. (2020). Fecal microbiota transplantation improves metabolism and gut microbiome composition in db/db mice. *Acta Pharmacologica Sinica*, *41*(5), 678–685. https://doi.org/10.1038/s41401-019-0330-9 ⏎

Zhang, Q., Widmer, G., & Tzipori, S. (2013). A pig model of the human gastrointestinal tract. *Gut Microbes*, *4*(3), 193–200. https://doi.org/10.4161/gmic.23867 ⏎

Zhong, X., Li, J., Lu, F., Zhang, J., & Guo, L. (2022). Application of zebrafish in the study of the gut microbiome. *Animal Models and Experimental Medicine*, *5*(4), 323–336. https://doi.org/10.1002/ame2.12227 ⏎

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., & Goodman, A. L. (2019). Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature*, *570*(7762), 462–467. ⏎

4

Oral Microbiome Dysbiosis: Study Methods, Diseases, and Therapeutic Approaches for Restoration

Harshita Gupta, Shubham Sharma, Prerna Yadav, Rakesh Kumar Gupta, and Prerna Diwan

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4.1 Introduction

A complex microbial ecosystem that resides in or on the human body is referred to as the "Human Microbiome." Because of its vital role in human health and wellbeing, it has gained significant attention in recent years. The term "microbiome" was proposed by Nobel prize winner Joshua Lederberg (Lederberg & Mccray, 2001). The human microbiome is made up of microorganisms from every domain of life, including bacteria, viruses, eukarya, and archaea (Reynoso-García *et al.*, 2022). In humans, every individual has a distinct microbiome. It starts to develop at birth and stabilizes in the first few years of life as it gains exposure to environmental microbes (Dominguez-Bello *et al.*, 2010). There are around 3.8×10^{13} cells of microbes present in the human body, which make up about 0.2 kg of a 70 kg "reference man" (Sender *et al.*, 2016). Different body locations support different microbial communities due to variations in biological and physical features at each habitat (Costello *et al.*, 2009) (Figure 4.1). These microbial composition and diversity in each body niche are influenced by various factors like genetics, diet, lifestyle, and environmental exposure (Losasso *et al.*, 2018). Understanding the microbiome in

different body niches is important because it helps researchers to explore host microbiome interaction in health and disease conditions. For example, *Lactobacillus* species are predominantly found in the vaginal ecosystem. They produce antimicrobial molecules and lactic acid, which inhibit the growth of harmful microbes and maintain vaginal pH, respectively (Diop *et al.*, 2019). Different body niches have shown an approximately tenfold difference in estimated species richness. Stool samples have the highest estimated microbial richness, followed by samples from the mouth, throat, gums, skin, nasal, and vagina (Deo & Deshmukh, 2019; Huse *et al.*, 2012).

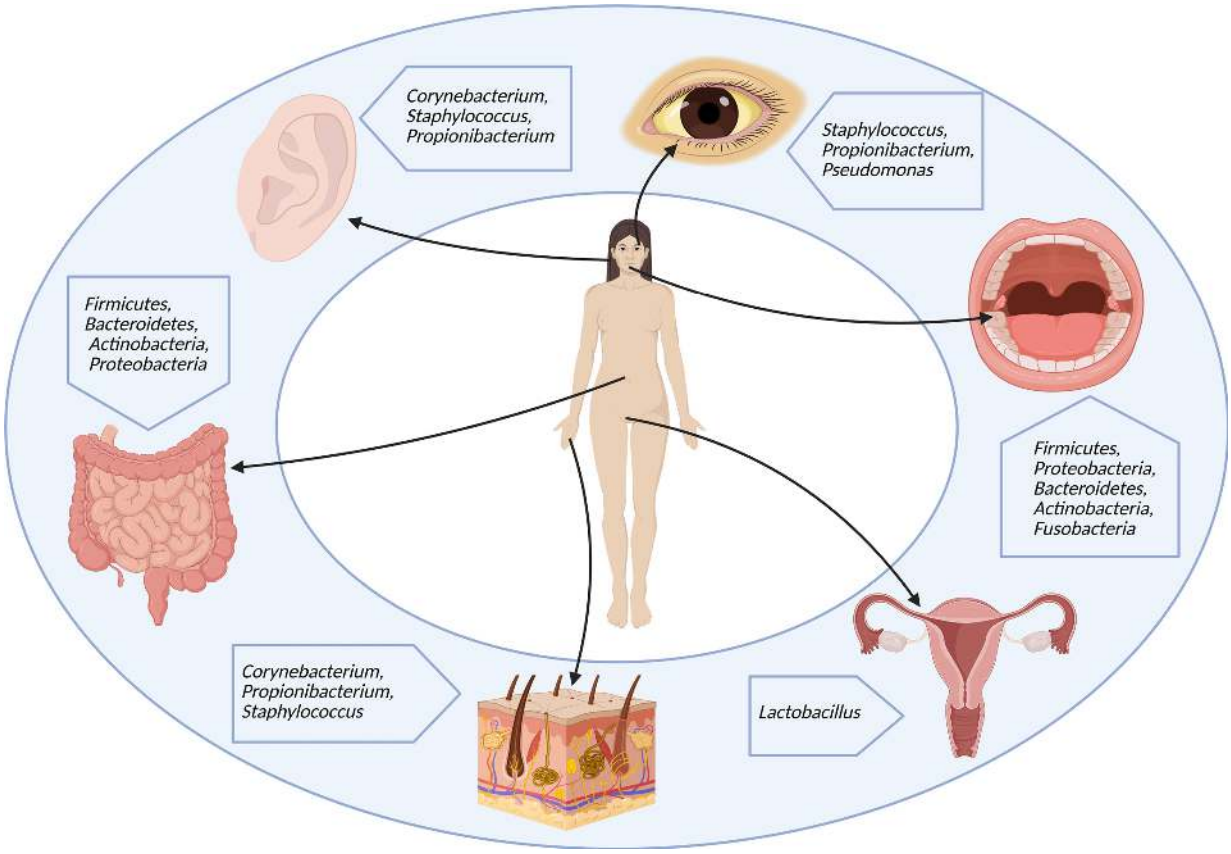

FIGURE 4.1 Bacterial genera across different sites of the human body (Ceccarani *et al.*, 2019; Chen *et al.*, 2021; Das *et al.*, 2018; Grice *et al.*, 2009; King *et al.*, 2019; Smythe & Wilkinson, 2023). ⏎

4.2 Oral Microbiome

Oral microbiome refers to the microorganisms found in the oral cavity. The oral cavity provides a variety of unique environments for the colonization of

microorganisms, including the tongue, cheek, lip, gingival sulcus, connected gingiva, hard palate, and soft palate (Dewhirst *et al*., 2010). The tongue has the most diverse range of microorganisms among all these areas. The warm, moist, and nutrient-rich environment in the oral cavity facilitates the growth of diverse microorganisms, including bacteria, fungi, viruses, archaea, and protozoa. The Human Oral Microbiome Database (HOMD) reports that more than 700 bacterial species inhabit various ecological niches of the oral cavity. These microbes interact symbiotically with the host and promote various health benefits (Lan *et al*., 2023a). Each site of the oral cavity has its own unique bacterial composition that interacts with the host cell and regulates various physiological processes in the human body (Ma *et al*., 2023). Saliva provides nutrients such as glycoproteins, proteins, and gingival crevicular fluid to support the growth of microbial communities (van't Hof *et al*., 2014). Bacterial species bind to these habitats' surfaces through different receptors and adhesion molecules, such as fimbriae (*Porphyromonas gingivalis* FimA), glucan-binding proteins (*Streptococcus mutans* GbpC), and LraI family proteins (*Streptococci*), which contribute to biofilm formation and microbial interactions (Jenkinson, 1994; Matsumoto-Nakano, 2018; Nakagawa *et al*., 2006).

The oral microbiome undergoes constant changes from birth to adulthood due to various factors such as delivery mode, feeding habits, hormonal changes, and environmental conditions. However, irrespective of changes, a resilient core microbial community typically persists to maintain oral health (Corrêa *et al*., 2024). The healthy human oral microbial community is dominated by members from the phyla *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes*, and *Fusobacteria*, while spirochaetes are present in smaller amounts. Among them, Streptococcus bacteria from the phylum Firmicutes are most abundantly present in the oral cavity (Lee *et al*., 2021; Verma *et al*., 2018; Zaura *et al*., 2009). The oral microbiome shows site-specific distribution. Bacterial species belonging to the genus *Neisseria* were found more abundant in saliva and tongue dorsum than in supragingival plaque. *Porphyromonas, Rothia*, and *Actinomyces* were found in abundance in saliva samples, whereas *Streptococcus, Veillonella, Haemophilus, Granulicatella, Oribacterium*, and *Bulleidia* were found predominantly on the tongue. *Capnocytophaga* was present only in supragingival plaque, whereas *Fusobacterium* was more abundant on the tongue and plaque than in saliva (Dong *et al*., 2018). The oral microbiome is inhabited by both indigenous (resident) and exogenous (transient) bacterial communities. Indigenous communities consist of species that are prevalent in most of the adults as they are very well adapted to the oral environment and maintain oral health, whereas the exogenous communities are temporary residents of

the oral cavity that may be introduced through food, contact, or environmental exposure. Though they are not permanent residents of the oral cavity, they can interact with the resident oral microbiome and potentially influence oral health (Dahlén, 2009).

To better understand the microbial communities within the human body and their impact on health and disease, several databases has been created. The Human Microbiome Project (HMP) (www.hmpdacc.org) is a large-scale effort within the scientific community to better understand the communities of microbes within the human body and their role in health and disease. It was launched in 2017 by the National Institute of Health (NIH). It provides many resources for researchers, such as genomic information, datasets, and bioinformatic tools, to understand the dynamics of human microbiome-related health conditions. By 2017, HMP researchers published more than 650 publications and created a foundation for ongoing human microbiome research across various institutes within the NIH. HMP contributed to the development of organ-specific databases, including HOMD and Metagenomics of Human Intestinal Tract (MetaHIT). HOMD provides information of microbiome present in the pharynx, sinuses, nasal passages, esophagus, and oral cavity. CORE is another 16S rRNA-based database of the oral microbiome, which is used to characterize the bacterial taxa (Griffen *et al.*, 2011). These initiatives were made to create a map, understand, and share human microbiome knowledge, which could help us to improve human healthcare, like diagnostics, therapies, and a deeper understanding of human biology.

4.3 Oral Dysbiosis

Oral dysbiosis refers to the disruption or imbalance of the microbiota within the oral cavity, resulting from environmental changes in the mouth caused by factors such as oral hygiene, smoking, chewing betel quid/nut, alcohol consumption, and other lifestyle habits (Lee *et al.*, 2021; Sudhakara *et al.*, 2018). These factors favor the colonization of pathogenic bacteria in the oral cavity which influence critical metabolic pathways linked to immune activation, cell growth oxidative stress (Praveen *et al.*, 2024), and lead to development of many systemic and oral diseases including periodontitis (Di Stefano *et al.*, 2022), dental caries (Zhang *et al.*, 2022), oral cancers (Gholizadeh *et al.*, 2016), other cancers (Fan, Alekseyenko, *et al.*, 2018), diabetes (Long *et al.*, 2017), cardiovascular disease (CVD) (Li *et al.*, 2022),

rheumatoid arthritis (RA) (Bingham & Moni, 2013), and Alzheimer's disease (Poole *et al*., 2013) (Figure 4.2).

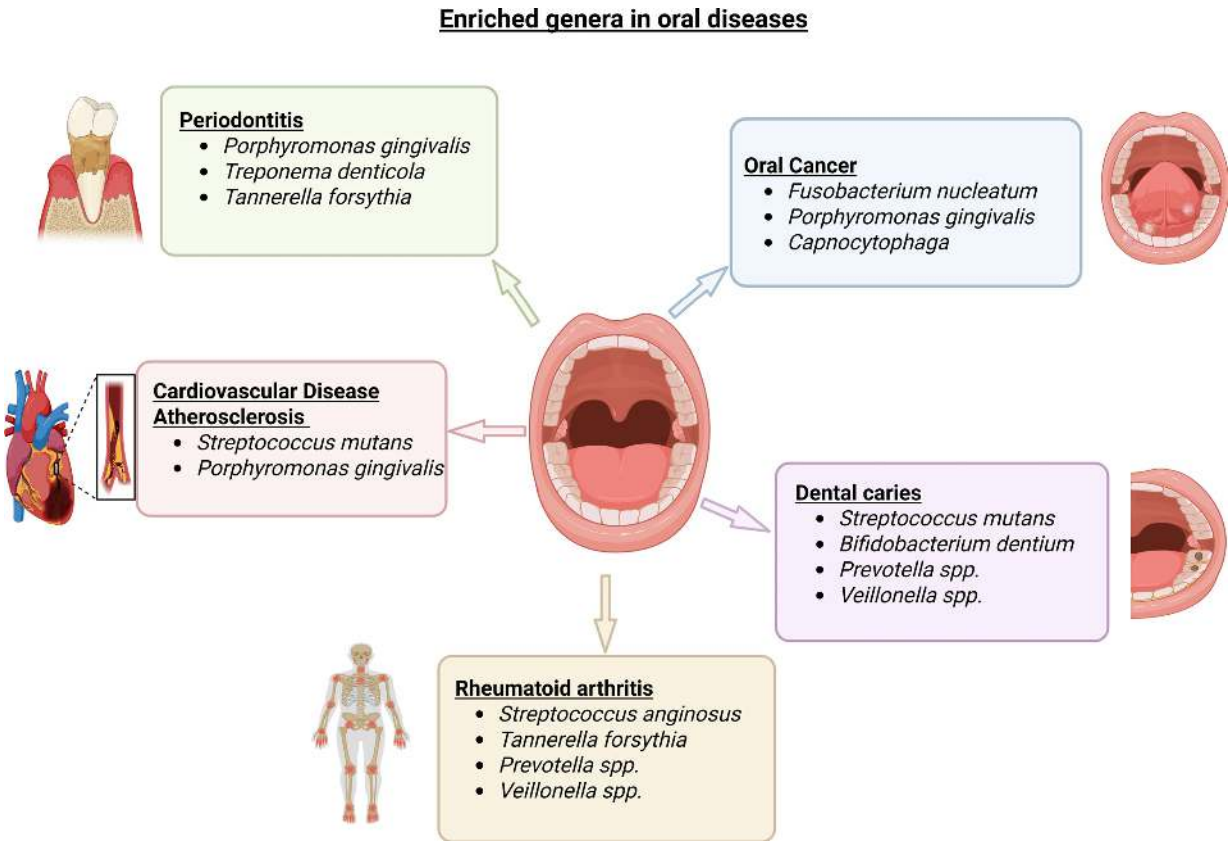

FIGURE 4.2 Dysbiosis of oral microbiome contributes to onset of oral and systemic diseases. ⏎

4.4 Oral Microbiome and Dental Caries

Dental caries, commonly known as cavities or tooth decay, is the most prevalent chronic condition of the oral cavity worldwide. It is a complex, multifactorial disease that develops from the interplay between tooth structure, microbial biofilm dominated by acid-producing bacteria, sugars, salivary, and genetic factors, and is closely linked to the oral microbiome (Alshayeb & Dashash, 2025; Zhang *et al*., 2022). Microscopy and cultivation technology have found that the bacterial species *S. mutans* is a key pathogen responsible for dental caries (Adler *et al*., 2017), but advancements in sequencing technologies have revealed that the presence of *S. mutans* is not the only causative factor of dental caries, and other bacterial genera such as *Lactobacillus, Actinomyces, Bifidobacterium, Veillonella*, and

Propionibacteria also play a crucial role in the infection (Liu *et al.*, 2025). An *in vivo* experiment using 16S rRNA gene Illumina sequencing reported that Gram-negative anaerobes, including *Veillonella* and *Prevotella*, were predominant in active caries lesions (da Costa Rosa *et al.*, 2021). Another study analyzed the composition of microbiota depending on different locations in root caries lesions, and found that *Prevotella* was the most dominant genus of caries lesions that extended to the subgingival margin, while *Streptococcus* was dominant in the supragingival site of the lesion (Takenaka *et al.*, 2021).

4.5 Oral Microbiome and Periodontitis

Gingivitis is a reversible inflammatory disease caused by poor hygiene. It is the most prevalent form of periodontal disease among adult individuals. The clinical symptoms and signs of gingivitis are the continuous formation of microbial biofilm at the gingival margin. *Leptotrichia, TM7, Streptococcus, Selenomonas, Prevotella, Veillonella, Lautropia*, and *Haemophilus* are the most dominant taxa found in gingivitis and may serve as its biomarker (Huang *et al.*, 2011). Untreated gingivitis will progress into irreversible inflammatory chronic periodontitis disease of the oral cavity, which causes tooth loss in adults. Gingival inflammation, radiographic assessment, resorption of alveolar bone, clinical attachment loss, presence of periodontal pocket, teeth mobility, premature teeth loss, and gingival bleeding upon probing are clinical signals of periodontal disease (Di Stefano *et al.*, 2022). An unbalanced diet, smoking, and poor oral hygiene are the most important factors that promote dysbiosis of the oral microbiome and the development of periodontitis (Di Stefano *et al.*, 2022; Nowicki *et al.*, 2018). This disease is initiated by the accumulation of primary colonizers, predominantly facultative anaerobes and Gram-positive aerobes such as *Streptococci*, on the tooth surface. This early colonization is followed by late colonizers, which are predominantly Gram-negative members of the "red complex", that is, *Tannerella forsythia, Treponema denticola*, and *P. gingivalis* (Sudhakara *et al.*, 2018). This red complex is one of the groups of bacteria known to increase in number as periodontitis develops, being the cause of this disease. However, they do not act alone, but with involvement of other groups of bacteria, known as the orange (*Fusobacterium, Prevotella, Peptostreptococcus, Streptococcus, Eubacterium*, and *Campylobacter*), purple (*Veillonella* and *Actinomyces*), blue (*Actinomyces israelii, A. naeslundii, A. odontolyticus, A. gerencseriae, A. oris*), green (*Eikenella corrodens, Capnocytophaga, Aggregatibacter actinomycetemcomitans*),

and yellow complexes (*Streptococcus mitis, S. oralis, S. sanguinis*), and others often found alongside them (Łasica *et al*., 2024).

4.6 Oral Microbiome and Cardiovascular Disease

Cardiovascular disease (CVD) refers to the disease of the circulatory system, including endocarditis, myocardial infection, atherosclerotic diseases, heart failure, rheumatic heart disease, and stroke (Nabel, 2003; Tonelli *et al*., 2023). Oral dysbiosis has been increasingly recognized as a contributing factor in the development of CVD (Men *et al*., 2024). According to Tonelli *et al*. (2023), oral dysbiosis may cause CVD through the following mechanisms: endothelial dysfunction, altered immune response, platelet activation and aggregation, thrombosis, arterial invasion, molecular mimicry and antibody cross-reactivity, protein citrullination, and systemic inflammation (Tonelli *et al*., 2023). Oral pathogens such as *P. gingivalis, Fusobacterium nucleatum, A. actinomycetemcomitans, T. denticola*, and *Tannerella* may cause systemic inflammation, leading to vascular damage due to the virulence factor of the pathogen, which further contribute to the development of atherosclerosis and endothelial dysfunction (Gualtero *et al*., 2023). Research has implicated a higher incidence of severe CVD (e.g., stroke, endocarditis, myocardial infection, and atherosclerosis) in individuals suffering from periodontitis (Carra *et al*., 2023; Zheng *et al*., 2023). It has been found that specific oral bacteria, including *S. mutans* and *P. gingivalis*, significantly increase in patients with periodontitis and atherosclerosis (Lucchese, 2017; Xie *et al*., 2020). Managing oral dysbiosis in a clinical setting may play a valuable role in the management of CVD (Tonelli *et al*., 2023).

4.7 Oral Microbiome and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune, inflammatory disease (Pisano *et al*., 2023). Bone deterioration, inflammation in the joint lining, immunological activation, and production of autoantibodies are some of the characteristics of RA (Chu *et al*., 2021). It is well established that oral microbial dysbiosis increases a person's risk of developing RA (Tong *et al*., 2020). Multiple studies consistently report an increased abundance of genus *Prevotella* in patients with RA, pointing to its potential role in disease development (Gao *et al*., 2022; Kroese *et al*., 2021). Several anaerobic bacteria that are typically found in the oral cavity and that are associated with periodontal disease, such as *Lactobacillus salivarius,*

Cryptobacterium curtum, Atopobium, various *Prevotella* species (*P. melaninogenica, P. denticola, P. histicola, P. nigrescens, P. oulorum, P. maculosa*), *Selenomonas noxia, Selenomonas sputigena, Anaeroglobus geminatus*, and *Leptotrichia*, were found to be higher in the saliva of RA patients than healthy individuals (Pisano *et al.*, 2023). The literature also suggests that *P. gingivalis* may have a significant role in RA development (Kulkarni *et al.*, 2022). These findings raise the possibility of these bacteria being more important in exacerbating RA in individuals who are not yet RA patients.

4.8 Oral Microbiome and Cancer

Cancer is the leading cause of mortality worldwide, with an approximate 47% increase in cancer cases over the next 20 years (from 2020 to 2040) (Lan *et al.*, 2023b; Zhong *et al.*, 2025). Recent evidence suggests that the composition and diversity of the oral microbiome may be involved in the onset and progression of various types of cancer, including oral, oro digestive, breast, lung, colorectal, and even distant cancers (Atanasova & Yilmaz, 2014; Martínez *et al.*, 2022). For example, *P. gingivalis*, and some members of the *Prevotella, Fusobacterium*, and *Streptococcus* group have been suggested as potential contributors to this association (Atanasova & Yilmaz, 2014; Bowland & Weyrich, 2022; Sudhakara *et al.*, 2018). These specific microbial species have been found to contribute to the development of chronic inflammatory conditions, such as periodontal disease. This chronic inflammation condition has the potential to create a pro-tumorigenic environment through the release of inflammatory mediators, growth factors, and enzymes (Atanasova & Yilmaz, 2014; Bowland & Weyrich, 2022). Microorganisms of the oral cavity can contribute to the development of tumors through different means: first, they induce chronic inflammation, which facilitates tumor initiation and progression; second, they may disrupt the normal regulation of cell cycle and signaling pathways; and third, they are capable of metabolizing various substances involved in tumor development and recurrence. Furthermore, they can modulate the host immune response (Sawant *et al.*, 2023).

Oral cancers refer to cancers that occur in the lips and mouth (such as the tongue, gums, floor of the mouth, and palate). It is the 13th most common cancer type globally, with 377,713 new cases diagnosed in 2020, resulting in 177,757 deaths in the same year (Sung *et al.*, 2021). Smoking and alcohol consumption are the leading risk factors for oral cancer in many parts of the world; however, in several Southeast

Asian countries, as well as many Pacific islands, smokeless tobacco and areca nut chewing are the predominant etiological agents for oral cancer (Rumgay *et al.*, 2024). Approximately 90% of oral cancers arise from oral squamous cell carcinomas (OSCCs). Other types of oral cancers, such as lymphomas and melanomas, make up a smaller percentage of cases (Smędra & Berent, 2023). Several literature reviews and meta-analyses of case-control studies showed that periodontal disease increases the risk of oral cancer development (Farhad *et al.*, 2024; Kavarthapu & Gurumoorthy, 2021). Multiple studies reported compositional variation in oral microbiome in OSCC patients and healthy individuals (Table 4.1). In their research, Saxena *et al*. employed 16S rRNA gene sequencing and explored oral microbiome changes in both healthy and OSCC patients. They found that several Gram-negative genera, including *Prevotella, Capnocytophaga*, and *Fusobacterium*, were significantly differentially abundant in OSCC patients and could be used as a potential microbial biomarker for OSCC (Saxena *et al.*, 2022). Another study also analyzed alterations of the oral microbiome; it revealed that genus *Capnocytophaga, Fusobacterium, Leptotrichia, Neisseria, Bergeyella, Mycoplasma, Johnsonella*, and *Staphylococcus* were enriched in OSCC patient samples (Herreros-Pomares *et al.*, 2023). Beyond genus-level associations, multiple studies have identified specific bacterial species, such as *Fusobacterium nucleatum, P. gingivalis*, and *Prevotella intermedia*, which are enriched in OSCC samples (Sukmana *et al.*, 2024; van Dijk *et al.*, 2024). A few studies have analyzed the alterations of the oral microbiome during the progression of oral cancer; they found that Stage 4 patients have significantly higher complexity than healthy individuals, and *Fusobacteria* significantly increase with the progression of oral cancer from stages 1 to 4 (Yang *et al.*, 2018). While in another study, *Capnocytophaga* was strongly associated with late-stage cancer and showed high predictive accuracy, whereas *Fusobacterium* was linked to early cancer stages (Pratap Singh *et al.*, 2023). Table 4.1 summarizes key studies that have reported increased and decreased bacterial microorganisms with OSCC, highlighting common dysbiosis patterns.

TABLE 4.1

Altered Diversity of Oral Bacteria in OSCC in Human Studies

Type of Sample	Sample Size and Categories	Method of Sequencing	Enriched Phyla and Genus in OSCC	Decreased Phylum and Genus in OSCC	Reference

Type of Sample	Sample Size and Categories	Method of Sequencing	Enriched Phyla and Genus in OSCC	Decreased Phylum and Genus in OSCC	Reference
Oral swabs	34 OSCC and 32 healthy	16S rRNA gene sequencing V3 hypervariable region	Phylum: Bacteroidetes, Fusobacteria	Phylum: Firmicutes	Saxena *et al.* (2022)
Biopsies	Homogeneous leucoplakia (9), proliferative verrucous leukoplakia (PVL (12), PVL- OSCC (8), OSCC (10), healthy (11)	16S rRNA gene sequencing V3–V4 hypervariable region.	Phylum: Fusobacteriota, Bacteroidota Campylobacterota Genus: *Fusobacterium, Prevotella, Capnocytophaga, Campylobacter*	Phylum: Proteobacteria, Firmicutes, Spirochaetota Genus: *Porphyromonas, Haemophilus, Streptococcus, Treponema*	Herreros-Pomares *et al.* (2023)
Oral swabs	Oral benign tumours (5), OSCC (22) and healthy (15)	16S rRNA gene sequencing V3–V5 hypervariable region.	Phylum: Proteobacteria, Actinobacteria	Phylum: Bacteroidetes, Firmicutes, Fusobacteria	Yang *et al.* (2022)

Type of Sample	Sample Size and Categories	Method of Sequencing	Enriched Phyla and Genus in OSCC	Decreased Phylum and Genus in OSCC	Reference
Oral swabs	Surface of OSCC lesion and its contralateral normal tissue from discovery (*n* = 74) and validation (*n* = 42) cohort of male patients with cancers of the buccal mucosa.	16S rRNA gene sequencing V3 hypervariable region	Genus: *Fusobacterium, Peptostreptococcus, Campylobacter Prevotella, Capnocytophaga*	Genus: *Streptococcus*	Su *et al.* (2021)
Saliva	Healthy (10), OSCC (10) and long-term tobacco chewers (10)	16S rRNA gene sequencing V6–V8 hypervariable region	Phylum: Bacteroidetes Genus: *Prevotella, Neisseria, Porphyromonas, Fusobacterium*	Phylum: Firmicutes Genus: *Streptococcus, Rothia, Haemophilus*	Sawant, Dugad, Parikh, and Srinivasan, *et al.* (2021)

Type of Sample	Sample Size and Categories	Method of Sequencing	Enriched Phyla and Genus in OSCC	Decreased Phylum and Genus in OSCC	Reference
Tissue from cancerous and Para cancerous lesions	Patients at different stage of OSCC (24) and healthy (15)	16S rRNA gene sequencing. V3–V4 region	Phylum: Firmicutes, Bacteroidetes, Fusobacteria, Spirochaetes Genus: *Fusobacterium, Treponema, Streptococcus, Peptostreptococcus, Carnobacterium, Tannerella, Parvimonas, Filifactor*	Phylum: Proteobacteria, Actinobacteria, Cyanobacteria Genus: *Streptophyta, Brevundimonas, Paenibacillus, Microbacterium, Desulfovibrio, Mucispirillum*, *Arthrobacter*	Zhou *et al.* (2020)
Saliva	OSCC (41), Leukoplakia (25), postoperative OSCC (20)	16S rRNA gene sequencing V4 hypervariable region	Phylum: Fusobacteria, Bacteroidetes Genus: *Fusobacterium*	Phylum: Firmicutes Genus: *Streptococcus*	Hashimoto *et al.* (2022)

4.9 Causative Agents of Oral Microbiome Dysbiosis

Although oral microbiome dysbiosis can result from a variety of factors, this section specifically focuses on tobacco, alcohol, and betel nut or betel quid discussion, as these are among the most widely consumed psychoactive substances globally. Their extensive use by a variety of groups and geographical areas contributes significantly to the burden of disease associated with alterations of the oral microbiota.

4.9.1 Tobacco

Tobacco is used in two forms, the most common way is to consume it by smoking, and another is to consume without burning as smokeless tobacco (Halboub *et al.*, 2020). The chemical constituents and bacterial communities of tobacco alter the oral microbiome, which leads to dysbiosis and increases the risk of oral cancer (Al Kawas

et al., 2021; Chattopadhyay *et al.*, 2024; Suzuki *et al.*, 2022). Oral microbiome alterations have been consistently observed in individuals who consume tobacco products. The effects of smokeless tobacco products (STPs) on oral bacteria have shown that STPs encourage the growth of harmful bacteria, including *S. mutans, S. salivarius*, and *S. sanguinis* (Sajid *et al.*, 2021). The changes are drastic; some of the bacterial species get depleted, and some of them increase, which are generally pathogenic (Kadam *et al.*, 2021). A study on tobacco use revealed that its consumption is linked to the depletion of beneficial bacteria such as *Lactobacillus* and *Haemophilus* while increasing the abundance of opportunistic pathogens like *Neisseria subflava* and *Porphyromonas endodontalis* (Senaratne *et al.*, 2024). Tobacco use is found to induce dysbiosis that can create a pro-inflammatory microenvironment in the oral cavity, influencing key carcinogenic processes (Senaratne *et al.*, 2024). The change in the oral environment is very evident because of tobacco use that these microbial changes can be a potential biomarker. Specific bacterial genera, such as *Leptotrichia, Treponema, Lautropia*, and *Cardiobacterium*, are enriched in tobacco chewers, while others like *Pseudomonas, Capnocytophaga, Bifidobacterium, Peptostreptococcus, Paludibacter* and *Mycoplasma* are enriched in patients with oral cancer, suggesting potential biomarkers for oral cancer (Sawant *et al.*, 2023). In another study, smokeless tobacco use, such as Toombak, modifies the oral microbiome into a dysbiotic state, increasing the risk for oral diseases like cancer (Sami *et al.*, 2023). Cigarette smoking also significantly alters the oral microbiome, leading to a pro-inflammatory environment and changes in bacterial profiles, which may contribute to various oral health issues (Al Kawas *et al.*, 2021). Another study explored the composition of the tongue and salivary microbial communities of smokers and non-smokers in healthy adults (Suzuki *et al.*, 2022). Therefore, it is important to understand the changes occurring and the relationship between tobacco use, oral microbiome, and oral cancer development for developing targeted strategies for the prevention and treatment (Kadam *et al.*, 2021).

4.9.2 Alcohol

As the initial site of alcohol exposure, the oral cavity is vulnerable to its effects, potentially leading to conditions such as reduced saliva production, decreased oral pH, enamel disruption, cavities, and periodontal inflammation. The main component of alcohol is ethanol, which is known to have cytotoxic effects on bacteria and affect the oral microflora (Liao *et al.*, 2022). Alcohol influences the oral microbiota through various mechanisms, such as its direct toxic effects on bacteria, disruption of

saliva–bacterium interactions, and the toxic effects of ethanol (Fan, Peters, *et al.*, 2018; Fiore *et al.*, 2023; O'Grady *et al.*, 2020). A study of 1,044 US adults revealed that heavy drinkers had altered oral microbiome diversity, reduced *Lactobacillales*, and increased *Actinomyces, Leptotrichia, Cardiobacterium, Neisseria*, and some other oral pathogens (Fan, Peters, *et al.*, 2018). A meta-analysis of 4,636 individuals concluded that people who consume alcohol have more red-complex bacteria (*P. gingivalis*) and orange-complex bacteria (*F. nucleatum*) in their subgingival microbiota compared to non-consumers (Oliveira *et al.*, 2023).

4.9.3 Betel Nut and Betel Quid

Another causative agent and the most widely used substance is betel quid. It specifically consists of betel leaf combined with areca nut and slaked lime, with or without tobacco (Diwan *et al.*, 2023). The components of betel quid interact with the oral epithelium and induce molecular alterations that can lead to the initiation and progression of oral cancer. The high prevalence of oral cancer in South Asia is strongly associated with betel quid chewing, a major etiological factor and a serious public health concern. Studies estimate that approximately 10% of the global population consumes betel quid, with 20–40% of individuals in South Asian countries engaged in this habit (Islam *et al.*, 2019).

Chewing betel quid has been shown to change the composition of the oral microbiome, altering the relative abundance of many different genera of bacteria. For example, Hernandez *et al.* (2017) showed that with betel nut chewing, there is a decrease in microbial richness and evenness with significant changes in the number of bacteria from the genera *Streptococcus* and *Actinomyces* (Hernandez *et al.*, 2017). In contrast, Drucker (2016) showed that the diversity of microorganisms in the oral cavity increases significantly when chewing betel quid. Additionally, recent research demonstrates that there are significant changes in the composition of the oral microbiome of betel quid users, with changes in genera such as *Prevotella* and *Bacteroides*, which may correlate with an increased risk for developing oral cancer (Bahuguna *et al.*, 2023). Furthermore, the oral microbiome dysbiosis caused by betel quid chewing has been suggested as a potential driver and biomarker for oral cancer (Diwan *et al.*, 2023). According to research carried out in Sri Lanka, a notable decrease in the prevalence of *Streptococcus* in betel quid chewers was observed, which may be associated with oral diseases such as oral cancer and periodontitis (Uehara *et al.*, 2021). Moreover, the dysbiosis of the oral microbiome caused by betel

quid chewing has been suggested as both a possible contributing factor and a potential indicator of increased risk of oral cancer.

4.10 Methodological Approaches to Oral Microbiome Analysis

As discussed above, oral microflora influenced by the lifestyle of an individual has a significant impact on health and disease progression. The impact is not limited to oral conditions like dental caries, periodontitis, and oral cancer; moreover, its vital role has been studied in systemic conditions such as CVD and RA. Therefore, it is important to study oral microflora to gain a deeper understanding of the microbiome, associated genes, and their regulation. Such studies will help in identifying biomarkers and developing therapeutics. Different methods have been used to characterize the composition of the oral microbiome, including culture-based analysis on artificial media, biochemical assays, microscopy, immunoassays, gel-based and PCR-based techniques, and next-generation sequencing (NGS; Table 4.2). This section presents a review of these methods.

4.10.1 Culture-dependent Approaches

The principal and traditional method of characterizing the oral microbiome is a culture-dependent technique. The approach requires a specific substrate for culturing oral bacteria, and further characterization is based on differential staining, biochemical tests, antibiotic sensitivity tests, sugar utilization, examination of metabolic end products, and analysis of cell membrane composition (Krishnan *et al.*, 2017; Paster & Dewhirst, 2009).

This method is used to examine the differences in microbial flora between individuals with OSCC and healthy controls, finding distinct microbial profiles in both mucosal and saliva samples (Metgud *et al.*, 2014) as well as to explore the microbial diversity in different sites of the oral cavity in oral cancer patients before and after radiation therapy (Anjali *et al.*, 2020). A study utilized blood agar supplemented with selective Campylobacter medium to detect and compare the prevalence of *Helicobacter pylori* in oral samples from patients who consumed betel quid (Fernando *et al.*, 2009). However, so far, only about 50% of the 700 bacterial species that exist in the human mouth have been successfully cultured, and the rest remain difficult to grow due to complex biofilm interactions and specific nutrient needs (Benn *et al.*, 2018). Hence, the culture-based methods independently cannot reveal the full diversity of the oral microbiome. Additionally, these methods are

costly and time-consuming, as diverse growth requirements need to be met and many microbial species cannot grow in laboratory conditions, therefore making this approach unsuitable for studies that aim to reveal the whole microbiome profile (Pozhitkov *et al*., 2011).

4.10.2 Gel-based Approaches

Temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE), and restriction fragment length polymorphism (RFLP) techniques are the gel-based approaches used to explore the diversity of bacteria, fungi, and protozoa in the samples (Deng *et al*., 2008). DGGE and TGGE can separate DNA of equal size and then can be sequenced for identification (Krishnan *et al*., 2017). Another advantage of DGGE is the simultaneous analysis of multiple samples, enabling monitoring of community changes over time. Unlike RFLP, DGGE separates fragments based on melting behavior, making it more discriminating (Deng *et al*., 2008). Fujimoto *et al*. used DGGE for the analysis of the microbial community of subgingival plaque of the oral cavity (Fujimoto *et al*., 2003). Another study used the DGGE technique for the analysis of microbial community in the pocket of periodontitis before and after the treatment (Zijnge *et al*., 2003). However, DGGE is limited in detecting the complete profile of the sample, and such an approach requires careful interpretation (Deng *et al*., 2008). The terminal restriction fragment length polymorphism (T-RFLP) technique is useful for the assessment and comparison of oral microbiota between healthy subjects and patients with periodontitis (Sakamoto *et al*., 2005). A study in 2003 used T-RFLP for the characterization of oral microflora from periodontitis patients and healthy individuals and it suggested that T-RFLP analysis is useful for rapid comparison of the microbial community between individuals with and without periodontitis (Sakamoto *et al*., 2005).

4.10.3 PCR-based Approaches

Conventional PCR, real-time PCR (RT-PCR), and multiplex PCR methods are available for the identification of microbes in clinical samples. Many oral samples have been studied through the PCR method to detect specific species related to disease. Species or phylotype-specific PCR primers were used in PCR reactions to detect the prevalence of target species in healthy and diseased subjects (Kumar *et al*., 2003).

In multiplex PCR, more than one pair of species-specific primers is used in a single PCR reaction, which allows for the simultaneous detection of multiple species. It is rapid, cost-effective, and a valuable tool for the clinical diagnosis of pathogenic bacterial infection in the oral cavity (Mohammed, 2023). Such an assay has been used for simultaneously detection of *Actinobacillus actinomycetemcomitans, P. gingivalis*, and *P. intermedia* in subgingival plaque samples of patients with periodontitis (García *et al*., 1998). Lochman and colleagues used a multiplex RT-PCR method to detect and quantify the 10 bacterial species associated with periodontal disease or caries and used as a tool for intervention and thus prevention of periodontal and caries diseases (Lochman *et al*., 2019). Another study identified and quantified five bacterial species: *A. actinomycetemcomitans*, *F. nucleatum*, *P. gingivalis*, *T. denticola*, and *T. forsythia* relevant to periodontal diseases (Coffey *et al*., 2016).

RT-PCR method is used to detect as well as quantify the genes/bacteria in clinical samples. It uses the SYBR Green dye or TaqMan probe to quantify the bacteria in microbial samples. Using RT- PCR six periodontal bacteria have been identified and quantified: *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, P. intermedia*, and *Prevotella nigrescens* (Tanaka *et al*., 2004). Quantification of eight oral bacteria in oral cancer patients, healthy, and tobacco chewers was possible by the SYBR green-based RT-PCR assay, and the method appeared to be highly sensitive, specific, and reproducible to quantify bacterial population and to understand the progression and prognosis of cancer (Sawant, Dugad, Parikh, & Singh, 2021). In another study, TaqMan RT-PCR assays with specific primers detected and quantified oral treponemes in periodontitis patients (Asai *et al*., 2002). qPCR specificity is determined by primer design, which controls the amplification of target genes. It also identifies taxonomic or functional gene markers, from broad domains to specific species or phylotypes in a mixed community (Sawant, Dugad, Parikh, & Singh, 2021).

4.10.4 Hybridization-based Approach

Fluorescence in situ hybridization (FISH), checkerboard hybridization, and oligonucleotide microarray are some of the DNA–DNA hybridization technologies used to characterize bacterial association with oral health and disease (Paster & Dewhirst, 2009).

FISH can be used to quantify, determine the spatial distribution, and identify the morphology of individual bacterial cells in complex microbial communities (Amann

et al., 1995). It can also be used as a prognostic and diagnostic tool in oral cancer treatment (Sunil *et al.*, 2013). FISH method enabled the identification of *P. gingivalis* and *Bacteroides forsythus* bacteria in subgingival plaque samples (Gersdorf, Meissner, *et al.*, 1993; Gersdorf, Pelz, *et al.*, 1993).

Checkerboard hybridization is also referred to as reverse capture, 16S rRNA-based oligonucleotide checkerboard hybridization. It involves the hybridization of DNA from an oral sample against the labelled probes representing whole genomes or 16S ribosomal RNA genes of known microbes (Pozhitkov *et al.*, 2011). This method is used to examine the type, number, and composition of microbiota in saliva and supragingival and subgingival plaque from healthy and periodontitis patients (Darout *et al.*, 2002; Ximénez-Fyvie *et al.*, 2000).

Microarrays contain oligonucleotide probe which target DNA and RNA genes in a biological sample to identify microbes in the oral cavity (Colombo *et al.*, 2009; Ma *et al.*, 2015) and investigate the expression of genes (Paster *et al.*, 2006).

4.10.5 Omics Approaches

Omics approaches provide comprehensive information of the whole microbiome and have opened opportunities for oral-health-related research. With the advancement of sequencing technologies, we can analyze the genes and their expression in healthy and diseased individuals induced by the oral microbiome. Though microarray is a targeted approach for characterizing the genes, there are several advantages of using sequencing instead of microarray hybridization. First, sequencing does not require knowledge of the targets, and second, it gives a digital signal rather than analog signal, which only tells about the presence and absence of gene sequence in a sample. Third, we can compare the sequencing result to the reference DNA databases for the putative identification of genes (Pozhitkov *et al.*, 2011). For more than a decade, NGS has been used to sequence the 16S rRNA gene, but more recently, metagenomics, metatranscriptomics, metaproteomics, and metabolomics approaches have provided greater evidence regarding microbial functionality in the oral cavity.

4.10.5.1 Metagenomics

Metagenomics involves the isolation and purification of DNA in a sample, and it reveals all bacteria in a sample, including the unculturable bacteria, based on 16S rRNA. 16S rRNA is present in all bacteria and archaea, containing nine hypervariable regions (V1–V9), out of which V3–V4 regions have the maximum discriminating ability for characterizing various bacterial groups, revealing the

taxonomic composition at the genus or species level (Chattopadhyay *et al.*, 2019). It is used to investigate the specific oral microbiome differences associated with different sites when transition of healthy mucosa to oral dysplasia occurs (Radaic *et al.*, 2023; Bahuguna *et al.*, 2023) and also unveil the microbial profile in the saliva of oral cancer patients before and after treatment (Mäkinen *et al.*, 2023). A study in China used 16S rRNA sequencing to know the distribution of oral microbiota in healthy individuals and children with early childhood caries (ECC), and they found that the genera *Actinomyces, Bifidobacterium*, and *Abiotrophia* play a crucial role in the transition from healthy to ECC states (Xu *et al.*, 2023). This approach is used extensively for clinical and research applications, but the major disadvantage is the short length of the V3–V4 region, which is around 460 base pairs, resulting in low taxonomic resolution (Zaura, 2012). In addition, biases can get introduced at various steps of the workflow, including sample collection, DNA extraction, PCR amplification, and sequencing (Statnikov *et al.*, 2013). Other factors like primer selection, PCR conditions, and the sequencing platform used can also create biasness leading to the over- or under-representation of certain taxa (D'Amore *et al.*, 2016). Additionally, the limitation of 16S rRNA as a phylogenetic marker is to distinguish closely related species reliably, that might complicate the interpretation of community composition data (Boers *et al.*, 2019; D'Amore *et al.*, 2016; Johnson *et al.*, 2019).

Compared to the 16S rRNA sequencing method, Whole Metagenome Sequencing (WMS) approach provides a more accurate taxonomic and functional profile (Qian *et al.*, 2020). This involves sequencing the whole DNA present in a sample which can be derived from bacteria, viruses, fungi, protozoa, archaea, and the host genome. WMS has emerged as a powerful tool for the comprehensive analysis of the microbial communities within the human oral cavity, providing valuable insights into the complex and dynamic ecosystem of the oral microbiome (Fakhruddin *et al.*, 2019; Liu *et al.*, 2012). This method enables the characterization of the entire genomic content of a microbial community (Oh *et al.*, 2014), allowing the identification of both known and previously uncharacterized microorganisms, their genes, functional capabilities, and metabolic pathways (Chaudhari *et al.*, 2023). WMS is being used for the identification of undiagnosed and rare diseases, and to identify cancer drivers and biomarkers (Bagger *et al.*, 2024).

4.10.5.2 Metatranscriptomics

Metatranscriptomes represent the total mRNA encoded by the microbial community of the sample and tell about the metabolic activities of the microbiome in a host.

Compared to amplicon sequencing, it focuses on transcriptionally active genes, providing a more comprehensive analysis of microbiomes and their interactions with host cells (Jain *et al*., 2023). Clinical samples can be compared through a metatranscriptomics approach to see the difference in gene expression between healthy and diseased individuals. Examining the collective gene expression of the oral microbiome through metatranscriptomics also helps us pinpoint the environmental factors that trigger a change in the microbiome's metabolic activity, leading to a shift from a healthy to a disease-associated state (Duran-Pinedo, 2021). A metatranscriptomics study analyzed mRNA expression profiles in the entire oral microbiome in OSCC patients and healthy controls to predict the molecular function of this disease, and they found that *Fusobacteria* transcripts were significantly more abundant at the tumor site of OSCC patients compared to healthy individuals (Yost *et al*., 2018).

4.10.5.3 Metaproteomics

Metaproteomics is used to identify and quantify all proteins present in a sample, enabling the study of host microbiome interactions by cataloguing the abundance of microbial and host proteins within a sample. It provides direct insight into microbial phenotypes at the molecular level (Kleiner, 2019) and offers direct evidence of active biological processes, thereby linking microbial composition to physiological activity. This functional information is particularly valuable for the oral cavity, where changes in protein expression often precede shifts in microbial taxa. In the context of oral lichen planus, functional annotation revealed that most expressed proteins were associated with core metabolic functions, such as DNA/RNA metabolism, 30S and 50S ribosomal subunits, or ABC transporters, whereas the presence of numerous peptidases implicated potential virulence activity (Bankvall *et al*., 2022). A metaproteomic study of ECC found that the majority of identified salivary proteins were derived from oral microbes, particularly ribosomal proteins, reflecting altered microbial metabolic activity (Ruan *et al*., 2021). These findings highlight how metaproteomic complements sequencing technologies by revealing the metabolically active microbiome and its potential role in disease. Metaproteomics is increasingly being used for gut microbiome studies. However, its application to the oral microbiome is limited by significant host interference and the low concentration of bacteria in the oral microbiome. A specialized sample pretreatment approach is necessary to improve the accuracy of bacterial identification. Furthermore, existing metaproteomic methods might not allow sufficient quantification of bacteria present at low abundance in complex microbiomes (Jiang *et al*., 2022).

4.10.5.4 Metabolomics

Metabolomics involves the study of metabolites and identifies the shift in metabolism, which is a promising way to investigate metabolic changes linked to diseases. The oral environment significantly impacts microbial metabolic activity, and changes can increase bacterial pathogenicity and promote microbial selection, resulting in a more pathogenic oral microbiome (Tsuchida *et al.*, 2025). Therefore, metabolomics data are essential to understand the impact of metabolites in the disease progression, revealing the key pathogenic mechanisms, and can be potential biomarkers and therapeutic targets. The two commonly used techniques in metabolic studies are nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy. These methods can be applied independently or together with other separation approaches, such as lipid chromatography, gas chromatography, and capillary electrophoresis. Compared to other approaches, NMR offers outstanding analytical precision for identifying cancer metabolomic signatures as it yields quantified data of high reproducibility and accuracy despite being relatively less sensitive (Mohd Faizal *et al.*, 2024).

Spearman's correlation analysis, in order to find correlation between differentially abundant microorganisms and metabolites, found a strong association between *Veillonella, Staphylococcus, Neisseria*, and *Porphyromonas* and specific differential metabolites (Li *et al.*, 2023). Metabolomics analysis has revealed the presence of metabolites linked to glucose metabolism and oxidative stress byproducts with positive correlations found between dominant bacteria like *Streptococcus* and organic acids. It suggests functional discrepancies in the oral microbiome may be more pronounced than compositional ones in active dental caries (Wang *et al.*, 2022). A pilot study that examined salivary microbiome and metabolome in periodontitis patients revealed significant differences in microbial and metabolite profiles compared to healthy individuals. Metabolites showed marked differences between aggressive and chronic periodontitis, including altered pathways for pyrimidine, alanine, aspartate, and glutamate metabolism. Differential metabolites like urea and beta-alanine correlated with specific bacterial genera (Wei *et al.*, 2022). Salivary metabolomics has identified new metabolite biomarkers capable of distinguishing between oral cancer and healthy samples, using various detection and separation technologies. For instance, N-acetyl-D-glucosamine, L-pipecolic acid, and L-carnitine have been identified as signature diagnostic biomarkers for oral tongue squamous cell carcinoma (Vimal *et al.*, 2023). Another meta-analysis also highlights N-acetylglucosamine as a distinctive metabolite having the potential to serve as an

early diagnostic marker for OSCC (Panneerselvam *et al*., 2024). Additionally, in another comparison study, 36 metabolites were found to be differentially abundant, among which 13(S)-hydroxyoctadecatrienoic acid and 13(S)-hydroxy-9Z,11E-octadecadienoic acid showed significant downregulation, whereas docosanamide was significantly upregulated in the oral cancer group. Furthermore, six bacteria-specific metabolites, including indole, were also found to be downregulated (Wei *et al*., 2025). However, despite its promising results, limitations of this approach remain in standardizing sample collection and analysis methods, and also validating these biomarkers across diverse populations for robust clinical application is difficult.

4.11 Potential Therapeutic Approaches for Reversing Oral Microbiome Dysbiosis

As discussed above, the changes in oral microflora lead to various oral diseases. The transition of healthy microflora to a disturbed state requires targeted strategies to restore oral health and prevent disease. While probiotics and prebiotics effectively restore healthy microflora, advanced approaches like oral microbiome transplantation (OMT) and bacteriophage therapy provide additional solutions (Figure 4.3). This section discusses new interventions in the field.

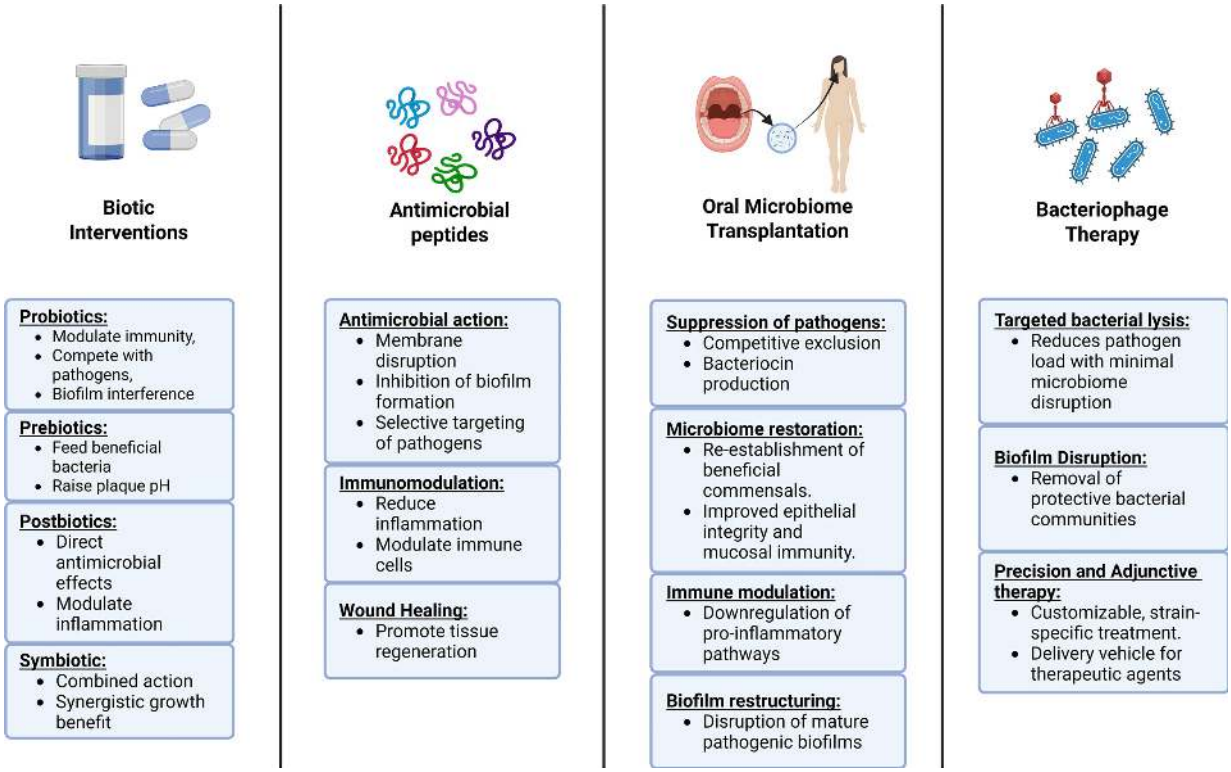

FIGURE 4.3 The role of different therapeutic approaches in restoring oral health. ⏎

4.11.1 Biotic Interventions

Probiotics, prebiotics, synbiotics, and postbiotics represent promising therapeutic strategies for managing oral health. The studies related to these have been reviewed in the section below.

4.11.1.1 Probiotics

According to research, probiotics may help prevent cancer by contributing to and strengthening the immune system (Banna *et al*., 2017). Probiotics are defined as live microorganisms (non-pathogenic beneficial bacteria) that, when administered in adequate amounts, confer a health benefit on the host (Zhong *et al*., 2025). These interventions can help prevent dental caries, reduce inflammation linked with gum disease, and improve overall oral hygiene. Probiotics are mostly present in fermented vegetables like cucumbers and cabbage, as well as fermented dairy products like kefir and yoghurt (Lekshmi Priya *et al*., 2025). These beneficial microorganisms operate by forming biofilms, competing for adhesion sites or nutrients with other pathogens, toxin-degrading substances, and producing antimicrobial substances, and local or systemic immunomodulation that inhibits pathogenic bacteria and promotes

the development of beneficial species (Mohd Fuad *et al*., 2022). Numerous studies have shown that probiotics positively impact the treatment of periodontitis, gingivitis, dental caries, and plaque build-up, while significantly reducing periodontopathogens and carcinogenic microorganisms (Homayouni Rad *et al*., 2023; Shirbhate *et al*., 2023). They function by lowering salivary pH and producing antioxidants that inhibit the growth of harmful bacteria, thus preventing plaque formation. Probiotics effectively reduce biochemical markers associated with inflammation, such as interleukin (IL)-1β, tissue inhibitor of metalloproteinase (TIMP)-1, and matrix metalloproteinase (MMP)-8. Probiotics such as *Lactobacillus* and *Bifidobacterium* enhance various periodontal health indicators, demonstrating their potential as a beneficial adjunct in managing periodontal disease and dental caries (Homayouni Rad *et al*., 2023; Shirbhate *et al*., 2023). Scientific research indicates that probiotics, especially *Lactobacillus* and *Bifidobacterium* strains, possess anticancer properties (Lekshmi Priya *et al*., 2025; Zhong *et al*., 2025). These strains show antitumor activities such as promoting apoptosis, inhibiting angiogenesis, and modulating the immune responses (Zhong *et al*., 2025). A promising probiotic strain *Lactiplantibacillus plantarum* Y33 exhibited a high probiotic score and potential health advantages. The bacteriocin extracted from Y33 exhibited significant cytotoxic activity against OSCC and KB cell lines, indicating its potential application as an anti-oral cancer agent (Nami *et al*., 2024). A study suggests that *L. acidophilus* La5 may modify gingival epithelial cells' production of inflammatory mediators (CXCL-8, GM-CSF, IL-1β), modulating the inflammatory response and reducing pathogen adhesion (*A. actinomycetemcomitans*), which ultimately manages periodontitis (Bueno *et al*., 2022). Another study showed the antiproliferative effects of *L. acidophilus* strain, against OSCC cells, potentially through a TRAIL (TNF-related apoptosis inducing ligand) induced apoptosis mechanism (Al-Asfour *et al*., 2024). Additionally, *Limosilactobacillus reuteri* (known as *Lactobacillus reuteri*) serves as an effective probiotic in bacteriotherapy against dental caries by preventing the growth of cariogenic bacteria like *S. mutans* and *Streptococcus sobrinus*, inhibiting biofilm formation, and reducing bacterial viability. It produces reuterin, a broad-spectrum antimicrobial compound that inhibits both Gram-positive and Gram-negative bacteria across a wide pH range while inhibiting proteolytic and lipolytic enzymes (Félix-Sicairos *et al*., 2024). Probiotics can suppress pathogenic bacteria by counteracting biofilm formation. A study shows *L. plantarum 14917* suppresses *S. mutans* and *Candida albicans* growth by reducing the growth medium's pH, decreasing biofilm's dry weight, and protein content (Li *et al*., 2025). Furthermore, a study shows *Bifidobacterium animalis* subsp. *Lactis*

HN019 enhances expression of β-defensin 3 and TLR4 in gingival tissues, thus increasing CD-4+ T-cell activity and consequently reduces expressive pro-inflammatory cytokine production. This anti-inflammatory effect promotes oral microenvironmental homeostasis by inhibiting the growth of pathogenic bacteria under inflammatory conditions (Invernici *et al*., 2020). Probiotics also show antimutagenic properties by binding to, degrading, and inhibiting mutagens, as well as preventing and converting cancer-causing compounds. They also lower intestinal pH through the production of short-chain fatty acids (SCFAs) and enhance the host's innate immunity by producing anti-inflammatory molecules (Lekshmi Priya *et al*., 2025). Several other studies have revealed advanced delivery systems such as Sprayed multi-adsorbed-droplet reposing technology (SMART) encapsulation, microgels, bioresponsive gels increase probiotic survival, storage stability, and targeted release. Study shows alginate-based microcapsules and mucoadhesive carriers like alginate-Ca^{2+} hydrogels or chitosan enhanced probiotic effectiveness in oral applications, particularly against biofilms and periodontal inflammation (Cheng *et al*., 2021; Choudhary *et al*., 2023; Garcia-Brand *et al*., 2022; Quan *et al*., 2025; Yeung *et al*., 2016). These findings highlight the potential of these probiotics in oral cancer treatment and encourage further exploration of their therapeutic applications.

4.11.1.2 Prebiotics

Prebiotics are non-digestive food components that specifically stimulate the growth, activity, and quality of host's beneficial microorganisms (Vazquez-munoz *et al*., 2023). Prebiotics are now defined as "a substrate that is selectively utilized by host microorganisms, conferring a health benefit, which includes components that stimulate growth and activity of bacteria in the oral cavity (Gibson *et al*., 2017), thereby maintaining a healthy balance of microorganisms through various metabolic pathways, which can enhance oral microbiome stability. Most of the prebiotics are a subset of carbohydrate groups, mainly oligosaccharides such as fructo-oligosaccharides and galacto-oligosaccharides, amino acids, and nitrogen species (Mohd Fuad *et al*., 2022). Research has identified specific compounds, such as N-acetyl-D-mannosamine and beta-methyl-D-galactoside, that selectively boost beneficial bacteria and inhibit the co-aggregation of harmful pathogens. Additionally, nitrate has been recognized as a potential prebiotic that can influence the oral environment by being metabolized by commensal bacteria to produce nitric oxide, which is crucial for oral and systemic health (Yu *et al*., 2024). Some studies have shown that xylitol, arginine, and urea, the well-known prebiotics, serve to raise oral

pH by promoting alkali production and reducing *S. mutans* levels as it produces acidic plaque, which is responsible for many oral diseases (Beattie, 2024).

4.11.1.3 Synbiotics

Probiotic bacteria often struggle to colonize in the adult oral cavity effectively. The combination of probiotics and prebiotics forms synbiotics which helps to create a more favorable environment for these beneficial bacteria, improving their survival and colonization rates in the oral cavity for synergistic effects (Ohshima *et al.*, 2016) and showing greater efficacy than either component alone in promoting health benefits. In 2020, International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the definition of synbiotics as a combination of live microorganisms and substrates specifically utilized by the host's microorganisms to provide a health benefit (Luo *et al.*, 2024). For managing oral potentially malignant disorders (OPMDs) synbiotics show promising potential. It has been widely studied in preventing and alleviating the severity of oral mucositis in cancer patients (Zhong *et al.*, 2025). For example, studies have demonstrated that synbiotics containing *Lactobacillus acidophilus* combined with fructo-oligosaccharides effectively reduce *S. mutans* levels and biofilm formation by supporting beneficial bacteria and favorable oral pH maintenance, which are key factors in preventing dental caries. This synergistic effect arises from the ability of prebiotics to nourish beneficial bacteria, thereby enhancing the survival and colonization of probiotics in the oral cavity (Khammissa *et al.*, 2025; Mohd Fuad *et al.*, 2022). A study reveals that symbiotic supplementation with non-surgical periodontal therapy appears to enhance inflammatory response, antioxidant capacity, and periodontal health in type 2 diabetes mellitus patients with chronic periodontitis as compared to patients who receive non-surgical periodontal therapy only (Bazyar *et al.*, 2020). Synbiotics composed of *Lactobacillus rhamnosus* and 2% L-arginine decrease lactate content in spent media and reduce the biomass of *S. mutans* biofilm, resulting in a decline in pH within 24 hours (Luo *et al.*, 2024), thus suggesting promising potential for synbiotics, as a novel approach in improving oral health.

4.11.1.4 Postbiotics

Postbiotics are beneficial microbial products or components highlighted from probiotics – either produced during their viability or after their breakdown and provide health benefits to the host (Homayouni Rad *et al.*, 2023). They result from the metabolic activities of beneficial microorganisms, such as those involved in

fermentation, and typically include dead cells, cell wall fragments, metabolites, and bioactive molecules (Mohd Fuad *et al*., 2022). Common postbiotic components include SCFAs like acetic, lactic, butanoic, and propionic acids, along with antimicrobial compounds (e.g., bacteriocins), amino acids, extracellular polysaccharides (EPS), peptides, enzymes, flavonoids, and phenolic compounds (Rebelo *et al*., 2025; Scott *et al*., 2022). Postbiotics offer therapeutic advantages by maintaining the integrity and promoting the balance of the host microbiome (Peluzio *et al*., 2021), making them a promising approach for preventing oral dysbiosis (Rebelo *et al*., 2025). Based on their antimicrobial properties, postbiotics are considered a suitable alternative option to antibiotics (Homayouni Rad *et al*., 2023). Unlike probiotics, postbiotics do not contain live bacteria, thus reducing associated risks while still providing beneficial effects. As food supplements, they can mimic probiotic benefits without introducing new microbial species, thereby supporting the function of native probiotics and maintaining microbiome balance (Liu *et al*., 2024). Probiotics, primarily represented by *Lactobacillus* and *Bifidobacterium* species, produce SCFAs that inhibit biofilm formation by harmful oral microbes, such as *P. gingivalis* and *F. nucleatum*. This activity effectively reduces the growth and colonization potential of these pathogenic bacteria, contributing to improved oral health (Rui *et al*., 2024). A study reveals bacterial lysate derived from *L. plantarum* and *L. rhamnosus* GG can help combat factors leading to periodontal disease caused by *P. gingivalis*. This study observed that lysate inhibits *P. gingivalis* lipopolysaccharides-induced inflammatory mediators through the regulation of MAPK and NF- κB, while this lysate significantly inhibits the biofilm-forming ability of *S. mutans* by downregulating the genes associated with glycosyltransferases (gtf's), and did not show an effect upon existing biofilms (Kim *et al*., 2022). Additionally, postbiotics from *L. plantarum 226V* and *Lacticaseibacillus paracasei L26* were found effective against biofilm formation and strong antimicrobial effects against *S. mutans* (Rebelo *et al*., 2025). Another study on postbiotics derived from *L. plantarum* EIR/IF-1 strain demonstrated their effect in periodontal regeneration by enhancing viability, migration, and collagen production in human periodontal ligament mesenchymal stromal cells (hPDL-MSCs). Also, they demonstrated antioxidant properties by lowering reactive oxygen species (ROS) levels in cells that had been exposed to oxidative stress and anti-inflammatory effects by downregulating pro-inflammatory cytokines and upregulating IL-10 (Demirhan *et al*., 2025). Thus, postbiotics may exert antimicrobial, immunomodulatory, and anti-inflammatory effects in the oral cavity, which can support the oral microbiome to balance and boost mucosal immunity.

4.11.2 Antimicrobial Peptides

The misuse and overuse of antibiotics have resulted in the rise of antibiotic resistance. However, recent research indicates that antimicrobial peptides (AMPs) represent a promising class of antibacterial agents effective against oral pathogenic bacteria and have a lower risk of developing antimicrobial resistance (Lin *et al.*, 2021; Morio *et al.*, 2023). Some of these peptides, such as human cathelicidin LL-37, PAC113/Nal-P-113, Azurocidin, Histatins, Defensins, GH12, and C16G2, show potential against oral pathogens (Gradinaru *et al.*, 2022; Lin *et al.*, 2021). In the oral cavity, various cells secrete AMPs that play a vital role in the innate immune defense against pathogens. These peptides, including human neutrophil peptides (HNP-1, -2, and 3), human beta defensins (HBD-1, -2, and 3), and cathelicidin AMP (LL-37), are expressed in various oral tissues such as the tongue, tonsils, salivary glands, gingival epithelium, and dental pulp. Their presence in saliva and gingival crevicular fluid enables them to combat microbial infections while also modulating inflammatory responses (Morio *et al.*, 2023). Among these, LL-37 exhibits antimicrobial activity, modulates inflammation, and promotes wound healing, making it suitable for periodontal therapy (Dale & Fredericks, 2006). In addition to substantial promising results obtained using naturally occurring AMPs, recent studies have also explored synthetic AMPs, developed from both human and non-human sources. These synthetic AMPs are often modified through techniques like polymerization, PEGylation, incorporation of D-amino acids, unnatural amino acids, cyclization, and lipidation to enhance their antimicrobial properties and bioavailability. One such example is [W7]KR12-KAEK, a modified variant of KR-12, which itself is a fragment (residues 18–29) of the human cathelicidin LL-37. It exhibits enhanced antimicrobial activity against *Enterococcus faecalis* due to alterations in peptide charge and amphipathicity. Additionally, DJK-5, a D-enantiomeric cationic peptide, has demonstrated potent antibiofilm capabilities, targeting intracellular stress responses in bacteria and outperforming commercial mouthwashes in disrupting oral biofilms. Furthermore, lactotransferrin-derived peptides such as LF-1 selectively inhibit *S. mutans* while preserving oral commensal bacteria, offering a precision approach to oral microbiome modulation. Synthetic AMPs provide promising therapeutic options for oral health applications, including dental caries prevention, periodontitis management, and wound healing, while mitigating the risks associated with antimicrobial resistance (Czarnowski *et al.*, 2024). PAC113 is also a synthetic analogue of histatin-5, specifically targeting *C. albicans* and *S. mutans*, offering protection against oral candidiasis and caries while preserving beneficial microbiota

(Dale & Fredericks, 2006). C16G2, designed to selectively inhibit *S. mutans*, provides a targeted approach to prevent and treat dental caries without affecting non-pathogenic flora (Namburu *et al.*, 2022). These peptides represent advanced alternatives to traditional antibiotics, addressing oral dysbiosis with precision, reducing antibiotic resistance risks, and supporting oral health through antimicrobial, anti-inflammatory, and tissue-regenerative properties (Morio *et al.*, 2023). This suggests that AMPs may have significant future applications in dentistry and other fields.

4.11.3 Oral Microbiome Transplantation

Microbiome transplantation involves the transfer of a diverse microbial community from a healthy individual known as a donor, to a diseased individual, with the aim to change the microbiome dysbiosis into eubiosis. Researchers have explored microbiota transplants for various diseases, such as *Clostridioides difficile* infections, ulcerative colitis, Crohn's disease, cancer, disorders affecting the skin, and vaginal microbiota (Junca *et al.*, 2022). OMT has the potential to treat oral dysbiosis-related oral diseases. Like fecal microbiome transplantation, OMT involves the transfer of a healthy oral microbial community from a healthy donor to an oral disease patient, and offers a promising way to restore oral balance and improve oral health (Lindo *et al.*, 2024; Nath *et al.*, 2021). A study by Beikler *et al.* (2021) aimed to evaluate the safety and efficiency of OMT treatment for naturally occurring periodontitis in beagle dogs found that it has potential for modulating microbiota composition of dogs with naturally occurring periodontitis disease. Additionally, in a related recent study, researchers developed and characterized effectiveness, feasibility, and safety of OMT therapy for the treatment of dental caries and periodontal disease. The study was conducted using an interdisciplinary approach, with the goal of identifying a healthy microbiome donor that could be used as a therapeutic source for transplantation through hydrogel-mediated delivery. The research then evaluates the safety and effectiveness of the OMT delivery system in rodent models of caries and periodontal disease. The results were promising, showing that OMT could effectively restore microbial balance and reduce disease symptoms in these models. This research provides an important foundational data that can guide future human clinical trials for OMT therapy in managing oral diseases (Nath *et al.*, 2021). Recently, a clinical study reported that transplantation of maternal saliva significantly reduced the severity of oral mucositis in a pediatric patient undergoing chemotherapy for neuroblastoma, effectively preventing the development of severe mucositis during

chemotherapy cycles (Goloshchapov *et al.*, 2024). In parallel, a research team developed a novel in vitro oral biofilm model that maintained a diverse oral microbiome with an average of 252 species over 14 days, compared to existing biofilm model, this model demonstrated improve microbial stability and viability, thereby providing a valuable platform for studying oral microbiome dynamics and for developing future OMT-based therapeutic strategies (Ketagoda *et al.*, 2024). A single oral microbiota transplant may not be sufficient to achieve lasting changes in highly resistant oral microbial populations. Instead, multiple administrations of OMT might be necessary to maintain beneficial changes in the recipient's oral microbiota and enhance clinical outcomes (Lindo *et al., 2024*).

4.11.4 Bacteriophage Therapy

Bacteriophage (phage) therapy has emerged as one alternative possibility with some successful antimicrobial applications during trials. People have considered the therapeutic use of phages as an effective treatment for some infectious diseases, such as oral infectious diseases. Phage therapy includes phage-derived enzymes, phage-antibiotic combinations, synthetic phages, single phages, and phage cocktails (Li *et al.*, 2025). Studies have reported the potential of phages targeting *S. mutans*. For instance, phage SMHBZ8 has a narrow host range that specifically interact with *S. mutans* type c, while phage ϕAPCM01 has a major impact on metabolic activity of *S. mutans* biofilm, and highlighting their antimicrobial efficacy (Zhu *et al.*, 2025). A study by Mwila Kabwe's group shows a full morphology and genetic characterization of novel lytic bacteriophage FNU1 against *F. nucleatum*, a critical oral bacterium associated with chronic periodontitis. A phage FNU1 (genome size 130 kb) demonstrates the ability to degrade biofilm of *F. nucleatum* and lyses bacterial cells composing the biofilm. They demonstrated that bacteriophages formulated into dosage forms such as lozenges and pastes allow slower release of phages in the oral cavity, enabling effective delivery of phage FNU1 for the treatment of periodontitis and other *F. nucleatum*-associated diseases (Kabwe *et al.*, 2019). Another study demonstrated that the synthetic phage ϕEf11/ϕFL1C(Δ36) PnisA has the ability to disrupt *E. faecalis* biofilms, as well as antibiotic-resistant strains in a vitro study (Tinoco *et al.*, 2016). Bacteriophages often produce enzymes that break down polysaccharides of bacterial biofilms, such as sialidases, levanases, and xylosidases, thus enhancing phage penetration into the biofilm and effectiveness. The probability of microorganisms developing resistance toward phages is less in comparison to antibiotics. Phages hold a greater potential in treating bacterial

infections than antibiotics. Even though microorganisms may be able to develop resistance against phages through factors such as phase variation, epigenetic changes, and mutations within receptor genes. To reduce the chances of microorganisms developing resistant isolates, phage cocktails are used (Li *et al.*, 2025).

Phage therapy offers several advantages that make it a promising alternative or supplement to traditional therapies. The high specificity enables precise targeting of pathogenic bacteria, biofilms, or even tumor cells by synthetic mechanisms such as antigen display, while reducing harm to beneficial microbiota and healthy tissues. Furthermore, besides the direct antibacterial properties, phages contribute to immune modulation, functioning as immunomodulatory molecular vectors or cancer vaccines, and can be synthesized to lyse cancer cells. Synthetic bacteriophages are emerging as innovative diagnostic tools for oral cancer, enabling early and non-invasive detection because of their specificity for tumor-associated biomarkers. Furthermore, real-time monitoring of diseases during progression and recurrence through phage-based systems is supporting precision oncology and improving treatment outcomes (Panneerselvam *et al.*, 2025).

4.12 Conclusion

The oral microbiome plays a critical role in maintaining oral health by establishing a balanced microbial ecosystem, and its influence has been observed in oral as well as systemic diseases. Understanding the complex interactions within the oral microbiome is crucial, as it offers insights into disease progression and therapeutic interventions. Until now, most of the studies have explored microbial communities based on amplicon sequencing, such as 16S rRNA. Such an approach limits the understanding to the bacterial genera, failing to decipher the species-level information, correlation of microbial communities, and the genes involved in the disease progression. To have a better understanding of this extent, new omics approaches such as WMS, metatranscriptomics, metabolomics, and metaproteomics must be incorporated in future studies. These techniques have the potential to provide a clear picture of how microbial diversity, composition, and functional shift occur during health and disease. Probiotics, prebiotics, postbiotics, AMPs, bacteriophage therapy, and OMT hold potential for reversing oral dysbiosis and significantly improving oral health. Probiotics have shown potential in promoting beneficial microbial growth, while prebiotics enhance the proliferation of health-associated bacteria by providing nutritional support. A combination of probiotics and prebiotics

creates a more favorable environment for oral-health-associated beneficial bacteria and shows greater effectiveness than either of the compounds consumed alone. Bacteriophage therapy serves as a target-specific bacterial lysis and biofilm disruptor, thereby facilitating innovative intervention better than traditional antibiotic treatment. OMT, although an emerging approach, demonstrates remarkable potential for reversing oral microbiome dysbiosis.

References

Adler, C. J., Browne, G. V., Sukumar, S., & Hughes, T. (2017). Evolution of the oral microbiome and dental caries. *Current Oral Health Reports*, *4*(3), 264–269. ⏎

Al-Asfour, A., Bhardwaj, R. G., & Karched, M. (2024). Growth suppression of oral squamous cell carcinoma cells by *Lactobacillus acidophilus*. *International Dental Journal*, *74*(5), 1151–1160. https://doi.org/10.1016/J.IDENTJ.2024.03.017 ⏎

Al Kawas, S., Al-Marzooq, F., Rahman, B., Shearston, J. A., Saad, H., Benzina, D., & Weitzman, M. (2021). The impact of smoking different tobacco types on the subgingival microbiome and periodontal health: a pilot study. *Scientific Reports*, *11*(1), 1–16. https://doi.org/10.1038/s41598-020-80937-3 ⏎

Alshayeb, L., & Dashash, M. (2025). Prevalence and clinical risk factors of dental caries in Syrian children: a cross-sectional study. *Scientific Reports*, *15*(1), 10721. https://doi.org/10.1038/s41598-025-95534-5 ⏎

Amann, R. I., Ludwig, W., & Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, *59*(1), 143–169. https://doi.org/10.1128/MR.59.1.143-169.1995 ⏎

Anjali, K., Arun, A., Bastian, T., Parthiban, R., Selvamani, M., & Adarsh, H. (2020). Oral microbial profile in oral cancer patients before and after radiation therapy in a cancer care center – a prospective study. *Journal of Oral and Maxillofacial Pathology*, *24*(1), 117. https://doi.org/10.4103/JOMFP.JOMFP_213_19 ⏎

Asai, Y., Jinno, T., Igarashi, H., Ohyama, Y., & Ogawa, T. (2002). Detection and quantification of oral treponemes in subgingival plaque by real-time PCR. *Journal of Clinical Microbiology*, *40*(9), 3334–3340. https://doi.org/10.1128/JCM.40.9.3334-3340.2002 ⏎

Atanasova, K. R., & Yilmaz, O. (2014). Looking in the *Porphyromonas gingivalis* cabinet of curiosities: the microbium, the host and cancer association. *Molecular Oral Microbiology*, *29*(2), 55–66. https://doi.org/10.1111/OMI.12047 ⏎

Bagger, F. O., Borgwardt, L., Jespersen, A. S., Hansen, A. R., Bertelsen, B., Kodama, M., & Nielsen, F. C. (2024). Whole genome sequencing in clinical practice. *BMC Medical Genomics*, *17*(1), 39. https://doi.org/10.1186/S12920-024-01795-W ⏎

Bahuguna, M., Hooda, S., Mohan, L., Gupta, R. K., & Diwan, P. (2023). Identifying oral microbiome alterations in adult betel quid chewing population of Delhi, India. *PloS One*, *18*, 1–24. https://doi.org/10.1371/journal.pone.0278221 ⏎

Banna, G. L., Torino, F., Marletta, F., Santagati, M., Salemi, R., Cannarozzo, E., Falzone, L., Ferraù, F., & Libra, M. (2017). *Lactobacillus rhamnosus GG: an overview to explore the rationale of its use in cancer*. *Frontiers in Pharmacology*, *8*, 603. https://doi.org/10.3389/fphar.2017.00603 ⏎

Bazyar, H., Maghsoumi-Norouzabad, L., Yarahmadi, M., Gholinezhad, H., Moradi, L., Salehi, P., Haghighi-Zadeh, M. H., & Zare Javid, A. (2020). The impacts of synbiotic supplementation on periodontal indices and biomarkers of oxidative stress in type 2 diabetes mellitus patients with chronic periodontitis under non-surgical periodontal therapy. A double-blind, placebo-controlled trial. *Diabetes, Metabolic Syndrome and Obesity*, *13*, 19–29. https://doi.org/10.2147/DMSO.S230060 ⏎

Beattie, R. E. (2024). Probiotics for oral health: a critical evaluation of bacterial strains. *Frontiers in Microbiology*, *15*, 1430810. https://doi.org/10.3389/fmicb.2024.1430810 ⏎

Beikler, T., Bunte, K., Chan, Y., Weiher, B., Selbach, S., Peters, U., Klocke, A., Watt, R. M., & Flemmig, T. F. (2021). Oral microbiota transplant in dogs with naturally occurring periodontitis. *Journal of Dental Research*, *100*(7), 764–770. https://doi.org/10.1177/0022034521995423 ⏎

Benn, A. M. L., Heng, N. C. K., Broadbent, J. M., & Thomson, W. M. (2018). Studying the human oral microbiome: challenges and the evolution of solutions. *Australian Dental Journal*, *63*(1), 14–24. https://doi.org/10.1111/ADJ.12565 ⏎

Bingham, C. O., & Moni, M. (2013). Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions. *Current Opinion in Rheumatology*, *25*(3), 345. https://doi.org/10.1097/BOR.0B013E32835FB8EC ⏎

Boers, S. A., Jansen, R., & Hays, J. P. (2019). Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. *European Journal of*

Clinical Microbiology & Infectious Disease, *38*(6), 1059–1070. https://doi.org/10.1007/S10096-019-03520-3 ⏎

Bowland, G. B., & Weyrich, L. S. (2022). The oral-microbiome-brain axis and neuropsychiatric disorders: an anthropological perspective. *Frontiers in Psychiatry*, *13*, 810008. https://doi.org/10.3389/FPSYT.2022.810008 ⏎

Bueno, M. R., Ishikawa, K. H., Almeida-Santos, G., Ando-Suguimoto, E. S., Shimabukuro, N., Kawamoto, D., & Mayer, M. P. A. (2022). *Lactobacilli* attenuate the effect of *Aggregatibacter actinomycetemcomitans* infection in gingival epithelial cells. *Frontiers in Microbiology*, *13*, 846192. https://doi.org/10.3389/FMICB.2022.846192 ⏎

Carra, M. C., Rangé, H., Caligiuri, G., & Bouchard, P. (2023). Periodontitis and atherosclerotic cardiovascular disease: a critical appraisal. *Periodontology 2000*, 1–34. https://doi.org/10.1111/PRD.12528 ⏎

Ceccarani, C., Foschi, C., Parolin, C., D'Antuono, A., Gaspari, V., Consolandi, C., Laghi, L., Camboni, T., Vitali, B., Severgnini, M., & Marangoni, A. (2019). Diversity of vaginal microbiome and metabolome during genital infections. *Scientific Reports*, *9*(1), 14095. https://doi.org/10.1038/s41598-019-50410-x ⏎

Chattopadhyay, I., Verma, M., & Panda, M. (2019). Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. *Technology in Cancer Research & Treatment*, *18*. https://doi.org/10.1177/1533033819867354 ⏎

Chattopadhyay, S., Malayil, L., Chopyk, J., Smyth, E., Kulkarni, P., Raspanti, G., Thomas, S. B., Sapkota, A., Mongodin, E. F., & Sapkota, A. R. (2024). Oral microbiome dysbiosis among cigarette smokers and smokeless tobacco users compared to non-users. *Scientific Reports*, *14*(1), 10394. https://doi.org/10.1038/s41598-024-60730-2 ⏎

Chaudhari, H. G., Prajapati, S., Wardah, Z. H., Raol, G., Prajapati, V., Patel, R., Shati, A. A., Alfaifi, M. Y., Elbehairi, S. E. I., & Sayyed, R. Z. (2023). Decoding the microbial universe with metagenomics: a brief insight. *Frontiers in Genetics*, *14*, 1119740. https://doi.org/10.3389/FGENE.2023.1119740 ⏎

Chen, X., Lu, Y., Chen, T., & Li, R. (2021). The female vaginal microbiome in health and bacterial vaginosis. *Frontiers in Cellular and Infection Microbiology*, *11*, 631972. https://doi.org/10.3389/FCIMB.2021.631972 ⏎

Cheng, Q., Liu, L., Xie, M., Li, H., Ma, D., & Xue, W. (2021). A colon-targeted oral probiotics delivery system using an enzyme-triggered fuse-like microcapsule. *Advanced Healthcare Materials*, *10*(8), e2001953. https://doi.org/10.1002/ADHM.202001953, ⏎

Choudhary, P., Kraatz, H. B., Lévesque, C. M., & Gong, S. G. (2023). Microencapsulation of probiotic *Streptococcus salivarius* LAB813. *ACS Omega*, *8*(13), 12011–12018. https://doi.org/10.1021/ACSOMEGA.2C07721 ⏎

Chu, X. J., Cao, N. W., Zhou, H. Y., Meng, X., Guo, B., Zhang, H. Y., & Li, B. Z. (2021). The oral and gut microbiome in rheumatoid arthritis patients: a systematic review. *Rheumatology*, *60*(3), 1054–1066. https://doi.org/10.1093/RHEUMATOLOGY/KEAA835 ⏎

Coffey, J., Choudhry, M., Shlossman, M., Makin, I. R. S., & Singh, V. K. (2016). Multiplex real-time PCR detection and relative quantification of periodontal pathogens. *Clinical and Experimental Dental Research*, *2*(3), 185–192. https://doi.org/10.1002/CRE2.37 ⏎

Colombo, A. P. V., Boches, S. K., Cotton, S. L., Goodson, J. M., Kent, R., Haffajee, A. D., Socransky, S. S., Hasturk, H., Van Dyke, T. E., Dewhirst, F., & Paster, B. J. (2009). Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. *Journal of Periodontology*, *80*(9), 1421–1432. https://doi.org/10.1902/JOP.2009.090185 ⏎

Corrêa, J. D., Carlos, P. P. S., Faria, G. A., Pacheco, L. C. R., da Costa, V. S., Mendes, I. R. R., de Oliveira, A. B., & Colombo, A. P. V. (2024). The healthy oral microbiome: a changing ecosystem throughout the human lifespan. *Journal of Dental Research*, 104(3), 235–242. https://doi.org/10.1177/00220345241297583 ⏎

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science (New York, N.Y.)*, *326*(5960), 1694–1697. https://doi.org/10.1126/SCIENCE.1177486 ⏎

Czarnowski, M., Wnorowska, U., Łuckiewicz, M., Dargiewicz, E., Spałek, J., Okła, S., Sawczuk, B., Savage, P. B., Bucki, R., & Piktel, E. (2024). Natural antimicrobial peptides and their synthetic analogues for effective oral microflora control and oral infection treatment – the role of ceragenins in the development of new therapeutic methods. *Pharmaceuticals*, *17*(12), 1725. https://doi.org/10.3390/PH17121725 ⏎

da Costa Rosa, T., de Almeida Neves, A., Azcarate-Peril, M. A., Divaris, K., Wu, D., Cho, H., Moss, K., Paster, B. J., Chen, T., B. Freitas-Fernandes, L., Fidalgo, T. K. S., Tadeu Lopes, R., Valente, A. P., R. Arnold, R., & de Aguiar Ribeiro, A. (2021). The bacterial microbiome and metabolome in caries progression and arrest.

Journal of Oral Microbiology, *13*(1), 1886748. https://doi.org/10.1080/20002297.2021.1886748 ⏎

Dahlén, G. (2009). Bacterial infections of the oral mucosa. *Periodontology 2000*, *49*(1), 13–38. https://doi.org/10.1111/J.1600-0757.2008.00295.X ⏎

Dale, B. A., & Fredericks, L. P. (2005). Antimicrobial peptides in the oral environment: expression and function in health and disease. *Curr Issues Mol Biol*. 2005 Jul;7(2):119–33. doi:10.1093/jac/dki103 ⏎

D'Amore, R., Ijaz, U. Z., Schirmer, M., Kenny, J. G., Gregory, R., Darby, A. C., Shakya, M., Podar, M., Quince, C., & Hall, N. (2016). A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics*, *17*(1), 1–20. https://doi.org/10.1186/S12864-015-2194-9 ⏎

Darout, I. A., Albandar, J. M., Skaug, N., & Ali, R. W. (2002). Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. *Journal of Clinical Periodontology*, *29*(5), 411–420. https://doi.org/10.1034/J.1600-051X.2002.290505.X ⏎

Das, B., Ghosh, T. S., Kedia, S., Rampal, R., Saxena, S., Bag, S., Mitra, R., Dayal, M., Mehta, O., Surendranath, A., Travis, S. P. L., Tripathi, P., Nair, G. B., & Ahuja, V. (2018). Analysis of the gut microbiome of rural and urban healthy Indians living in sea level and high altitude areas. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-018-28550-3 ⏎

Demirhan, H. K., Omer Oglou, E., Aksoy, Z. B., & Kiran, F. (2025). Evaluation of the anti-inflammatory, antioxidant and regenerative effects of microbiota-derived postbiotics in human periodontal ligament mesenchymal stromal cells. *Clinical Oral Investigations*, *29*(5). 262. https://doi.org/10.1007/S00784-025-06341-1, ⏎

Deng, W., Xi, D., Mao, H., & Wanapat, M. (2008). The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. *Molecular Biology Reports*, *35*(2), 265–274. https://doi.org/10.1007/S11033-007-9079-1 ⏎

Deo, P. N., & Deshmukh, R. (2019). Oral microbiome: unveiling the fundamentals. *Journal of Oral and Maxillofacial Pathology*, *23*(1), 122–128. https://doi.org/10.4103/JOMFP.JOMFP_304_18 ⏎

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*(19), 5002. https://doi.org/10.1128/JB.00542-10 ⏎

Diop, K., Dufour, J. C., Levasseur, A., & Fenollar, F. (2019). Exhaustive repertoire of human vaginal microbiota. *Human Microbiome Journal*, *11*, 100051. https://doi.org/10.1016/J.HUMIC.2018.11.002 ⏎

Di Stefano, M., Polizzi, A., Santonocito, S., Romano, A., Lombardi, T., & Isola, G. (2022). Impact of oral microbiome in periodontal health and periodontitis: a critical review on prevention and treatment. *International Journal of Molecular Sciences*, *23*(9), 5142. https://doi.org/10.3390/IJMS23095142 ⏎

Diwan, P., Nirwan, M., Bahuguna, M., Kumari, S. P., Wahlang, J., & Gupta, R. K. (2023). Evaluating alterations of the oral microbiome and its link to oral cancer among Betel Quid Chewers: prospecting reversal through probiotic intervention. *Pathogens*, *12*(8), 996. https://doi.org/10.3390/pathogens12080996 ⏎

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975. https://doi.org/10.1073/PNAS.1002601107 ⏎

Dong, L., Yin, J., Zhao, J., Ma, S. R., Wang, H. R., Wang, M., Chen, W., & Wei, W. Q. (2018). Microbial similarity and preference for specific sites in healthy oral cavity and esophagus. *Frontiers in Microbiology*, *9*, 379685. https://doi.org/10.3389/FMICB.2018.01603 ⏎

Drucker, S. D., (2016). *Impact of Chewing Betel Nut on the Oral Microbiome*. M.Sc Thesis. University of Illinois at Chicago, /articles/thesis/Impact_of_Chewing_Betel_Nut_on_the_Oral_Microbiome/10816898/1 ⏎

Duran-Pinedo, A. E. (2021). Metatranscriptomic analyses of the oral microbiome. *Periodontology 2000*, *85*(1), 28–45. https://doi.org/10.1111/PRD.12350 ⏎

Fakhruddin, K. S., Ngo, H. C., & Samaranayake, L. P. (2019). Cariogenic microbiome and microbiota of the early primary dentition: a contemporary overview. *Oral Diseases*, *25*(4), 982–995. https://doi.org/10.1111/ODI.12932 ⏎

Fan, X., Alekseyenko, A. V., Wu, J., Peters, B. A., Jacobs, E. J., Gapstur, S. M., Purdue, M. P., Abnet, C. C., Stolzenberg-Solomon, R., Miller, G., Ravel, J., Hayes, R. B., & Ahn, J. (2018). Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut*, *67*(1), 120–127. https://doi.org/10.1136/GUTJNL-2016-312580 ⏎

Fan, X., Peters, B. A., Jacobs, E. J., Gapstur, S. M., Purdue, M. P., Freedman, N. D., Alekseyenko, A. V., Wu, J., Yang, L., Pei, Z., Hayes, R. B., & Ahn, J. (2018).

Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. *Microbiome*, *6*(1), 59. https://doi.org/10.1186/S40168-018-0448-X ⏎

Farhad, S. Z., Karbalaeihasanesfahani, A., Dadgar, E., Nasiri, K., Esfahaniani, M., & Nabi Afjadi, M. (2024). The role of periodontitis in cancer development, with a focus on oral cancers. *Molecular Biology Reports 51*(1), 1–22. https://doi.org/10.1007/S11033-024-09737-6 ⏎

Félix-Sicairos, B. K., Martinez-Martinez, R. E., Aranda-Romo, S., Gonzalez-Amaro, R., Salgado-Bustamante, M., Zavala-Alonso, N. V., & Alpuche-Solís, Á. G. (2024). *Limosilactobacillus reuteri* and its probiotic potential against cariogenic bacteria. *Microbiology Research 2024*, *15*(3), 1178–1188. https://doi.org/10.3390/MICROBIOLRES15030079 ⏎

Fernando, N., Jayakumar, G., Perera, N., Amarasingha, I., Meedin, F., & Holton, J. (2009). Presence of *Helicobacter pylori* in betel chewers and non betel chewers with and without oral cancers. *BMC Oral Health*, *9*(1), 1–6. https://doi.org/10.1186/1472-6831-9-23 ⏎

Fiore, M., Minni, A., Cavalcanti, L., Raponi, G., Puggioni, G., Mattia, A., Gariglio, S., Colizza, A., Meliante, P. G., Zoccali, F., Tarani, L., Barbato, C., Lucarelli, M., Ceci, F. M., Francati, S., Ferraguti, G., Ceccanti, M., & Petrella, C. (2023). The impact of alcohol consumption and oral microbiota on upper aerodigestive tract carcinomas: a pilot study. *Antioxidants*, *12*(6), 1233. https://doi.org/10.3390/ANTIOX12061233 ⏎

Fujimoto, C., Maeda, H., Kokeguchi, S., Takashiba, S., Nishimura, F., Arai, H., Fukui, K., & Murayama, Y. (2003). Application of denaturing gradient gel electrophoresis (DGGE) to the analysis of microbial communities of subgingival plaque. *Journal of Periodontal Research*, *38*(4), 440–445. https://doi.org/10.1034/J.1600-0765.2003.02607.X ⏎

Gao, L., Cheng, Z., Zhu, F., Bi, C., Shi, Q., & Chen, X. (2022). The oral microbiome and its role in systemic autoimmune diseases: a systematic review of big data analysis. *Frontiers in Big Data*, *5*, 927520. https://doi.org/10.3389/FDATA.2022.927520 ⏎

García, L., Tercero, J. C., Legido, B., Ramos, J. A., Alemany, J., & Sanz, M. (1998). Rapid detection of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromona gingivalis* by multiplex PCR. *Journal of Periodontal Research*, *33*(1), 59–64. https://doi.org/10.1111/J.1600-0765.1998.TB02292.X

Garcia-Brand, A. J., Quezada, V., Gonzalez-Melo, C., Bolaños-Barbosa, A. D., Cruz, J. C., & Reyes, L. H. (2022). Novel developments on stimuli-responsive probiotic encapsulates: from smart hydrogels to nanostructured platforms. *Fermentation*, *8*(3), 117. https://doi.org/10.3390/FERMENTATION8030117 ⏎

Gersdorf, H., Meissner, A., Pelz, K., Krekeler, G., & Gobel, U. B. (1993). Identification of *Bacteroides forsythus* in subgingival plaque from patients with advanced periodontitis. *Journal of Clinical Microbiology*, *31*(4), 941–946. https://doi.org/10.1128/JCM.31.4.941-946.1993 ⏎

Gersdorf, H., Pelz, K., & Göbel, U. B. (1993). Fluorescence in situ hybridization for direct visualization of gram-negative anaerobes in subgingival plaque samples. *FEMS Immunology and Medical Microbiology*, *6*(2–3), 109–114. https://doi.org/10.1111/J.1574-695X.1993.TB00312.X ⏎

Gholizadeh, P., Eslami, H., Yousefi, M., Asgharzadeh, M., Aghazadeh, M., & Kafil, H. S. (2016). Role of oral microbiome on oral cancers, a review. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *84*, 552–558. https://doi.org/10.1016/J.BIOPHA.2016.09.082 ⏎

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews. Gastroenterology & Hepatology*, *14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75 ⏎

Goloshchapov, O. V., Chukhlovin, A. B., Bug, D. S., Polev, D. E., Kosarev, O. V., Klementeva, R. V., Izmailova, E. A., Kazantsev, I. V., Khalipskaia, M. S., Goloshchapova, M. O., Yudintseva, O. S., Barkhatov, I. M., Petukhova, N. V., Zubarovskaya, L. S., Kulagin, A. D., & Moiseev, I. S. (2024). Safety, feasibility, and advantages of oral microbiota transplantation: the first clinical case. *Journal of Pediatric Hematology/Oncology*, *46*(6), 287–296. https://doi.org/10.1097/MPH.0000000000002896 ⏎

Gradinaru, V., Copolovici, D. M., Luong, A. D., Buzid, A., & Luong, J. H. T. (2022). Important roles and potential uses of natural and synthetic antimicrobial peptides (AMPs) in oral diseases: cavity, periodontal disease, and thrush. *Journal of Functional Biomaterials*, *13*(4), 175. https://doi.org/10.3390/JFB13040175 ⏎

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., Bouffard, G. G., Blakesley, R. W., Murray, P. R., Green, E. D., Turner, M. L., & Segre, J. A. (2009). Topographical and temporal diversity of the human skin

microbiome. *Science (New York, N.Y.)*, *324*(5931), 1190–1192. https://doi.org/10.1126/SCIENCE.1171700 ⏎

Griffen, A. L., Beall, C. J., Firestone, N. D., Gross, E. L., DiFranco, J. M., Hardman, J. H., Vriesendorp, B., Faust, R. A., Janies, D. A., & Leys, E. J. (2011). CORE: a phylogenetically-curated 16S rDNA database of the core oral microbiome. *PloS One*, *6*(4), e19051. https://doi.org/10.1371/JOURNAL.PONE.0019051 ⏎

Gualtero, D. F., Lafaurie, G. I., Buitrago, D. M., Castillo, Y., Vargas-Sanchez, P. K., & Castillo, D. M. (2023). Oral microbiome mediated inflammation, a potential inductor of vascular diseases: a comprehensive review. *Frontiers in Cardiovascular Medicine*, *10*, 1250263. https://doi.org/10.3389/FCVM.2023.1250263 ⏎

Halboub, E., Al-Ak'hali, M. S., Alamir, A. H., Homeida, H. E., Baraniya, D., Chen, T., & Al-Hebshi, N. N. (2020). Tongue microbiome of smokeless tobacco users. *BMC Microbiology*, *20*(1), 1–9. https://doi.org/10.1186/S12866-020-01883-8 ⏎

Hashimoto, K., Shimizu, D., Ueda, S., Miyabe, S., Oh-Iwa, I., Nagao, T., Shimozato, K., & Nomoto, S. (2022). Feasibility of oral microbiome profiles associated with oral squamous cell carcinoma. *Journal of Oral Microbiology*, *14*(1), 2105574. https://doi.org/10.1080/20002297.2022.2105574 ⏎

Hernandez, B. Y., Zhu, X., Goodman, M. T., Gatewood, R., Mendiola, P., Quinata, K., & Paulino, Y. C. (2017). Betel nut chewing, oral premalignant lesions, and the oral microbiome. *PloS One*, *12*(2), e0172196. https://doi.org/10.1371/JOURNAL.PONE.0172196 ⏎

Herreros-Pomares, A., Hervás, D., Bagan-Debón, L., Jantus-Lewintre, E., Gimeno-Cardona, C., & Bagan, J. (2023). On the oral microbiome of oral potentially malignant and malignant disorders: dysbiosis, loss of diversity, and pathogens enrichment. *International Journal of Molecular Sciences*, *24*(4), 3466. https://doi.org/10.3390/IJMS24043466 ⏎

Homayouni Rad, A., Pourjafar, H., & Mirzakhani, E. (2023). A comprehensive review of the application of probiotics and postbiotics in oral health. *Frontiers in Cellular and Infection Microbiology*, *13*, 1120995. https://doi.org/10.3389/FCIMB.2023.1120995 ⏎

Huang, S., Yang, F., Zeng, X., Chen, J., Li, R., Wen, T., Li, C., Wei, W., Liu, J., Chen, L., Davis, C., & Xu, J. (2011). Preliminary characterization of the oral microbiota of Chinese adults with and without gingivitis. *BMC Oral Health*, *11*(1), 33. https://doi.org/10.1186/1472-6831-11-33 ⏎

Huse, S. M., Ye, Y., Zhou, Y., & Fodor, A. A. (2012). A core human microbiome as viewed through 16S rRNA sequence clusters. *PloS One*, *7*(6), e34242. https://doi.org/10.1371/JOURNAL.PONE.0034242 ⏎

Invernici, M. M., Furlaneto, F. A. C., Salvador, S. L., Ouwehand, A. C., Salminen, S., Mantziari, A., Vinderola, G., Ervolino, E., Santana, S. I., Silva, P. H. F., & Messora, M. R. (2020). Bifidobacterium animalis subsp lactis HN019 presents antimicrobial potential against periodontopathogens and modulates the immunological response of oral mucosa in periodontitis patients. *PloS One*, *15*, e0238425. https://doi.org/10.1371/JOURNAL.PONE.0238425, ⏎

Islam, S., Muthumala, M., Matsuoka, H., Uehara, O., Kuramitsu, Y., Chiba, I., & Abiko, Y. (2019). How each component of betel quid is involved in oral carcinogenesis: mutual interactions and synergistic effects with other carcinogens – a review article. *Current Oncology Reports*, *21*(6), 1–13. https://doi.org/10.1007/S11912-019-0800-8 ⏎

Jain, V., Baraniya, D., El-Hadedy, D. E., Chen, T., Slifker, M., Alakwaa, F., Cai, K. Q., Chitrala, K. N., Fundakowski, C., & Al-Hebshi, N. N. (2023). Integrative metatranscriptomic analysis reveals disease-specific microbiome–host interactions in oral squamous cell carcinoma. *Cancer Research Communications*, *3*(5), 807–820. https://doi.org/10.1158/2767-9764.CRC-22-0349 ⏎

Jenkinson, H. F. (1994). Cell surface protein receptors in oral streptococci. *FEMS Microbiology Letters*, *121*(2), 133–140. https://doi.org/10.1111/J.1574-6968.1994.TB07089.X ⏎

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1 ⏎

Junca, H., Pieper, D. H., & Medina, E. (2022). The emerging potential of microbiome transplantation on human health interventions. *Computational and Structural Biotechnology Journal*, *20*, 615–627. https://doi.org/10.1016/J.CSBJ.2022.01.009 ⏎

Kabwe, M., Brown, T. L., Dashper, S., Speirs, L., Ku, H., Petrovski, S., Chan, H. T., Lock, P., & Tucci, J. (2019). Genomic, morphological and functional characterisation of novel bacteriophage FNU1 capable of disrupting *Fusobacterium nucleatum* biofilms. *Scientific Reports*, *9(1)*, 9107. https://doi.org/10.1038/s41598-019-45549-6 ⏎

Kadam, S., Vandana, M., Patwardhan, S., & Kaushik, K. S. (2021). Looking beyond the smokescreen: can the oral microbiome be a tool or target in the management of tobacco-associated oral cancer? *Ecancermedicalscience*, *15*, 1179. https://doi.org/10.3332/ECANCER.2021.1179 ⏎

Kavarthapu, A., & Gurumoorthy, K. (2021). Linking chronic periodontitis and oral cancer: a review. *Oral Oncology*, *121*, 105375. https://doi.org/10.1016/J.ORALONCOLOGY.2021.105375 ⏎

Ketagoda, D. H. K., Varga, P., Fitzsimmons, T. R., Moore, N. E., Weyrich, L. S., & Zilm, P. S. (2024). Development of an in vitro biofilm model of the human supra-gingival microbiome for oral microbiome transplantation. *Journal of Microbiological Methods*, *223*, 106961. https://doi.org/10.1016/J.MIMET.2024.106961 ⏎

Khammissa, G. G., Twetman, S., & Belstrøm, D. (2025). Effect of synbiotic and postbiotic supplements on dental caries and periodontal diseases – a comprehensive review. *International Journal of Environmental Research and Public Health*, *22*(1), 72. https://doi.org/10.3390/IJERPH22010072 ⏎

Kim, M. Y., Hyun, I. K., An, S., Kim, D., Kim, K. H., & Kang, S. S. (2022). In vitro anti-inflammatory and antibiofilm activities of bacterial lysates from lactobacilli against oral pathogenic bacteria. *Food and Function*, *13*(24), 12755–12765. https://doi.org/10.1039/D2FO00936F, ⏎

King, C. H., Desai, H., Sylvetsky, A. C., LoTempio, J., Ayanyan, S., Carrie, J., Crandall, K. A., Fochtman, B. C., Gasparyan, L., Gulzar, N., Howell, P., Issa, N., Krampis, K., Mishra, L., Morizono, H., Pisegna, J. R., Rao, S., Ren, Y., Simonyan, V., … Mazumder, R. (2019). Baseline human gut microbiota profile in healthy people and standard reporting template. *PloS One*, *14*(9), e0206484. https://doi.org/10.1371/JOURNAL.PONE.0206484 ⏎

Krishnan, K., Chen, T., & Paster, B. J. (2017). A practical guide to the oral microbiome and its relation to health and disease. *Oral Diseases*, *23*(3), 276–286. https://doi.org/10.1111/ODI.12509 ⏎

Kroese, J. M., Brandt, B. W., Buijs, M. J., Crielaard, W., Lobbezoo, F., Loos, B. G., van Boheemen, L., van Schaardenburg, D., Zaura, E., & Volgenant, C. M. C. (2021). Differences in the oral microbiome in patients with early rheumatoid arthritis and individuals at risk of rheumatoid arthritis compared to healthy individuals. *Arthritis and Rheumatology*, *73*(11), 1986–1993. https://doi.org/10.1002/ART.41780, ⏎

Kulkarni, A., Beckler, M. D., Amini, S. S., & Kesselman, M. M. (2022). Oral microbiome in pre-rheumatoid arthritis: the role of *Aggregatibacter actinomycetemcomitans* in bacterial composition. *Cureus*, *14*(12), e32201. https://doi.org/10.7759/CUREUS.32201 ⏎

Kumar, P. S., Griffen, A. L., Barton, J. A., Paster, B. J., Moeschberger, M. L., & Leys, E. J. (2003). New bacterial species associated with chronic periodontitis. *Journal of Dental Research*, *82*(5), 338–344. https://doi.org/10.1177/154405910308200503 ⏎

Lan, Z., Liu, W. J., Cui, H., Zou, K. L., Chen, H., Zhao, Y. Y., & Yu, G. T. (2023a). The role of oral microbiota in cancer. *Frontiers in Microbiology*, *14*(October), 1–8. https://doi.org/10.3389/fmicb.2023.1253025 ⏎

Lan, Z., Liu, W. J., Cui, H., Zou, K. L., Chen, H., Zhao, Y. Y., & Yu, G. T. (2023b). The role of oral microbiota in cancer. *Frontiers in Microbiology*, *14*, 1253025. https://doi.org/10.3389/FMICB.2023.1253025 ⏎

Łasica, A., Golec, P., Laskus, A., Zalewska, M., Gędaj, M., & Popowska, M. (2024). Periodontitis: etiology, conventional treatments, and emerging bacteriophage and predatory bacteria therapies. *Frontiers in Microbiology*, *15*, 1469414. https://doi.org/10.3389/FMICB.2024.1469414 ⏎

Lederberg, J., & McCray, A. T. (2001). "Ome Sweet" omics – a genealogical treasury of words. *The Scientist*, *15*(7). https://go.gale.com/ps/i.do?p=AONE&sw=w&issn=08903670&v=2.1&it=r&id=GALE%7CA73535513&sid=googleScholar&linkaccess=fulltext ⏎

Lee, Y. H., Chung, S. W., Auh, Q. S., Hong, S. J., Lee, Y. A., Jung, J., Lee, G. J., Park, H. J., Shin, S. I., & Hong, J. Y. (2021). Progress in oral microbiome related to oral and systemic diseases: an update. *Diagnostics*, *11*(7), 1283. https://doi.org/10.3390/DIAGNOSTICS11071283 ⏎

Lekshmi Priya, K. S., Maheswary, D., Ravi, S. S. S., Leela, K. V., Lathakumari, R. H., & Malavika, G. (2025). The impact of probiotics on oral cancer: mechanistic insights and therapeutic strategies. *Oral Oncology Reports*, *13*, 100715. https://doi.org/10.1016/J.OOR.2025.100715 ⏎

Li, K., Wang, J., Du, N., Sun, Y., Sun, Q., Yin, W., Li, H., Meng, L., & Liu, X. (2023). Salivary microbiome and metabolome analysis of severe early childhood caries. *BMC Oral Health*, *23*(1), 1–18. https://doi.org/10.1186/S12903-023-02722-8 ⏎

Li, Y., Zhu, M., Liu, Y., Luo, B., Cui, J., Huang, L., Chen, K., & Liu, Y. (2022). The oral microbiota and cardiometabolic health: a comprehensive review and emerging

insights. *Frontiers in Immunology*, *13*, 1010368. https://doi.org/10.3389/FIMMU.2022.1010368 ⏎

Liao, Y., Tong, X. T., Jia, Y. J., Liu, Q. Y., Wu, Y. X., Xue, W. Q., He, Y. Q., Wang, T. M., Zheng, X. H., Zheng, M. Q., & Jia, W. H. (2022). The effects of alcohol drinking on oral microbiota in the Chinese population. *International Journal of Environmental Research and Public Health*, *19*(9), 5729. https://doi.org/10.3390/IJERPH19095729 ⏎

Lin, B., Li, R., Handley, T. N. G., Wade, J. D., Li, W., & O'Brien-Simpson, N. M. (2021). Cationic antimicrobial peptides are leading the way to combat oropathogenic infections. *ACS Infectious Diseases*, *7*(11), 2959–2970. https://doi.org/10.1021/ACSINFECDIS.1C00424 ⏎

Lindo, C. E., Sebastian, J., Kuntjoro, K. N., Halim, V. A., Tadjoedin, F. M., Kuswandani, S. O., & Sulijaya, B. (2024). Microbiota transplantation as an adjunct to standard periodontal treatment in periodontal disease: a systematic review. *Medicina*, *60*(4), 672. https://doi.org/10.3390/MEDICINA60040672 ⏎

Liu, B., Faller, L. L., Klitgord, N., Mazumdar, V., Ghodsi, M., Sommer, D. D., Gibbons, T. R., Treangen, T. J., Chang, Y. C., Li, S., Stine, O. C., Hasturk, H., Kasif, S., Segrè, D., Pop, M., & Amar, S. (2012). Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PloS One*, *7*(6), e37919. https://doi.org/10.1371/JOURNAL.PONE.0037919 ⏎

Liu, Q., Ma, T., Feng, C., Li, Y., Jin, H., Shi, X., Kwok, L.-Y., Shi, Y., Chen, T., & Zhang, H. (2024). Adjuvant postbiotic administration improves dental caries prognosis by restoring the oral microbiota. *Food Science and Human Wellness*, *13*(5), 2690–2702. https://doi.org/10.26599/FSHW.2022.9250217 ⏎

Liu, R., Liu, Y., Yi, J., Fang, Y., Guo, Q., Cheng, L., He, J., & Li, M. (2025). Imbalance of oral microbiome homeostasis: the relationship between microbiota and the occurrence of dental caries. *BMC Microbiology*, *25*(1), 46. https://doi.org/10.1186/S12866-025-03762-6 ⏎

Lochman, J., Zapletalova, M., Poskerova, H., Holla, L. I., & Linhartova, P. B. (2019). Rapid multiplex real-time PCR method for the detection and quantification of selected cariogenic and periodontal bacteria. *Diagnostics 2020*, *10*(1), 8. https://doi.org/10.3390/DIAGNOSTICS10010008 ⏎

Long, J., Cai, Q., Steinwandel, M., Hargreaves, M. K., Bordenstein, S. R., Blot, W. J., Zheng, W., & Shu, X. O. (2017). Association of oral microbiome with type 2 diabetes risk. *Journal of Periodontal Research*, *52*(3), 636–643. https://doi.org/10.1111/JRE.12432 ⏎

Losasso, C., Eckert, E. M., Mastrorilli, E., Villiger, J., Mancin, M., Patuzzi, I., Di Cesare, A., Cibin, V., Barrucci, F., Pernthaler, J., Corno, G., & Ricci, A. (2018). Assessing the influence of vegan, vegetarian and omnivore oriented westernized dietary styles on human gut microbiota: a cross sectional study. *Frontiers in Microbiology*, *9*, 317. https://doi.org/10.3389/FMICB.2018.00317 ⏎

Lucchese, A. (2017). *Streptococcus mutans* antigen I/II and autoimmunity in cardiovascular diseases. *Autoimmunity Reviews*, *16*(5), 456–460. https://doi.org/10.1016/J.AUTREV.2017.03.009 ⏎

Luo, S., Wei, S., Luo, X., Yang, Q., Wong, K., Cheung, P. C. K., & Zhang, B. (2024). How probiotics, prebiotics, synbiotics, and and postbiotics prevent dental caries: an oral microbiota perspective. *NPJ Biofilms Microbiomes*, *10*(1), 14. https://doi.org/10.1038/s41522-024-00488-7 ⏎

Ma, C., Chen, F., Zhang, Y., Sun, X., Tong, P., Si, Y., & Zheng, S. (2015). Comparison of oral microbial profiles between children with severe early childhood caries and caries-free children using the human oral microbe identification microarray. *PloS One*, *10*(3), e0122075. https://doi.org/10.1371/JOURNAL.PONE.0122075 ⏎

Ma, Q., Li, X., Jiang, H., Fu, X., You, L., You, F., & Ren, Y. (2023). Mechanisms underlying the effects, and clinical applications, of oral microbiota in lung cancer: current challenges and prospects. *Critical Reviews in Microbiology*, 50(5), 631–652. https://doi.org/10.1080/1040841X.2023.2247493 ⏎

Mäkinen, A. I., Pappalardo, V. Y., Buijs, M. J., Brandt, B. W., Mäkitie, A. A., Meurman, J. H., & Zaura, E. (2023). Salivary microbiome profiles of oral cancer patients analyzed before and after treatment. *Microbiome*, *11*(1), 171. https://doi.org/10.1186/S40168-023-01613-Y ⏎

Martínez, M., Postolache, T. T., García-Bueno, B., Leza, J. C., Figuero, E., Lowry, C. A., & Malan-Müller, S. (2022). The role of the oral microbiota related to periodontal diseases in anxiety, mood and trauma- and stress-related disorders. *Frontiers in Psychiatry*, *12*, 814177. https://doi.org/10.3389/FPSYT.2021.814177 ⏎

Matsumoto-Nakano, M. (2018). Role of *Streptococcus mutans* surface proteins for biofilm formation. *Japanese Dental Science Review*, *54*(1), 22–29. https://doi.org/10.1016/J.JDSR.2017.08.002 ⏎

Men, B., Li, Y., & Jiang, S. (2024). Updates on the role of periodontitis-related epigenetics, inflammation, oral microbiome, and treatment in cardiovascular risk.

Journal of Inflammation Research, *17*, 837–851. https://doi.org/10.2147/JIR.S449661 ⏎

Metgud, R., Gupta, K., & Gupta, J. (2014). Exploring bacterial flora in oral squamous cell carcinoma: a microbiological study. *Biotechnic & Histochemistry: Official Publication of the Biological Stain Commission*, *89*(2), 153–159. https://doi.org/10.3109/10520295.2013.831120 ⏎

Mohammed, W. K. (2023). Using multiplex polymerase chain reaction (m-PCR) methods to detect six pathogenic bacteria in human oral cavity. *Journal of University of Anbar for Pure Science (JUAPS) Open Access*, *17*, 48–56. https://doi.org/10.37652/juaps.2023.140052.1060 ⏎

Mohd Faizal, N. F., Vincent-Chong, V. K., Ramanathan, A., Paterson, I. C., Karen-Ng, L. P., & Zaini, Z. M. (2024). Metabolomic profiling of oral potentially malignant disorders and its clinical values. *Biomedicines*, *12*(12), 2899. https://doi.org/10.3390/BIOMEDICINES12122899 ⏎

Mohd Fuad, A. S., Amran, N. A., Nasruddin, N. S., Burhanudin, N. A., Dashper, S., & Arzmi, M. H. (2022). The mechanisms of probiotics, prebiotics, synbiotics, and postbiotics in oral cancer management. *Probiotics and Antimicrobial Proteins*, *15*(5), 1. https://doi.org/10.1007/S12602-022-09985-7 ⏎

Morio, K. A., Sternowski, R. H., & Brogden, K. A. (2023). Induction of endogenous antimicrobial peptides to prevent or treat oral infection and inflammation. *Antibiotics*, *12*(2), 361. https://doi.org/10.3390/ANTIBIOTICS12020361 ⏎

Nabel, E. G. (2003). Cardiovascular disease. *New England Journal of Medicine*, *349*(1), 60–72. https://doi.org/10.1056/NEJMRA035098 ⏎

Nakagawa, I., Inaba, H., Yamamura, T., Kato, T., Kawai, S., Ooshima, T., & Amano, A. (2006). Invasion of epithelial cells and proteolysis of cellular focal adhesion components by distinct types of *Porphyromonas gingivalis* fimbriae. *Infection and Immunity*, *74*(7), 3773–3782. https://doi.org/10.1128/IAI.01902-05 ⏎

Namburu, J. R., Babu, A., Sanosh, R., Shekar, C., Manthapuri, S., Pinnaka, M., Ramana, V., & Baddam, R. (2022). Frontiers in dentistry *Streptococcus mutans*-specific antimicrobial peptide C16G2-mediated caries prevention: a review. *Frontiers in Dental Medicine*, *19*, 17. ⏎

Nami, Y., Tavallaei, O., Kiani, A., Moazami, N., Samari, M., Derakhshankhah, H., Jaymand, M., & Haghshenas, B. (2024). Anti-oral cancer properties of potential probiotic lactobacilli isolated from traditional milk, cheese, and yogurt. *Scientific Reports 14*(1), 1–15. https://doi.org/10.1038/s41598-024-57024-y ⏎

Nath, S., Zilm, P., Jamieson, L., Kapellas, K., Goswami, N., Ketagoda, K., & Weyrich, L. S. (2021). Development and characterization of an oral microbiome transplant among Australians for the treatment of dental caries and periodontal disease: a study protocol. *PloS One*, *16*(11), e0260433. https://doi.org/10.1371/JOURNAL.PONE.0260433 ⏎

Nowicki, E. M., Shroff, R., Singleton, J. A., Renaud, D. E., Wallace, D., Drury, J., Zirnheld, J., Colleti, B., Ellington, A. D., Lamont, R. J., Scott, D. A., & Whiteley, M. (2018). Microbiota and metatranscriptome changes accompanying the onset of gingivitis. *MBio*, *9*(2), e00575-18. https://doi.org/10.1128/MBIO.00575-18 ⏎

O'Grady, I., Anderson, A., & O'Sullivan, J. (2020). The interplay of the oral microbiome and alcohol consumption in oral squamous cell carcinomas. *Oral Oncology*, *110*, 105011. https://doi.org/10.1016/J.ORALONCOLOGY.2020.105011 ⏎

Oh, J., Byrd, A. L., Deming, C., Conlan, S., Kong, H. H., Segre, J. A., Barnabas, B., Blakesley, R., Bouffard, G., Brooks, S., Coleman, H., Dekhtyar, M., Gregory, M., Guan, X., Gupta, J., Han, J., Ho, S. L., Legaspi, R., Maduro, Q., … Young, A. (2014). Biogeography and individuality shape function in the human skin metagenome. *Nature*, *514*(7520), 59–64. https://doi.org/10.1038/nature13786 ⏎

Ohshima, T., Kojima, Y., Seneviratne, C. J., & Maeda, N. (2016). Therapeutic application of synbiotics, a fusion of probiotics and prebiotics, and biogenics as a new concept for oral Candida infections: a mini review. *Frontiers in Microbiology*, *7*, 168868. https://doi.org/10.3389/FMICB.2016.00010 ⏎

Oliveira, L. M., Antoniazzi, R. P., Demarco, F. F., & Zanatta, F. B. (2023). Differences in the subgingival microbial composition associated with alcohol intake: a systematic review. *Journal of Oral Biology and Craniofacial Research*, *13*(2), 259–266. https://doi.org/10.1016/J.JOBCR.2023.02.002 ⏎

Panneerselvam, K., Rajkumar, K., Kumar, S., Mohan, A. M., Arockiam, A. S., & Sugimoto, M. (2024). Salivary metabolomics in early detection of oral squamous cell carcinoma – a meta-analysis. *Expert Review of Proteomics*, *21*(7–8), 317–332. https://doi.org/10.1080/14789450.2024.2395398 ⏎

Panneerselvam, V. P., Kagithakara Vajravelu, L., Harikumar Lathakumari, R., Baskar Vimala, P., M Nair, D., & Thulukanam, J. (2025). Bacteriophage-based therapies in oral cancer: a new frontier in oncology. *Cancer Pathogenesis and Therapy*, *3*(6), 453–465. https://doi.org/10.1016/J.CPT.2025.03.003 ⏎

Paster, B. J., & Dewhirst, F. E. (2009). Molecular microbial diagnosis. *Periodontology 2000*, *51*(1), 38. https://doi.org/10.1111/J.1600-

0757.2009.00316.X ⏎

Paster, B. J., Olsen, I., Aas, J. A., & Dewhirst, F. E. (2006). The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology 2000*, *42*(1), 80–87. https://doi.org/10.1111/J.1600-0757.2006.00174.X ⏎

Peluzio, M. C. G., Martinez, J. A., & Milagro, F. I. (2021). Postbiotics: metabolites and mechanisms involved in microbiota-host interactions. *Trends in Food Science & Technology*, *108*, 11–26. https://doi.org/10.1016/J.TIFS.2020.12.004 ⏎

Pisano, M., Giordano, F., Sangiovanni, G., Capuano, N., Acerra, A., & D'Ambrosio, F. (2023). The interaction between the oral microbiome and systemic diseases: a narrative review. *Microbiology Research*, *14*(4), 1862–1878. https://doi.org/10.3390/MICROBIOLRES14040127 ⏎

Poole, S., Singhrao, S. K., Kesavalu, L., Curtis, M. A., & Crean, S. J. (2013). Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *Journal of Alzheimer's Disease*, *36*(4), 665–677. https://doi.org/10.3233/JAD-121918 ⏎

Pozhitkov, A. E., Beikler, T., Flemmig, T., & Noble, P. A. (2011). High-throughput methods for analysis of the human oral microbiome. *Periodontology 2000*, *55*(1), 70–86. https://doi.org/10.1111/J.1600-0757.2010.00380.X ⏎

Pratap Singh, R., Kumari, N., Gupta, S., Jaiswal, R., Mehrotra, D., Singh, S., Mukherjee, S., & Kumar, R. (2023). Intratumoral microbiota changes with tumor stage and influences the immune signature of oral squamous cell carcinoma. *Microbiology Spectrum*, *11*(4), e0459622. https://doi.org/10.1128/SPECTRUM.04596-22 ⏎

Praveen, Z., Choi, S.-W., Lee, J. H., Park, J. Y., Oh, H. J., Kwon, I. J., Park, J. H., & Kim, M. K. (2024). Oral microbiome and CPT1A function in fatty acid metabolism in oral cancer. *International Journal of Molecular Sciences*, *25*(20), 10890. https://doi.org/10.3390/IJMS252010890 ⏎

Qian, X. B., Chen, T., Xu, Y. P., Chen, L., Sun, F. X., Lu, M. P., & Liu, Y. X. (2020). A guide to human microbiome research: study design, sample collection, and bioinformatics analysis. *Chinese Medical Journal*, *133*(15), 1844–1855. https://doi.org/10.1097/CM9.0000000000000871 ⏎

Quan, Y., Shao, H., Wang, N., Gao, Z., & Jin, M. (2025). Microenvironment-sensitive hydrogels as promising drug delivery systems for co-encapsulating microbial homeostasis probiotics and anti-inflammatory drugs to treat periodontitis. *Materials Today Bio*, *32*, 101711. https://doi.org/10.1016/J.MTBIO.2025.101711 ⏎

Radaic, A., Shamir, E. R., Jones, K., Villa, A., Garud, N. R., Tward, A. D., Kamarajan, P., & Kapila, Y. L. (2023). Specific oral microbial differences in proteobacteria and bacteroidetes are associated with distinct sites when moving from healthy mucosa to oral dysplasia – a microbiome and gene profiling study and focused review. *Microorganisms*, *11*(9), 2250. https://doi.org/10.3390/MICROORGANISMS11092250 ⏎

Rebelo, M. B., Oliveira, C. S., & Tavaria, F. K. (2025). Development of a postbiotic-based orodispersible film to prevent dysbiosis in the oral cavity. *Frontiers in Bioscience-Elite*, *17*(1), 26987. https://doi.org/10.31083/FBE26987 ⏎

Reynoso-García, J., Miranda-Santiago, A. E., Meléndez-Vázquez, N. M., Acosta-Pagán, K., Sánchez-Rosado, M., Díaz-Rivera, J., Rosado-Quiñones, A. M., Acevedo-Márquez, L., Cruz-Roldán, L., Tosado-Rodríguez, E. L., Figueroa-Gispert, M. D. M., & Godoy-Vitorino, F. (2022). A complete guide to human microbiomes: body niches, transmission, development, dysbiosis, and restoration. *Frontiers in Systems Biology*, *2*, 951403. https://doi.org/10.3389/FSYSB.2022.951403 ⏎

Rui, W., Zhong, S., Li, X., Tang, X., Wang, L., & Yang, J. (2024). Evaluating the role of postbiotics in the modulation of human oral microbiota: a randomized controlled clinical trial. *Probiotics and Antimicrobial Proteins*, *17*, 2942–2952. https://doi.org/10.1007/S12602-024-10238-Y ⏎

Rumgay, H., Nethan, S. T., Shah, R., Vignat, J., Ayo-Yusuf, O., Chaturvedi, P., Guerra, E. N. S., Gupta, P. C., Gupta, R., Liu, S., Magnusson, C., Parascandola, M., Paulino, Y. C., Rezaei, N., Siddiqi, K., Warnakulasuriya, S., Lauby-Secretan, B., & Soerjomataram, I. (2024). Global burden of oral cancer in 2022 attributable to smokeless tobacco and areca nut consumption: a population attributable fraction analysis. *Lancet Oncology*, *25*(11), 1413–1423. https://doi.org/10.1016/S1470-2045(24)00458-3 ⏎

Sajid, M., Srivastava, S., Joshi, L., & Bharadwaj, M. (2021). Impact of smokeless tobacco-associated bacteriome in oral carcinogenesis. *Anaerobe*, *70*, 102400. https://doi.org/10.1016/J.ANAEROBE.2021.102400 ⏎

Sakamoto, M., Umeda, M., & Benno, Y. (2005). Molecular analysis of human oral microbiota. *Journal of Periodontal Research*, *40*(3), 277–285. https://doi.org/10.1111/J.1600-0765.2005.00793.X ⏎

Sami, A., Elimairi, I., Ryan, C. A., Stanton, C., Patangia, D., & Ross, R. P. (2023). Altered oral microbiome in Sudanese Toombak smokeless tobacco users carries a

newly emerging risk of squamous cell carcinoma development and progression. *Scientific Reports*, *13*(1), 6645. https://doi.org/10.1038/s41598-023-32892-y ⏎

Sawant, S., Dugad, J., Parikh, D., & Singh, H. (2021). Absolute quantitation of oral bacteria involved in oral cancer by real-time PCR. *Medicine in Microecology*, *7*, 100034. https://doi.org/10.1016/J.MEDMIC.2021.100034 ⏎

Sawant, S., Dugad, J., Parikh, D., Srinivasan, S., & Singh, H. (2021). Identification & correlation of bacterial diversity in oral cancer and long-term tobacco chewers – a case-control pilot study. *Journal of Medical Microbiology*, *70*(9). https://doi.org/10.1099/JMM.0.001417 ⏎

Sawant, S., Dugad, J., Parikh, D., Srinivasan, S., & Singh, H. (2023). Oral microbial signatures of tobacco chewers and oral cancer patients in India. *Pathogens*, *12*(1), 78. https://doi.org/10.3390/PATHOGENS12010078 ⏎

Saxena, R., Vishnu Prasoodanan, P. K., Gupta, S. V., Gupta, S., Waiker, P., Samaiya, A., Sharma, A. K., & Sharma, V. K. (2022). Assessing the effect of smokeless tobacco consumption on oral microbiome in healthy and oral cancer patients. *Frontiers in Cellular and Infection Microbiology*, *12*, 841465. https://doi.org/10.3389/FCIMB.2022.841465 ⏎

Scott, E., De Paepe, K., & Van de Wiele, T. (2022). Postbiotics and their health modulatory biomolecules. *Biomolecules*, *12*(11), 1640. https://doi.org/10.3390/BIOM12111640 ⏎

Senaratne, N. L. M., Yung on, C., Shetty, N. Y., & Gopinath, D. (2024). Effect of different forms of tobacco on the oral microbiome in healthy adults: a systematic review. *Frontiers in Oral Health*, *5*, 1310334. https://doi.org/10.3389/FROH.2024.1310334 ⏎

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PloS Biology*, *14*(8), e1002533. https://doi.org/10.1371/JOURNAL.PBIO.1002533 ⏎

Shirbhate, U., Bajaj, P., Chandak, M., Jaiswal, P., Sarangi, S., Suchak, D., & Bharti, L. (2023). Clinical implications of probiotics in oral and periodontal health: a comprehensive review. *Cureus*, *15*(12), e51177. https://doi.org/10.7759/CUREUS.51177 ⏎

Smędra, A., & Berent, J. (2023). The influence of the oral microbiome on oral cancer: a literature review and a new approach. *Biomolecules*, *13*(5), 815. https://doi.org/10.3390/BIOM13050815 ⏎

Smythe, P., & Wilkinson, H. N. (2023). The skin microbiome: current landscape and future opportunities. *International Journal of Molecular Sciences*, *24*(4), 3950.

https://doi.org/10.3390/IJMS24043950 ⏎

Statnikov, A., Henaff, M., Narendra, V., Konganti, K., Li, Z., Yang, L., Pei, Z., Blaser, M. J., Aliferis, C. F., & Alekseyenko, A. V. (2013). A comprehensive evaluation of multicategory classification methods for microbiomic data. *Microbiome*, *1*(1), 11. https://doi.org/10.1186/2049-2618-1-11 ⏎

Su, S. C., Chang, L. C., Huang, H. D., Peng, C. Y., Chuang, C. Y., Chen, Y. T., Lu, M. Y., Chiu, Y. W., Chen, P. Y., & Yang, S. F. (2021). Oral microbial dysbiosis and its performance in predicting oral cancer. *Carcinogenesis*, *42*(1), 127–135. https://doi.org/10.1093/CARCIN/BGAA062 ⏎

Sudhakara, P., Gupta, A., Bhardwaj, A., & Wilson, A. (2018). Oral dysbiotic communities and their implications in systemic diseases. *Dentistry Journal*, *6*(2), 10. https://doi.org/10.3390/DJ6020010 ⏎

Sukmana, B. I., Saleh, R. O., Najim, M. A., AL-Ghamdi, H. S., Achmad, H., Al-Hamdani, M. M., Taher, A. A. Y., Alsalamy, A., Khaledi, M., & Javadi, K. (2024). Oral microbiota and oral squamous cell carcinoma: a review of their relation and carcinogenic mechanisms. *Frontiers in Oncology*, *14*, 1319777. https://doi.org/10.3389/FONC.2024.1319777 ⏎

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660 ⏎

Sunil, P. M., Ramachandran, C. R., Gokul, S., & Jaisanghar, N. (2013). Fluorescence in-situ hybridization technique as a diagnostic and prognostic tool in oral squamous cell carcinoma. *Journal of Oral and Maxillofacial Pathology*, *17*(1), 61–64. https://doi.org/10.4103/0973-029X.110731 ⏎

Suzuki, N., Nakano, Y., Yoneda, M., Hirofuji, T., & Hanioka, T. (2022). The effects of cigarette smoking on the salivary and tongue microbiome. *Clinical and Experimental Dental Research*, *8*(1), 449–456. https://doi.org/10.1002/CRE2.489 ⏎

Takenaka, S., Edanami, N., Komatsu, Y., Nagata, R., Naksagoon, T., Sotozono, M., Ida, T., & Noiri, Y. (2021). Periodontal pathogens inhabit root caries lesions extending beyond the gingival margin: a next-generation sequencing analysis. *Microorganisms*, *9*(11), 2349. https://doi.org/10.3390/MICROORGANISMS9112349 ⏎

Tinoco, J. M., Buttaro, B., Zhang, H., Liss, N., Sassone, L., & Stevens, R. (2016). Effect of a genetically engineered bacteriophage on *Enterococcus faecalis*

biofilms. *Archives of Oral Biology*, *71*, 80–86. https://doi.org/10.1016/J.ARCHORALBIO.2016.07.001 ⏎

Tonelli, A., Lumngwena, E. N., & Ntusi, N. A. B. (2023). The oral microbiome in the pathophysiology of cardiovascular disease. *Nature Reviews Cardiology*, *20*(6), 386–403. https://doi.org/10.1038/s41569-022-00825-3 ⏎

Tong, Y., Zheng, L., Qing, P., Zhao, H., Li, Y., Su, L., Zhang, Q., Zhao, Y., Luo, Y., & Liu, Y. (2020). Oral microbiota perturbations are linked to high risk for rheumatoid arthritis. *Frontiers in Cellular and Infection Microbiology*, *9*, 475. https://doi.org/10.3389/FCIMB.2019.00475 ⏎

Tsuchida, S., Umemura, H., Iizuka, K., Yamamoto, H., Shimazaki, I., Shikata, E., & Nakayama, T. (2025). Recent findings on metabolomics and the microbiome of oral bacteria involved in dental caries and periodontal disease. *World Journal of Microbiology and Biotechnology*, *41*(1), 1–10. https://doi.org/10.1007/S11274-024-04224-3 ⏎

Uehara, O., Hiraki, D., Kuramitsu, Y., Matsuoka, H., Takai, R., Fujita, M., Harada, F., Paudel, D., Takahashi, S., Yoshida, K., Muthumala, M., Nagayasu, H., Chiba, I., & Abiko, Y. (2021). Alteration of oral flora in betel quid chewers in Sri Lanka. *Journal of Microbiology, Immunology and Infection*, *54*(6), 1159–1166. https://doi.org/10.1016/J.JMII.2020.06.009 ⏎

van Dijk, M. C., Petersen, J. F., Raber-Durlacher, J. E., Epstein, J. B., & Laheij, A. M. G. A. (2024). Diversity and compositional differences in the oral microbiome of oral squamous cell carcinoma patients and healthy controls: a scoping review. *Frontiers in Oral Health*, *5*, 1366153. https://doi.org/10.3389/FROH.2024.1366153 ⏎

van't Hof, W., Veerman, E. C. I., Amerongen, A. V. N., & Ligtenberg, A. J. M. (2014). Antimicrobial defense systems in saliva. *Monographs in Oral Science*, *24*, 40–51. https://doi.org/10.1159/000358783 ⏎

Vazquez-munoz, R., Thompson, A., Sobue, T., Dongari-bagtzoglou, A., (2024). *results in the attenuation of oropharyngeal candidiasis in mice*. *July*, 1–14. https://doi.org/10.1128/aem.01713-23 ⏎

Verma, D., Garg, P. K., & Dubey, A. K. (2018). Insights into the human oral microbiome. *Archives of Microbiology*, *200*(4), 525–540. https://doi.org/10.1007/S00203-018-1505-3 ⏎

Vimal, J., George, N. A., Kumar, R. R., Kattoor, J., & Kannan, S. (2023). Identification of salivary metabolic biomarker signatures for oral tongue squamous

cell carcinoma. *Archives of Oral Biology*, *155*, 105780. https://doi.org/10.1016/j.archoralbio.2023.105780 ⏎

Wang, Y., Zhang, Y., Pan, T., Lin, H., & Zhou, Y. (2022). Metabolic differences of the oral microbiome related to dental caries – a pilot study. *Archives of Oral Biology*, *141*, 105471. https://doi.org/10.1016/J.ARCHORALBIO.2022.105471 ⏎

Wei, K., Ma, Y., Xu, J., Zheng, H., Xue, L., Chu, Y., Shi, Y., Sun, Z., & Sun, Q. (2025). Potential changes in microorganisms and metabolites associated with oral cancer: a preliminary study. *BMC Cancer*, *25*(1), 611. https://doi.org/10.1186/S12885-025-13680-5 ⏎

Wei, Y., Shi, M., Nie, Y., Wang, C., Sun, F., Jiang, W., Hu, W., & Wu, X. (2022). Integrated analysis of the salivary microbiome and metabolome in chronic and aggressive periodontitis: a pilot study. *Frontiers in Microbiology*, *13*, 959416. https://doi.org/10.3389/FMICB.2022.959416 ⏎

Xie, M., Tang, Q., Nie, J., Zhang, C., Zhou, X., Yu, S., Sun, J., Cheng, X., Dong, N., Hu, Y., & Chen, L. (2020). BMAL1-downregulation aggravates *Porphyromonas gingivalis*-induced atherosclerosis by encouraging oxidative stress. *Circulation Research*, *126*(6), E15–E29. https://doi.org/10.1161/CIRCRESAHA.119.315502 ⏎

Ximénez-Fyvie, L. A., Haffajee, A. D., & Socransky, S. S. (2000). Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. *Journal of Clinical Periodontology*, *27*(9), 648–657. https://doi.org/10.1034/J.1600-051X.2000.027009648.X ⏎

Xu, X., Shan, B., Zhang, Q., Lu, W., Zhao, J., Zhang, H., & Chen, W. (2023). Oral microbiome characteristics in children with and without early childhood caries. *Journal of Clinical Pediatric Dentistry*, *47*(2), 58–67. https://doi.org/10.22514/JOCPD.2023.012 ⏎

Yang, C. Y., Yeh, Y. M., Yu, H. Y., Chin, C. Y., Hsu, C. W., Liu, H., Huang, P. J., Hu, S. N., Liao, C. T., Chang, K. P., & Chang, Y. L. (2018). Oral microbiota community dynamics associated with oral squamous cell carcinoma staging. *Frontiers in Microbiology*, *9*(MAY), 349233. https://doi.org/10.3389/FMICB.2018.00862 ⏎

Yang, J., He, P., Zhou, M., Li, S., Zhang, J., Tao, X., Wang, A., & Wu, X. (2022). Variations in oral microbiome and its predictive functions between tumorous and healthy individuals. *Journal of Medical Microbiology*, *71*(8). https://doi.org/10.1099/JMM.0.001568 ⏎

Yeung, T. W., Üçok, E. F., Tiani, K. A., McClements, D. J., & Sela, D. A. (2016). Microencapsulation in alginate and chitosan microgels to enhance viability of *Bifidobacterium longum* for oral delivery. *Frontiers in Microbiology*, *7*, 494. https://doi.org/10.3389/FMICB.2016.00494 ⏎

Yost, S., Stashenko, P., Choi, Y., Kukuruzinska, M., Genco, C. A., Salama, A., Weinberg, E. O., Kramer, C. D., & Frias-Lopez, J. (2018). Increased virulence of the oral microbiome in oral squamous cell carcinoma revealed by metatranscriptome analyses. *International Journal of Oral Science*, *10*(4), 32. https://doi.org/10.1038/s41368-018-0037-7 ⏎

Yu, X., Devine, D. A., & Vernon, J. J. (2024). Manipulating the diseased oral microbiome: the power of probiotics and prebiotics. *Journal of Oral Microbiology*, *16*(1), 2307416. https://doi.org/10.1080/20002297.2024.2307416 ⏎

Zaura, E., Keijser, B. J., Huse, S. M., & Crielaard, W. (2009). Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiology*, *9*(1), 259. https://doi.org/10.1186/1471-2180-9-259 ⏎

Zhang, J. S., Chu, C. H., & Yu, O. Y. (2022). Oral microbiome and dental caries development. *Dentistry Journal*, *10*(10), 184. https://doi.org/10.3390/DJ10100184 ⏎

Zheng, X., Li, X., Zhen, J., Xue, D., Hu, J., Cao, Q., Xu, A., Cheung, B. M. Y., Wu, J., & Li, C. (2023). Periodontitis is associated with stroke. *Journal of Translational Medicine*, *21*(1), 697. https://doi.org/10.1186/S12967-023-04545-1 ⏎

Zhong, W., Faruque Ahmad, M., Ali Ahmad, F., Alsayegh, A. A., Zeyaullah, M., Babalghith, A. O., Faidah, H., Ahmed, F., Khanam, A., Mozaffar, B., Kambal, N., & Bantun, F. (2025). Probiotics and cancer: mechanistic insights and organ-specific impact. *Biomolecules*, *15*(6), 879. https://doi.org/10.3390/BIOM15060879 ⏎

Zhou, J., Wang, L., Yuan, R., Yu, X., Chen, Z., Yang, F., Sun, G., & Dong, Q. (2020). Signatures of mucosal microbiome in oral squamous cell carcinoma identified using a random forest model. *Cancer Management and Research*, *12*, 5353–5363. https://doi.org/10.2147/CMAR.S251021 ⏎

Zhu, M., Hao, C., Zou, T., Jiang, S., & Wu, B. (2025). Phage therapy as an alternative strategy for oral bacterial infections: a systematic review. *BMC Oral Health, 25(1), 44*. https://doi.org/10.1186/s12903-024-05399-9 ⏎

Zijnge, V., Harmsen, H. J. M., Kleinfelder, J. W., Van der Rest, M. E., Degener, J. E., & Welling, G. W. (2003). Denaturing gradient gel electrophoresis analysis to study

bacterial community structure in pockets of periodontitis patients. *Oral Microbiology and Immunology*, *18*(1), 59–65. https://doi.org/10.1034/J.1399-302X.2003.180110.X ⏎

5

Early Diet and the Infant Gut Microbiome: How Breastfeeding and Complementary Feeding Shape the Microbiome

S. Bikram Singh, Kalimuthusamy Natarajaseenivasan, Sumit Aggarwal, Hanna Yumnam, Yashmin Choudhury, and Indu Sharma

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5.1 Introduction

The human gut microbiome is a diverse and lively home for microorganisms, which is necessary for host physiology, metabolism, and immunology from the beginning of life. Colonization begins at or even before birth, and up to 3 years of life constitute a vital doorway for microbial establishment and modulation of the immune system (Jeong, 2021). Until then, host-related and environmental factors are included in the microbial diversity, and functionality is largely determined by maternal microbiota components, the mode of delivery, exposure to antibiotics, and particularly the feeding habits of newborns (O'Neill et al., 2020).

The gut of an infant is yet more active in metabolic processes than that of adults, and its maturation requires a regular ecological succession. Early colonizers such as *Bifidobacterium* and *Lactobacillus* dominate during milk feeding; however, the addition of solid meals results in enrichment *of Bacteroides, Faecalibacterium*, and *Clostridium* species (Laursen, 2021). Necrotizing enterocolitis in infancy and chronic illnesses, and inflammatory bowel disease (IBD) later in adulthood are linked to disruptions in this pattern, known as gut dysbiosis (Jeong, 2021).

Diet, especially during breastfeeding, is one of the earliest-life influences in the development of the infant's gut microbiome. Mother's milk provides essential nutrients and bioactive phytochemicals, particularly encouraging the development of *Bifidobacterium* and *Lactobacillus* species, including human milk oligosaccharides (HMOs), immunoglobulins, lactoferrin, and lysozyme (Penders, 2017). HMOs serve as prebiotics for good bacteria; however, they remain indigestible to infants, resulting in a less diverse but more stable community with anti-inflammatory and barrier-protective properties (Lawson et al., 2019). Additionally, the microbiota may directly seed the infant's stomach and microbial metabolites found in breast milk (Xu & Wan, 2025).

A more proteolytic and less saccharolytic metabolism is indicated by the increased microbial diversity in formula-fed infants, which is frequently enriched with *Enterobacteriaceae* and *Clostridium* species. Such shifts are linked to a higher burden of antibiotic resistance genes (ARGs) and inflammatory potential (Pärnänen et al., 2022). The lack of HMOs and lower levels of immunomodulatory molecules in the formula limit the selective advantage of *Bifidobacterium* species, potentially predisposing infants to infections, allergies, and metabolic dysfunction later in adult life (Doré & Blottière, 2015).

5.2 Maternal and Perinatal Effects on Gut Microbiota

The maternal diet plays an important part in immune system development and in reducing the risk of noncommunicable disease in progeny due to epigenetic changes. Perinatal influences on the development of the baby microbiome are complex, with many underlying mechanisms still unknown, and the type and intensity of these connections have not been thoroughly assessed (Grech et al., 2021).

5.2.1 Maternal Health, Diet, and Microbial Inheritance

Maternal malnutrition, including both undernutrition and overnutrition, is a global issue. In today's high-income nations, severe maternal undernutrition is uncommon, but there are growing worries about women who become pregnant while dieting due to social media and celebrity pressure. Furthermore, some women have crippling chronic disorders like hyperemesis gravidarum, and only because of advancements in prenatal care are viable babies now attainable for them (Williams et al., 2014; Valles-Colome et al., 2023). Factors that can affect preterm birth have been identified by the categorization of maternal nutrition as "prudent" (mostly composed of fresh food), "Western" (composed of processed food), or "traditional" diet (Ojha et al., 2015). The maintenance of microbe–host interactions that may support gut health is mostly dependent on dietary components. As a result, diet is an important tool that can be used to improve gut integrity and health by restoring beneficial microbe–host interactions (Forgie et al., 2020). Recent studies mentioned that dietary fiber consumption affects the health of maternal offspring. However, β-glucan supplementation has been demonstrated to improve the cognitive growth of children. A low-fiber diet has been proven to increase vulnerability to inflammation, diet-induced obesity, and acute infections in the lower respiratory tract. Immune development and longevity are both greatly affected by the early-life microbiome, and the mother has an impact on the development and functionality of the infant microbiome (Huang et al., 2025).

5.2.2 Delivery Mode: Vaginal versus Cesarean Birth

The gut microbial community varies with the mode of delivery and also differs between vaginally delivered (VD) infants and cesarean section (CS) delivered infants. The recent cohort study revealed that *Bacteroides, Escherichia coli*, and *Shigella* species predominated in fecal samples from VD infants. The most fecal metabolites connected to the VD groups were substantially associated with the *Bacteroides, Bifidobacterium*, and *Parabacteroides*. However, stool specimens of CS-delivered neonates demonstrated a lower presence of these taxa. *Blautia, Klebsiella*, and *Megasphaera* species, which are mostly associated with hospital environments (Li, Liang et al., 2021). Additionally, the microbiome of the VD babies was predominantly formed of bacteria from the mother's vagina, whereas neonates delivered by CS revealed a larger frequency of species connected to the maternal oral microbiota. The newborn gut microbiome changes later in life due to dietary and lifestyle changes (Weng et al., 2023). The pattern of infant gut colonization is altered by delivery mode, and the composition of the *Firmicutes* genus is independently linked to the mode of delivery (**Fig. 5.1**) (Rutayisire et al., 2016). According to recent metagenomic investigations, some CS-born newborns temporarily acquire Bacteroides species soon after birth but are unable to sustain them over time, suggesting that the stability of early microbial colonization may be more important than initial exposure (Mitchell et al., 2020).

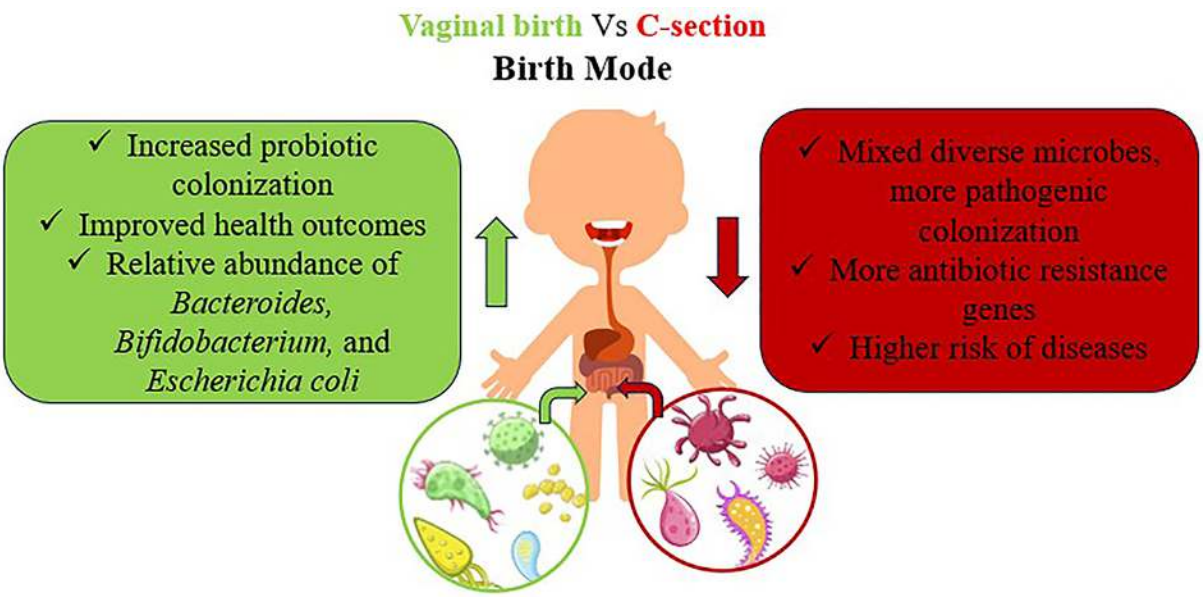

FIGURE 5.1 Birth mode factors shaping the development of the gut microbiome. ⏎

A metabolomic study analysis revealed that VD infants have fecal profiles enriched in metabolites associated with tryptophan and pyruvate metabolism. In contrast, CS-born infants exhibit elevated levels of lipid and amino acid intermediates, indicating metabolic dysbiosis (Li, Liang et al., 2021). These metabolic alterations may have a higher prevalence of asthma, allergies, and obesity among individuals later in life. Another recent microbiome study also confirmed that approximately 95% of neonatal microbes originate from the maternal body; however, CS-born infants acquire a microbiota resembling the maternal oral and skin microbiomes, rather than vaginal sources (Weng et al., 2023; Ma et al., 2024).

5.2.3 Antibiotic Exposure and Microbial Disruptions

Maternal antibiotic exposure during pregnancy and after delivery increases the *Enterobacteriaceae* and decreases *Lactobacillus* in the microbiomes of mothers and newborns (Zhou et al., 2020). Metagenomic study data demonstrated that exposure to antibiotics affects microbial diversity and functional pathways, affecting immunological regulation and food

metabolism among the infants (Yadav, 2024). Additionally, only 25% of bacterial species are vertically transmitted in antibiotic-treated compared to 72% in untreated ones, indicating that antibiotic-mediated dysbiosis hinders maternal-to-infant microbial inheritance (Li, Tapiainen et al., 2021). Mothers who administered antibiotics soon before or after giving birth, exposing the baby to antibiotics indirectly through breast milk or placental transfer. At one year of age, infants exposed to antibiotics, both directly and indirectly, had a more diverse microbiome. Additionally, the phylum *Proteobacteria* was found to be relatively abundant, while that of the phylum Bacteroidetes was found to be lower among newborns; however, a decrease in Bacteroidetes was observed among breastfed infants. Similar patterns emerged even after accounting for delivery mode, early membrane rupture, breastfeeding, and postnatal antibiotic use (Persaud et al., 2014). Antibiotic exposure also increases the ARGs and mobile genetic elements of the infant gut, many of which originate from the breast milk and mother's gut microbiota (Pärnänen et al., 2018). According to a few recent studies, antibiotic-driven disruption during this critical developmental window alters both inherited and microbe-to-microbe gene exchange processes, changing the microbiome and resistance patterns in early life (Bernabeu et al., 2024).

5.3 Early-life Nutrition and the Development of the Microbiome Development

A well-balanced and nutritious diet is the cornerstone of nutrition in order to support children's growth and development. Since birth, breastfeeding ensures that they receive a nutrient-rich diet uniquely adapted to their needs, strengthening their immunity and providing essential nutrients. After 6 months, semisolid home-cooked foods are introduced as part of complementary feeding, while nursing lasts until the child is two to three years old to prevent malnutrition and improve health outcomes (Isolauri et al., 2019).

5.3.1 The Function of Human Milk Oligosaccharides

HMOs are the most prevalent particulate elements of human breast milk, and they are crucial in forming the infant's gut microbiota and overall immune system health (**Fig. 5.2**). Additionally, some fatty acids and oligosaccharides that affect early microbial colonization and pathogen and other microbe adherence to the gut barrier, as well as particular microbiota and molecules involved in host–microbe interaction, are human milk protective chemicals. *Bifidobacterium breve, B. longum, Lactobacillus fermentum*, and *L. gasseri* are the predominant bacteria in human breast milk, followed by *Staphylococcus* and *Streptococcus* species. Breast milk has also been associated with containing butyrate-producing organisms, including *Coprococcus* and *Faecalibacterium*, as well as strictly anaerobic gut-associated germs such as *Blautia, Clostridium, Collinsella*, and *Veillonella* (Gomez-Gallego et al., 2016). However, evidence of a large and varied microbial community in breast milk has been revealed by the latest sequencing techniques (Gomez-Gallego et al., 2016). Optimal growth factors and HMOs, which include more than 200 prebiotic oligosaccharide isomers, are given to the health-promoting microorganisms. Differences in the oligosaccharide composition of breast milk help shape how microbes establish within an infant's gut. Oligosaccharides, scientifically shown to support the development of some gut bacterial genera, such as *Bifidobacterium* spp. and *Staphylococcus*, which are also found in breast milk (Aakko, Kumar et al., 2017). The HMO profile shapes infant gut microbiota by promoting specific bacteria and acting as decoys for certain pathogens (Wang, Li et al., 2015). A study demonstrated that infants fed breast milk from nonsecretor mothers with low 2′-fucosyllactose (2′ FL) levels show deferred and reduced *Bifidobacterium* colonization compared to those getting milk from secretor mothers with higher 2′ FL levels (Lewis, Totten et al., 2015, Isolauri, Rautava et al., 2019).

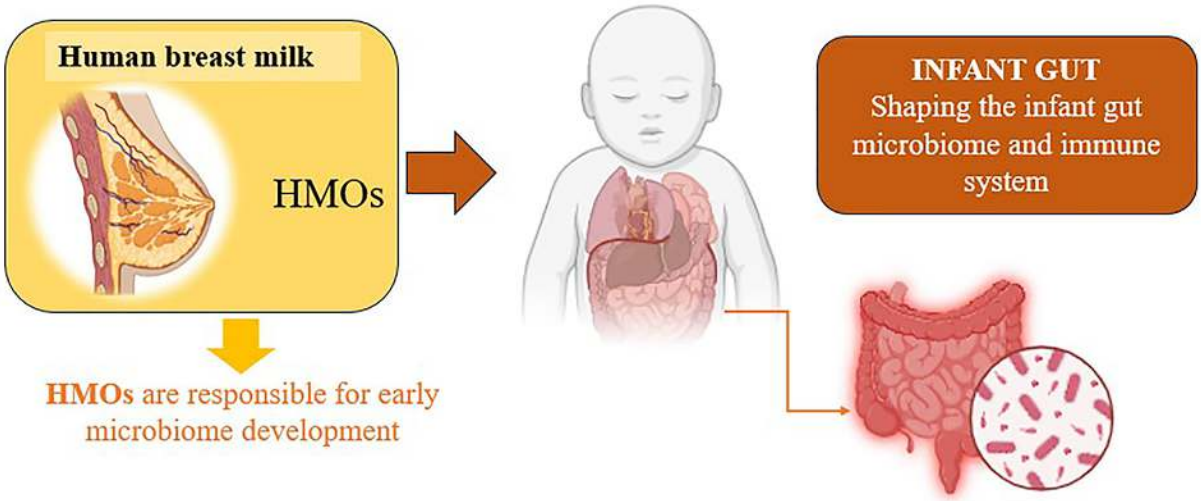

FIGURE 5.2 HMOs from breast milk shape early infant gut microbiome development. ⏎

Recent metagenomic and metabolomic studies demonstrated that specific fucosylated and sialylated HMO structures guide microbial succession in a structure-dependent manner. For example, 2′-FL and *difucosyllactose* (di-FL) encourage syntrophic relationships between *Eubacterium hallii* and *B. infantis*, enhancing short-chain fatty acid (SCFA) production and gut community stability (Dedon et al., 2023). Infants fed formula supplemented with major HMOs exhibited higher abundance of *B. infantis* and decreased *Clostridium difficile*, gut microbiota resembling that of breastfed infants, as well as improved gut barrier and immunological maturation (Bosheva et al., 2022). It suggests that HMOs facilitate the sharing of resources between bacteria, allowing *Bifidobacteria* and other commensals to cooperatively degrade and feed on one another, which is crucial for maintaining microbiome stability (Ioannou et al., 2024).

5.3.2 Breast Milk Microbiota as a Microbial Inoculum

Breast milk is commonly known to influence immune, metabolic, and programming in infants. Breast milk's microbiome, which is influenced by the mother's health and delivery method, is crucial in guiding the colonization of the infant's gut (Rautava, 2016). Breast milk contains a

variety of microbial taxa, including *Lactobacillus, Bifidobacterium, Corynebacterium, Streptococcus*, and *Staphylococcus*, which serve as a starter culture, helping to develop the healthy gut microbiota in the breastfed infant (**Table 5.1**) (Fernández et al., 2020). Although formula-fed children had a more varied gut flora, breastfed infants' intestinal flora is primarily dominated by *Bifidobacteria* (Harmsen et al., 2000). A study linking microbial communities in infants, particularly those of the genera *Streptococcus, Veillonella*, and *Bifidobacterium*, suggests long-term microbial transfer through breastfeeding (Lundgren et al., 2023). Breast milk contains a significant amount of energy in the form of indigestible HMOs, which can be ingested by intestinal microbes but do not directly give the infant energy. HMOs also operate as decoy receptors for prospective pathogens, defending against infections and influencing the growth of *Bifidobacteria* (Roger et al., 2010; Bode, 2012; Rautava, 2016). In order to make up for their lack of touch with the mother's vaginal microorganisms during delivery, CS-born newborns seem to be more affected by microbial transmission. A study on metagenomics and metabolomics has shown that breast milk changes the microbiota dynamically at the time of lactation, with early colostrum being particularly rich in *Staphylococcus* and *Streptococcus*. In contrast, mature milk harbors more *Lactobacillus* and *Bifidobacterium* (Karampatsas et al., 2022). These microbial populations work synergistically with HMOs and immune factors such as lysozyme and lactoferrin, promoting the proliferation of beneficial taxa and preventing pathogen colonization (Wong & Xiao, 2018).

TABLE 5.1

Key Bacterial Genera in Human Breast Milk and Their Roles in Infant Gut Colonization ⏎

Microbial Taxa	Role in Infant Gut Colonization	References
Streptococcus	Dominant genus in milk; contributes to overall microbial diversity in milk and initial gut colonizers	Lopez Leyva, Gonzalez et al. (2022)

Microbial Taxa	Role in Infant Gut Colonization	References
Staphylococcus	Part of the core milk microbiome, potentially seeds the gut or interacts with other microbes	Notarbartolo, Giuffrè et al. (2022)
Bifidobacterium	Specific species, including *Bifidobacterium longum* and *Bifidobacterium infantis*, present in both human breast milk and the gastrointestinal tract of infants, correlated with gut SCFA profiles production, metabolic programming	Laursen, Pekmez et al. (2021), Qi, Zhou et al. (2022)
Lactobacillus	Maternal probiotic (Lactobacilli) supplementation raises the amount of SCFAs in milk and the baby's gut.	Lopez Leyva, Gonzalez et al. (2022)
Enterococcus	Potentially participates in early colonization or cross-feeding interactions in the gut	Li et al. (2022)
Proteobacteria (Pseudomonas, Acinetobacter)	Genera like Enterobacteriaceae found in milk may influence early gut immune maturation, indicating possible seeding from human breast milk	Wang et al. (2023)

5.3.3 Formula Feeding and Altered Microbiome Trajectories

Infant feeding practices have a profound influence on gut microbial assembly during early life. In early life, the gut microbiome undergoes dynamic changes that gradually lead to the formation of diverse microbial species (Roswall et al., 2021; Yatsunenko et al., 2012). The foundation for future gains in taxonomic and functional diversity is laid at birth when pioneering bacteria infiltrate the sterile gut (Sprockett et al., 2018; Arrieta, 2025). Both host-related variables, such as oxygen, intestinal pH availability, and glycan expression on both breast milk and gut lining, and external factors, such as delivery mode, the introduction of solid foods, breastfeeding, and antibiotic exposure, have an impact on these early changes in succession (Mercer et al., 2024; Arrieta, 2025). Formula-fed

infants initially exhibit more bacterial diversity but reduced dominance of *Bifidobacterium* and *Lactobacillus*, which are essential commensals encouraged by HMOs (Odiase et al., 2023). Formula feeding frequently causes intestinal inflammation and metabolic disorders by encouraging the growth of *Clostridium, Enterobacteriaceae*, and *Streptococcus* (Ma et al., 2020).

A metagenomic study by Di Guglielmo et al. (2019) exhibited that complementary-fed infants altered microbial functional gene profiles, including an enrichment of genes involved in autotransporter adhesins with a decreased abundance of CRISPR/Cas9-related genes, signaling diminished microbial defense mechanisms and stability (Di Guglielmo et al., 2019). Furthermore, infants receiving formula usually have higher fecal pH, which inhibits the growth of acid-tolerant *Bifidobacteria* and encourages the early formation of an adult-like microbial community (Usman et al., 2024).

Although modern formulas are increasingly supplemented with prebiotics, probiotics, and HMOs to mimic breast milk, significant compositional gaps persist. For example, supplementation with *B. longum* and galacto-oligosaccharides (GOS) can partially restore a bifidogenic profile. However, the metabolic and immunological benefits remain lower than those achieved through exclusive breastfeeding (Fabiano et al., 2021). One systematic review confirmed that prebiotic- and probiotic-enriched formulas approximate the microbial ecology of breastfed infants and immunomodulatory effects of mother's milk (Chong et al., 2022).

5.4 Complementary Feeding and Microbiome Maturation

Early newborn development is greatly influenced by the period from breast milk to formula feeding until the introduction of solid foods during the weaning phase (Shi et al., 2024).

5.4.1 Influence of Dietary Macronutrients (Fiber, Fat, Protein, and Carbs)

During weaning, the early-life microbiome is supported by the introduction of plant-based, high-fiber meals. Notably, fiber sources from pulses, fruits, and vegetables commonly included in early purees are associated with infant-related bacterial species of *Bifidobacterium, Bacteroides, Lactobacillus, Streptococcus*, and *Enterococcus*. Immune regulation is significantly influenced by these microorganisms (Lalli et al., 2025). Fiber consumption during the weaning phase modifies microbial populations and increases the production of SCFAs, especially butyrate and acetate, which are critical for intestinal epithelium health and immunological modulation (McDonald et al., 2025).

On the other hand, changes toward bile-tolerant species, such as *Bacteroides*, have been associated with excessive dietary fat, particularly from animal sources, which may limit microbial diversity and low-grade inflammation (Yang et al., 2020). Meanwhile, eating protein, especially from meats and dairy, influences the microbiome's composition by promoting proteolytic fermentation, which depends on the kind and amount of protein, and can result in both beneficial and harmful metabolites (Laursen et al., 2016).

When infants start eating solid foods during the crucial complementary feeding window, the gut microbiota undergoes changes that might have long-term effects on the microbiome development of the microbiome and the infants' outcomes. During the supplement feeding period, the gut microbiome may influence outcomes such as growth trajectories, obesity risk, immune function, and the development of allergies (Noles et al., 2025). Overconsumption of animal protein and saturated fat during weaning may cause microbial metabolism to be skewed toward pathways linked to inflammation and proteolysis. It could make metabolic dysregulation more likely in later life (Laursen, 2021).

5.4.2 Introduction of Plant-based versus Animal-based Foods

The establishment of the early life microbiota during the weaning phase is supported by introducing plant-based and high-fiber foods. Fiber-rich foods, such as oats, fruits, and vegetables, which are common in purees, are linked to infant-associated bacteria genera, including *Bifidobacterium, Bacteroides*, *Lactobacillus, Enterococcus*, and *Veillonella*, which contribute to immunomodulation (Lalli et al., 2024). During the weaning process, these microbial metabolites improve immunological tolerance and intestinal epithelial integrity. Greater plant dietary diversity has been demonstrated to expedite microbial maturation, which is relatively abundant in *Ruminococcaceae* and *Bacteroides* species (McDonald et al., 2025). At approximately six months, plant-based foods are typically fed to newborns to help with their growth and development needs. Human gut carbohydrases primarily convert plant starches with 1–4-alpha glycosidic bonds into glucose for absorption in the small intestine, and they do not break down plant cell wall polysaccharides. The two main polysaccharides are xyloglucan and cellulose (present in leafy greens), gelatin (present in veggies and fruits), glucans (present in oats), mucilage (present in seeds), and chitin (such as in mushrooms) (Parkar et al., 2021).

Complementary foods derived from animals, such as dairy, meat, and fish, provide vital micronutrients like zinc and iron, which enhance the growth of an infant's gut. When an infant is introduced to complementary food, there might be an increase in microbial community and an alteration toward a more developed gut microbiota. (Tang et al., 2023). These variations imply that animal protein sources promote different microbiological and metabolic results, which may have consequences for growth patterns and the regulation of inflammation (Tang et al., 2019). However, an excessive reliance on animal-based meals, particularly those heavy in saturated fats, might decrease microbial diversity and change metabolism to bile acid modification and proteolysis, which may raise the risk of metabolic diseases (**Table 5.2**) (Lin, 2023).

TABLE 5.2

Comparative Effects of Plant-based and Animal-based Complementary Foods on Infant Gut Microbiome and Metabolic Function ⏎

Parameters	Plant-based Complementary Food	Animal-based Complementary Food	References
Major nutritional component	Rich in carbohydrates, fibers, and antioxidants	Rich in iron, zinc, lipids, and proteins	Lalli et al. (2024)
Dominant microbial genera	*Bifidobacterium, Bacteroides, Faecalibacterium, Ruminococcaceae*	*Ruminococcus, Roseburia*	McDonald et al. (2025), Tang et al. (2023)
Microbial components	SCFAs, particularly acetate, butyrate	Secondary bile acids, branched-chain fatty acids, and butyrate	Lalli et al. (2025), Tang et al. (2023)
Influence on the gut microbiome	Encourages increased microbial diversity and development similar to that of adults	Consuming an excessive amount of saturated fat may increase richness but decrease variety	McDonald et al. (2025), Lin et al. (2023)
Functional effects	Improves immunological tolerance, intestinal epithelial integrity, and anti-inflammatory potential	Enhances the development and absorption of micronutrients and affects proteolytic pathways	Lalli et al. (2025; Tang et al. (2019)
Possible health concerns	Promotes resilience and long-term metabolic health	Excess consumption may lead to bile acid imbalance and metabolic risk	Lin (2023)

5.4.3 Effects of Early Dietary Diversity, Sugar, and Processed Foods on the Gut Microbiome of Infants

Gut microbial carbohydrate utilization shapes the gut diversity and is connected to the conditions of metabolism of glycolipid. Large intake of sugar could cause alterations in the composition and colonization of the infant's gut microbiota and is known to cause particular common diseases, such as obesity, diabetes, cardiovascular disease, and impaired cognitive issues. Excessive consumption of sugar can alter the carbohydrate stores and create conditions that are conducive to dangerous bacteria. Different forms of carbohydrates influence the gut flora, which helps to alleviate problems associated with glycolipid metabolism. During complementary feeding, SCFAs, which are byproducts of the breakdown of dietary fiber, mediate interactions between the microbiota and the host (Mokhtari et al., 2023). Important producers of SCFAs that preserve the gut integrity and regulate the immune function include *Faecalibacterium* and *Coprococcus*. Microbiome dysbiosis can be accelerated and its abundance reduced by early exposure to ultra-processed meals, which are often heavy in added sugars, salt, and emulsifiers (Shi et al., 2024).

Dietary diversity during the supplementary feeding phase is ultimately linked to the maturation and solidity of the infant gut microbiome, boosting the establishment of essential bacteria families that are involved in fiber fermentation and SCFA synthesis (McDonald et al., 2025). Early colonization and more microbial diversity are shown in infants who eat a variety of supplemental plant-based diets, which encourages immunological tolerance and metabolic flexibility. Additionally, the early dietary exposures, even in modest amounts, alter host and microbial gene expression, suggesting that microbes respond quickly to food diversity (Ferro et al., 2024).

Energy-dense, low-fiber meals may inhibit alpha diversity and change metabolic potential toward lipolytic and proteolytic fermentation processes, which are associated with insulin resistance and obesity in later life (Laursen, 2021). During complementary feeding, balanced exposure to whole foods high in phytonutrients and natural fibers improves microbial richness and functional maturity (Noles et al., 2025).

5.5 Host–Microbiota Interactions and Functional Consequences

A significant implication of host–microbiota interactions in the treatment and prevention of diseases is based on therapies that restore altered microbiota, highlighting the crucial role of host–microbiota equilibrium and how disruption of this homeostasis can result in changes from healthy to pre-disease and disease states. A significant percentage of people who lead Western lifestyles are in a pre-disease state, which could account for the recent sharp rise in obesity and inflammatory disorders (Simon et al., 2019).

5.5.1 Infant Immune System and Microbiota

Infant gut microbiota shapes the immune development, leading to lifelong immunological tolerance and disease risk. The gut microbiota delivers essential microbial antigens and metabolites that instruct the innate and adaptive immunity, supporting intestinal epithelial integrity, secretory immunoglobulin A (IgA) production, and T-cell differentiation (Zheng et al., 2020). Immune tolerance in mouse models and human infant cohorts depends on early colonization by commensal taxa like *Bifidobacterium* and *Bacteroides*. Increased T helper (Th) 2 and Th17 responses brought on by abnormal colonization, such as that caused by CS-born children, put people at risk for allergies and autoimmune diseases (Ansaldo et al., 2021).

A new investigation revealed that T-cell-deficient infants had altered metabolomic profiles and irregular microbiota profiles, which indicates that microbial-derived markers are critical for neonatal T-cell development and demonstrate a mutually beneficial relationship between microbial and immune development (Caspani et al., 2022). Immunological competence and resistance may rely on microbiota immunological mutual growth, since early exposure to a variety of commensals has been linked to better mucosal immunity and vaccine responses (Ciabattini et al., 2019).

5.5.2 Early Neurodevelopmental and Gut–Brain Axis Implications

The infant gut–brain axis is a direct channel that links to the immune, neurological, endocrine, and gut microbiota systems. This connection has a substantial influence on the structure, function, and behavior of the brain throughout infancy, which is a pivotal period for both microbial colonization and neurodevelopment. Microbial metabolites, such as tryptophan metabolites and neurotransmitter precursors, help to build a healthy gut microbiota, which supports neurogenesis, synaptic plasticity, and blood–brain barrier development (Vaher et al., 2022). Early dysbiosis caused by antibiotic treatment affects microbial activation, neuroinflammation, and delayed mental growth, demonstrating that signals from the infant gut microbiota are critical for the standardization of connections through the brain (**Fig. 5.3**) (Bettag et al., 2023).

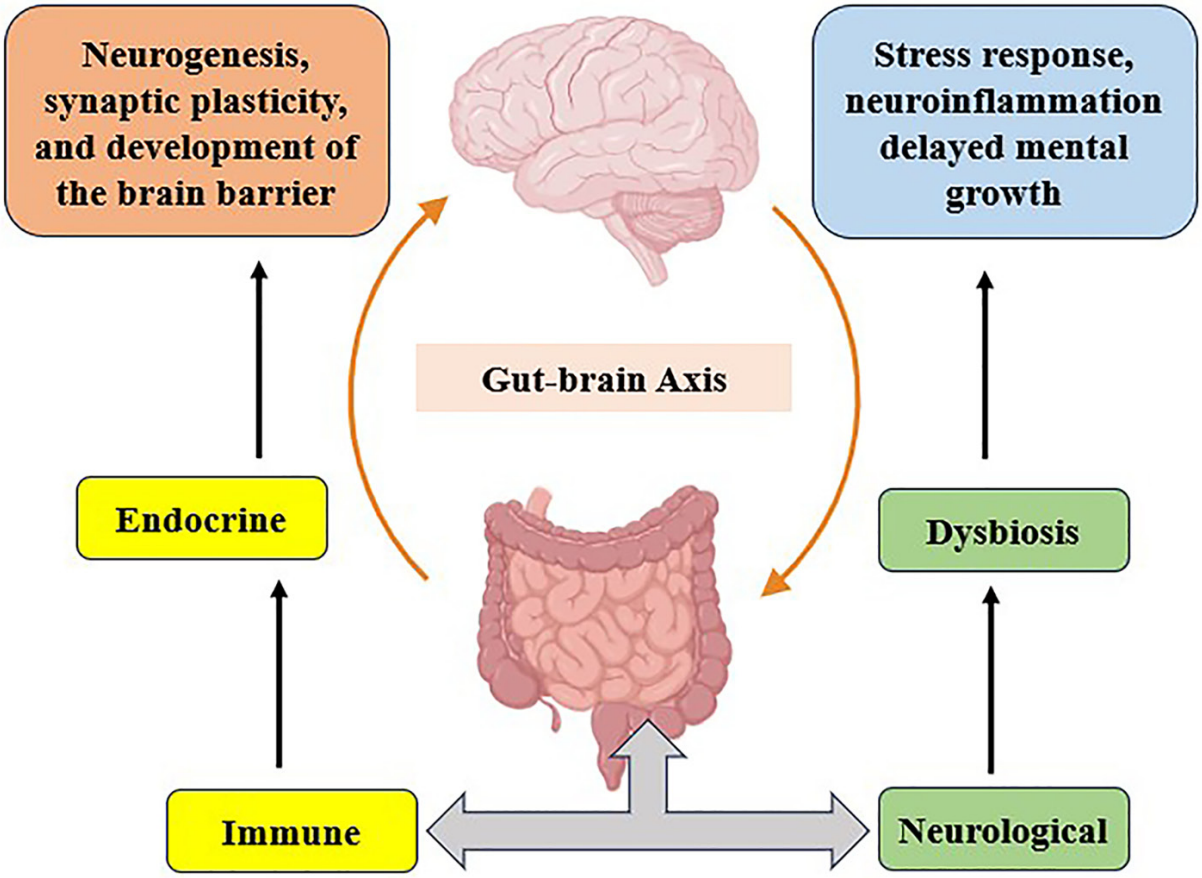

FIGURE 5.3 Gut–brain axis in infants. ⏎

The infant gut microbiota has a variety of interactions with the brain and nervous system, including neuroimmune control, endocrine signaling, and vagus nerve activity. Bacterial metabolites regulate neurotransmission by altering and generating gamma-aminobutyric acid (GABA) and serotonin, influencing social behavior and stress response (Sherwin et al., 2016). Animal studies indicate altered hippocampal gene expression and anxiety-like behaviors that are reversible with probiotic colonization, demonstrating the causal involvement of microbiota in behavioral and mental growth (Cryan, 2018). In addition, alterations in early microbial community colonization caused by immunological and metabolic dysregulation are linked to neurodevelopmental disorders, such as hyperactivity and autism spectrum disorder (ASD) (Wang et al., 2023).

5.5.3 Gut Microbial Metabolites and Host Signaling Pathways

Gut microbial enzymes interact with host physiology, affecting immune response, metabolism, and neuroendocrine signaling. SCFAs, which use G-protein-coupled receptors (GPCRs) to regulate intestinal barrier function, inflammation, and other functions, are the most extensively studied of these. SCFAs influence gene expression by inhibiting histone deacetylase, linking microbial metabolism to host epigenetic regulation (Xie et al., 2013). Lipid metabolism and bile acid transformations are important for immunological tolerance and energy regulation (Ouyang et al., 2023). The bacterial breakdown of aromatic amino acids (AAAs) creates phenolic compounds and interacts with host receptors, which regulate mucosal immunity and oxidative stress responses (Liu, Hou et al., 2020). Similarly, the microbiota's fatty acid and lipid metabolites control host lipid signaling pathways, which influence how inflammation and metabolism interact (Zhang et al., 2024).

Metabolomics demonstrated that infant gut microbial metabolites interact with host receptors that regulate multiorgan signaling networks, notably related to gut–liver and gut–brain axis (Chang, 2024). These metabolites impact systemic metabolic pathways essential for growth and neurodevelopment in infants, as well as immunological tolerance and intestinal development (Roager et al., 2023).

5.5.4 Systemic and Developmental Implications for Gut Microbial Metabolism and Host Signaling Pathways

Beyond their effects on the intestines, gut microbial metabolites act as systemic signaling molecules that affect the liver, brain, and immune system, thereby establishing the biochemical foundation for long-term health trajectories and metabolites, including SCFAs, bile acid derivatives, and polyamines, that regulate important host signaling pathways linked to immunological homeostasis, energy metabolism, and neurodevelopment from early life (Roager et al., 2023). *Bifidobacterium* and *Faecalibacterium*

create acetate and butyrate that interact with GPCRs to activate AMP-activated protein kinase (AMPK) and gamma peroxisome proliferator-activated receptor (PPAR-γ), which regulate the metabolism of glucose and lipids (Zhang et al., 2024).

Metabolites increase intestinal epithelial integrity and control mucosal immunological tolerance (Liu, Hou et al., 2020). Those substances are known to be neuroactive, influencing microglial development and neuroinflammation through the gut–brain axis (Vaher et al., 2022). Similarly, bile acids generated by microbial enzymes such as bile salt hydrolases bind to the farnesoid X receptor (FXR) and TGR5, which influence hepatic lipid metabolism and anti-inflammatory responses in infants (Ouyang et al., 2023).

5.6 Dysbiosis and Long-term Health Implications

The newborn gut microbiota plays a key role in healthy aging, as emphasized by its relationship to human physiology. This relationship stands out as a possible issue for future treatments due to its dynamic and sensitive character (Tseng & Wu, 2025).

5.6.1 Early-life Dysbiosis and Its Causes

Rapid changes in the infant's gut microbial establishment throughout infancy are due to maternal gut microbiome, delivery mode, breastfeeding, antibiotic use, and a variety of external factors. A comprehensive investigation of nutrition, microbiota, and transcriptome-level genetic activity might shed light on how diet influences the human–microbiome interaction. The gut dysbiosis linked to necrotizing enterocolitis in infancy as well as several chronic conditions, may affect in later life, such as overweight, cancer, allergies, asthma, and diabetes (Jeong, 2021). Infants born with VD often develop a good microbiota that is predominated by *Bifidobacterium* and *Lactobacillus*. While CS-born are mostly colonized by

environmental microbes, such as *Corynebacterium* and *Staphylococcus*, which can increase their susceptibility to dysbiosis (van Best et al., 2015).

By eliminating beneficial microbes, decreasing community diversity, and encouraging the growth of resistant strains, antibiotic exposure in maternal and newborns interferes with the early colonization process (Hong et al., 2017). Breast milk promotes the development of a protective microbiome through HMOs and bioactive substances, whereas formula feeding restricts colonization by *Bifidobacterium* and *Lactobacillus*, favoring the *Enterobacteriaceae* family (Turroni et al., 2020).

Gut dysbiosis is represented by how opportunistic microorganisms, such as *Staphylococcus aureus*, can lower the quantity of anaerobes such as *Bifidobacterium* and disturb the adult human microbiome. According to Graspeuntner et al. (2019), suggested that the absence of these anaerobic microbes is crucial for preventing bacterial translocation and maintaining epithelial integrity, which increases the risk of gut-derived sepsis (Graspeuntner et al., 2018). Additionally, dysbiosis modifies the regulation of metabolism and immunological tolerance, and it may be caused by lifestyle variables like early-life stress that limit exposure to a variety of bacteria (Cong et al., 2016; Casaburi et al., 2021).

5.6.2 Links with Allergic Reactions, Asthma, and Atopy

The World Health Organization (WHO) encourages breastfeeding during the first six months to reduce allergies and asthma among babies. Complementary meals must be introduced beyond this time, and breastfeeding must continue. Dairy, eggs, peanuts, and fish are among the foods associated with allergies that should not be eaten until the child is three years old (Nwaru et al., 2013). Changing microbial colonization throughout infancy affects immunological homeostasis, especially by influencing Th cell responses toward Th2-mediated inflammation, according to several birth cohort studies (Panzer & Lynch, 2015). A lot of emphasis has been placed on the role that nursing plays in reducing allergy disorders. Breastmilk contributes to creating the infant's gut microbiota by

feeding beneficial microbes, prebiotic oligosaccharides that stimulate their growth, and immunactive components that boost immunological development (Pettersen & Arrieta, 2020; Chiu et al., 2019). Although there is much discussion about how breastfeeding influences the development of allergy disorders, research shows that early microbial patterns may contribute to risk. For example, children who subsequently acquired allergies exhibited dramatically lower *Firmicutes* and increased *Clostridium* species, which was associated with higher levels of fecal immunoglobulin E (IgE; Chiu et al., 2019).

Furthermore, metagenomic research revealed a decreased genetic potential for butyrate synthesis in children who later had allergy diseases, underscoring the importance of SCFAs formation in maintaining immunological tolerance (Cait et al., 2019; Chiu et al., 2019). This suggests that changes in microbial networks during important early immune development windows may put people at risk for persistent allergy and atopic illnesses. Early gut microbial composition, notably low levels of *Bifidobacterium* and *Escherichia* or *Shigella*, predicts allergic rhinitis and atopic asthma during infancy or adolescence (Kallio et al., 2024).

5.6.3 Links to Obesity, Type 1 Diabetes, and Metabolic Syndrome

Due to its impacts on energy collection, inflammation, and metabolic control, Obesity, type 1 diabetes, and metabolic syndrome have been closely linked with early life dysbiosis. Infants showing a higher *Firmicutes* to *Bacteroidetes* ratio, reduced *Bifidobacterium* abundance, enhanced dietary energy extraction, and inflammation exhibit microbiome patterns linked to risk for obesity later in life (Tilg & Moschen, 2015). Formula feeding, antibiotic exposure, maternal obesity, and CS delivery all interfere with early microbial colonization, resulting in a pro-inflammatory gut milieu that changes lipid metabolism and insulin sensitivity (Duca et al., 2018).

In type 1 diabetes, gut dysbiosis precedes colonization by modulating permeability in the intestinal and immune activation, allowing microbial antigens and endotoxins to stimulate β-cell-targeting immune responses (Ianiro et al., 2021). A reduced abundance of taxa, including *Faecalibacterium* and *Roseburia intestinalis*, has been associated with compromised gut barrier function and heightened systemic inflammation, ultimately promoting insulin resistance and broader metabolic dysfunction (Tilg & Moschen, 2014).

Maternal consumption of non-nutritive sweeteners is a risk factor for metabolic disturbances in offspring, acting through multiple pathways. Among these, gut microbiota and its metabolites serve as a key link between early-life exposure to non-nutritive sweeteners and the development of metabolic syndrome (Yin et al., 2025). Additionally, dysbiosis affects adipokine signaling, especially through the enrichment of *Erysipelotrichaceae* and *Lachnospiraceae*, which promotes metabolic inefficiency and inflammation (Armutcu & McCloskey, 2024).

5.6.4 Microbiota Restoration and Interventions

Restoring gut microbial balance is now considered clinically important, as correcting the dysbiosis microbiota has been shown to aid in the management of several illnesses. Current strategies include pre- and probiotics, dietary modifications, phage treatment, extracellular vesicles, metabolites, fecal microbiota transplantation (FMT), and immunomodulatory approaches (Dixit et al., 2021). Supplementing with probiotics containing species like *Bifidobacterium* and *Lactobacillus* strengthens the gut barrier, lowers inflammation, and increases the production of SCFA, all of which benefit host metabolism and immunity (Álvarez-Mercado et al., 2019).

Treatments based on microbiota, including FMT, probiotics, and prebiotics, are becoming more and more popular as innovative treatment approaches. FMT may help those with autoimmune diseases and IBD. In order to have a therapeutic effect, FMT has been demonstrated to

reconstruct the intestinal microecosystem, restore altered gut microbiota composition, and mediate innate and adaptive immunity responses (Yang et al., 2023). Restoration of FMT results in gut microbial diversity and reestablishes normal metabolite production, leading to improved insulin sensitivity, reduced intestinal inflammation, and modulation of immune pathways (Daliri et al., 2018). Additionally, tailored FMT techniques and donor-to-recipient microbiota matching are enhancing safety and efficacy results (Wu et al., 2023; García-Mateo et al., 2024). Precision probiotics, phage treatment, and postbiotics are examples of novel strategies being investigated to selectively treat dysbiosis without negative side effects. Microbiome-targeted treatments have great potential, but long-term efficacy, safety, and donor screening are still major obstacles (Facciotti, 2022; Todor & Ichim, 2025).

5.7 Modern Omics Approaches and Future Perspectives

The expanding use of advanced high-throughput sequencing, including transcriptomics, proteomics, metabolomics, and genomics, now produces data sets that could reach terabyte to petabyte scales. Integrating these molecular layers into a biological framework is challenging due to massive file sizes and inconsistent terminology across omics fields. Further complications include variations in verification of data, molecular characterization, management of data, biological interpretation, statistical evaluation, and long-term preservation. These challenges define the broader domain known as integrated omics, multiomics, and polyomics research (Misra et al., 2019).

5.7.1 Advances in Metagenomics

Metagenomic techniques and resources have deepened our understanding of the origin and establishment of the newborn microbiome and its influence on health and disease across the lifespan.

Microbiome research has benefited from technological developments in sequencing of the next generation, especially 16S rRNA gene amplicon, and shotgun metagenomic sequencing significantly advanced knowledge of gut microbiota development, marking a shift from the earlier unculturable period of microbiome research toward a fully multidisciplinary field. The relationship between the microbiome and infant illnesses may be better understood by investigating the neonatal microbiome using omics techniques. Metagenomics has deepened our understanding of the genes and newborn infant gut microbiome development and its influence on health and disease across the lifespan (Boudar et al., 2022). Recent research using shotgun metagenomics has shown dynamic microbial succession patterns in early life, finding dominating taxa that are essential for metabolic and immunological development, such as *Bifidobacterium*, *Bacteroides*, and *Lactobacillus* (Bäckhed et al., 2015; Lou et al., 2021).

The Metagenome-Assembled Genome Inventory for Children (MAGIC) is a high-resolution metagenomic database inventory that has revealed thousands of child-distinct bacterial and viral genomes, greatly improving the characterization of infant microbial gut communities (Peng, Zhu et al., 2024). These explain the identification of keystone species and microbial groupings that influence health outcomes, including allergy susceptibility and metabolic programming. The ecology of viruses in infancy is studied through the Early-Life Gut Virome (ELGV) database, highlighting the fact that over 68% of the viral species in newborns differ from those in adults (Zeng et al., 2024). Functional metagenomics has made it possible to investigate microbial metabolic pathways, including vitamin metabolism, carbohydrate use, and SCFA production, and to connect microbial gene expression to newborn health outcomes (Lugli et al., 2023; Xiong et al., 2017).

5.7.2 Personalized Nutrition and Microbiome Modeling

A new era of personalized nutrition has been brought about by developments in systems biology and computational modelling. This era

uses data from the individual's microbiome to predict how they will react to dietary treatments and to maximize health outcomes starting in infancy. The personalized metagenomic, metabolomic, and nutritional data enable precise modelling of host–microbe interactions, facilitating tailored dietary strategies that support optimal microbial and metabolic development (Shaaban et al., 2024). The personalized metabolic modeling of infant gut microbiomes, utilizing resources like the reconstruction and analysis of gut microbiota, indicates that CS-born infants exhibit lower metabolic potential and decreased SCFA synthesis, suggesting that customized nutritional interventions could help restore functional deficiencies in microbial activity (Shaaban et al., 2024).

The dietary microbiome on host gene expression occurs through personalized dietary modifications, including DNA methylation and histone acetylation. This process regulates immune maturation, metabolic homeostasis, and neurodevelopment (Nauta et al., 2013). Early-life microbiome modeling enables precise therapies to prevent allergy, metabolic, and neurodevelopmental issues in newborns by revealing how the mother's diet, nursing, and supplemental feeding affect microbial function and nutrient metabolism (Dogra et al., 2021).

5.7.3 Microbial Biomarkers of Healthy Infant Development

Identifying microbial biomarkers in infancy is crucial for assessing how a healthy gut microbiome develops, providing information on the risk of disorders in early life, and guiding microbiome-targeting therapies. High relative abundances of *Bifidobacterium, Lactobacillus*, and *Bacteroides* species, which are connected to better immunological maturation, gut barrier function, and metabolic function, are generally indicative of a healthy baby microbiome (Milani et al., 2017). Different microbial community state types in early infancy, particularly those enriched in *B. longum* subsp. *Infantis* have been shown by shotgun metagenomic analyses to be biomarkers of healthy immune and metabolic development,

correlating with increased production of SCFA and anti-inflammatory activity (Lugli et al., 2023).

Microbial biomarkers, including *Eubacterium rectale* and *Faecalibacterium prausnitzii*, have been connected to epithelial barrier integrity and anti-inflammatory signaling. On the other hand, dysbiotic babies who are at risk of allergies or necrotizing enterocolitis frequently have lower abundances of these taxa (Koleilat, 2017). Additionally, because of their capacity to control T-regulatory cell development and IgA release, *B. breve* and *Lactobacillus rhamnosus* have been suggested as predictive markers of mucosal immunoregulation (van Best et al., 2015). Functional biomarkers, such as genes for butyrate and acetate production, have also been found by metagenomic and metabolomic integration as indications of a robust microbiome linked to decreased inflammation and better neurodevelopmental outcomes (Bélteky et al., 2023).

5.7.4 Knowledge Gaps and Future Research Directions

There are still a lot of unanswered questions about how early-life microbial habitats affect long-term health, despite the quick advancements in microbiome research and omics technologies. In particular, the precise ways in which microbial succession affects immunological, metabolic, and neurodevelopmental effects are still not fully understood (Laursen et al., 2017). Research on infant gut microbiome is growing quickly, according to recent bibliometric analyses, but the field is still marked by methodological flaws, a dearth of longitudinal cohort data, and a focus on diversity representation (Yang et al., 2024).

Gut immune colonization mechanistic pathways have been demonstrated to be mediated by metabolites like SCFAs. Further research is needed to understand the role of microbial metabolites within newborn physiology (Bui et al., 2025). Additionally, research highlights the significance of merging multi-omics approaches, such as transcriptomics, metagenomics, metabolomics, and host proteomics, to clarify how signals coming from the microbiome coordinate developmental programming (Boudar et al., 2022).

Precision nutrition, targeted probiotics, and microbiota restoration therapy are examples of another translational microbiome science that connects bench discoveries to clinical applications. Research emphasizes the lack of population-specific data and contextual factors that affect microbial resilience and response to interventions (Mudhluli et al., 2024).

5.8 Conclusion

The establishment of the early infant gut microbiome is a crucial process influencing long-term health. From birth through early childhood, the infant gut experiences microbial colonization and maturation, shaped by maternal factors, delivery mode, and diet. Among these, early nutrition plays the most decisive role. Colonization, which involves feeding formula, breast milk, or a mix of the two, affects the variety, stability, and microbial function, thereby setting the foundation for immune development and metabolic balance throughout life.

Breastfeeding provides the optimal environment for healthy microbial growth. Human milk contains beneficial bacteria, immune-modulating proteins, and complex carbohydrates, such as HMOs, that nourish key microbes, including *Bifidobacterium* and *Lactobacillus*. These species help establish a stable, protective gut ecosystem that supports digestion, strengthens the intestinal barrier, and guides immune tolerance. In contrast, Infants fed formula frequently grow up to have more varied but less functionally specialized microbiota dominated by *Enterobacteriaceae* and *Clostridium* species, which tends to be more inflammatory and higher risks of allergies in later life.

The complementary feeding mode represents another milestone in the establishment of the microbiome. A gradual shift from milk-based to solid diets between 6 and 24 months leads to increased microbial communities and the emergence of SCFA-producing bacteria. Diets rich in fiber and plant-based foods support these beneficial populations, while excessive

intake of sugar or protein can disrupt microbial balance and promote pro-inflammatory species.

VD infants acquire beneficial microbes, such as *Lactobacill*us and *Bacteroides*, while those born via CS often harbor more skin-associated bacteria, which can delay microbial diversification. Maternal diet, antibiotic exposure, and health status during pregnancy further influence this microbial inheritance, influencing the immune development and metabolism among infants.

Advances in modern omics techniques have led to a new understanding of the interaction between diet and microbes during infancy. These approaches open doors for personalized nutrition and targeted interventions using probiotics, prebiotics, and postbiotics to promote optimal microbial development. Therefore, understanding and supporting this delicate microbial ecosystem from the outset is crucial for preventing disease and promoting overall well-being throughout the lifespan**.**

References

Álvarez-Mercado, A. I., Navarro-Oliveros, M., Robles-Sánchez, C., Plaza-Díaz, J., Sáez-Lara, M. J., Muñoz-Quezada, S., … & Abadía-Molina, F. (2019). Microbial population changes and their relationship with human health and disease. *Microorganisms*, *7*(3), 68. ⏎

Ansaldo, E., Farley, T. K., & Belkaid, Y. (2021). Control of immunity by the microbiota. *Annual Review of Immunology*, *39*(1), 449–479. ⏎

Armutcu, F., & McCloskey, E. (2024). Signaling modifications associated with obesity and dysbiosis and their impact on metabolic disorders. *Journal of Cellular Signaling*, *5*(2), 57–64. ⏎

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., … & Wang, J. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host & Microbe*, *17*(5), 690–703. ⏎

Bélteky, M., Milletich, P. L., Ahrens, A. P., Triplett, E. W., & Ludvigsson, J. (2023). Infant gut microbiome composition correlated with type 1 diabetes acquisition in the general population: the ABIS study. *Diabetologia*, *66*(6), 1116–1128. ⏎

Bernabeu, M., Cabello-Yeves, E., Flores, E., Samarra, A., Summers, J. K., Marina, A., & Collado, M. C. (2024). Role of vertical and horizontal microbial transmission of antimicrobial resistance genes in early life: insights from maternal-infant dyads. *Current Opinion in Microbiology*, *77*, 102424. ⏎

Bettag, J., Goldenberg, D., Carter, J., Morfin, S., Borsotti, A., Fox, J., … & Jain, A. K. (2023). Gut microbiota to microglia: microbiome influences neurodevelopment in the CNS. *Children*, *10*(11), 1767. ⏎

Bosheva, M., Tokodi, I., Krasnow, A., Pedersen, H. K., Lukjancenko, O., Eklund, A. C., … & 5 HMO Study Investigator Consortium. (2022). Infant formula with a specific blend of five human milk oligosaccharides drives the gut microbiota development and improves gut maturation markers: a randomized controlled trial. *Frontiers in Nutrition*, *9*, 920362. ⏎

Boudar, Z., Sehli, S., El Janahi, S., Al Idrissi, N., Hamdi, S., Dini, N., … & Ghazal, H. (2022). Metagenomics approaches to investigate the neonatal gut microbiome. *Frontiers in Pediatrics*, *10*, 886627. ⏎

Bui, T. N., Paul, A., Guleria, S., O'Sullivan, J. M., & Toldi, G. (2025). Short-chain fatty acidsa key link between the gut microbiome and T-lymphocytes in neonates?. *Pediatric Research*, 99(5):1–9. doi: 10.1038/s41390-025-04075-0 ⏎

Cait, A., Cardenas, E., Dimitriu, P. A., Amenyogbe, N., Dai, D., Cait, J., … & Mohn, W. W. (2019). Reduced genetic potential for butyrate fermentation in the gut microbiome of infants who develop allergic sensitization. *Journal of Allergy and Clinical Immunology*, *144*(6), 1638–1647. ⏎

Casaburi, G., Duar, R. M., Brown, H., Mitchell, R. D., Kazi, S., Chew, S., … & Freeman, S. L. (2021). Metagenomic insights of the infant

microbiome community structure and function across multiple sites in the United States. *Scientific Reports*, *11*(1), 1472. ⏎

Caspani, G., Green, M., Swann, J. R., & Foster, J. A. (2022). Microbe-immune crosstalk: evidence that T cells influence the development of the brain metabolome. *International Journal of Molecular Sciences*, *23*(6), 3259. ⏎

Chang, P. V. (2024). Microbial metabolite-receptor interactions in the gut microbiome. *Current Opinion in Chemical Biology*, *83*, 102539. ⏎

Chiu, C. Y., Chan, Y. L., Tsai, M. H., Wang, C. J., Chiang, M. H., & Chiu, C. C. (2019). Gut microbial dysbiosis is associated with allergen-specific IgE responses in young children with airway allergies. *World Allergy Organization Journal*, *12*(3), 100021. ⏎

Chong, H. Y., Tan, L. T. H., Law, J. W. F., Hong, K. W., Ratnasingam, V., Ab Mutalib, N. S., … & Letchumanan, V. (2022). Exploring the potential of human milk and formula milk on infants' gut and health. *Nutrients*, *14*(17), 3554. ⏎

Ciabattini, A., Olivieri, R., Lazzeri, E., & Medaglini, D. (2019). Role of the microbiota in the modulation of vaccine immune responses. *Frontiers in Microbiology*, *10*, 1305. ⏎

Cong, X., Xu, W., Romisher, R., Poveda, S., Forte, S., Starkweather, A., & Henderson, W. A. (2016). Gut microbiome and infant health: brain-gut-microbiota axis and host genetic factors. *Yale Journal of Biology and Medicine*, *89*(3), 299. ⏎

Cryan, J. (2018). The gut microbiome: a key regulator of neurodevelopment and behaviour. *Schizophrenia Bulletin*, *44*(Suppl 1), S59. ⏎

Daliri, E. B. M., Tango, C. N., Lee, B. H., & Oh, D. H. (2018). Human microbiome restoration and safety. *International Journal of Medical Microbiology*, *308*(5), 487–497. ⏎

Dedon, L. R., Hilliard, M. A., Rani, A., Daza-Merchan, Z. T., Story, G., Briere, C. E., & Sela, D. A. (2023). Fucosylated human milk oligosaccharides drive structure-specific syntrophy between

Bifidobacterium infantis and *Eubacterium hallii* within a modeled infant gut microbiome. *Molecular Nutrition & Food Research*, *67*(11), 2200851. ⏎

Di Guglielmo, M. D., Franke, K., Cox, C., & Crowgey, E. L. (2019). Whole genome metagenomic analysis of the gut microbiome of differently fed infants identifies differences in microbial composition and functional genes, including an absent CRISPR/Cas9 gene in the formula-fed cohort. *Human Microbiome Journal*, *12*, 100057. ⏎

Dixit, K., Chaudhari, D., Dhotre, D., Shouche, Y., & Saroj, S. (2021). Restoration of dysbiotic human gut microbiome for homeostasis. *Life Sciences*, *278*, 119622. ⏎

Dogra, S. K., Chung, C. K., Wang, D., Sakwinska, O., Colombo Mottaz, S., & Sprenger, N. (2021). Nurturing the early life gut microbiome and immune maturation for long term health. *Microorganisms*, *9*(10), 2110. ⏎

Doré, J., & Blottière, H. (2015). The influence of diet on the gut microbiota and its consequences for health. *Current Opinion in Biotechnology*, *32*, 195–199. ⏎

Duca, I., Rusu, F., Chira, A., & Dumitrascu, D. L. (2018). Gut microbiota and body weight – a review. *Psihologijske Teme*, *27*(1), 33–53. ⏎

Fabiano, V., Indrio, F., Verduci, E., Calcaterra, V., Pop, T. L., Mari, A., … & Goulet, O. (2021). Term infant formulas influencing gut microbiota: an overview. *Nutrients*, *13*, 4200. ⏎

Facciotti, F. (2022). Modulation of intestinal immune cell responses by eubiotic or dysbiotic microbiota in inflammatory bowel diseases. *PharmaNutrition*, *21*, 100303. ⏎

Fernández, L., Pannaraj, P. S., Rautava, S., & Rodríguez, J. M. (2020). The microbiota of the human mammary ecosystem. *Frontiers in Cellular and Infection Microbiology*, *10*, 586667. ⏎

Ferro, L. E., Bittinger, K., Trudo, S. P., Beane, K. E., Polson, S. W., Kim, J. K., & Trabulsi, J. C. (2024). A short-term, randomized, controlled, feasibility study of the effects of different vegetables on the gut

microbiota and microRNA expression in infants. *Frontiers in Microbiomes*, *3*, 1342464. ⏎

Forgie, A. J., Drall, K. M., Bourque, S. L., Field, C. J., Kozyrskyj, A. L., & Willing, B. P. (2020). The impact of maternal and early life malnutrition on health: a diet-microbe perspective. *BMC Medicine*, *18*(1), 135. ⏎

García-Mateo, S., Rondinella, D., Ponziani, F. R., Miele, L., Gasbarrini, A., Cammarota, G., … & Gomollón, F. (2024). Gut microbiome and metabolic dysfunction-associated steatotic liver disease: pathogenic role and potential for therapeutics. *Best Practice & Research Clinical Gastroenterology*, *72*, 101924. ⏎

Golshany, H., Helmy, S. A., Morsy, N. F. S., Kamal, A., Yu, Q., & Fan, L. (2025). The gut microbiome across the lifespan: how diet modulates our microbial ecosystem from infancy to the elderly. *International Journal of Food Sciences and Nutrition*, *76*(2), 95–121.

Gomez-Gallego, C., Garcia-Mantrana, I., Salminen, S., & Collado, M. C. (2016). The human milk microbiome and factors influencing its composition and activity. *Seminars in Fetal and Neonatal Medicine*, *21*(6), 400–405. ⏎

Grech, A., Collins, C. E., Holmes, A., Lal, R., Duncanson, K., Taylor, R., & Gordon, A. (2021). Maternal exposures and the infant gut microbiome: a systematic review with meta-analysis. *Gut Microbes*, *13*(1), 1897210. ⏎

Hong, Y. W., Si, T. S., Zhen, Y. Y., Xiu, Y. W., & Guo, Z. W. (2017). *Impact of early-life antibiotic use on gut microbiota of infants*. Journal of Microbial & Biochemical Technology 9(5): 227–231. ⏎

Huang, R., Zhou, G., Cai, J., Cao, C., Zhu, Z., Wu, Q., … & Ding, Y. (2025). Maternal of urbanized diet compromises early-life health in association with gut microbiota. *Gut Microbes*, *17*(1), 2483783. ⏎

Ioannou, A., Berkhout, M. D., Scott Jr, W. T., Blijenberg, B., Boeren, S., Mank, M., … & Belzer, C. (2024). Resource sharing of an infant gut microbiota synthetic community in combinations of human milk oligosaccharides. *ISME Journal*, *18*(1), wrae209. ⏎

Jeong, S. (2021). Factors influencing development of the infant microbiota: from prenatal period to early infancy. *Clinical and Experimental Pediatrics*, *65*(9), 438. ⏎

Kallio, S., Jian, C., Korpela, K., Kukkonen, A. K., Salonen, A., Savilahti, E., … & M. de Vos, W. (2024). Early-life gut microbiota associates with allergic rhinitis during 13-year follow-up in a Finnish probiotic intervention cohort. *Microbiology Spectrum*, 12(6), e04135–23. ⏎

Karampatsas, K., Faal, A., Jaiteh, M., Garcia-Perez, I., Aller, S., Shaw, A. G., … & Le Doare, K. (2022). Gastrointestinal, vaginal, nasopharyngeal, and breast milk microbiota profiles and breast milk metabolomic changes in Gambian infants over the first two months of lactation: a prospective cohort study. *Medicine*, *101*(46), e31419. ⏎

Koleilat, A. (2017). Healthy newborn microbiome. *Journal of Molecular Biomarkers & Diagnosis*, *8*(347), 2. ⏎

Lalli, M. K., Salo, T. E., Hakola, L., Knip, M., Virtanen, S. M., & Vatanen, T. (2025). Associations between dietary fibers and gut microbiome composition in the EDIA longitudinal infant cohort. *American Journal of Clinical Nutrition*, *121*(1), 83–99. ⏎

Laursen, M. F. (2021). Gut microbiota development: influence of diet from infancy to toddlerhood. *Annals of Nutrition and Metabolism*, *77*(Suppl. 3), 21–34. ⏎

Laursen, M. F., Andersen, L. B., Michaelsen, K. F., Mølgaard, C., Trolle, E., Bahl, M. I., & Licht, T. R. (2016). Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. *Msphere*, *1*(1), 10–1128. ⏎

Laursen, M. F., Bahl, M. I., Michaelsen, K. F., & Licht, T. R. (2017). First foods and gut microbes. *Frontiers in Microbiology*, *8*, 356. ⏎

Lawson, M. A., O'Neill, I. J., Kujawska, M., Wijeyesekera, A., Flegg, Z., Chalklen, L., & Hall, L. J. (2019). Infant diet promotes *Bifidobacterium* community cooperation within a single ecosystem. *bioRxiv*, 711234. ⏎

Li, J., Dizzell, S. E., Jones, S. L., Kanji, S., Lau, J. T., Mousseau, A., … & Hutton, E. K. (2019). Early life exposures and the development of the

infant gut microbiome: a review: expositions lors des premiers stades de vie et développement du microbiome intestinal du nourrisson: examen. *Canadian Journal of Midwifery Research and Practice*, *18*(2), 8–24.

Li, N., Liang, S., Chen, Q., Zhao, L., Li, B., & Huo, G. (2021). Distinct gut microbiota and metabolite profiles induced by delivery mode in healthy Chinese infants. *Journal of Proteomics*, *232*, 104071. ⏎

Li, W., Tapiainen, T., Brinkac, L., Lorenzi, H. A., Moncera, K., Tejesvi, M. V., … & Nelson, K. E. (2021). Vertical transmission of gut microbiome and antimicrobial resistance genes in infants exposed to antibiotics at birth. *Journal of Infectious Diseases*, *224*(7), 1236–1246. ⏎

Lin, J. (2023). Effect of different diets on human gut microbiome health. *Highlights in Science, Engineering and Technology*, *36*, 702–708. ⏎

Liu, Y., Hou, Y., Wang, G., Zheng, X., & Hao, H. (2020). Gut microbial metabolites of aromatic amino acids as signals in host–microbe interplay. *Trends in Endocrinology & Metabolism*, *31*(11), 818–834. ⏎

Liu, Z., Subbaraj, A., Fraser, K., Jia, H., Chen, W., Day, L., … & Young, W. (2020). Human milk and infant formula differentially alters the microbiota composition and functional gene relative abundance in the small and large intestines in weanling rats. *European Journal of Nutrition*, *59*(5), 2131–2143.

Lou, Y. C., Olm, M. R., Diamond, S., Crits-Christoph, A., Firek, B. A., Baker, R., … & Banfield, J. F. (2021). Infant gut strain persistence is associated with maternal origin, phylogeny, and traits including surface adhesion and iron acquisition. *Cell Reports Medicine*, *2*(9), 100393. ⏎

Louwies, T., Johnson, A. C., Orock, A., Yuan, T., & Greenwood-Van Meerveld, B. (2020). The microbiota-gut-brain axis: an emerging role for the epigenome. *Experimental Biology and Medicine*, *245*(2), 138–145.

Lu, J., Martin, C. R., & Claud, E. C. (2023, February). Neurodevelopmental outcome of infants who develop necrotizing enterocolitis: the gut-brain axis. *Seminars in Perinatology*, *47*(1), 151694. WB Saunders.

Lugli, G. A., Mancabelli, L., Milani, C., Fontana, F., Tarracchini, C., Alessandri, G., … & Ventura, M. (2023). Comprehensive insights from

composition to functional microbe-based biodiversity of the infant human gut microbiota. *npj Biofilms and Microbiomes*, *9*(1), 25. ⏎

Lundgren, S. N., Madan, J. C., Emond, J. A., Morrison, H. G., Christensen, B. C., Karagas, M. R., & Hoen, A. G. (2018). Maternal diet during pregnancy is related with the infant stool microbiome in a delivery mode-dependent manner. *Microbiome*, *6*(1), 109.

Lundgren, S. N., Madan, J. C., Karagas, M. R., Morrison, H. G., Christensen, B. C., & Hoen, A. G. (2023). Human milk-associated bacterial communities associate with the infant gut microbiome over the first year of life. *Frontiers in Microbiology*, *14*, 1164553. ⏎

Lyons, K. E., Ryan, C. A., Dempsey, E. M., Ross, R. P., & Stanton, C. (2020). Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients*, *12*(4), 1039.

Ma, J., Li, Z., Zhang, W., Zhang, C., Zhang, Y., Mei, H., … & Wu, D. (2020). Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. *Scientific Reports*, *10*(1), 15792. ⏎

Ma, G., Chen, Z., Li, Z., & Xiao, X. (2024). Unveiling the neonatal gut microbiota: exploring the influence of delivery mode on early microbial colonization and intervention strategies. *Archives of Gynecology and Obstetrics*, *310*(6), 2853–2861. ⏎

Maher, S. E., O'Brien, E. C., Moore, R. L., Byrne, D. F., Geraghty, A. A., Saldova, R., … & McAuliffe, F. M. (2023). The association between the maternal diet and the maternal and infant gut microbiome: a systematic review. *British Journal of Nutrition*, *129*(9), 1491–1499.

McDonald, T., Aqeel, A., Neubert, B., Bauer, A., Jiang, S., Osborne, O., … & David, L. (2025). Dietary plant diversity predicts early life microbiome maturation. *medRxiv*, 2025–02. ⏎

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., … & Ventura, M. (2017). The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut

microbiota. *Microbiology and Molecular Biology Reviews*, *81*(4), e00036-17. ⏎

Misra, B. B., Langefeld, C., Olivier, M., & Cox, L. A. (2019). Integrated omics: tools, advances and future approaches. *Journal of Molecular Endocrinology*, *62*(1), R21–R45. ⏎

Mitchell, C. M., Mazzoni, C., Hogstrom, L., Bryant, A., Bergerat, A., Cher, A., … & Yassour, M. (2020). Delivery mode affects stability of early infant gut microbiota. *Cell Reports Medicine*, *1*(9), 100156. ⏎

Mokhtari, P., Holzhausen, E. A., Chalifour, B. N., Schmidt, K. A., Babaei, M., Machle, C. J., … & Goran, M. I. (2024). Associations between dietary sugar and fiber with infant gut microbiome colonization at 6 Mo of age. *Journal of Nutrition*, *154*(1), 152–162. ⏎

Mudhluli, T. E., Kujawska, M., Mueller, J., Felsl, A., Truppel, B. A., Hall, L. J., … & Zhou, D. T. (2024). Exploring the genomic traits of infant-associated microbiota members from a Zimbabwean cohort. *BMC Genomics*, *25*(1), 718. ⏎

Mulligan, C. M., & Friedman, J. E. (2017). Maternal modifiers of the infant gut microbiota: metabolic consequences. *Journal of Endocrinology*, *235*(1), R1–R12.

Nagpal, R., & Yamashiro, Y. (2018). Gut microbiota composition in healthy Japanese infants and young adults born by C-section. *Annals of Nutrition and Metabolism*, *73*(Suppl. 3), 4–11.

Nauta, A. J., Amor, K. B., Knol, J., Garssen, J., & Van der Beek, E. M. (2013). Relevance of pre-and postnatal nutrition to development and interplay between the microbiota and metabolic and immune systems123. *American Journal of Clinical Nutrition*, *98*(2), 586S–593S. ⏎

Noles, D. L., Matzeller, K. L., Frank, D. N., Krebs, N. F., & Tang, M. (2025). Complementary feeding and infant gut microbiota: *a narrative review. Nutrients*, *17*(5), 743. ⏎

Nwaru, B. I., Takkinen, H. M., Niemelä, O., Kaila, M., Erkkola, M., Ahonen, S., … & Virtanen, S. M. (2013). Timing of infant feeding in

relation to childhood asthma and allergic diseases. *Journal of Allergy and Clinical Immunology*, *131*(1), 78–86. ⏎

Odiase, E., Frank, D. N., Young, B. E., Robertson, C. E., Kofonow, J. M., Davis, K. N., … & Tang, M. (2023). The gut microbiota differ in exclusively breastfed and formula-fed United States infants and are associated with growth status. *Journal of Nutrition*, *153*(9), 2612–2621. ⏎

Ojha, S., Fainberg, H. P., Sebert, S., Budge, H., & Symonds, M. E. (2015). Maternal health and eating habits: metabolic consequences and impact on child health. *Trends in Molecular Medicine*, *21*(2), 126–133. ⏎

O'Neill, I. J., Sanchez Gallardo, R., Saldova, R., Murphy, E. F., Cotter, P. D., McAuliffe, F. M., & van Sinderen, D. (2020). Maternal and infant factors that shape neonatal gut colonization by bacteria. *Expert Review of Gastroenterology & Hepatology*, *14*(8), 651–664. ⏎

Ouyang, R., Ding, J., Huang, Y., Zheng, F., Zheng, S., Ye, Y., … & Xu, G. (2023). Maturation of the gut metabolome during the first year of life in humans. *Gut Microbes*, *15*(1), 2231596. ⏎

Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., … & Aldrovandi, G. M. (2017). Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatrics*, *171*(7), 647–654.

Panzer, A. R., & Lynch, S. V. (2015). Influence and effect of the human microbiome in allergy and asthma. *Current Opinion in Rheumatology*, *27*(4), 373–380. ⏎

Parkar, S. G., Frost, J. K., Rosendale, D., Stoklosinski, H. M., Jobsis, C. M., Hedderley, D. I., & Gopal, P. (2021). The sugar composition of the fibre in selected plant foods modulates weaning infants' gut microbiome composition and fermentation metabolites in vitro. *Scientific Reports*, *11*(1), 9292. ⏎

Pärnänen, K., Karkman, A., Hultman, J., Lyra, C., Bengtsson-Palme, J., Larsson, D. J., … & Virta, M. (2018). Maternal gut and breast milk

microbiota affect infant gut antibiotic resistome and mobile genetic elements. *Nature Communications*, *9*(1), 3891. ⏎

Pärnänen, K. M., Hultman, J., Markkanen, M., Satokari, R., Rautava, S., Lamendella, R., … & Virta, M. P. (2022). Early-life formula feeding is associated with infant gut microbiota alterations and an increased antibiotic resistance load. *American Journal of Clinical Nutrition*, *115*(2), 407–421. ⏎

Penders, J. (2017). Early diet and the infant gut microbiome: how breastfeeding and solid foods shape the microbiome. In *Microbiota in Health and Disease: From Pregnancy to Childhood* (pp. 105–118). ⏎

Peng, L. H., Tan, Y., & Bajinka, O. (2024). The influence of maternal diet on offspring's gut microbiota in early life. *Archives of Gynecology and Obstetrics*, *309*(4), 1183–1190.

Peng, Y., Zhu, J., Wang, S., Liu, Y., Liu, X., DeLeon, O., … & Tun, H. M. (2024). A metagenome-assembled genome inventory for children reveals early-life gut bacteriome and virome dynamics. *Cell Host & Microbe*, *32*(12), 2212–2230. ⏎

Persaud, R., Azad, M. B., Konya, T., Guttman, D. S., Chari, R. S., Sears, M. R., … & CHILD Study Investigators. (2014). Impact of perinatal antibiotic exposure on the infant gut microbiota at one year of age. *Allergy, Asthma & Clinical Immunology*, *10*(Suppl. 1), A31. ⏎

Pettersen, V. K., & Arrieta, M. C. (2020). Host–microbiome intestinal interactions during early life: considerations for atopy and asthma development. *Current Opinion in Allergy and Clinical Immunology*, *20*(2), 138–148. ⏎

Rani, K., Kumar, J., Sangwan, S., Masharing, N., Mitra, M. D., & Singh, H. B. (2021). Signaling pathways associated with metabolites of dietary fibers link to host health. In *Dietary Fibers*. IntechOpen.

Riazi-Rad, F., Behrouzi, A., Mazaheri, H., Katebi, A., & Ajdary, S. (2021). Impact of gut microbiota on immune system. *Acta Microbiologica et Immunologica Hungarica*, *68*(3), 135–144.

Roager, H. M., Stanton, C., & Hall, L. J. (2023). Microbial metabolites as modulators of the infant gut microbiome and host-microbial interactions in early life. *Gut Microbes*, *15*(1), 2192151. ⏎

Rodriguez, K. A., Gurung, M., Talatala, R., Rearick, J. R., Ruebel, M. L., Stephens, K. E., & Yeruva, L. (2024). The role of early life gut mycobiome on child health. *Advances in Nutrition*, *15*(3), 100185.

Rutayisire, E., Huang, K., Liu, Y., & Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterology*, *16*(1), 86. ⏎

Shaaban, R., Busi, S. B., Wilmes, P., Guéant, J. L., & Heinken, A. (2024). Personalized modeling of gut microbiome metabolism throughout the first year of life. *Communications Medicine*, *4*(1), 281. ⏎

Shapiro, H., Thaiss, C. A., Levy, M., & Elinav, E. (2014). The cross talk between microbiota and the immune system: metabolites take centre stage. *Current Opinion in Immunology*, *30*, 54–62.

Sherwin, E., Rea, K., Dinan, T. G., & Cryan, J. F. (2016). A gut (microbiome) feeling about the brain. *Current Opinion in Gastroenterology*, *32*(2), 96–102. ⏎

Shi, Y., Yin, R., Pang, J., Chen, Y., Li, Z., Su, S., & Wen, Y. (2024). Impact of complementary feeding on infant gut microbiome, metabolites and early development. *Food & Function*, *15*(21), 10663–10678. ⏎

Simon, J. C., Marchesi, J. R., Mougel, C., & Selosse, M. A. (2019). Host-microbiota interactions: from holobiont theory to analysis. *Microbiome*, *7*(1), 5. ⏎

Singh, R. P., Niharika, J., Kondepudi, K. K., Bishnoi, M., & Tingirikari, J. M. R. (2022). Recent understanding of human milk oligosaccharides in establishing infant gut microbiome and roles in immune system. *Food Research International*, *151*, 110884.

Tang, M., Frank, D., Hendricks, A., Ir, D., & Krebs, N. (2019). Protein intake during early complementary feeding affects the gut microbiota in

US formula-fed infants (FS04–03–19). *Current Developments in Nutrition*, *3*, nzz048-FS04. ⏎

Tang, M., Ma, C., Weinheimer-Haus, E. M., Robertson, C. E., Kofonow, J. M., Berman, L. M., … & Krebs, N. F. (2023). Different gut microbiota in US formula-fed infants consuming a meat vs. dairy-based complementary foods: a randomized controlled trial. *Frontiers in Nutrition*, *9*, 1063518. ⏎

Thomson, P., Medina, D. A., & Garrido, D. (2018). Human milk oligosaccharides and infant gut bifidobacteria: molecular strategies for their utilization. *Food Microbiology*, *75*, 37–46.

Tilg, H., & Moschen, A. R. (2014). Microbiota and diabetes: an evolving relationship. *Gut*, *63*(9), 1513–1521. ⏎

Todor, S. B., & Ichim, C. (2025). Microbiome modulation in pediatric leukemia: impact on graft-versus-host disease and treatment outcomes: *a narrative review. Children*, *12*(2), 166. ⏎

Tseng, C. H., & Wu, C. Y. (2025). From dysbiosis to longevity: a narrative review into the gut microbiome's impact on aging. *Journal of Biomedical Science*, *32*(1), 93. ⏎

Turroni, F., Milani, C., Duranti, S., Lugli, G. A., Bernasconi, S., Margolles, A., … & Ventura, M. (2020). The infant gut microbiome as a microbial organ influencing host well-being. *Italian Journal of Pediatrics*, *46*(1), 16. ⏎

Usman, A., Abdulkadir, B., Lawal, B., & Isah, M. B. (2024). Comparative effect of breast milk and infant formulae on neonatal gut microbiome within katsina metropolis. *UMYU Journal of Microbiology Research*, *9*(3), 22–29. ⏎

Vaher, K., Bogaert, D., Richardson, H., & Boardman, J. P. (2022). Microbiome-gut-brain axis in brain development, cognition and behavior during infancy and early childhood. *Developmental Review*, *66*, 101038. ⏎

Valles-Colomer, M., Blanco-Míguez, A., Manghi, P., Asnicar, F., Dubois, L., Golzato, D., … & Segata, N. (2023). The person-to-person

transmission landscape of the gut and oral microbiomes. *Nature*, *614*(7946), 125–135. ⏎

van Best, N., Hornef, M. W., Savelkoul, P. H., & Penders, J. (2015). On the origin of species: factors shaping the establishment of infant's gut microbiota. *Birth Defects Research Part C: Embryo Today: Reviews*, *105*(4), 240–251. ⏎

Wang, Q., Yang, Q., & Liu, X. (2023). The microbiota–gut–brain axis and neurodevelopmental disorders. *Protein & Cell*, *14*(10), 762–775. ⏎

Weng, T. H., Huang, K. Y., Jhong, J. H., Kao, H. J., Chen, C. H., Chen, Y. C., & Weng, S. L. (2023). Microbiome analysis of maternal and neonatal microbial communities associated with the different delivery modes based on 16S rRNA gene amplicon sequencing. *Taiwanese Journal of Obstetrics and Gynecology*, *62*(5), 687–696. ⏎

Williams, G., Hamm, M. P., Shulhan, J., Vandermeer, B., & Hartling, L. (2014). Social media interventions for diet and exercise behaviours: a systematic review and meta-analysis of randomised controlled trials. *BMJ Open*, *4*(2), e003926. ⏎

Wong, C. B., & Xiao, J. Z. (2018). Breast milk on the infant faecal microbiome: components of breast milk orchestrating the establishment of bifidobacteria species. *Life*, *2*, 3. ⏎

Wu, Q., Boonma, P., Badu, S., Yalcinkaya, N., So, S. Y., Garey, K. W., … & Savidge, T. C. (2023). Donor-recipient specificity and age-dependency in fecal microbiota therapy and probiotic resolution of gastrointestinal symptoms. *npj Biofilms and Microbiomes*, *9*(1), 54. ⏎

Xie, G., Zhang, S., Zheng, X., & Jia, W. (2013). Metabolomics approaches for characterizing metabolic interactions between host and its commensal microbes. *Electrophoresis*, *34*(19), 2787–2798. ⏎

Yadav, R. (2024). Effect of pharmaceutically active antibiotics on gut metagenome of mother and infant. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*, *3*(4), 284–295. ⏎

Yang, Q., Liang, Q., Balakrishnan, B., Belobrajdic, D. P., Feng, Q. J., & Zhang, W. (2020). Role of dietary nutrients in the modulation of gut

microbiota: a narrative review. *Nutrients*, *12*(2), 381. ⏎

Yang, R., Chen, Z., & Cai, J. (2023). Fecal microbiota transplantation: emerging applications in autoimmune diseases. *Journal of Autoimmunity*, *141*, 103038. ⏎

Yang, R., Shi, Z., Li, Y., Huang, X., Li, Y., Li, X., … & Li, X. (2024). Research focus and emerging trends of the gut microbiome and infant: a bibliometric analysis from 2004 to 2024. *Frontiers in Microbiology*, *15*, 1459867. ⏎

Zeng, S., Almeida, A., Li, S., Ying, J., Wang, H., Qu, Y., … & Wang, S. (2024). A metagenomic catalog of the early-life human gut virome. *Nature Communications*, *15*(1), 1864. ⏎

Zhang, H., Xie, Y., Cao, F., & Song, X. (2024). Gut microbiota-derived fatty acid and sterol metabolites: biotransformation and immunomodulatory functions. *Gut Microbes*, *16*(1), 2382336. ⏎

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, *30*(6), 492–506. ⏎

Zhou, P., Zhou, Y., Liu, B., Jin, Z., Zhuang, X., Dai, W., … & Zhang, L. (2020). Perinatal antibiotic exposure affects the transmission between maternal and neonatal microbiota and is associated with early-onset sepsis. *mSphere*, *5*(1), e00984-19. ⏎

6

Microbiota of the Gut–Brain Axis

Alex Muppasani and Sunil Thomas

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6.1 Introduction

The human body is increasingly understood not merely as a collection of genetically identical cells but as a "holobiont," a complex super-organism composed of the host and its trillions of resident microorganisms that function in a symbiotic relationship (Abildinova et al., 2024; Bordenstein & Theis, 2015). This conceptual shift has been most profound and disruptive in the field of neuroscience, where the traditional view of the central nervous system (CNS) as an autonomous, privileged site protected by the blood–brain barrier (BBB) has been systematically dismantled by the discovery and elucidation of the microbiota–gut–brain (MGB) axis (Mayer et al., 2014). This bidirectional communication highway physically and biochemically links the emotional and cognitive centers of the brain with the peripheral functions of the gastrointestinal tract, fundamentally altering our understanding of mental health, neurodevelopment, and neurodegenerative disease (Ramadan et al., 2025; Yassin et al., 2025). The gut microbiota, comprising a diverse ecosystem of bacteria, viruses, fungi,

and archaea, possesses a collective genomic repertoire—the microbiome—that vastly exceeds that of the human host, acting as a metabolic organ that regulates digestion, immunity, and even behavior through signaling mechanisms. Historical antecedents to this modern science date back to the early 20th century and even to antiquity with Hippocrates' prescient assertion that "all disease begins in the gut," yet it is only with the recent advent of high-throughput sequencing, metabolomics, and gnotobiotic animal models that science has begun to map the specific molecular pathways involved (Venkatakrishnan et al., 2021). The implications of this research are staggering, suggesting that the etiology of complex neuropsychiatric disorders such as depression, anxiety, autism spectrum disorder (ASD), and schizophrenia, as well as neurodegenerative conditions like Parkinson's disease (PD), may originate or be significantly modulated by events occurring within the intestinal lumen rather than solely within the cranial vault (Doenyas et al., 2025; Oliver et al., 2025). This review provides a granular, expert-level analysis of the anatomical infrastructure, molecular languages, and clinical manifestations of the MGB axis, synthesizing data from the most recent randomized controlled trials and mechanistic studies. We will explore how the gut acts as a sensory organ, detecting microbial metabolites and transmitting this information to the brain to modulate gene expression, neuroinflammation, and synaptic plasticity, thereby offering new targets for therapeutic intervention in conditions previously considered intractable.

6.2 Anatomy and Physiology of the Microbiota–Gut–Brain Axis

The anatomical and physiological foundations of the MGB axis constitute a highly interconnected system in which neural, endocrine, immune, and epithelial barrier components operate as overlapping channels of communication between the intestinal lumen and the CNS. Rather than functioning as isolated pathways, these systems form an integrated signaling matrix in which neural circuits are embedded within

immunologically active tissues, exposed to circulating hormones, and constrained by barrier properties that are themselves modulated by microbial metabolites (Bonaz et al., 2017). Contemporary frameworks therefore emphasize a distributed architecture with multiple parallel conduits, allowing both redundancy and fine-tuned regulation of how gut-derived signals shape brain function and behavior (Doenyas et al., 2025).

The neural backbone of the MGB axis is centered on the vagus nerve, the primary high-speed communication channel linking visceral organs to the brainstem. Structurally, the vagus is predominantly sensory—approximately 80% afferent and 20% efferent fibers—highlighting its principal role as a monitoring system for internal physiological states (Förster & Shityakov., 2026). Afferent terminals densely innervate the gastrointestinal mucosa, where they detect mechanical stretch, chemical cues, and microbial metabolites, although they rarely contact microbes directly (Powley et al., 2011). Instead, signal transduction occurs through specialized epithelial intermediaries, particularly enteroendocrine and enterochromaffin cells, which sense luminal contents and convert them into neuroactive outputs (Grundeken & El Aidy, 2025; Lorsch & Liddle, 2026). Upon stimulation by bacterial products such as short-chain fatty acids, bile acids, lipopolysaccharides (LPSs), or indole derivatives, these cells release mediators including serotonin, cholecystokinin, peptide YY, and glucagon-like peptide-1, activating receptors on vagal afferents and initiating neural transmission to the nucleus of the solitary tract (NTS; Jameson et al., 2024; Hwang et al., 2025; Ragozzino et al., 2020). Subsequent projections to limbic and cortical regions—such as the hypothalamus, amygdala, insula, and prefrontal cortex—allow visceral signals to influence mood, stress reactivity, appetite, and interoceptive awareness (Chang et al., 2025; Lopez-Martin et al., 2026).

Recent discoveries further refine this model by identifying "neuropod cells," a subset of enteroendocrine cells capable of forming synapse-like contacts with vagal fibers and transmitting information via rapid glutamatergic signaling (Souza, 2026; Kaelberer et al., 2020). This finding indicates that gut stimuli can be encoded through both slow endocrine

pathways and fast synaptic mechanisms, enabling the vagus nerve to integrate temporal layers of information—from sustained metabolic status to acute luminal changes—relevant for behaviors such as satiety, nausea, and aversive learning.

Efferent vagal fibers form the cholinergic anti-inflammatory pathway, a reflex mechanism that maintains immune equilibrium (Pavlov & Tracey, 2012). Acetylcholine released from these fibers binds α7 nicotinic receptors on immune cells, suppressing nuclear factor kappa B (NF-κB) activation and reducing pro-inflammatory cytokine production (Keever et al., 2023; Li et al., 2025b). This "inflammatory reflex" limits peripheral immune activation and protects the CNS from inflammatory spillover implicated in psychiatric and neurodegenerative conditions (Faraji et al., 2025). Consistently, diminished vagal tone—as indexed by reduced heart-rate variability—correlates with depression, anxiety, and cognitive decline, suggesting that impaired vagal regulation may permit chronic inflammation to become biologically embedded (Pavlov & Tracey, 2012).

Parallel to vagal signaling, the enteric nervous system (ENS) represents an intrinsic neural network of approximately 200–600 million neurons capable of autonomously regulating gastrointestinal function (Lorsch & Liddle, 2026). Organized into the myenteric and submucosal plexuses, the ENS coordinates motility, secretion, and blood flow while producing neurotransmitters such as serotonin, acetylcholine, dopamine, and gamma-aminobutyric acid (GABA; Hazart et al., 2025; Waxenbaum et al., 2025; Mittal et al., 2017). Enteric glial cells further contribute to barrier maintenance and immune surveillance (Bali & Grubišić, 2024). Microbial signals shape ENS excitability through metabolites and receptor-mediated interactions, and germ-free models demonstrate that microbiota absence disrupts neuronal density, neurochemical coding, and motility—deficits reversible by microbial colonization (Dong & Mayer, 2024; Tan et al., 2025; De Vadder et al., 2018). These findings position the microbiota as a developmental organizer of enteric circuitry.

The ENS may also serve as a gateway for pathological processes. Evidence that α-synuclein aggregation appears in the gut prior to

neurological symptoms, combined with experimental demonstrations of retrograde transport to the brain and epidemiological links between vagotomy and reduced PD risk, supports a gut-origin hypothesis for certain neurodegenerative disorders (Cui et al., 2023; Liu et al., 2017; Valdetaro et al., 2026). Thus, the ENS functions not only as a regulator of digestion but also as a critical interface integrating environmental exposures, microbial signals, and host proteins.

The hypothalamic–pituitary–adrenal (HPA) axis forms the principal neuroendocrine arm of the MGB axis, orchestrating stress responses through sequential release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), pituitary adrenocorticotropic hormone (ACTH) secretion, and adrenal glucocorticoid production (Nicolaides et al., 2014; Bertollo et al., 2025; Yamamoto & Kaur, 2025). While acute activation supports adaptation, chronic HPA hyperactivity is strongly linked to mood and anxiety disorders (Nunez et al., 2025). Microbiota presence during early development is essential for normal HPA calibration: germ-free animals exhibit exaggerated stress hormone responses that can be partially normalized by microbial colonization during critical windows (Foster et al., 2017; Sudo et al., 2004). Human studies similarly associate early-life microbiota disruptions with altered cortisol dynamics and increased psychiatric risk (Ortega et al., 2025).

Stress reciprocally alters gut physiology by promoting dysbiosis and increasing intestinal permeability, allowing microbial antigens to activate immune pathways that further stimulate HPA activity (Anand et al., 2022). Glucocorticoids influence mucus production, tight-junction expression, and immune cell distribution, reshaping the gut ecosystem and reinforcing a bidirectional stress–microbiota feedback loop (Marwaha et al., 2025; Bertollo et al., 2025). Consequently, interventions targeting stress pathways may exert therapeutic effects partly through microbiome and barrier modulation (Ring, 2025).

Additional structural components broaden this network perspective. Sympathetic innervation regulates vascular tone, motility, and immune trafficking and is associated with microbial shifts under chronic stress

(Marwaha et al., 2025). The intestinal epithelium acts simultaneously as a barrier and sensory interface, with tight-junction integrity dynamically regulated by cytokines, neuropeptides, and microbial metabolites (Worthington et al., 2018). At the CNS boundary, the BBB exhibits microbiota-dependent regulation, particularly through short-chain fatty acids that influence permeability (Fock & Parnova, 2023). Meningeal immune compartments and lymphatic vessels add further layers of communication, linking peripheral immune signals to microglial activity and CNS surveillance (Mokbel et al., 2024).

Collectively, this expanded view portrays the MGB axis as a distributed, multi-node network rather than a single linear pathway. Neural, endocrine, immune, and barrier systems operate as interlocking modules through which local perturbations—dietary changes, infection, pharmacological exposure, or chronic stress—can propagate to influence brain structure and function (Khawar et al., 2023). Recognizing this systems-level architecture provides a mechanistic framework for understanding disease pathogenesis and underscores why effective therapeutic strategies may need to target multiple levels of the axis simultaneously (Qu et al., 2024).

6.3 Molecular Signaling

The biochemical dialogue between the gut and the brain is mediated by a diverse array of signaling molecules, among which neurotransmitters, microbial metabolites, and immune mediators play pivotal and tightly interconnected roles (Qu et al., 2024). It is now recognized that the gut microbiota possesses the enzymatic machinery to synthesize, degrade, or modulate many of the same neurochemicals that regulate human mood, cognition, and behavior, effectively extending canonical neuropharmacology into the intestinal lumen (Mhanna et al., 2023). This distributed "chemosensory network" involves not only classical neurotransmitters such as serotonin, GABA, dopamine, and norepinephrine, but also short-chain fatty acids (SCFAs), bile acid derivatives, tryptophan

catabolites, indoles, and microbial-associated molecular patterns that act on neural, immune, and endocrine cells across the body (Agirman & Hsiao, 2021). The net central effect of these molecules depends on a complex integration of local gut actions (on motility, secretion, barrier integrity), systemic immune and endocrine responses, and direct or indirect actions within the CNS on neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells (Rusch et al., 2023).

A central component of this chemical language is the direct modulation of neurotransmitter systems by gut bacteria and their metabolites (Ataei et al., 2026). Serotonin (5-hydroxytryptamine, 5-HT) is perhaps the most widely cited example, with over 90% of the body's serotonin stores residing in the gastrointestinal tract, primarily within enterochromaffin cells rather than in central serotonergic neurons. Enterochromaffin cells synthesize serotonin from dietary tryptophan via the enzyme tryptophan hydroxylase 1 (TPH1), and this process is strongly influenced by microbial cues; for instance, spore-forming bacteria from the *Clostridia* class upregulate TPH1 expression, increasing colonic serotonin production and circulating 5-HT levels in germ-free mice after colonization (Shah et al., 2021). Peripheral serotonin acts locally to regulate gut motility, secretion, and platelet aggregation, but it also engages 5-HT receptors on vagal afferent fibers, thereby transmitting information about luminal and mucosal status to the NTS and higher-order autonomic and limbic centers (Hwang & Oh, 2025). In parallel, specific bacterial taxa such as *Streptococcus*, *Escherichia*, and *Enterococcus* can synthesize serotonin de novo from tryptophan, further augmenting the luminal pool of 5-HT that can signal to epithelial, immune, and neural cells (Moretti et al., 2025). Although 5-HT itself crosses the BBB poorly, microbiota-mediated shifts in peripheral serotonin availability and receptor expression can reshape gut–brain signaling and influence central serotonergic tone via vagal and immune pathways (Li et al., 2024).

In addition to serotonin, the inhibitory neurotransmitter GABA is produced in substantial quantities by various commensal bacteria, notably several species of *Lactobacillus* and *Bifidobacterium* (including *L. brevis, L. rhamnosus*, and *B. dentium*), which use the glutamate decarboxylase

system to convert glutamate into GABA (Yunes et al., 2016). Germ-free animals show markedly reduced luminal and serum GABA levels compared to conventionally colonized controls, demonstrating a basal dependence on microbial synthesis (Rusch et al., 2023). Importantly, ingestion of GABA-producing strains such as *L. rhamnosus* JB-1 has been shown to alter the mRNA expression of GABA receptor subunits in the brain and to reduce anxiety- and depression-like behaviors in mice; these behavioral and molecular effects are abolished by vagotomy, indicating that the relevant signals reach the CNS primarily via vagal pathways rather than by direct GABA transport across the BBB (Bravo et al., 2011). Mechanistically, *L. rhamnosus* JB-1 increases expression of GABA and certain GABA subunits in regions such as the prefrontal cortex, amygdala, and hippocampus, thereby reshaping inhibitory microcircuits and shifting the balance between excitation and inhibition in anxiety-related networks (Chudzik et al., 2022).

Catecholaminergic systems are similarly impacted by the gut microbiota. Dopamine and norepinephrine are synthesized by several bacterial genera, including *Bacillus*, *Serratia*, and *Morganella*, and the microbiota can modulate precursor availability (e.g., phenylalanine, tyrosine, levodopa) and host enzyme expression along the catecholamine biosynthetic pathway (Barbu et al., 2025). Experimental depletion of gut microbiota alters striatal dopamine turnover, dopaminergic receptor expression, and reward-related behaviors in rodents, while colonization with selected strains can normalize these parameters (Winters et al., 2025). In the periphery, microbial metabolites regulate sympathetic ganglion excitability and adrenal catecholamine release, influencing systemic norepinephrine levels and stress responses (Winters et al., 2025). These effects are highly relevant for disorders such as PD and attention-deficit/hyperactivity disorder (ADHD), where dopaminergic signaling and reward circuitry are central, and they also suggest that gut–brain interventions may alter the pharmacokinetics and pharmacodynamics of catecholamine-targeting medications (Barbu et al., 2025). Early human work links specific microbiota compositions to altered dopamine metabolites in plasma and cerebrospinal fluid (CSF),

though causal directions and mechanisms remain under active investigation (Zhang et al., 2025d).

6.4 Short-Chain Fatty Acids Act as Vital Signaling Molecules in the Gut–Brain Axis

SCFAs, primarily acetate, propionate, and butyrate, represent a crucial class of signaling molecules derived from bacterial fermentation of indigestible dietary fibers and resistant starches (Nireeksha et al., 2025). SCFAs, the main metabolites produced in the colon by bacterial fermentation of dietary fibers and resistant starch, are speculated to play a key role in neuro-immunoendocrine regulation. At the level of the intestinal epithelium, SCFAs act as primary energy substrates for colonocytes, support mucus production, and strengthen tight junctions, thereby maintaining barrier integrity and preventing the translocation of pro-inflammatory endotoxins such as LPS into the systemic circulation (He et al., 2019). Their influence, however, extends well beyond the gut. Increasing evidence suggests that these metabolites play a central role in neuro-immunoendocrine regulation, linking gut microbial activity to systemic physiology and brain function (Silva et al., 2020).

SCFAs are small organic monocarboxylic acids containing up to six carbon atoms. They are primarily produced by anaerobic fermentation of indigestible polysaccharides—such as dietary fiber and resistant starch—by the gut microbiota in the large intestine. The three most abundant SCFAs are acetate (C2), propionate (C3), and butyrate (C4), typically present in an approximate molar ratio of 60:20:20, respectively. Daily production of SCFAs in the human gut is estimated at 500–600 mmol, although this amount varies depending on dietary fiber intake, microbiota composition, and intestinal transit time. While fiber fermentation is the dominant source of SCFAs, these metabolites can also arise from amino acid metabolism. However, less than 1% of colonic microbial species rely on amino acid–based pathways for SCFA production (Silva et al., 2020).

SCFAs exert numerous beneficial effects on gut health. Locally, they help maintain intestinal barrier integrity, stimulate mucus production, and protect against inflammation. Butyrate, in particular, serves as a primary energy source for colonocytes and contributes to epithelial repair. SCFAs have also been associated with a reduced risk of colorectal cancer through their anti-inflammatory and epigenetic regulatory properties (Correa Lopes et al., 2025). Although the full spectrum of SCFA signaling mechanisms remains under investigation, it is well established that SCFAs act through G protein–coupled receptors (GPCRs), such as GPR41 and GPR43, expressed on intestinal and immune cells. Activation of these receptors on enteroendocrine cells stimulates the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), hormones involved in appetite regulation and glucose metabolism. In pancreatic β-cells, SCFA signaling can enhance insulin secretion, further underscoring their endocrine influence (Silva et al., 2020).

Although the precise mechanisms governing microbiota–brain communication remain incompletely defined, several pathways have been identified. The gut microbiota can influence the CNS via neural, immune, endocrine, and metabolic routes. Neural communication occurs largely through the vagus nerve, which transmits signals from the gut to the brain. Microbial modulation of the immune system can alter cytokine production and inflammatory responses, thereby affecting brain function (Fülling et al., 2019). The HPA axis represents another major pathway linking stress responses to microbial activity. Additionally, gut microbes influence tryptophan metabolism, thereby regulating serotonin availability. Importantly, many microorganisms can synthesize or modulate neurotransmitters—including γ-aminobutyric acid (GABA), serotonin (5-HT), dopamine (DA), and noradrenaline (NA)—as well as produce neuroactive metabolites such as SCFAs, which can affect neuronal signaling and microglial activity.

While a definitive causal relationship between altered microbial composition and specific brain disorders is still being established, accumulating evidence indicates that disruptions in gut microbial

communities during prenatal and postnatal development may predispose individuals to neurodevelopmental and psychiatric disorders later in life. Maternal factors during pregnancy—including antibiotic or probiotic use, dietary changes, immune activation, and stress exposure—can modify the maternal microbiome, thereby influencing offspring neurodevelopment and behavior in both animal models and humans. Furthermore, early-life factors such as mode of delivery, feeding practices, infections, and antibiotic treatments significantly shape gut microbiota composition, with long-term consequences for brain function and behavior.

Under normal physiological conditions, immune activation and cytokine production exert minimal effects on the CNS. However, chronic systemic inflammation—often associated with persistent infections—has long been linked to behavioral alterations and cognitive impairment. Peripheral inflammatory insults can influence the CNS by activating microglia, promoting the production of inflammatory mediators, and facilitating the recruitment of peripheral immune cells into the brain. This creates a pro-inflammatory cerebral environment that can disrupt neuronal function. In the context of intestinal disorders characterized by increased gut permeability ("leaky gut"), translocation of bacterial products such as LPS into the circulation can amplify systemic cytokine production and compromise the integrity of the BBB, intensifying neuroinflammatory effects (Silva et al., 2020).

Communication between the gut microbiota and the CNS also involves enterochromaffin cells, which respond to microbial products by secreting serotonin into the lamina propria, thereby increasing both colonic and circulating 5-HT levels. The autonomic nervous system—particularly its sympathetic branch—plays a role in maintaining intestinal homeostasis and immune regulation. Meanwhile, gut-derived metabolites and endotoxins can directly enter the circulation, influencing distant organs, including the brain (Lu et al., 2024).

The vagus nerve represents a critical neural conduit in gut–brain signaling. Changes in enteric neuronal activity are detected by vagal afferents, which transmit signals involved in regulating satiety, stress

responses, and mood. Due to their close anatomical proximity, gut bacteria may directly or indirectly stimulate vagal pathways. Experimental studies have shown that oral administration of pathogens or probiotics activates vagal sensory neurons innervating the GI tract, leading to altered CNS function—effects that are abolished in vagotomized animals. Whether this activation results from direct microbial contact or soluble microbial components remains an area of ongoing investigation (Silva et al., 2020).

SCFAs enter the systemic circulation via monocarboxylate transporters and are capable of crossing the BBB, where they interact with neurons, astrocytes, microglia, and endothelial cells through both receptor-mediated and epigenetic mechanisms. SCFAs signal predominantly through GPCRs, including FFAR2, FFAR3, and GPR109A, which are expressed on immune cells, microglia, and, in certain contexts, neurons and vascular endothelial cells (Cao et al., 2025). Activation of these receptors modulates intracellular pathways such as ERK1/2 and influences microglial proliferation, morphology, and cytokine production. In microglia, SCFAs downregulate inducible nitric oxide synthase and cyclooxygenase-2, reduce pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, and enhance anti-inflammatory mediators such as IL-10, thereby promoting a neuroprotective phenotype that limits chronic neuroinflammation (Musazadeh et al., 2023).

Beyond GPCR signaling, SCFAs—particularly butyrate—function as potent histone deacetylase (HDAC) inhibitors, enabling epigenetic regulation of gene expression in both peripheral tissues and the brain (Bourassa et al., 2016). HDAC inhibition increases histone acetylation, relaxes chromatin structure, and enhances transcription of multiple genes, including neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Intlekofer et al., 2013). Experimental administration of butyrate or SCFA-enriched diets elevates hippocampal BDNF levels, enhances synaptic plasticity, and improves performance in learning and memory tasks in rodents, providing mechanistic support for the antidepressant and cognition-enhancing effects associated with high-fiber diets and SCFA supplementation (Intlekofer et al., 2013). These epigenetic actions are not

confined to neurons; SCFAs also regulate astrocytic antioxidant defenses, oligodendrocyte maturation, and endothelial gene expression, thereby influencing myelination, redox homeostasis, and BBB integrity. bIn models of neurodegeneration, SCFAs have been shown to promote beneficial plaque-associated microglial responses in some contexts, whereas in others they may accelerate amyloid deposition, highlighting that their effects are context dependent and shaped by disease stage, immune environment, and receptor expression patterns (Yang et al., 2025b).

SCFAs additionally act as entrainment signals for circadian rhythms, regulating the expression of core clock genes such as Bmal1 and Per2 in peripheral tissues and potentially in central structures (Tahara et al., 2018). Oscillations in microbial fermentation and SCFA production parallel feeding–fasting cycles and modulate host circadian machinery, thereby aligning metabolic and immune rhythms with dietary patterns (Bautista & López-Cortés, 2025). Disruption of circadian rhythms through irregular feeding schedules, jet lag, or shift work alters SCFA profiles and downstream clock gene expression, changes that have been implicated in metabolic and mood disturbances (Li et al., 2025). Thus, SCFAs form a functional bridge connecting diet, microbial metabolism, circadian biology, and brain function.

Tryptophan metabolism represents another major microbiota-regulated signaling pathway with significant implications for mental health, neuroinflammation, and neurodegeneration (Roth et al., 2021). Tryptophan, an essential amino acid, is metabolized through three principal pathways: the serotonin pathway, the kynurenine pathway, and the indole pathway (Hubková et al., 2022). Under physiological conditions, only a small fraction of dietary tryptophan is converted into serotonin, while the majority is metabolized through the hepatic and extrahepatic kynurenine pathway (Badawy et al., 2023). During inflammation or chronic stress, pro-inflammatory cytokines such as interferon-γ and TNF-α upregulate indoleamine 2,3-dioxygenase (IDO1) in immune cells and tryptophan 2,3-dioxygenase (TDO) in the liver, diverting tryptophan away from serotonin

synthesis toward kynurenine and its downstream metabolites (Salminen, 2022).

Within the CNS, kynurenine can be converted into quinolinic acid, a potent N-methyl-d-aspartate (NMDA) receptor agonist that promotes excitotoxicity and neuronal injury, or into kynurenic acid, an NMDA and α7 nicotinic acetylcholine receptor antagonist generally considered neuroprotective—although excessive kynurenic acid may impair cognition by dampening glutamatergic signaling (Pocivavsek et al., 2024). Dysregulation of the kynurenine pathway—often reflected in elevated kynurenine-to-tryptophan ratios and imbalances between quinolinic and kynurenic acid—is strongly associated with major depressive disorder (MDD), schizophrenia, and several neurodegenerative diseases (Staats Pires et al., 2025).

The gut microbiota modulates tryptophan metabolism at multiple levels: by competing for luminal tryptophan, generating indole derivatives, and shaping inflammatory tone that regulates IDO1 and TDO activity (Wyatt & Greathouse, 2021). Certain commensal and pathobiont species convert tryptophan into indoles that serve as ligands for the aryl hydrocarbon receptor (AhR), which is expressed on intestinal epithelial cells, innate lymphoid cells, astrocytes, and microglia. Activation of AhR by microbial indoles enhances epithelial barrier integrity, reduces local and systemic inflammation, and promotes anti-inflammatory astrocytic phenotypes in the brain (Coretti et al., 2024). Conversely, dysbiosis-associated loss of these signals correlates with barrier dysfunction, exaggerated inflammatory responses, and increased susceptibility to neuroinflammation. In germ-free animals, plasma tryptophan levels are often elevated while kynurenine levels are reduced, suggesting that microbiota are required for normal kynurenine pathway flux. Colonization restores these metabolic pathways, and transplantation of microbiota from depressed patients into rodents increases kynurenine levels and anxiety-like behaviors, supporting a causal role for microbiota-driven tryptophan shunting (Hou et al., 2023).

Clinically, patients with irritable bowel syndrome, depression, and stress-related disorders frequently exhibit a shift in tryptophan metabolism

toward the kynurenine pathway, driven by dysbiosis and low-grade inflammation. This shift leads to reduced serotonin availability and accumulation of neuroactive or neurotoxic metabolites (Liu et al., 2025). These alterations correlate with symptom severity and inflammatory markers and may interact with HPA axis hyperactivity to sustain a chronically pro-depressogenic biochemical milieu. Preclinical and early clinical studies indicate that probiotics, prebiotics, and dietary strategies increasing fermentable fiber and polyphenol intake can normalize tryptophan metabolites, lower kynurenine-to-tryptophan ratios, and ameliorate depressive-like behaviors (Du et al., 2025). These findings suggest that targeted modulation of the microbiota–tryptophan–kynurenine axis may represent a promising adjunctive strategy for mood and cognitive disorders (Zhu et al., 2025).

Collectively, the neurotransmitter, SCFA, and tryptophan pathways demonstrate how the MGB axis employs a chemically diverse signaling network to translate changes in the gut ecosystem into specific alterations in neural circuit function (Gao et al., 2020). These pathways operate in an integrated manner: microbial modulation of tryptophan availability affects both serotonin and kynurenine signaling; SCFAs shape microglial responses to kynurenine-driven inflammatory cues; and gut-derived neurotransmitter-like molecules influence vagal and spinal afferent pathways regulating HPA axis activity and cortical processing. Elucidating this interconnected molecular dialogue—and its context-dependent variation across development, sex, and disease states—will be critical for designing rational, mechanism-based interventions that target the gut to treat disorders of the brain.

6.5 Virome and Mycobiome

Although bacteria have historically dominated MGB axis research, it is increasingly evident that the broader microbial ecosystem—including the gut virome and mycobiome—represents a critical and functionally distinct

layer of host–microbe interaction (Dinesh et al., 2025). The virome comprises the full complement of gut-associated viruses, including bacteriophages that infect bacteria and eukaryotic viruses capable of infecting host tissues, whereas the mycobiome encompasses intestinal fungal communities that, despite their relatively low abundance, exert substantial effects on immune tone, epithelial barrier integrity, and metabolic signaling (Houshyar et al., 2026). These viral and fungal components interact extensively with bacterial populations and host physiology, forming a multi-kingdom network in which perturbations in one domain propagate through others to shape MGB communication (Che et al., 2026; Martin et al., 2018). Increasing evidence links specific viral and fungal signatures with psychiatric and neurodegenerative phenotypes, indicating that exclusion of these communities leaves key elements of pathophysiology unexplained (Hadrich et al., 2025).

The gut virome is extraordinarily abundant and diverse, with estimates of approximately 10^8–10^9 viral particles per gram of human feces, most of which are bacteriophages (Lathakumari et al., 2025). Phages influence bacterial community dynamics through both lytic and lysogenic life cycles. During lytic infection, phage-mediated bacterial lysis can rapidly alter the abundance of dominant taxa—including SCFA producers or mucin degraders—thereby modifying metabolic outputs relevant to gut–brain signaling (Salari et al., 2025). In lysogeny, prophage integration enables horizontal gene transfer that can introduce new metabolic traits, stress-response pathways, or virulence determinants, accelerating bacterial adaptation to environmental pressures such as diet, antibiotics, or immune activation (Zhang et al., 2025a). This phage-driven gene flow can substantially reshape the functional potential of gut microbial ecosystems over short timescales, particularly among taxa within groups such as Siphoviridae, Myoviridae, Microviridae, and Caudoviricetes (Rybicka & Kaźmierczak, 2025).

Metagenomic and metaviromic studies increasingly reveal reproducible associations between virome composition and neuropsychiatric conditions (Ren et al., 2025). In MDD, reduced viral diversity and enrichment of

specific bacteriophage families—particularly Siphoviridae and Myoviridae—have been reported, mirroring patterns of bacterial dysbiosis (Chen et al., 2025a). Certain phages, including those targeting *Stenotrophomonas* species, show promise as biomarkers, with predictive models achieving high diagnostic accuracy when combined with bacterial and clinical features (Gao et al., 2023). Mechanistically, virome effects likely operate through indirect pathways—such as phage-mediated shifts in microbial metabolites and inflammatory tone—and direct immune interactions, as viral nucleic acids can activate host receptors such as TLR9 to influence systemic interferon signaling and neuroimmune status (Kurilovich & Geva-Zatorsky, 2025).

Chronic stress offers a clear example of virome responsiveness within the gut–brain axis. Animal models of social defeat or restraint stress demonstrate substantial restructuring of gut phage communities, particularly within Caudoviricetes, alongside behavioral and immunological changes (Ritz et al., 2024). Altered phage–host interaction networks correlate with cytokine profiles and anxiety-like behaviors, indicating sensitivity of the virome to neuroendocrine signals (Dominguez et al., 2019). Notably, fecal virome transplantation from unstressed donors can partially reverse stress-related behavioral and transcriptional changes, even when bacterial composition remains largely stable, suggesting that viral communities can independently modulate neural and endocrine pathways (Ritz et al., 2024).

Beyond bacteriophages, the gut also harbors eukaryotic viruses—including members of Picornaviridae, Caliciviridae, and Adenoviridae—that infect host cells (Guerin et al., 2018). While often asymptomatic, persistent enteric viral colonization may sustain low-grade mucosal immune activation, influence microbial ecology, and affect barrier function, with downstream consequences for systemic inflammation and brain physiology (Gavkare et al., 2025). However, direct causal links between specific eukaryotic gut viruses and defined neuropsychiatric outcomes remain limited, highlighting a significant knowledge gap (Pargin et al., 2023).

The mycobiome, representing less than 1% of gut microbial biomass, exerts disproportionately large immunological and metabolic effects (Belvoncikova et al., 2022). Common genera include *Candida, Saccharomyces, Malassezia*, and *Cladosporium*, with composition shaped by diet, medications, and host immunity (Hallen-Adams & Suhr, 2016). Fungal cell walls contain β-glucans, mannans, and chitin that engage innate immune receptors such as Dectin-1, Dectin-2, MINCLE, and TLR2, eliciting cytokine responses often skewed toward Th1 and Th17 profiles (Kozłowska et al., 2025). These immune pathways influence epithelial permeability, systemic inflammation, and microglial activation, providing mechanistic links between mycobiome dynamics and brain function (Zheng & Luo, 2025).

Mycobiome alterations have been associated with several neuropsychiatric disorders. In schizophrenia, fungal dysbiosis characterized by *Candida albicans* overgrowth, reduced diversity, and altered fungal–bacterial interactions has been described (Hadrich et al., 2025). Elevated antifungal antibodies, including anti-*Candida* and anti-*Saccharomyces cerevisiae*, correlate with cognitive impairment and symptom severity, suggesting a role for chronic antifungal immune activation (Severance et al., 2017). Mechanistically, *Candida* species can transition to invasive hyphal forms that disrupt epithelial barriers, facilitating translocation of fungal components such as β-glucans into circulation and promoting systemic inflammation with potential neuroimmune consequences (Cheng et al., 2011).

Host genetics further modulate these interactions. Individuals with elevated polygenic risk for psychiatric disorders show increased prevalence of *Candida*-dominated fungal communities, implicating gene–environment interactions in shaping fungal ecology (Hadrich et al., 2025). Variants affecting antifungal immune pathways, including Dectin-1 or CARD9, may permit persistent colonization and chronic low-grade inflammation that influences microglial activation and synaptic function (Rosentul et al., 2011). Mycobiome changes have also been reported in depression, bipolar

disorder, ASD, and Alzheimer's disease (AD), though causal evidence remains limited (Hadrich et al., 2025).

Therapeutic modulation of the mycobiome is an emerging frontier. Preliminary clinical studies indicate that probiotics or synbiotics can reduce antifungal antibody titers and modestly improve symptoms in subsets of patients, possibly through competitive microbial interactions and restoration of barrier integrity (Severance et al., 2017). Preclinical data further suggest that antifungal interventions or manipulation of fungal–bacterial consortia can alter behavioral and neuroinflammatory outcomes, although careful strategies are needed to avoid disrupting beneficial interkingdom interactions (Ye et al., 2022).

Collectively, the virome and mycobiome expand the MGB axis beyond a bacteria-centric paradigm, adding layers of ecological complexity and regulatory capacity. Phages shape bacterial populations and metabolic potential while responding dynamically to host physiology, whereas fungi exert potent immunomodulatory effects through their structural components and barrier interactions. Integrating viral and fungal dimensions into microbiome research will be essential for a comprehensive understanding of how multi-kingdom microbial ecosystems influence neurodevelopment, stress resilience, and vulnerability to neuropsychiatric and neurodegenerative disease.

6.6 Clinical Implications in Psychiatric Disorders

6.6.1 Systemic Mechanisms Linking Dysbiosis to Psychiatric Pathophysiology

MDD is increasingly framed as a systemic inflammatory and metabolic condition in which gut microbiota alterations act as upstream drivers (Zhu et al., 2025). Across case–control and meta-analytic studies, individuals with MDD show reduced microbial alpha diversity and depletion of butyrate-producing genera such as *Faecalibacterium* and *Coprococcus*,

alongside enrichment of pro-inflammatory taxa including *Eggerthella, Alistipes*, and members of Enterobacteriaceae (Gao et al., 2023). Comparable microbial shifts are reported across bipolar disorder, schizophrenia, and anxiety disorders, suggesting a transdiagnostic signature characterized by loss of anti- inflammatory SCFA producers and expansion of inflammatory microbes (Hashimoto, 2023).

Functionally, reduced SCFA availability compromises epithelial barrier integrity through altered mucus production and tight-junction disruption, facilitating translocation of microbial products such as LPS into circulation (Cebi & Yilmaz, 2025). These molecules activate innate immune pathways involving TLR4, NOD-like receptors, and NF-κB signaling, leading to elevated cytokines including IL-1β, IL-6, and TNF-α (Ciesielska et al., 2020). Cytokine signaling then influences the brain through BBB modulation, vagal activation, and microglial priming, promoting oxidative stress, reduced neurogenesis, and impaired synaptic plasticity (Fang et al., 2023; Gryka-Marton et al., 2025). Concurrently, inflammation shifts tryptophan metabolism toward the kynurenine pathway, generating neuroactive metabolites such as quinolinic acid that disrupt glutamatergic transmission (Więdłocha et al., 2021). Persistent immune activation further stimulates the HPA axis, reinforcing a feedback loop between stress physiology and dysbiosis (Bertollo et al., 2025b).

6.6.2 Multi-Omics and Clinical Correlates

Integrative multi-omics studies link microbial alterations to metabolic and neuroimaging markers. Depressed individuals frequently show reduced SCFAs, altered bile acids, and lipid-metabolism disruptions that correlate with hippocampal atrophy, cortical thinning, and altered limbic connectivity (Xiao et al., 2024). Symptom severity and somatic complaints often parallel inflammatory markers and dysbiosis indices (Lee & Giuliani, 2019). Epidemiologic data further suggest that repeated antibiotic exposure increases risk of depression and anxiety, likely through loss of protective microbial metabolites (Dinan & Dinan, 2022).

6.6.3 Neurodevelopmental Disorders: Autism Spectrum Disorder

ASD illustrates developmental dimensions of microbiota–brain interactions. Gastrointestinal symptoms are common and correlate with behavioral severity, while microbial studies report shifts in *Clostridium, Sutterella, Desulfovibrio*, and *Bacteroides* (Coretti et al., 2018). Elevated microbial propionic acid can induce ASD-like behaviors in animal models via mitochondrial dysfunction, altered neurotransmission, and neuroinflammation (Meeking et al., 2020). Sulfur-metabolizing bacteria producing hydrogen sulfide may further damage epithelial barriers and promote immune activation (Petropoulos et al., 2025).

Maternal immune activation provides causal insight: maternal dysbiosis enhances Th17 responses and IL-17A signaling that alters fetal cortical development, producing persistent ASD-like phenotypes, while microbiota-targeted interventions mitigate these effects (Chen et al., 2025b; Taha et al., 2025). Early clinical trials of probiotics, diet, and fecal microbiota transplantation (FMT) show variable but promising improvements in gastrointestinal and behavioral outcomes.

6.6.4 Other Psychiatric Conditions

Across anxiety disorders, post-traumatic stress disorder (PTSD), bipolar disorder, schizophrenia, and ADHD, meta-analyses identify recurring reductions in *Faecalibacterium* and *Coprococcus* with increased inflammatory taxa (Nikolova et al., 2021). In anxiety and PTSD, microbial alterations correlate with increased permeability and altered emotion-regulation network connectivity, while experimental microbiota manipulation influences fear learning via GABAergic and glutamatergic pathways (Bravo et al., 2011).

Schizophrenia exemplifies the intersection of microbiota, immunity, and neurotransmission: reduced SCFA producers, altered kynurenine metabolism, and elevated kynurenic acid—an NMDA receptor antagonist—

are linked to cognitive deficits and negative symptoms (Mosquera et al., 2024; Zhou et al., 2025). Probiotic and prebiotic interventions show modest benefits for inflammation and gastrointestinal symptoms, though effects on core psychosis remain inconsistent (Yang et al., 2025a).

6.6.5 Clinical Translation

Overall, psychiatric evidence supports a model in which dysbiosis and barrier dysfunction act as upstream modifiers that manifest as distinct phenotypes depending on developmental timing, genetics, and environment (Eskandar, 2025). Clinically, this points to microbiota-based biomarkers, therapies targeting barrier integrity and microbial metabolism, and early-life interventions—while highlighting the need for longitudinal mechanistic trials (Guo et al., 2022).

6.7 Clinical Implications in Neurodegenerative Diseases

6.7.1 Parkinson's Disease: A Gut-First Paradigm

PD is widely viewed as a prototypical "gut-first" neurodegenerative disorder. Misfolded α-synuclein may originate in the ENS and propagate via the vagus nerve to the brainstem and substantia nigra, consistent with Braak staging (Menozzi et al., 2025). Gastrointestinal α-synuclein pathology and prodromal constipation often precede motor symptoms, and truncal vagotomy is associated with reduced PD risk.

Microbiome studies reveal depletion of SCFA-producing genera such as *Faecalibacterium* and *Roseburia* and enrichment of Enterobacteriaceae, *Akkermansia*, and *Desulfovibrio* (Mohammadi et al., 2025). Resulting SCFA deficits, increased permeability, and elevated LPS promote chronic inflammation and α-synuclein aggregation; bacterial amyloids can further cross-seed misfolding (Bhoite et al., 2022; Caetano-Silva et al., 2023;

Valdetaro et al., 2026). This framework links dysbiosis, enteric pathology, and progressive nigrostriatal degeneration.

6.7.2 Alzheimer's Disease: Inflammation and Microbial Triggers

AD is increasingly interpreted through both gut and oral microbial axes (Seaks & Wilcock, 2020). The periodontal pathogen *Porphyromonas gingivalis* and its gingipain proteases have been detected in AD brains, where they associate with tau pathology and neuroinflammation (Dominy et al., 2019). Amyloid-β may act as an antimicrobial peptide, implying plaque deposition could reflect chronic innate immune activation (Xie et al., 2025).

Gut dysbiosis in AD—marked by reduced diversity, SCFA depletion, and increased Gram-negative taxa—contributes to systemic inflammation and BBB compromise, allowing microbial products to exacerbate amyloid and tau aggregation (Manfredi et al., 2025). Multi-omics studies link microbial metabolites to amyloid burden, cortical atrophy, and hypometabolism, while Mediterranean-style microbiota profiles correlate with preserved cognition (Ji et al., 2025; Jiang et al., 2025).

6.7.3 Other Neurodegenerative and Demyelinating Disorders

MGB interactions also influence multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia. In MS, reduced SCFA producers and increased Th17-promoting taxa contribute to inflammation and BBB disruption; restoring commensals reduces disease severity in experimental models (Kujawa et al., 2023). In ALS, dysbiosis and barrier dysfunction may precede motor neuron loss, with microbiota-modulating interventions altering neuroinflammation in preclinical studies (Yang, 2025). Across disorders, dysbiosis acts as an amplifier of systemic inflammation and neurodegenerative cascades.

6.7.4 Therapeutic Implications and Future Directions

Viewing neurodegeneration through a microbiome lens highlights preventive and disease-modifying strategies, including dietary modulation, targeted probiotics or postbiotics, and microbiota transplantation (Cuffaro et al., 2024). Early trials suggest benefits for PD motor and non-motor symptoms and modest cognitive improvements in AD, though heterogeneity across patients indicates the need for precision approaches (Seo & Holtzman, 2024; Zhong et al., 2023).

Future research should employ longitudinal, integrative designs combining microbiome sequencing, metabolomics, immune profiling, and neuroimaging to clarify causality and identify responders. Such work will be essential for translating microbiota-targeted strategies into durable clinical interventions.

6.8 Therapeutic Modulation

The reversibility of gut dysbiosis and its downstream effects on the MGB axis has fueled growing interest in therapies that intervene upstream—at the level of microbial ecology, barrier function, and key metabolic pathways—rather than focusing solely on neurotransmission. Across psychiatric and neurodegenerative conditions, convergent evidence highlights a core set of shared mechanisms: depletion of SCFA-producing microbes, epithelial and BBB dysfunction, chronic low-grade inflammation, and inflammation-driven diversion of tryptophan from serotonin toward the kynurenine pathway (Kearns, 2024). Microbiota-focused treatments can therefore be viewed as attempts to restore four interconnected axes—SCFA availability, barrier integrity, inflammatory balance, and tryptophan metabolism—to re-establish a neuroprotective physiological state (Nagpal et al., 2025).

Psychobiotics—encompassing probiotics, prebiotics, and synbiotics—are the most extensively studied microbiota-targeted interventions for mood and stress-related disorders. Randomized controlled trials using strains such as *L. rhamnosus, L. helveticus* R0052, *Bifidobacterium longum* 1714 or R0175, and *L. plantarum* P-8 report modest but significant improvements in

depressive and anxiety symptoms, particularly as adjuncts to pharmacotherapy (Menni et al., 2025). Mechanistic studies indicate that these interventions increase SCFA production, improve epithelial tight-junction function, reduce markers of endotoxemia, dampen pro-inflammatory cytokines, and normalize HPA axis activity (Binda et al., 2024). Some trials also report increases in circulating BDNF and modulation of GABAergic or glutamatergic signaling, linking upstream metabolic and immune effects to downstream synaptic plasticity. These findings suggest that precision selection of strains based on individual microbial or metabolic deficits may enhance therapeutic efficacy (Binda et al., 2024).

FMT provides a broader ecological reset by transferring a complete microbial community from a healthy donor. Meta- analytic data from randomized trials indicate significant reductions in depressive symptoms, with large short-term effect sizes (Zhang et al., 2025c). FMT typically restores microbial diversity and replenishes key SCFA-producing taxa such as *Faecalibacterium* and *Roseburia*, leading to increased SCFA levels, improved barrier function, reduced systemic inflammation, and more favorable tryptophan–kynurenine ratios (Bertollo et al., 2025). In ASD and related conditions, open-label studies report sustained improvements in gastrointestinal symptoms and, in some cases, behavioral outcomes, though larger blinded trials remain necessary (Arizona State University, 2023). Limitations—including donor variability and durability of engraftment—are driving development of defined microbial consortia and next-generation live biotherapeutics designed to reproduce FMT's benefits with greater consistency and safety.

Neuromodulatory approaches such as vagus nerve stimulation (VNS) and transcutaneous auricular VNS (taVNS) represent top-down strategies for recalibrating the MGB axis. In addition to modulating neural circuits implicated in mood regulation, VNS activates the cholinergic anti-inflammatory pathway, reducing systemic cytokine production and indirectly supporting barrier integrity (Chen & Liu, 2025). Preclinical studies show that taVNS can reverse stress-induced behavioral changes,

reduce neuroinflammation, and remodel gut microbial communities—often increasing beneficial taxa and improving metabolic profiles (Zhang et al., 2025b). Early human studies suggest improvements in mood, autonomic regulation, and inflammatory markers, although detailed characterization of microbiome changes in clinical populations is still emerging (Marano et al., 2025).

Dietary interventions remain the most scalable long-term strategy for modulating upstream mechanisms. Mediterranean-style diets rich in fiber, polyphenols, and unsaturated fats consistently increase microbial diversity, enrich SCFA-producing taxa, and reduce pro-inflammatory microbes, thereby enhancing barrier function and lowering systemic inflammation (Kong et al., 2024). Microbial metabolism of polyphenols yields bioactive compounds with antioxidant and anti-inflammatory properties that can influence brain function, while SCFAs strengthen epithelial and BBB tight junctions and regulate microglial activity (Fock and Parnova, 2023). Clinical trials show that transitioning from a Western to a Mediterranean dietary pattern can significantly improve depressive symptoms, with microbiota and metabolite changes mediating part of the effect (Horn et al., 2022). Conversely, Western diets high in saturated fat and refined sugars promote dysbiosis, endotoxemia, and kynurenine-biased tryptophan metabolism, reinforcing the very disturbances these therapies aim to correct (Randeni & Xu, 2025).

Collectively, therapeutic strategies for the MGB axis can be understood as acting on a limited set of shared pathophysiological levers: restoring SCFA production, repairing barrier integrity, reducing chronic inflammation, and rebalancing tryptophan metabolism. Psychobiotics provide targeted modulation; FMT and engineered consortia offer ecosystem-level resets; neuromodulation recalibrates inflammatory and autonomic signaling; and diet supplies the substrate environment that determines durability of all other interventions (Bibolar et al., 2025). Future precision approaches will likely integrate these modalities, guided by microbiome, metabolomic, immune, and neuroimaging profiles to tailor

interventions that address the upstream drivers of psychiatric and neurodegenerative disease.

6.9 Conclusion

The MGB axis has emerged as a central biological system governing brain development, mental health, and neurodegeneration in humans. Across mood, anxiety, neurodevelopmental, psychotic, and neurodegenerative disorders, intestinal microbial communities function as essential metabolic, immunological, and neuroactive partners, shaping host physiology through continuous biochemical and neural communication with the CNS. These host–microbe interactions influence not only local gut function but also systemic inflammation, endocrine stress responses, and central processes such as synaptic plasticity, neurogenesis, and protein aggregation. Comparative evidence across diagnoses highlights both conserved and condition-specific microbial signatures that reflect interactions among diet, genetics, environment, and disease stage, underscoring the microbiota's pivotal role in SCFA production, barrier integrity, tryptophan metabolism, and immune–glial homeostasis.

Recent advances in high-throughput sequencing, multi-omics integration, and systems neuroscience have substantially deepened understanding of how microbiome structure–function relationships translate into brain-relevant phenotypes. This growing body of work converges on a small set of shared upstream mechanisms, including loss of SCFA-producing taxa, epithelial and BBB failure, chronic low-grade inflammation, and stress or inflammation-driven tryptophan shunting, that appear to modulate risk and progression across multiple psychiatric and neurodegenerative conditions. At the same time, it has opened translational avenues for microbiota-informed interventions, ranging from diet, psychobiotics, and postbiotics to FMT, neuromodulation, and rationally designed microbial consortia. Effective clinical translation will require integrated strategies that account for host genetics, baseline microbiome

configuration, diet, medication exposure, and environmental context, and that are evaluated in well-powered, longitudinal, and mechanistically rich human studies. Ultimately, strategically harnessing the MGB axis offers a powerful and potentially sustainable pathway to prevent and modify the trajectory of brain disorders, shifting practice from purely downstream symptom control toward upstream, systems-level interventions that support resilience across the lifespan.

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References

Abildinova, G.Z., Benberin, V.V., Vochshenkova, T.A., *et al*., 2024. The gut-brain-metabolic axis: Exploring the role of microbiota in insulin resistance and cognitive function. *Front. Microbiol*. 15, 1463958. ⏎

Agirman, G., Hsiao, E.Y., 2021. SnapShot: The microbiota-gut-brain axis. *Cell* 184, 2524–2524.e1. ⏎

Anand, N., Gorantla, V.R., Chidambaram, S.B., 2022. The role of gut dysbiosis in the pathophysiology of neuropsychiatric disorders. *Cells* 12, 54. ⏎

Arizona State University, 2023. *Microbiota Transfer Therapy for Adults with Autism Spectrum Disorder (ASD) Who Have Gastrointestinal Disorders (Clinical trial registration No. NCT03408886)*. clinicaltrials.gov. ⏎

Ataei, P., Kalantari, H., Bodnar, T.S., *et al*., 2026. The gut–brain connection: Microbes' influence on mental health and psychological disorders. *Front. Microbiomes* 4, 1701608. ⏎

Badawy, A.A.-B., Dawood, S., Bano, S., 2023. Kynurenine pathway of tryptophan metabolism in pathophysiology and therapy of major depressive disorder. *World J. Psychiatry* 13, 141–148. ⏎

Bali, V., Grubišić, V., 2024. Enteric glia as friends and foes of the intestinal epithelial barrier function. *Front. Immunol*. 15, 1394654. ⏎

Barbu, A.C., Stoleru, S., Zugravu, A., *et al*., 2025a. Dopamine and the gut microbiota: Interactions within the microbiota–gut–brain axis and therapeutic perspectives. *Int. J. Mol. Sci*. 27, 271. ⏎

Bautista, J., López-Cortés, A., 2025. Chronobiome medicine: Circadian regulation of host–microbiota crosstalk in systemic physiology. *Front. Endocrinol. (Lausanne)* 16, 1691172. ⏎

Belvoncikova, P., Splichalova, P., Videnska, P., *et al*., 2022. The human mycobiome: Colonization, composition and the role in health and disease. *J. Fungi (Basel)* 8, 1046. ⏎

Bertollo, A.G., Santos, C.F., Bagatini, M.D., *et al*., 2025. Hypothalamus-pituitary-adrenal and gut-brain axes in biological interaction pathway of the depression. *Front. Neurosci*. 19, 1541075. ⏎

Bhoite, S.S., Han, Y., Ruotolo, B.T., *et al*., 2022. Mechanistic insights into accelerated α-synuclein aggregation mediated by human microbiome-associated functional amyloids. *J. Biol. Chem*. 298, 102088. ⏎

Bibolar, A.C., Nechita, V.I., Lung, F.C., *et al*., 2025. Gut feelings: Linking dysbiosis to depression—a narrative literature review. *Medicina (Kaunas)* 61, 1360. ⏎

Binda, S., Tremblay, A., Iqbal, U.H., *et al*., 2024. Psychobiotics and the microbiota–gut–brain axis: Where do we go from here? *Microorganisms* 12, 634.

Bonaz, B., Sinniger, V., Pellissier, S., 2017. The vagus nerve in the neuro-immune axis: Implications in the pathology of the gastrointestinal tract. *Front. Immunol*. 8, 1452. ⏎

Bordenstein, S.R., Theis, K.R., 2015. Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biol*. 13, e1002226. ⏎

Bourassa, M.W., Alim, I., Bultman, S.J., *et al*., 2016. Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neurosci. Lett*. 625, 56–63. ⏎

Bravo, J.A., Forsythe, P., Chew, M.V., *et al*., 2011. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. U.S.A*. 108, 16050–16055. ⏎

Caetano-Silva, M.E., Rund, L., Hutchinson, N.T., *et al*., 2023. Inhibition of inflammatory microglia by dietary fiber and short-chain fatty acids. *Sci. Rep*. 13, 2819. ⏎

Cao, Q., Shen, M., Li, R., *et al*., 2025. Elucidating the specific mechanisms of the gut-brain axis: The short-chain fatty acids-microglia pathway. *J. Neuroinflammation* 22, 133. ⏎

Cao, Z., Sugimura, N., Burgermeister, E., *et al*., 2022. The gut virome: A new microbiome component in health and disease. *eBioMedicine* 81, 104113.

Cebi, M., Yilmaz, Y., 2025. Epithelial barrier hypothesis in the context of nutrition, microbial dysbiosis, and immune dysregulation in metabolic dysfunction-associated steatotic liver. *Front. Immunol*. 16, 1575770. ⏎

Chang, L., Thaiss, C.A., Knight, Z., *et al*., 2025. Gut feelings: The critical role of interoception in obesity and disorders of gut–brain interaction. *Gastroenterology* 169, 188–194. ⏎

Che, H., Gao, Y., Xu, Y., *et al*., 2026. Organ cross-talk: Molecular mechanisms, biological functions, and therapeutic interventions for diseases. *Signal Transduct. Target Ther*. 11, 8. ⏎

Chen, X., Wu, J., Fan, D., *et al*., 2025a. Gut viral metagenomics identifies viral signatures and their role in depression. *Front. Microbiol*. 16, 1573851.

Chen, Z., Liu, K., 2025. Mechanism and applications of vagus nerve stimulation. *Curr. Issues Mol. Biol*. 47, 122. ⏎

Chen, Z., Wang, X., Hu, Y., *et al*., 2025b. Effect of maternal diet on gut bacteria and autism spectrum disorder in offspring. *Front. Cell. Neurosci*.

19, 1623576. ⏎

Cheng, S.-C., van de Veerdonk, F.L., Lenardon, M., *et al*., 2011. The dectin-1/inflammasome pathway is responsible for the induction of protective T-helper 17 responses that discriminate between yeasts and hyphae of *Candida albicans*. *J. Leukoc. Biol*. 90, 357–366. ⏎

Chudzik, A., Słowik, T., Kochalska, K., *et al*., 2022. Continuous ingestion of *Lacticaseibacillus rhamnosus* JB-1 during chronic stress ensures neurometabolic and behavioural stability in rats. *Int. J. Mol. Sci*. 23, 5173. ⏎

Ciesielska, A., Matyjek, M., Kwiatkowska, K., 2020. TLR4 and CD14 trafficking and its influence on LPS-induced pro- inflammatory signaling. *Cell. Mol. Life Sci*. 78, 1233–1261. ⏎

Coretti, L., Buommino, E., Lembo, F., 2024. The aryl hydrocarbon receptor pathway: A linking bridge between the gut microbiome and neurodegenerative diseases. *Front. Cell. Neurosci*. 18, 1433747. ⏎

Coretti, L., Paparo, L., Riccio, M.P., *et al*., 2018. Gut microbiota features in young children with autism spectrum disorders. *Front. Microbiol*. 9, 3146. ⏎

Correa Lopes, B., Turck, J., Tolbert, M.K., *et al*., 2025. Prolonged storage reduces viability of *Peptacetobacter (Clostridium) hiranonis* and core intestinal bacteria in fecal microbiota transplantation preparations for dogs. *Front. Microbiol*. 15, 1502452. ⏎

Cuffaro, F., Lamminpää, I., Niccolai, E., *et al*., 2024. Nutritional and microbiota-based approaches in amyotrophic lateral sclerosis: From prevention to treatment. *Nutrients* 17, 102. ⏎

Cui, H., Elford, J.D., Alitalo, O., *et al*., 2023. Nigrostriatal 6-hydroxydopamine lesions increase alpha-synuclein levels and permeability in rat colon. *Neurobiol. Aging* 129, 62–71. ⏎

De Vadder, F., Grasset, E., Mannerås Holm, L., *et al*., 2018. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc. Natl. Acad. Sci. U.S.A*. 115, 6458–6463. ⏎

Dinan, K., Dinan, T., 2022. Antibiotics and mental health: The good, the bad and the ugly. *J. Intern. Med*. 292, 858–869. ⏎

Dinesh, D., Morgan, X., Scott, T.M., *et al*., 2025. Variations in the gut bacteriome and virome associated with cognitive function in Puerto Rican adults. *Alzheimers Dement*. 21, e098660. ⏎

Doenyas, C., Clarke, G., Cserjési, R., 2025. Gut–brain axis and neuropsychiatric health: Recent advances. *Sci. Rep*. 15, 3415. ⏎

Dominguez, G., Henkous, N., Prevot, T., *et al*., 2019. Sustained corticosterone rise in the prefrontal cortex is a key factor for chronic stress-induced working memory deficits in mice. *Neurobiol. Stress* 10, 100161. ⏎

Dominy, S.S., Lynch, C., Ermini, F., *et al*., 2019. *Porphyromonas gingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv*. 5, eaau3333. ⏎

Dong, T.S., Mayer, E., 2024. Advances in brain–gut–microbiome interactions: A comprehensive update on signaling mechanisms, disorders, and therapeutic implications. *Cell. Mol. Gastroenterol. Hepatol*. 18, 1–13. ⏎

Du, J., Li, J., Liu, P., *et al*., 2025. Mechanisms by which the gut microbiota regulates depressive disorder via the tryptophan metabolic pathway. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 50, 1263–1270. ⏎

Eskandar, K., 2025. The gut–brain axis in depression, anxiety, and schizophrenia: A scoping review of mechanisms, biomarkers, and therapeutic implications. *Middle East Curr. Psychiatry* 32, 87. ⏎

Fang, S., Wu, Z., Guo, Y., *et al*., 2023. Roles of microglia in adult hippocampal neurogenesis in depression and their therapeutics. *Front. Immunol*. 14, 1193053. ⏎

Faraji, N., Payami, B., Ebadpour, N., *et al*., 2025. Vagus nerve stimulation and gut microbiota interactions: A novel therapeutic avenue for neuropsychiatric disorders. *Neurosci. Biobehav. Rev*. 169, 105990. ⏎

Fock, E., Parnova, R., 2023. Mechanisms of blood–brain barrier protection by microbiota-derived short-chain fatty acids. *Cells* 12, 657. ⏎

Förster, C.Y., Shityakov, S., 2026. A possible role for the vagus nerve in physical and mental health. *Biomolecules* 16, 0121. ⏎

Foster, J.A., Rinaman, L., Cryan, J.F., 2017. Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiol. Stress* 7, 124–136. ⏎

Fülling, C., Dinan, T.G., Cryan, J.F., 2019. Gut microbe to brain signaling: What happens in vagus…. *Neuron* 101, 998–1002. ⏎

Gao, K., Mu, C., Farzi, A.,*et al*., 2020. Tryptophan metabolism: A link between the gut microbiota and brain. *Adv. Nutr*. 11, 709–723. ⏎

Gao, M., Wang, J., Liu, P., *et al*., 2023. Gut microbiota composition in depressive disorder: A systematic review, meta-analysis, and meta-regression. *Transl. Psychiatry* 13, 379. ⏎

Gavkare, A.M., Nanaware, N.L., Sonar, M.N., *et al*., 2025. Gut microbiome and viral infections: A hidden nexus for immune protection. *World J. Virol*. 14, 111912. ⏎

Grundeken, E., El Aidy, S., 2025. Enteroendocrine cells: The gatekeepers of microbiome-gut-brain communication. *NPJ Biofilms Microbiomes* 11, 179. ⏎

Gryka-Marton, M., Grabowska, A.D., Szukiewicz, D., 2025. Breaking the barrier: The role of proinflammatory cytokines in BBB dysfunction. *Int. J. Mol. Sci*. 26, 3532. ⏎

Guerin, E., Shkoporov, A., Stockdale, S.R., *et al*., 2018. Biology and taxonomy of crAss-like bacteriophages, the most abundant virus in the human gut. *Cell Host Microbe* 24, 653–664.e6. ⏎

Guo, C., Huo, Y.-J., Li, Y., *et al*., 2022. Gut-brain axis: Focus on gut metabolites short-chain fatty acids. *World J. Clin. Cases* 10, 1754–1763. ⏎

Hadrich, I., Turki, M., Chaari, I., *et al*., 2025. Gut mycobiome and neuropsychiatric disorders: Insights and therapeutic potential. *Front. Cell. Neurosci*. 18, 1495224. ⏎

Hallen-Adams, H.E., Suhr, M.J., 2016. Fungi in the healthy human gastrointestinal tract. *Virulence* 8, 352–358. ⏎

Hashimoto, K., 2023. Emerging role of the host microbiome in neuropsychiatric disorders: Overview and future directions. *Mol. Psychiatry* 28, 3625–3637. ⏎

Hazart, D., Moulzir, M., Delhomme, B., *et al*., 2025. Imaging the enteric nervous system. *Front. Neuroanat*. 19, 1532900. ⏎

He, T., Zhu, Y.-H., Yu, J., *et al*., 2019. *Lactobacillus johnsonii* L531 reduces pathogen load and helps maintain short-chain fatty acid levels in the intestines of pigs challenged with *Salmonella enterica* Infantis. *Vet. Microbiol*. 230, 187–194. ⏎

Horn, J., Mayer, D.E., Chen, S., *et al*., 2022. Role of diet and its effects on the gut microbiome in the pathophysiology of mental disorders. *Transl. Psychiatry* 12, 164. ⏎

Hou, Y., Li, J., Ying, S., 2023. Tryptophan metabolism and gut microbiota: A novel regulatory axis integrating the microbiome, immunity, and cancer. *Metabolites* 13, 1166. ⏎

Hubková, B., Valko-Rokytovská, M., Čižmárová, B., *et al*., 2022. Tryptophan: Its metabolism along the Kynurenine, Serotonin, and Indole pathway in malignant melanoma. *Int. J. Mol. Sci*. 23, 9160. ⏎

Hwang, Y.K., Oh, J.S., 2025. Interaction of the vagus nerve and serotonin in the gut-brain axis. *Int. J. Mol. Sci*. 26, 1160. ⏎

Intlekofer, K.A., Berchtold, N.C., Malvaez, M., *et al*., 2013. Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. *Neuropsychopharmacology* 38, 2027–2034. ⏎

Jameson, K.G., Kazmi, S.A., Ohara, T.E., *et al*., 2024. Select microbial metabolites in the small intestinal lumen regulate vagal activity via receptor-mediated signaling. *iScience* 28, 111699. ⏎

Ji, X., Wang, J., Lan, T., *et al*., 2025. Gut microbial metabolites and the brain–gut axis in Alzheimer's disease: A review. *Biomol. Biomed*. 26, 240–250. ⏎

Jiang, Y., Chen, Y., Chen, Y., *et al*., 2025. Ketogenic diet and gut microbiota: Exploring new perspectives on cognition and mood. *Foods*

14, 1215. ⏎

Kaelberer, M.M., Rupprecht, L.E., Liu, W.W., *et al*., 2020. Neuropod cells: Emerging biology of the gut-brain sensory transduction. *Annu. Rev. Neurosci*. 43, 337–353. ⏎

Kearns, R., 2024. Gut–brain axis and neuroinflammation: The role of gut permeability and the kynurenine pathway in neurological disorders. *Cell. Mol. Neurobiol*. 44, 64. ⏎

Keever, K.R., Yakubenko, V.P., Hoover, D.B., 2023. Neuroimmune nexus in the pathophysiology and therapy of inflammatory disorders: Role of α7 nicotinic acetylcholine receptors. *Pharmacol. Res*. 191, 106758. ⏎

Khawar, M.M., Ijaz, S., Goyal, P., *et al*., n.d. The gut-brain axis in autoimmune diseases: Emerging insights and therapeutic implications. *Cureus* 15, e48655.

Kong, J., Yang, J., He, C., *et al*., 2024. Regulation of endotoxemia through the gut microbiota: The role of the Mediterranean diet and its components. *APMIS* 132, 948–955. ⏎

Kozłowska, E., Agier, J., Różalska, S., *et al*., 2025. Fungal β-glucans shape innate immune responses in human peripheral blood mononuclear cells (PBMCs): An in vitro study on PRR regulation, cytokine expression, and oxidative balance. *Int. J. Mol. Sci*. 26, 6458. ⏎

Kujawa, D., Laczmanski, L., Budrewicz, S., *et al*., 2023. Targeting gut microbiota: New therapeutic opportunities in multiple sclerosis. *Gut Microbes* 15, 2274126.

Kurilovich, E., Geva-Zatorsky, 2025. Effects of bacteriophages on gut microbiome functionality. *Gut Microbes* 17, 2481178.

Lathakumari, R.H., Vajravelu, L.K., Gopinathan, A., *et al*., 2025. The gut virome and human health: From diversity to personalized medicine. *Eng. Microbiol*. 5, 100191. ⏎

Lee, C.-H., Giuliani, F., 2019. The role of inflammation in depression and fatigue. *Front. Immunol*. 10, 1696. ⏎

Li, G., Dong, S., Liu, C., *et al*., 2024. Serotonin signaling to regulate energy metabolism: A gut microbiota perspective. *Life Metab*. 4,

loae039. ⏎

Li, J., Yu, K., Sui, X., *et al*., 2025a. Gut jet lag: How circadian rhythm disruption undermines the Chrono-Microbiota-Motility axis and induces functional constipation. *Front. Nutr*. 12, 1678482.

Li, Y., Ding, S., Wang, Y., 2025b. Targeting the cholinergic anti-inflammatory pathway: An innovative strategy for treating diseases. *Mol. Biol. Rep*. 52, 199. ⏎

Liu, B., Fang, F., Pedersen, N.L., *et al*., 2017. Vagotomy and Parkinson disease. *Neurology* 88, 1996–2002. ⏎

Liu, P., Ouyang, Y., Gao, Z., *et al*., 2025. Decoding the gut-brain axis in depression: Mechanistic insights and functional microbiota-based interventions. *J. Funct. Foods* 133, 107029. ⏎

Liu, Z., Ma, A., Mathé, E., *et al*., 2020. Network analyses in microbiome based on high-throughput multi-omics data. *Brief. Bioinform*. 22, 1639–1655.

Lopez-Martin, G., Caparco, A., van Steenoven, C., *et al*., 2026. Interoceptive rhythms and perceptual experience: Mechanisms, contexts, and strategies for real-world research. *NeuroImage* 325, 121650. ⏎

Lorsch, Z.S., Liddle, R.A., 2026. Mechanisms and clinical implications of gut-brain interactions. *J. Clin. Invest*. 136, 196346.

Lu, S., Zhao, Q., Guan, Y., *et al*., 2024. The communication mechanism of the gut-brain axis and its effect on central nervous system diseases: A systematic review. *Biomed. Pharmacother*. 178, 117207. ⏎

Manfredi, J.N., Gupta, S.K., Vyavahare, S., *et al*., 2025. Gut microbiota dysbiosis in Alzheimer's disease (AD): Insights from human clinical studies and the mouse AD models. *Physiol. Behav*. 290, 114778. ⏎

Martin, C.R., Osadchiy, V., Kalani, A., *et al*., 2018. The brain-gut-microbiome axis. *Cell. Mol. Gastroenterol. Hepatol*. 6, 133–148. ⏎

Marwaha, K., Cain, R., Asmis, K., *et al*., 2025. Exploring the complex relationship between psychosocial stress and the gut microbiome: Implications for inflammation and immune modulation. *J. Appl. Physiol*. 138, 518–535. ⏎

Mayer, E.A., Knight, R., Mazmanian, S.K., *et al*., 2014. Gut microbes and the brain: Paradigm shift in neuroscience. *J. Neurosci*. 34, 15490–15496. ⏎

Meeking, M.M., MacFabe, D.F., Mepham, J.R., *et al*., 2020. Propionic acid induced behavioural effects of relevance to autism spectrum disorder evaluated in the hole board test with rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 97, 109794. ⏎

Menni, A.-E., Theodorou, H., Tzikos, G., *et al*., 2025. Rewiring mood: Precision psychobiotics as adjunct or stand-alone therapy in depression using insights from 19 randomized controlled trials in adults. *Nutrients* 17, 2022. ⏎

Menozzi, E., Schapira, A.H.V., Borghammer, P., 2025. The gut-brain axis in Parkinson disease: Emerging concepts and therapeutic implications. *Mov. Disord. Clin. Pract*. 12, 904–916. ⏎

Mhanna, A., Martini, N., Hmaydoosh, G., *et al*., 2023. The correlation between gut microbiota and both neurotransmitters and mental disorders: A narrative review. *Medicine (Baltimore)* 103, e37114. ⏎

Mittal, R., Debs, L.H., Patel, A.P., *et al*., 2017. Neurotransmitters: The critical modulators regulating gut-brain axis. *J. Cell. Physiol*. 232, 2359–2372. ⏎

Mohammadi, K., Zhang, D., Erik Joakim Saris, P., 2025. Strain-specific effects of *Desulfovibrio* on neurodegeneration and oxidative stress in a *Caenorhabditis elegans* PD model. *npj Parkinsons Dis*. 11, 236. ⏎

Mokbel, A.Y., Burns, M.P., Main, B.S., 2024. The contribution of the meningeal immune interface to neuroinflammation in traumatic brain injury. *J. Neuroinflammation* 21, 135. ⏎

Moretti, C.H., Grasset, E., Zhu, J., *et al*., 2025. Identification of human gut bacteria that produce bioactive serotonin and promote colonic innervation. *Cell Rep*. 44, 116434. ⏎

Mosquera, F.E.C., Guevara-Montoya, M.C., Serna-Ramirez, V., *et al*., 2024. Neuroinflammation and schizophrenia: New therapeutic strategies

through psychobiotics, nanotechnology, and artificial intelligence (AI). *J. Pers. Med*. 14, 391. ⏎

Musazadeh, V., Faghfouri, A.H., Zarezadeh, M., *et al*., 2023. Remarkable impacts of probiotics supplementation in enhancing of the antioxidant status: Results of an umbrella meta-analysis. *Front. Nutr*. 10, 1117387. ⏎

Nagpal, D., Nema, S., Nagpal, S., *et al*., 2025. Management and prevention of neurodegenerative disorders: Can antioxidant-rich dietary interventions help? *Antioxidants (Basel)* 14, 1078. ⏎

Nicolaides, N.C., Charmandari, E., Chrousos, G.P., *et al*., 2014. Circadian endocrine rhythms: The hypothalamic–pituitary–adrenal axis and its actions. *Ann. N. Y. Acad. Sci*. 1318, 71–80. ⏎

Nikolova, V.L., Smith, M.R.B., Hall, L.J., *et al*., 2021. Perturbations in gut microbiota composition in psychiatric disorders: A review and meta-analysis. *JAMA Psychiatry* 78, 1343–1354. ⏎

Nireeksha, Maniangat Luke, A., Kumari N.S., *et al*., 2025. Metabolic interplay of SCFA's in the gut and oral microbiome: A link to health and disease. *Front. Oral Health* 6, 1646382. ⏎

Nunez, S.G., Rabelo, S.P., Subotic, N., *et al*., 2025. Chronic stress and autoimmunity: The role of HPA axis and cortisol dysregulation. *Int. J. Mol. Sci*. 26, 9994. ⏎

Oliver, P.J., Civitelli, L., Hu, M.T., 2025. The gut–brain axis in early Parkinson's disease: From prodrome to prevention. *J. Neurol*. 272, 413. ⏎

Ortega, V.A., Asbury, M.R., Mackenzie, C., *et al*., 2025. Early-life gut microbiome and stress-axis perturbations dysregulate systemic, mucosal, and brain immunity. *bioRxiv* 2025.03.19.644259. ⏎

Pargin, E., Roach, M.J., Skye, A., *et al*., 2023. The human gut virome: Composition, colonization, interactions, and impacts on human health. *Front. Microbiol*. 14, 963173. ⏎

Pavlov, V.A., Tracey, K.J., 2012. The vagus nerve and the inflammatory reflex—linking immunity and metabolism. *Nat. Rev. Endocrinol*. 8, 743–754. ⏎

Petropoulos, A., Stavropoulou, E., Tsigalou, C., *et al*., 2025. Microbiota gut–brain axis and autism spectrum disorder: Mechanisms and therapeutic perspectives. *Nutrients* 17, 2984. ⏎

Pocivavsek, A., Schwarcz, R., Erhardt, S., 2024. Neuroactive kynurenines as pharmacological targets: New experimental tools and exciting therapeutic opportunities. *Pharmacol. Rev*. 76, 978–1008. ⏎

Powley, T.L., Spaulding, R.A., Haglof, S.A., 2011. Vagal afferent innervation of the proximal gastrointestinal tract mucosa: Chemoreceptor and mechanoreceptor architecture. *J. Comp. Neurol*. 519, 644–660. ⏎

Qu, S., Yu, Z., Zhou, Y., *et al*., 2024. Gut microbiota modulates neurotransmitter and gut-brain signaling. *Microbiol. Res*. 287, 127858. ⏎

Ragozzino, F.J., Arnold, R.A., Kowalski, C.W., *et al*., 2020. Corticosterone inhibits vagal afferent glutamate release in the nucleus of the solitary tract via retrograde endocannabinoid signaling. *Am. J. Physiol. Cell Physiol*. 319, C1097–C1106. ⏎

Ramadan, Y.N., Alqifari, S.F., Alshehri, K., *et al*., 2025. Microbiome gut-brain-axis: Impact on brain development and mental health. *Mol. Neurobiol*. 62, 10813–10833. ⏎

Randeni, N., Xu, B., 2025. Critical review of the cross-links between dietary components, the gut microbiome, and depression. *Int. J. Mol. Sci*. 26, 614. ⏎

Ren, Y., Zhang, P., Yu, H., *et al*., 2025. Metagenome-based characterization of the gut virome in patients with schizophrenia. *J. Transl. Med*. 23, 895. ⏎

Ring, M., 2025. An integrative approach to HPA axis dysfunction: From recognition to recovery. *Am. J. Med*. 138, 1451–1463. ⏎

Ritz, N.L., Draper, L.A., Bastiaanssen, T.F.S., *et al*., 2024. The gut virome is associated with stress-induced changes in behaviour and immune responses in mice. *Nat. Microbiol*. 9, 359–376. ⏎

Rosentul, D.C., Plantinga, T.S., Oosting, M., *et al*., 2011. Genetic variation in the Dectin-1/CARD9 recognition pathway and susceptibility to candidemia. *J. Infect. Dis*. 204, 1138–1145. ⏎

Roth, W., Zadeh, K., Vekariya, R., *et al*., 2021. Tryptophan metabolism and gut-brain homeostasis. *Int. J. Mol. Sci*. 22, 2973. ⏎

Rusch, J.A., Layden, B.T., Dugas, L.R., 2023. Signalling cognition: The gut microbiota and hypothalamic-pituitary-adrenal axis. *Front. Endocrinol. (Lausanne)* 14, 1130689.

Rybicka, I., Kaźmierczak, Z., 2025. The human phageome: Niche-specific distribution of bacteriophages and their clinical implications. *Appl. Environ. Microbiol*. 91, e01788–24.

Salari, V., Parrella, E., Mengoni, F., *et al*., 2025. Phage-microbiota crosstalk: Implications for central nervous system disorders. *Int. J. Mol. Sci*. 26, 6183. ⏎

Salminen, A., 2022. Role of indoleamine 2,3-dioxygenase 1 (IDO1) and kynurenine pathway in the regulation of the aging process. *Ageing Res. Rev*. 75, 101573. ⏎

Seaks, C.E., Wilcock, D.M., 2020. Infectious hypothesis of Alzheimer disease. *PLoS Pathog*. 16, e1008596. ⏎

Seo, D., Holtzman, D.M., 2024. Current understanding of the Alzheimer's disease-associated microbiome and therapeutic strategies. *Exp. Mol. Med*. 56, 86–94. ⏎

Severance, E.G., Gressitt, K.L., Stallings, C.R., *et al*., 2017. Probiotic normalization of *Candida albicans* in schizophrenia: A randomized, placebo-controlled, longitudinal pilot study. *Brain Behav. Immun*. 62, 41–45.

Shah, P.A., Park, C.J., Shaughnessy, M.P., *et al*., 2021. Serotonin as a mitogen in the gastrointestinal tract: Revisiting a familiar molecule in a new role. *Cell. Mol. Gastroenterol. Hepatol*. 12, 1093–1104. ⏎

Souza, H.F., 2026. The gut is not just digestive and new evidence shows it actively controls brain function. *News-Medical*. ⏎

Staats Pires, A., Krishnamurthy, S., Sharma, S., *et al*., 2025. Dysregulation of the kynurenine pathway in relapsing remitting multiple sclerosis and its correlations with progressive neurodegeneration. *Neurol. Neuroimmunol. Neuroinflammation* 12, e200372. ⏎

Sudo, N., Chida, Y., Aiba, Y., *et al*., 2004. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J. Physiol*. 558, 263–275. ⏎

Taha, H., Issa, A., Muhanna, Z., *et al*., 2025. Microbiota-based interventions for autism spectrum disorder: A systematic review of efficacy and clinical potential. *Front. Microbiol*. 16, 1648118. ⏎

Tahara, Y., Yamazaki, M., Sukigara, H., *et al*., 2018. Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue. *Sci. Rep*. 8, 1395. ⏎

Tan, P., Jaiswal, A., Murphy, S.P., *et al*., 2025. Regional encoding of enteric nervous system responses to microbiota and type 2 inflammation. *Science* 390, eadr3545. ⏎

Valdetaro, L., Ricciardi, M.C., Almeida, P.P., *et al*., 2026. The enteric nervous system as a mediator of microbiota-gut-brain interactions in Parkinson's disease. *J. Neurochem*. 170, e70339.

Venkatakrishnan, A., Holzknecht, Z.E., Holzknecht, R., *et al*., 2021. Evolution of bacteria in the human gut in response to changing environments: An invisible player in the game of health. *Comput. Struct. Biotechnol. J*. 19, 752–758. ⏎

Waxenbaum, J.A., Reddy, V., Das, J.M., 2025. *Anatomy, Autonomic Nervous System*. StatPearls Publishing. ⏎

Więdłocha, M., Marcinowicz, P., Janoska-Jaździk, M., *et al*., 2021. Gut microbiota, kynurenine pathway and mental disorders—review. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 106, 110145. ⏎

Winters, A.D., Francescutti, D.M., Kracht, D.J., *et al*., 2025. The gut microbiome regulates the psychomotor effects and context-dependent rewarding responses to cocaine in germ-free and antibiotic-treated animal models. *Microorganisms* 13, 0077. ⏎

Worthington, J.J., Reimann, F., Gribble, F.M., 2018. Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. *Mucosal Immunol*. 11, 3–20. ⏎

Wyatt, M., Greathouse, K.L., 2021. Targeting dietary and microbial tryptophan-indole metabolism as therapeutic approaches to colon cancer. *Nutrients* 13, 1189. ⏎

Xiao, S., Yang, Z., Yan, H., *et al*., 2024. Gut proinflammatory bacteria is associated with abnormal functional connectivity of hippocampus in unmedicated patients with major depressive disorder. *Transl. Psychiatry* 14, 292. ⏎

Xie, M., Huang, X., Tang, Q., *et al*., 2025. *Porphyromonas gingivalis* impairs microglial Aβ clearance in a mouse model. *J. Dent. Res*. 104, 408–418. ⏎

Yamamoto, R., Kaur, J., 2025. *Physiology, Adrenocorticotropic Hormone (ACTH)*. StatPearls Publishing. ⏎

Yang, E.J., 2025. The emerging role of the brain–gut axis in amyotrophic lateral sclerosis: Pathogenesis, mechanisms, and therapeutic perspectives. *Int. J. Mol. Sci*. 26, 8419. ⏎

Yang, M., Cui, X., Kong, D., *et al*., 2025a. The efficacy of *Lactobacillus* and *Bifidobacterium* in patients with schizophrenia: A meta-analysis. *Eur. Arch. Psychiatry Clin. Neurosci*. 275, 1437–1451.

Yang, Y., Wang, Q., Wang, Z., *et al*., 2025b. The role of fatty acids in neurodegenerative diseases: Mechanistic insights and therapeutic strategies. *J. Lipid Res*. 66, 100944. ⏎

Yassin, L.K., Skrabulyte-Barbulescu, J., Alshamsi, S.H., *et al*., 2025. The microbiota–gut–brain axis in mental and neurodegenerative disorders: Opportunities for prevention and intervention. *Front. Aging Neurosci*. 17, 1667448. ⏎

Ye, T., Yuan, S., Kong, Y., *et al*., 2022. Effect of probiotic fungi against cognitive impairment in mice via regulation of the fungal microbiota-gut-brain axis. *J. Agric. Food Chem*. 70, 9026–9038. ⏎

Yunes, R.A., Poluektova, E.U., Dyachkova, M.S., *et al*., 2016. GABA production and structure of gadB/gadC genes in *Lactobacillus* and *Bifidobacterium* strains from human microbiota. *Anaerobe* 42, 197–204. ⏎

Zhang, S., Easwaran, M., Elafify, M., *et al*., n.d.2025a Prophages and their interactions with lytic phages in the human gut microbiota and their impact on microbial diversity, gut health, and disease. *Appl. Environ. Microbiol*. 91, e01899–25.

Zhang, X., He, X., Zhao, Y., *et al*., 2025b. Transcutaneous auricular vagus nerve stimulation regulates gut microbiota mediated peripheral inflammation and metabolic disorders to suppress depressive-like behaviors in CUMS rats. *Front. Microbiol*. 16, 1576686.

Zhang, X., Li, Y., Guo, Y., *et al*., 2025c. Clinical efficacy of fecal microbiota transplantation in alleviating depressive symptoms: A meta-analysis of randomized trials. *Front. Psychiatry* 16, 1656969. ⏎

Zhang, Y., Yan, Y., Kong, X., *et al*., 2025d. Potential cerebrospinal fluid metabolomic biomarkers and early prediction model for Parkinson's disease. *Front. Aging Neurosci*. 17, 1582362.

Zheng, M.-Y., Luo, L.-Z., 2025. The role of IL-17A in mediating inflammatory responses and progression of neurodegenerative diseases. *Int. J. Mol. Sci*. 26, 2505. ⏎

Zhong, Z., Ye, M., Yan, F., 2023. A review of studies on gut microbiota and levodopa metabolism. *Front. Neurol*. 14, 1046910. ⏎

Zhou, L., Wu, Q., Jiang, L., *et al*., 2025. Role of the microbiota in inflammation-related related psychiatric disorders. *Front. Immunol*. 16, 1613027. ⏎

Zhu, Z., Cheng, Y., Liu, X., *et al*., 2025. The microbiota-gut-brain axis in depression: Unraveling the relationships and therapeutic opportunities. *Front. Immunol*. 16, 1644160. ⏎

7

The Gut–Kidney Axis: Mechanism of Host–Microbiome Interactions and Renal Pathophysiology

Sumana Saseevan and Dhammika N. Magana-Arachchi

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7.1 The Gut Microbiome: Composition and Functional Roles

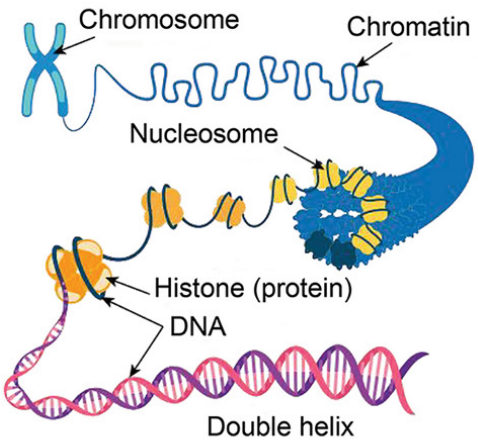
7.1.1 Gut Microbiome

The "gut microbiome" refers as the complex ecosystem of microorganisms inhabiting in the gastrointestinal tract. This diverse microbial community is composed primarily by bacteria and viruses along with fungi and archaea. Gut microbiota interact with the host in term of digestion and absorption, immune system development, and metabolic processes which maintain the body homeostasis.

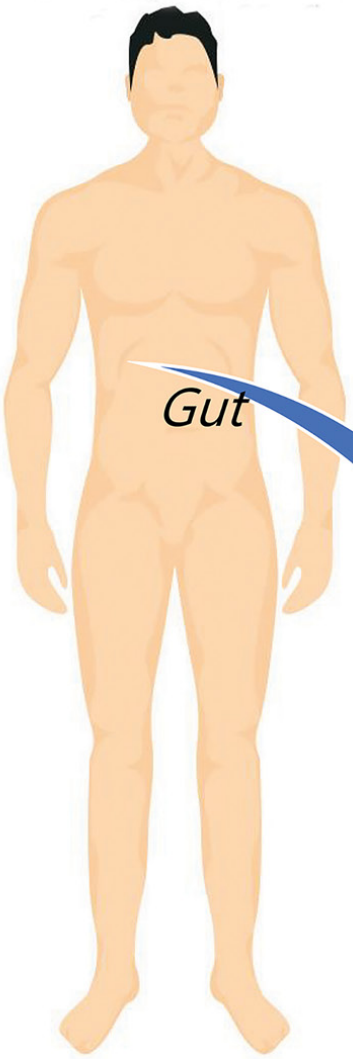
The gastrointestinal tract, being a major interface between the internal body and the external environment, becomes a primary site for microbial settlement. Disruption of the microbial composition of gut, known as "gut dysbiosis" has been linked to several conditions, including metabolic

disorders, inflammatory diseases, and immune dysfunction (Heintz-Buschart & Wilmes, 2018). The Human Genome Project, initiated in 1998 and completed in 2003, revealed that the human gut microbiota contributes approximately 100–150 times more genes than the human genome itself. It is estimated that the intestinal tract harbors around 1,000 distinct bacterial species (Figure 7.1). This chapter will discuss about the understanding of the role of the gut microbiome and gut–kidney axis on how it develops and interacts with the host throughout life.

Human genome project



20,000-25,000 coded genes



Gut

3 million genes comprising more than 1000 bacterial species

FIGURE 7.1 The gut microbiome encodes approximately 100–150 times more gene than the human genome. ⏎

7.1.2 Gut Microbial Shift during Human Development

The composition and diversity of the human gut microbiota undergo dynamic changes across different stages of life from infancy through adulthood and into older age (Table 7.1). These microbial shifts are influenced by various intrinsic and extrinsic factors, including in utero transmission via the placenta or amniotic fluid, the mode of delivery, feeding practices, dietary habits, host genetics, environmental exposures, and immune system of the host (Sanidad & Zeng, 2020).

At birth, the mode of delivery is a major determinant of the initial microbial colonization. Vaginally delivered newborns are exposed to the maternal vaginal and fecal microbiota, resulting in early colonization by maternal microbiota. In contrast, newborns delivered via cesarean section are more likely to be colonized by skin-associated microbes, leading to distinct microbial profiles. *Bifidobacterium* was predominantly reported during vaginal birth while this trend was decreased in c-section birth (Korpela, 2021).

During the neonatal period, the gut is predominantly colonized by facultative anaerobes, including the members of the Enterobacteriaceae and Lactobacillaceae families. During the development of gut with age, the gut becomes increasingly anaerobic, and there is a gradual transition toward obligate anaerobes, including Bifidobacteriaceae and Clostridiaceae. These shifts are driven by changes in oxygen availability within the intestinal lumen and the metabolic changes associated with early development (Sanidad & Zeng, 2020).

Breastfeeding plays a pivotal role in harboring infant gut microbiota. This selectively enriches beneficial bacteria, particularly *Bifidobacterium* species, through the presence of human milk oligosaccharides (Korpela, 2021; Sanidad & Zeng, 2020). In contrast, formula feeding results in a more

diverse microbial community, which may include a higher proportion of potentially pathogenic organisms.

In later stages, particularly during adulthood, families such as Bacteroidaceae, Porphyromonadaceae, Lachnospiraceae, and Ruminococcaceae become increasingly abundant.

TABLE 7.1

Microbial Shift and Its Potential Implications during the Human Developmental Stage ⏎

Developmental Stage	Predominant Microbes	Key Factors for Microbial Dysbiosis	Potential Health Implication	References
In utero	Limited or transient microbial exposure (possibly Micrococcaceae, Lactobacillaceae)	Antibiotic usage Maternal diet Intrauterine infection	Modulate prenatal and postnatal immune cell development	Rackaityte et al. (2020)
At birth (to few days of life)	*Vaginal delivery*: *Bifidobacterium*, Enterobacteriaceae, *Lactobacillus*; *C-section*: decreased *Bacteroides*, *Lactobacillus*, *Bifidobacterium* and increased *Clostridium difficile*, *Staphylococcus*, *Streptococcus*, *Propionibacterium*	Hospital settings, Length of gestation, Mode of delivery (c-section or vaginal delivery)	Delay in the development of immune cells including regulatory T cells, gut epithelium and mucus layer. Potential risk for inflammation and allergic reaction	Sanidad and Zeng (2020)

Developmental Stage	Predominant Microbes	Key Factors for Microbial Dysbiosis	Potential Health Implication	References
Neonatal (after first week to 28 days)	Vaginal birth: increase *Bacteroides*; C-section: decrease *Bifidobacterium*, increase *Klebsiella*, *Haemophilus*, *Veillonella*	Mode of delivery, early feeding practices, antibiotic exposure, hospital environment	Delayed colonization by beneficial microbes in C-section infants may influence immune development, metabolism, and susceptibility to infections	Hesla et al. (2014)
Infancy (up to 1 year of age)	Mode of delivery influence gut microbiome even after 6 months of age. Breastfed infants: *Bifidobacterium* spp. dominate and Clostridiaceae, Bacteroidaceae appear with age	Environmental factors, prebiotics and probiotics, breast feeding versus formula feeding	Formula feeding and antibiotic exposure increase pathogenic taxa and reduce immune tolerance; risk of obesity and allergy	Moore and Townsend (2019)

Developmental Stage	Predominant Microbes	Key Factors for Microbial Dysbiosis	Potential Health Implication	References
Childhood	Increased diversity of Firmicutes (e.g., Lachnospiraceae, Ruminococcaceae), *Bacteroidetes*	Dietary transition, infections, hygiene, antibiotic use	Diet-induced dysbiosis affects nutrient metabolism and may predispose to obesity and inflammatory disorders	Yan et al. (2022)
Adults	Stable communities of Bacteroidaceae, Lachnospiraceae, Ruminococcaceae	High-fat or high-protein diet, stress, alcohol, smoking, low physical activity	Dysbiosis linked to obesity, type 2 diabetes, metabolic syndrome, depression	Yan et al. (2022), Martinez et al. (2021)

Developmental Stage	Predominant Microbes	Key Factors for Microbial Dysbiosis	Potential Health Implication	References
Older age	*Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Clostridium bolteae*, *Escherichia coli*, *Escherichia* (unclassified), *Parabacteroides distasonis*, Parabacteroides (unclassified), *Ruminococcus gnavus*, and *Klebsiella pneumoniae*	Dietary changes, medication use (e.g., antidiabetic agents, antibiotics, PPIs), immune senescence	Reduced SCFA production, chronic inflammation, frailty, infection risk	O'Toole and Jeffery (2018)

This shift is associated with decreased oxygen levels in the intestinal lumen, increased production of short-chain fatty acids (SCFAs), and the introduction of solid foods.

As the individual grows from childhood to adulthood, long-term dietary patterns become a dominant factor in the composition of gut microbiota. Diets rich in fibers and complex carbohydrates promote the growth of beneficial commensals, whereas high-fat or high-protein diet favors the proliferation of potentially pathogenic species. Young individuals often exhibit higher abundances of *Alistipes putredinis*, *Barnesiella intestinihominis*, *Megamonas funiformis*, *Parabacteroides merdae*, and Subdoligranulum (unclassified). Conversely, older populations tend to have increased levels of *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Clostridium bolteae*, *Escherichia coli*, *Escherichia*

(unclassified), *Parabacteroides distasonis*, Parabacteroides (unclassified), *Ruminococcus gnavus*, and *Klebsiella pneumoniae* (Yan et al., 2022).

In addition to age and diet, various lifestyle and behavioral factors including smoking, alcohol consumption, psychological stress, and levels of physical activity have been shown to influence gut microbial diversity and composition across the lifespan. These factors can modulate microbial ecology either directly by altering the gut environment or indirectly by influencing host physiology and immunity (Martinez et al., 2021).

7.1.3 Functional Role of Gut Microbiome

The human gastrointestinal tract, particularly the small intestine and colon, provides a vast and dynamic environment for microbial colonization. The surface area of the small intestine, estimated to be roughly the size of a basketball court, which facilitates efficient digestion and absorption of nutrients and supports a diverse microbial ecosystem. Remarkably, the colon harbors the highest concentration of gut microbiota, with approximately 100 billion microbial cells per gram of content, making it the most densely populated microbial niche in the human body (De Vos et al., 2022).

The gut microbiota plays a central role in maintaining host physiological homeostasis. Table 7.2 summarizes the functional role of gut microbiota in humans. It contributes significantly to the digestion of indigestible carbohydrates and dietary fibers, leading to the production of SCFAs such as acetate, propionate, and butyrate. These SCFAs serve as critical energy sources for colonocytes, maintain gut epithelial barrier function, and anti-inflammatory properties (Andoh, 2016). Additionally, the gut microbiota synthesizes essential vitamins, including vitamin K and several B vitamins, which are important for coagulation, neurological health, and cellular metabolism (Tarracchini et al., 2024).

Intestinal microflora is extensively involved in immune modulation and inflammatory responses. Through interaction with host immune receptors (e.g., Toll-like receptors (TLRs)), the microbiota educates and modulates

the immune system, promoting immune tolerance, and protecting against pathogenic invasion (immune modulation). The immune interactions will be discussed in detail under the section on host–microbiome interactions in this chapter.

TABLE 7.2
Overview of Gut Microbiome and Its Functions

Role of Gut Microbiome	Function
Metabolic function	Fermentation of indigestible carbohydrates and dietary fibers, producing SCFAs such as acetate, propionate, and butyrate
Vitamin synthesis	Synthesis of essential vitamins including vitamin K and several B vitamins
Immune modulation	Interaction with immune receptors (e.g., TLRs) to regulate immune responses and maintain tolerance
Prevents pathogen colonization and infection	Competitive exclusion of pathogenic microbes through nutrient competition and antimicrobial compound production
Homeostatic regulation	Continuous signaling between microbes and host tissues to sustain metabolic and immune balance

7.2 Microbiome–Host Interaction Mechanisms

The interaction between the gut microbiome and the host plays a significant role in the regulation of physiological and pathological processes in the human body, particularly within the context of the gut–kidney axis. Disruptions in the gut microbiota, often referred to as dysbiosis, could compromise gut barrier integrity, increase intestinal permeability, and lead to the systemic dissemination of microbial components and metabolites. Modulation of immune responses through receptors such as TLRs and cytokines, and the generation of microbial metabolites such as SCFAs,

trimethylamine N-oxide (TMAO), and indoxyl sulfate, which have significant implications for kidney function and inflammation (Pérez-Reytor et al., 2021).

7.2.1 Microbial Metabolite

The gut microbiota produces a wide range of metabolites through the fermentation of dietary components and the metabolism of host-derived substrates. These microbial metabolites play a central role in host physiology, influencing not only gut health but also distant organs such as the liver, lungs, brain, and kidneys. Also, it plays a major role in immune modulation. Among the microbial metabolites, the following has a major role in host–microbiome interaction. Those are (1) SCFAs such as propionic acid (PA), butyrate, acetate and N-(1-carbamoyl-2-phenyl-ethyl) butyramide (FBN); (2) bile acid derivatives including tauroursodeoxycholic acid (TUDCA), tauro-β-muricholic acid (TβMC), glycoursodeoxycholic acid (GUDCA) and glycodeoxycholic acid (GDCA); and (3) various organic and inorganic compounds including ethanol, ammonia, indole-3 aldehyde, and ceramide (Rahman et al., 2023).

Primary bile acids are synthesized in the liver from cholesterol and are secreted into the intestine. Once in the colon, they undergo extensive biotransformation by gut microbiota into secondary bile acids (e.g., deoxycholic acid and lithocholic acid). These secondary bile acids serve as important signaling molecules that interact with host nuclear receptors such as the farnesoid X receptor (FXR), pregnane X receptor (PXR), and G-protein-coupled bile acid receptor 1 (TGR5), influencing lipid and glucose metabolism (J. Wu et al., 2021).

SCFAs are the metabolites produced resulting from anaerobic fermentation of dietary products by gut microbiota, especially those from the phylum Firmicutes, such as members of the Clostridiales order. The production of SCFAs is influenced by several factors, including diet and its associated microbiome. These metabolites serve as key energy sources for colonocytes and play essential roles in maintaining intestinal epithelial

integrity, regulating immune responses, and reducing inflammation by binding to G-protein-coupled receptors (e.g., GPR41, GPR43). Moreover, SCFAs protect the gut by preventing the colonization of enteric pathogens (Pérez-Reytor et al., 2021).

7.2.2 Gut Dysbiosis and Intestinal Permeability

Integrity of intestinal permeability plays a pivotal role in digestion and absorption of nutrients, maintaining water and electrolyte balance, and restricting the entry of harmful microorganisms and toxic substances into the bloodstream (Farhadi et al., 2003). Various factors could contribute to leaky gut, such as inflammation, oxidative stress, gut dysbiosis, allergic reactions, stress, certain drugs like non-steroidal anti-inflammatory (NSAID) drugs, alcohol intake, poor dietary practices and certain chronic diseases (Di Vincenzo et al., 2024). As the gut dysbiosis is caused due to several causes mentioned above, it plays a significant role in gut leakage and causes the development of multiple intestinal disorders, including inflammatory bowel disease, celiac disease, irritable bowel syndrome, and Crohn's disease. Additionally, extraintestinal disorders related to the lungs, brain, liver, and kidneys also affect the intestinal permeability (Camilleri, 2019). Emerging evidence indicates that various interrelated mechanisms are involved in the disruption of intestinal integrity. The primary mechanism involves the alteration of tight junction proteins. Bacterial dysbiosis decreases the expression of transmembrane proteins in tight junctions, such as occludin, claudins, and zonula occludens, which regulate paracellular permeability between epithelial cells. Further, inflammatory mediators, including tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2), could lead to increased paracellular leakage (Camilleri, 2019; Priadko et al., 2022). Additionally, gut dysbiosis induces the formation of reactive oxygen species (ROS) and causes the disruption in generation of epithelial cells and lipid membranes in the gut causing increase permeability (L. Li et al., 2023).

7.2.3 Role of Gut Microbiota in Immune Modulation

Apart from their role in maintaining intestinal barrier integrity, microbial metabolites, particularly SCFAs, play a crucial role in immune regulation. Emerging evidence suggests that intestinal permeability and immune modulation are closely interconnected. Microbial dysbiosis compromises intestinal barrier function, contributing to chronic inflammation and immune dysregulation. Among SCFAs, butyrate has been extensively studied for its immunomodulatory effects. It is produced predominantly by butyrate-producing bacteria from the order Clostridiales in the presence of dietary fibers. Butyrate supports immune homeostasis by inducing the activation of regulatory T cells. These regulatory T cells secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which help to suppress pro-inflammatory responses and maintain immune tolerance, thereby mitigating inflammation and preventing autoimmune reactions (Andoh, 2016).

In addition to promoting regulatory T-cell function, SCFAs like butyrate also exert anti-inflammatory effects at the molecular level by inhibiting the NF-κB signaling pathway, a central regulator of inflammatory gene expression. This inhibition reduces the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, further dampening systemic inflammation. Collectively, these findings highlight the pivotal role of gut microbiota-derived metabolites in modulating host immune responses, linking dietary composition, microbial activity, and immune health, especially in the context of chronic diseases related to the respiratory system, central nervous system, and urinary system (Adjei-Fremah et al., 2023)

7.3 Gut–Kidney Axis and the Potential Role in Renal Pathology

The gut–kidney axis refers to a bidirectional communication between the gastrointestinal tract to and the gut microbiota to the kidneys. This interaction involves a complex mechanism of microbial metabolites,

immune signaling pathways, and metabolic processes that influence the function and health of both systems. In this axis, the gut microbiota contributes to the generation of metabolites such as SCFAs, bile acid derivatives and other metabolomics.

Gut dysbiosis also alters the metabolism and tends to produce certain harmful metabolites as a result of proteolytic fermentation. These products, including indoxyl sulfate and p-cresyl sulfate, and uremic toxins, including TMAO, can enter systemic circulation and affect kidney function, particularly in chronic renal impairment (Evenepoel et al., 2017). Conversely, renal dysfunction in chronic kidney disease (CKD) leads to increased urea concentration, which promotes the accumulation of uremic toxins derived from microbial metabolism of choline and carnitine. Figure 7.2 explains the gut–kidney axis and its potential implications on gut and kidney function. Eventually, this disrupts the intestinal barrier and induces dysbiosis, further exacerbating inflammation and disease progression (Stavropoulou et al., 2021).

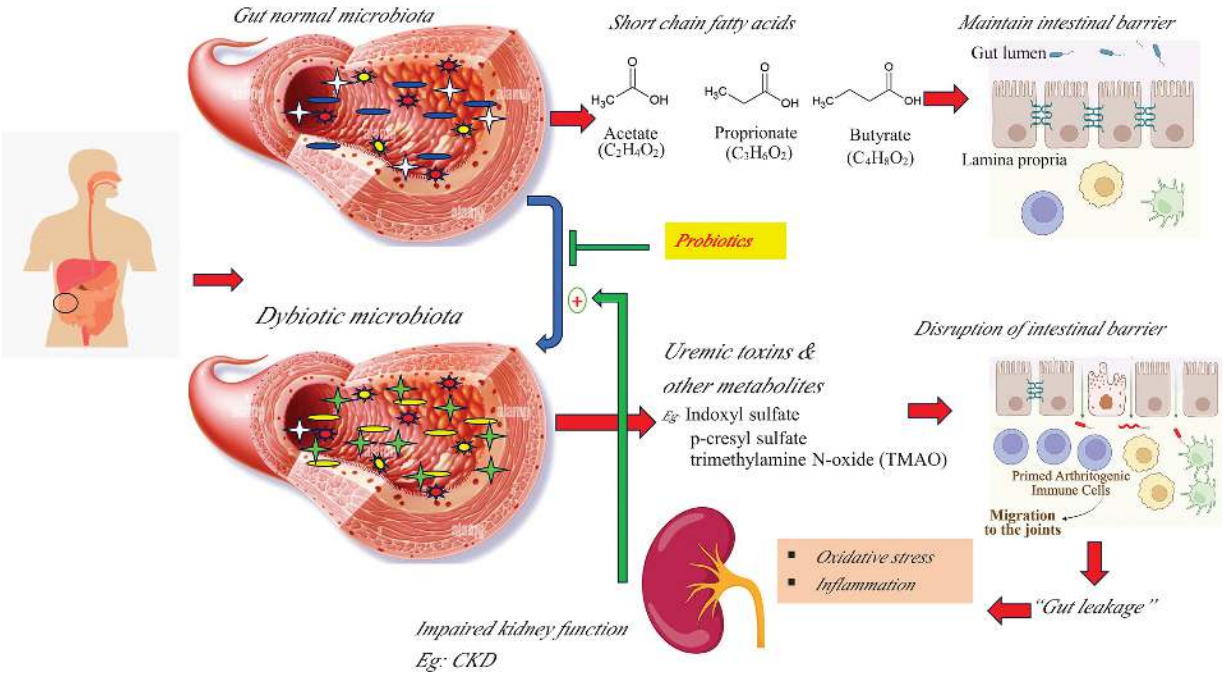


FIGURE 7.2 Schematic diagram of gut–kidney axis and its potential impact on gut and kidney function. ⏎

7.4 Gut and Urine Dysbiosis and Its Role in Renal Pathologies

With the advancement of high-throughput next-generation sequencing (NGS) technologies and bioinformatics tools, the study of microbial communities has undergone a major transformation. These innovations have enabled comprehensive, culture-independent profiling of microbial populations in various body sites, most notably the gut and, more recently, the urinary tract. Such tools have been instrumental in exploring the role of microbial dysbiosis in both health and chronic diseases, including CKD. NGS offers a highly sensitive and efficient approach to identifying microbial communities compared to traditional culture-based methods. This allows researchers to detect both abundant and rare species, analyze microbial diversity, and even predict metabolic functions using metagenomic or 16S rRNA sequencing data. These techniques apply broadly to both gut and urinary microbiome studies, providing critical insights into microbial shifts associated with renal pathologies. However, the adoption of NGS is not without limitations: high cost, need for specialized bioinformatics expertise, and the risk of contamination during sample processing must be carefully managed. Despite these challenges, the depth of analysis, speed, and functional prediction power make NGS the gold standard in modern microbiome research (Chen et al., 2019a).

In a healthy individual, the gut microbiota is composed of a diverse and balanced community of microorganisms that contribute to immune regulation, maintenance of the intestinal barrier, and metabolic functions. Microbiome and its dysbiosis have bidirectional effect on the pathophysiology of CKD. Metabolites produced by the certain microbes such as *Bifidobacterium, Lactobacillus, Ruminococcus, Faecialibacterium*, and *Blatibacterium* have a renopreotective effect on kidney and prevent the CKD progression. Conversely, harmful bacteria, including Fusobacterium nucleatum and Bacteroides which produce the harmful metabolites could accelerate further damage of the kidney (Wehedy et al., 2022).

The alterations in the gut microbiome may lead to the production of harmful metabolites, increased gut permeability, and systemic inflammation, contributing to the progression of CKD and its associated complications. Accumulation of uremic toxins due to impaired renal function and the result of medications might influence the gut microbial ecosystem, leading to a dysbiotic state (Zhao et al., 2021).

The urinary tract was historically believed to be sterile; however, recent studies enabled by NGS have revealed a complex urinary microbiome even in healthy individuals. The use of NGS offers several advantages over traditional culture-based methods, including the ability to identify both dominant and rare bacteria without being limited by growth conditions or media. This has expanded our understanding of microbial communities in the urinary tract and their potential implications in CKD.

In healthy individuals, the urine microbiome exhibits sex- specific differences, as shown by Pohl et al.(2020). *Lactobacillus* and *Prevotella* were predominant in healthy females, while *Staphylococcus and Streptococcus* were more common in males. An investigation using 16S rRNA gene sequencing of urine samples from CKD patients reported the presence of *Lactobacillus, Corynebacterium, Staphylococcus, Gardnerella, Streptococcus, Prevotella, and Escherichia–Shigella* in more than 50% of the tested individuals (Kramer et al., 2018). We show urine dysbiosis in CKD compared to the healthy control in Figure 7.3, Figure 7.4 and Figure 7.5.

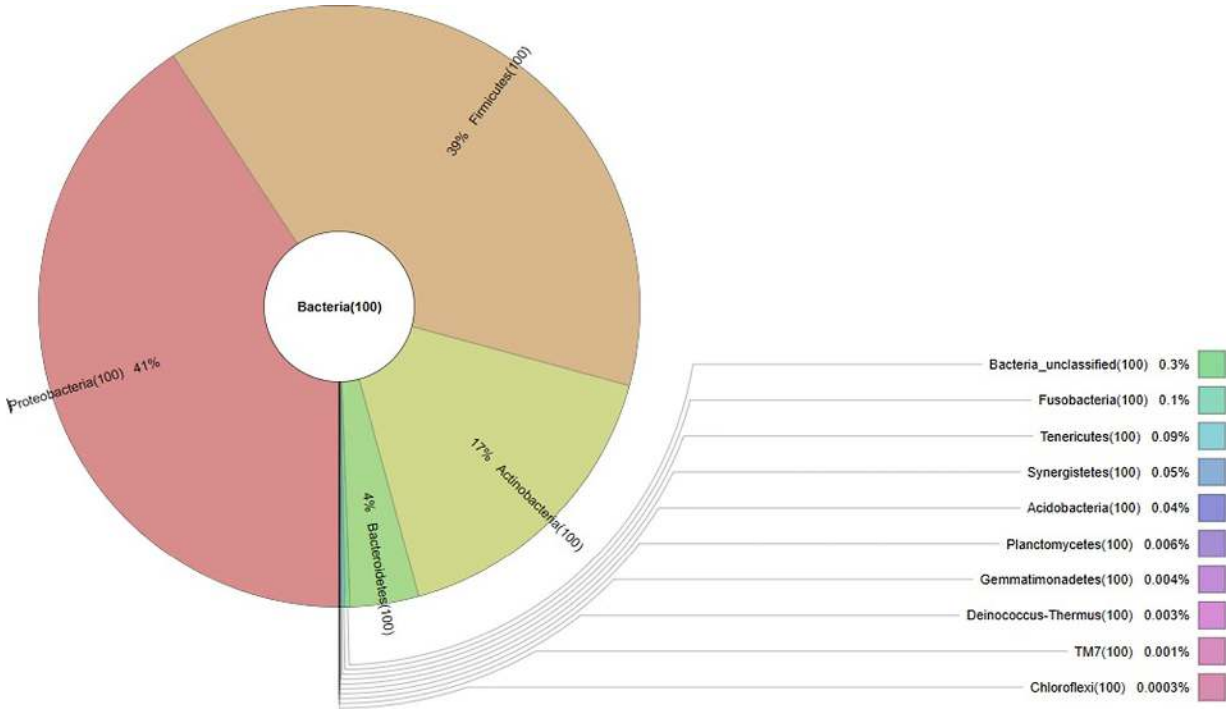


FIGURE 7.3 Relative abundance of bacterial phyla identified in healthy controls (n = 6). ⏎

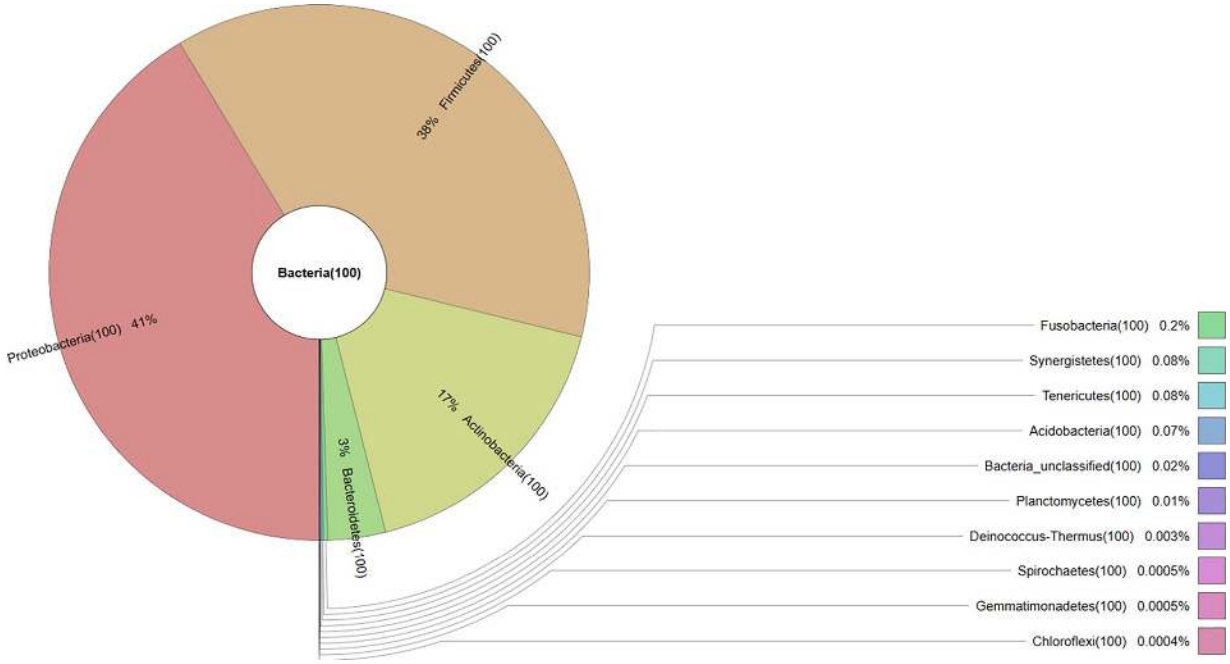


FIGURE 7.4 Relative abundance of bacterial phyla identified in diabetic nephropathy (n = 8). ⏎

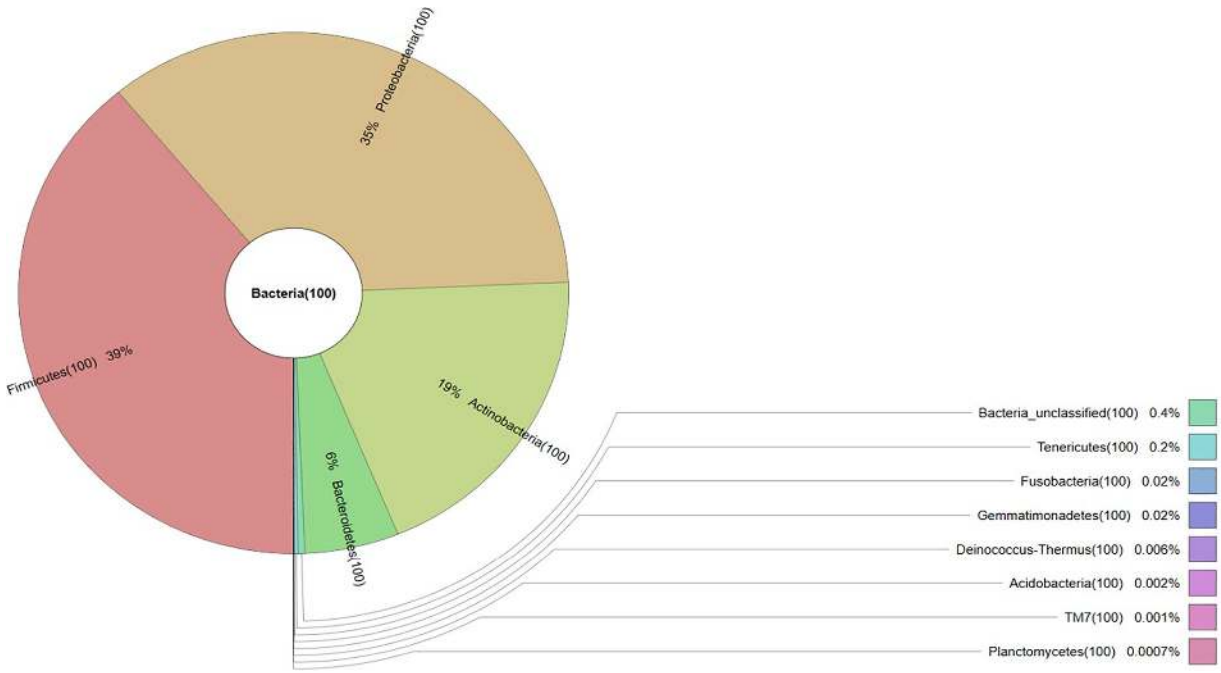


FIGURE 7.5 Relative abundance of bacterial phyla identified in hypertensive nephropathy (n = 6). ⏎

Moreover, microbial community structures were observed to shift significantly following renal transplantation, with opportunistic pathogens such as *Escherichia coli* and *Enterococcus faecalis* becoming more dominant (Campbell et al., 2020). This shift to the effects of immunosuppressive therapy, which alters host immune responses and creates favorable conditions for pathogenic bacteria to colonize the urinary tract. Thus, the gut–kidney axis and the bladder–kidney axis are interconnected pathways where dysbiosis in either compartment can amplify pathological signals to the kidneys. Understanding these microbial networks and their metabolic outputs is essential for identifying potential microbiome-based biomarkers and therapeutic targets.

7.5 Uremic Toxins

7.5.1 Uremic Toxins and Metabolic Crosstalk

Uremic toxins (UT) are classified based either on the physicochemical characteristics affecting their clearance during dialysis or depending on their site of origin: endogenic (host metabolism), exogenic (diet) or microbial (Chen et al., 2019b). Uremia or uremic syndrome is, in simple terms, urea in the blood, a clinical condition due to the gradual failure of kidney function. Dysbiosis in gut microbiota, metabolites (bile acids, SCFAs, neurotransmitters, inflammatory factors and excessive production of UTs, including p-cresyl sulfate (PCS), indoxyl sulfate (IS), TMAO, indole 3 acetic acid (IAA), and phenylacetyl glutamine, contribute to CKD and even lead to cardiovascular disease (CVD) and neurocognitive disorders. Hence, gut microbiota's role is not limited to the gut and displays the interrelatedness among gut, brain, and kidney, maintaining the homeostasis among these organs. According to research in nephropathy patients, when the gut microbiome gets imbalanced, there is increased production of nephrotoxic substances, resulting in damage to kidney function (Li et al., 2025). CKD raises the absorptivity of the colonic epithelial membranes increasing the swelling and causing oxidative stress. This high permeability causes dysbiosis in gut microbiome building up UTs in the plasma with vascular alterations (Cedillo-Flores et al., 2025).

The secondary metabolite TMAO is a well-established inducer for metabolic and cardiovascular abnormalities, a derivative of colonic bacterial metabolism of quaternary amines (Li et al., 2025). TMAO and phenylacetylglutamine produced from phenylalanine fermentation are dialyzable (Chen et al., 2019b). The uremic toxins indoxyl sulfate (IS) with a molecular weight of 251 Da, and p-cresyl sulfate (PCS) with a molecular weight of 108 Da, are derived exclusively from fermentation of proteins by gut microbiota. Primarily derived from gut microbial metabolism, the protein-bound uremic toxins (PBUTs) are ultra-large and hence PBUTs are difficult to clear using conventional dialysis (Cozzolino et al., 2025). Anaerobic bacteria in the gut catabolize phenylalanine and tyrosine into p-cresol, which is further converted to PCS and p-cresyl glucuronide by the intestinal bacteria. The conjugated process, known as detoxification, makes compounds more water soluble for excretion.

A study conducted on aryl hydrocarbon receptor (AhR) knockout mice has shown that AhR activation by IS with the increase level of IS in serum leading to cerebral impairment (blood–brain barrier/BBB damage) (Bobot et al., 2020). The unbalanced gut microbiome can cause the production of SCFAs, thereby affecting blood pressure, the main cause for both acute and CKDs. For more understanding of the renal physiology and the SCFAs, readers can refer the review by Pluznick (2016).

7.5.2 Uremic Toxins' Dynamics with Specific Changes in Microbiome Composition

Many studies have confirmed that variations occur in composition and function of the gut microbiota, which play a critical role in causing the production of UTs and the progression of CKD (Beau et al., 2025). The bacterial genera are accountable for the production of UTs, and for the IS, the genera identified belonged to *Citrobacter*, *Proteus*, and *Escherichia*, and for PCS, genera include *Bacteroides*, *Lactobacillus*, *Clostridium*, and *Bifidobacterium*. Studies conducted to determine association between the genus *Desulfovibrio* and estimated glomerular filtration rate (eGFR) had recorded significant variations, but the main consensus is that *Desulfovibrio* might be able to break down renoprotective SCFAs. A recent review on CKD, UTs and microbiota, mainly focusing on bacterial taxa, grouping them according to UTs production, provides a comprehensive understanding of the gut microbiome and the crucial role of UTs in the pathogenesis of CKD (Rumanli et al., 2025).

7.6 Therapeutic Interventions Targeting the Gut–Kidney Axis

The dysbiosis of the gut microbiome is capable of providing potential therapeutic targets and at the same time can be prompting complications for diseases (Chen et al., 2019b). The probiotic, prebiotic, and nutritional interventions can be given to diseased individuals targeting their gut microbiota, thereby lowering the systemic swelling, reducing the uremic

toxins and possibly curing (Zhu et al., 2025). Mostly found in either fermented food or food supplements, probiotics consist of living microbes, mainly bacteria and yeast. These microbes assist in digestion, immunity, and to balance the gut microbiome. A prebiotic (dietary prebiotics) is defined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus presenting benefit(s) upon host health" (Gibson et al., 2010). In comparison to probiotics, prebiotics are considered more advantageous due to their health benefits, safety in use, production processes, and also due to storage (Davani-Davari et al., 2019).

A randomised controlled trial of synbiotic therapy (a combination of prebiotics and probiotics) done on 40 patients with CKD has shown that dietary fiber was associated with free and total serum PCS but not with IS, and that the dietary protein-fiber index is associated with serum IS and PCS levels, demonstrating the importance of the interactions between nutrients (Rossi et al., 2015). Furthermore, it has been speculated that IS and PCS could be reduced in CKD patients with a dietary modification toward a lower protein-fiber index. Researchers investigated the gut microbiota composition in patients with end-stage renal disease (ESRD) who were treated with a synbiotic gel containing Bifidobacterium (a supplement combining a probiotic (*Bifidobacterium*) with a prebiotic (like inulin), by real-time polymerase chain reaction from fecal samples. The study recorded an increase in *Bifidobacterium* counts while maintaining the gut microbial balance (Cruz-Mora et al., 2014). Another synbiotic treatment considerably improved gut microbiome with *Bifidobacteria*, *Lactobacillus*, and *Subdoligranulum* genera enrichment and accordingly reduced serum IS level, improving eGFR and decreasing the level of high-sensitivity C-reactive protein in treated patients, except for two patients of the intervention arm complaining of increased flatulence, without any other recorded safety issues (Mitrović et al., 2023).

Many researchers had conducted meta-analysis to determine the possible beneficial utility of biotics in patients with CKD across a range of kidney dysfunction. A systematic review and a network meta-analysis of

randomized clinical trials (RCTs) performed to evaluate the effect of probiotics, prebiotics, and synbiotics in reducing uremic toxins produced by the gut microbiota in CKD patients concluded that gut microbiota could be altered through probiotics, prebiotics, and synbiotics ensuing an effective reduction of certain uremic toxins (Cedillo-Flores et al., 2025).

A meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, by Liu et al. (2022) to evaluate the therapeutic benefits of biotic supplements given to patients with CKD observed a positive impact from biotics on circulating markers of creatinine, oxidant stress (malondialdehyde, total antioxidative capacity), inflammation (interleukin-6), and UTs (PCS). However, biotics had no effect on eGFR, albumin, indole-3-acetic acid, or lipids in either predialysis or dialysis patients (Liu et al., 2022).

7.6.1 Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) is a method to re-form the diseased gut microbiota by transplanting stool from a healthy donor. FMT has been provided to patients suffering from several inflammatory bowel diseases (IBD) and the technique has the potential to be utilized for a variety of kidney diseases in reducing harmful uremic toxins, swelling, and systemic effects.

In advanced treatment protocol, the research evidences have been showed that FMT is directed to treat the patients infected with *Clostridium difficile* infection and also prevent the colonization of multi-drug resistance organism in the gut. Moreover, the evidence suggests that it is useful to treat recurrent UTI caused by *Clostridium difficile* (Priadko et al., 2022).

With a systematic approach, it has been shown how a specifically designed multi-biotic could decrease generation of UTs and improve the kidney function in CKD patients. In a study, researchers investigated the precise involvement of gut microbiota in generating p-cresol and indoles post-amino acid enrichment by using an ex vivo Simulator of the Human Intestinal Microbial Ecosystem (SHIME) colonized with fecal samples

collected from healthy individuals and from CKD patients (Beau et al, 2025). Next step involved a selection of a probiotic strain (without genes involved in UTs) and in vitro, confirmation of the potential of cellobiose as a prebiotic supporting the growth for the strain. Researchers further designed a novel specific multi-biotic for CKD (SynCKD) [containing a probiotic Lactobacillus johnsonii NCC533, a prebiotic (1% cellobiose), and a postbiotic (1% short and medium chain triglycerides C4–C8, a source of butyrate)]. They demonstrated how SynCKD effectively cut UT precursor generation ex vivo and the in vivo efficacy of SynCKD with two uremic rodent models, indicating lower plasma levels of UTs, associated with a healthy gut microbiome. The findings confirm the utility of multi-biotics as a potential therapy to alleviate CKD progression.

7.6.2 Diet Modulation

A recent review has shown the involvement of the gut–kidney axis in mediating the adverse health effects of high-altitude hypoxia, discussing how gut microbiota composition alters at high altitudes, citing studies conducted on humans and also on animal models, and also targeting the gut–kidney axis through dietary modifications and probiotic supplementation, potential strategies for preventing and treating high-altitude-related renal and intestinal disorders (P. Wu et al., 2025).

As summarized by Chen et al. (2019b), the impact of probiotics, prebiotics, and synbiotics in reducing uremic toxins is well documented and dysbiosis in gut microbiota provides a potential therapeutic target for preventing or harnessing complications observed with kidney patients.

Research has demonstrated associations between urinary microbiome dysbiosis and disorders of the urinary system (Jayalath & Magana-Arachchi, 2022); however, studies examining urine microbiota–host interactions in the context of diverse kidney diseases remain limited. A review by Jayalath and Magana-Arachchi (2022) emphasized that future investigations should move beyond merely correlating urinary microbiome alterations with disease states and instead focus on elucidating host–

microbiome interactions. This includes understanding how urobiome dysbiosis influences host functions—such as immune responses and creatinine clearance—as well as how host-related factors, including demographics and clinical characteristics, shape the urinary microbiome. Consequently, in exploring the microbiome–kidney axis and host–microbiome interactions in renal pathophysiology, it is important not to focus exclusively on the gut microbiota. Greater attention should also be directed toward urobiome dysbiosis in patients with CKD, an area that remains relatively understudied.

References

Adjei-Fremah, S., Worku, M., & Ibrahim, S. (2023). Immune-modulation and gut microbiome. *The Gut Microbiota in Health and Disease*, 179–191. ⏎

Andoh, A. (2016). Physiological role of gut microbiota for maintaining human health. *Digestion*, *93*(3), 176–181. ⏎

Beau, A., Natividad, J., Benoit, B., Delerive, P., Duboux, S., Feng, Y., Jammes, M., Barnel, C., Sequino, G., & Pinteur, C. (2025). A specifically designed multi-biotic reduces uremic toxin generation and improves kidney function. *Gut Microbes*, *17*(1), 2531202. ⏎

Bobot, M., Thomas, L., Moyon, A., Fernandez, S., McKay, N., Balasse, L., Garrigue, P., Brige, P., Chopinet, S., & Poitevin, S. (2020). Uremic toxic blood–brain barrier disruption mediated by AhR activation leads to cognitive impairment during experimental renal dysfunction. *Journal of the American Society of Nephrology*, *31*(7), 1509–1521. ⏎

Camilleri, M. (2019). Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut*, *68*(8), 1516–1526. ⏎

Cedillo-Flores, R., Cuevas-Budhart, M. A., Cavero-Redondo, I., Kappes, M., Ávila-Díaz, M., & Paniagua, R. (2025). Impact of gut microbiome modulation on uremic toxin reduction in chronic kidney disease: a systematic review and network meta-analysis. *Nutrients*, *17*(7), 1247. ⏎

Chen, Y.-Y., Chen, D.-Q., Chen, L., Liu, J.-R., Vaziri, N. D., Guo, Y., & Zhao, Y.-Y. (2019a). Microbiome–metabolome reveals the contribution of gut–kidney axis on kidney disease. *Journal of Translational Medicine*, *17*(1), 5. ⏎

Chen, Z., Hui, P. C., Hui, M., Yeoh, Y. K., Wong, P. Y., Chan, M. C. W., Wong, M. C. S., Ng, S. C., Chan, F. K. L., & Chan, P. K. S. (2019b). Impact of preservation method and 16S rRNA hypervariable region on gut microbiota profiling. *Msystems*, *4*(1), 10–1128. ⏎

Cozzolino, M., Magagnoli, L., & Ciceri, P. (2025). From physicochemical classification to multidimensional insights: a comprehensive review of uremic toxin research. *Toxins*, *17*(6), 295. ⏎

Cruz-Mora, J., Martínez-Hernández, N. E., del Campo-López, F. M., Viramontes-Hörner, D., Vizmanos-Lamotte, B., Muñoz-Valle, J. F., García-García, G., Parra-Rojas, I., & Castro-Alarcón, N. (2014). Effects of a symbiotic on gut microbiota in Mexican patients with end-stage renal disease. *Journal of Renal Nutrition*, *24*(5), 330–335. ⏎

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92. ⏎

De Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut*, *71*(5), 1020–1032. ⏎

Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L. R., & Scaldaferri, F. (2024). Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Internal and Emergency Medicine*, *19*(2), 275–293. ⏎

Evenepoel, P., Poesen, R., & Meijers, B. (2017). The gut–kidney axis. *Pediatric Nephrology*, *32*(11), 2005–2014. ⏎

Farhadi, A., Banan, A. L. I., Fields, J., & Keshavarzian, A. L. I. (2003). Intestinal barrier: an interface between health and disease. *Journal of Gastroenterology and Hepatology*, *18*(5), 479–497. ⏎

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E. F., Saulnier, D., & Loh, G. (2010). Dietary prebiotics: current status and new definition. *Food Science & Technology Bulletin: Functional Foods*, *7*(1), 1–19. ⏎

Heintz-Buschart, A., & Wilmes, P. (2018). Human gut microbiome: function matters. *Trends in Microbiology*, *26*(7), 563–574. ⏎

Hesla, H. M., Stenius, F., Jäderlund, L., Nelson, R., Engstrand, L., Alm, J., & Dicksved, J. (2014). Impact of lifestyle on the gut microbiota of healthy infants and their mothers—the ALADDIN birth cohort. *FEMS Microbiology Ecology*, *90*(3), 791–801. ⏎

Jayalath, S., & Magana-Arachchi, D. (2022). Dysbiosis of the human urinary microbiome and its association to diseases affecting the urinary system. *Indian Journal of Microbiology*, *62*(2), 153–166. ⏎

Korpela, K. (2021). Impact of delivery mode on infant gut microbiota. *Annals of Nutrition and Metabolism*, *77*(Suppl. 3), 11–19. ⏎

Li, D., Li, Z., & Liu, W. (2025). The gut-kidney axis in urolithiasis: roles of 1 gut microbiota, metabolites, and 2 therapeutic implications 3. *Frontiers in Microbiology*, *16*, 1655808. ⏎

Li, L., Peng, P., Ding, N., Jia, W., Huang, C., & Tang, Y. (2023). Oxidative stress, inflammation, gut dysbiosis: what can polyphenols do in inflammatory bowel disease? *Antioxidants*, *12*(4), 967. ⏎

Liu, J., Zhong, J., Yang, H., Wang, D., Zhang, Y., Yang, Y., Xing, G., & Kon, V. (2022). Biotic supplements in patients with chronic kidney disease: meta-analysis of randomized controlled trials. *Journal of Renal Nutrition*, *32*(1), 10–21. ⏎

Martinez, J. E., Kahana, D. D., Ghuman, S., Wilson, H. P., Wilson, J., Kim, S. C. J., Lagishetty, V., Jacobs, J. P., Sinha-Hikim, A. P., & Friedman, T. C. (2021). Unhealthy lifestyle and gut dysbiosis: a better understanding of the effects of poor diet and nicotine on the intestinal microbiome. *Frontiers in Endocrinology*, *12*, 667066. ⏎

Mitrović, M., Stanković-Popović, V., Tolinački, M., Golić, N., Bajić, S. S., Veljović, K., Nastasijević, B., Soldatović, I., Svorcan, P., & Dimković,

N. (2023). The impact of synbiotic treatment on the levels of gut-derived uremic toxins, inflammation, and gut microbiome of chronic kidney disease patients—a randomized trial. *Journal of Renal Nutrition*, *33*(2), 278–288. ⏎

Moore, R. E., & Townsend, S. D. (2019). Temporal development of the infant gut microbiome. *Open Biology*, *9*(9), 190128. ⏎

O'Toole, P. W., & Jeffery, I. B. (2018). Microbiome–health interactions in older people. *Cellular and Molecular Life Sciences*, *75*(1), 119–128. ⏎

Pérez-Reytor, D., Puebla, C., Karahanian, E., & García, K. (2021). Use of short-chain fatty acids for the recovery of the intestinal epithelial barrier affected by bacterial toxins. *Frontiers in Physiology*, *12*, 650313. ⏎

Pluznick, J. L. (2016). Gut microbiota in renal physiology: focus on short-chain fatty acids and their receptors. *Kidney International*, *90*(6), 1191–1198. ⏎

Priadko, K., Romano, L., Olivieri, S., Romeo, M., Barone, B., Sciorio, C., Spirito, L., Morelli, M., Crocetto, F., & Arcaniolo, D. (2022). Intestinal microbiota, intestinal permeability and the urogenital tract: is there a pathophysiological link? *Journal of Physiology & Pharmacology*, *73*(5), 575–585. ⏎

Rackaityte, E., Halkias, J., Fukui, E. M., Mendoza, V. F., Hayzelden, C., Crawford, E. D., Fujimura, K. E., Burt, T. D., & Lynch, S. V. (2020). Viable bacterial colonization is highly limited in the human intestine in utero. *Nature Medicine*, *26*(4), 599–607. ⏎

Rahman, S., O'connor, A. L., Becker, S. L., Patel, R. K., Martindale, R. G., & Tsikitis, V. L. (2023). Gut microbial metabolites and its impact on human health. *Annals of Gastroenterology*, *36*(4), 360. ⏎

Rossi, M., Johnson, D. W., Xu, H., Carrero, J. J., Pascoe, E., French, C., & Campbell, K. L. (2015). Dietary protein–fiber ratio associates with circulating levels of indoxyl sulfate and p-cresyl sulfate in chronic kidney disease patients. *Nutrition, Metabolism and Cardiovascular Diseases*, *25*(9), 860–865. ⏎

Rumanli, Z., Vural, I. M., & Avci, G. A. (2025). Chronic kidney disease, uremic toxins and microbiota. *Microbiota and Host*, *3*(1). ⏎

Sanidad, K. Z., & Zeng, M. Y. (2020). Neonatal gut microbiome and immunity. *Current Opinion in Microbiology*, *56*, 30–37. doi: 10.1016/j.mib.2020.05.011 ⏎

Stavropoulou, E., Kantartzi, K., Tsigalou, C., Konstantinidis, T., Romanidou, G., Voidarou, C., & Bezirtzoglou, E. (2021). Focus on the gut–kidney axis in health and disease. *Frontiers in Medicine*, *7*, 620102. ⏎

Tarracchini, C., Lugli, G. A., Mancabelli, L., van Sinderen, D., Turroni, F., Ventura, M., & Milani, C. (2024). Exploring the vitamin biosynthesis landscape of the human gut microbiota. *Msystems*, *9*(10), e00929–24. ⏎

Wu, J., Wang, K., Wang, X., Pang, Y., & Jiang, C. (2021). The role of the gut microbiome and its metabolites in metabolic diseases. *Protein & Cell*, *12*(5), 360–373. ⏎

Wu, P., Li, X., Long, W., Ma, Y., Xue, B., Xie, W., & Ma, X. (2025). The gut–kidney axis in high-altitude hypoxia: pathophysiological mechanisms and the central role of hypoxia inducible factor. *Annals of Medicine*, *57*(1), 2557514. ⏎

Yan, H., Qin, Q., Yan, S., Chen, J., Yang, Y., Li, T., Gao, X., & Ding, S. (2022). Comparison of the gut microbiota in different age groups in China. *Frontiers in Cellular and Infection Microbiology*, *12*, 877914. ⏎

Zhu, J., Fu, Y., Olovo, C. V., Xu, J., Wu, Q., Wei, W., Jiang, K., & Zheng, X. (2025). The influence of gut microbiota on the gut–brain–kidney axis and its implications for chronic kidney disease. *Frontiers in Microbiology*, *16*, 1535356. ⏎

8

The Gut Microbiome and Postmenopausal Bone Loss: Implications for Dietary Interventions

Briana M. Nosal, Alexander Aksenov, Catherine J. Andersen, Elaine Choung-Hee Lee, Yanjiao Zhou, and Ock K. Chun

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8.1 Introduction

The menopausal transition is a critical period for bone health in women during which the risk of osteoporosis and fractures significantly increases with aging (Riis et al., 1996). Hormonal fluctuations, particularly changes in estradiol and follicle-stimulating hormone, appear to drive changes in bone metabolism, with patterns of hormonal changes mirroring those in bone mineral density (BMD) (Karlamangla et al., 2018). Given that women spend approximately three decades of life post menopause, identifying effective strategies to preserve bone health during this life stage is essential. While pharmacological treatments such as hormone replacement therapy and bisphosphonates help reduce bone loss, their side effects and contraindications (Sindel, 2023) have prompted increasing interest in non-pharmacological approaches, particularly those targeting dietary and lifestyle modifications, especially those that modulate the gut microbiome.

The gut microbiome plays a vital role in human health and is influenced by numerous endogenous and exogenous factors. The composition and function of gut microbiota impact bone metabolism through immune and endocrine systems, and by affecting calcium homeostasis (Xu et al., 2017). Research has demonstrated that

the gut microbiome plays an important role in nutrient absorption, immune modulation, and the production of bioactive compounds, such as short-chain fatty acids (SCFAs), all of which can impact bone metabolism (Lu et al., 2021). For instance, SCFAs generated from dietary prebiotic sources can promote beneficial bone outcomes through their influence on inflammatory pathways, such as butyrate's ability to induce anti-inflammatory cytokine production and inhibit proinflammatory cytokines (Liu et al., 2018). Both butyrate and propionate are known to induce T regulatory cells (Tregs), while acetate modulates B cell function (Furusawa et al., 2013; Mariño et al., 2017). SCFAs may also enhance calcium absorption by modulating the expression of calcium transport proteins (Xu et al., 2017). In addition to SCFAs, other microbially derived metabolites, such as secondary bile acids, polyamines, and tryptophan-derived metabolites, have been implicated in bone health (Han et al., 2024). Secondary bile acids are recognized for their direct effects on bone, but also their ability to modulate gut and systemic inflammation. Certain types of secondary bile acids, such as lithocholic acid, can decrease the stimulatory effects of vitamin D on osteoblast activity (Ruiz-Gaspà et al., 2010) and seemingly have an overall negative impact on bone, while others, such as deoxycholic acids, can stimulate bone formation and suppress resorption (Dubreuil et al., 2013; Ahn et al., 2020). Furthermore, these metabolites can exacerbate inflammation, as explained by Fu et al. (2025), who showed that imbalances in bile acid conjugation and deconjugation can worsen inflammatory responses in the intestine. Polyamines, including spermidine, are essential for cellular growth and differentiation and have been linked to reduced bone resorption in an ovariectomized model of induced bone loss (Yamamoto et al., 2012). Tryptophan-derived metabolites, particularly kynurenine, have been shown to exhibit significant associations with bone metabolism (Zaiss et al., 2019). Thus, the gut–bone axis has gained attention as a potential target for interventions aimed at improving bone health outcomes in postmenopausal women.

The composition of the gut microbiome shifts throughout an individual's life, and sex hormones have been implicated in playing a crucial role in shaping the gut microbiome's structure from puberty onward (Yatsunenko et al., 2012). Menopause may alter the gut microbial composition, as evidenced by the estrobolome, a set of bacteria in the gut capable of deconjugating glucuronide and sulfate groups from sex hormones. This may affect the depletion of ovarian sex hormones that occurs during menopause (Kwa et al., 2016). However, relatively few studies have

evaluated the changes in gut microbiome observed in women during the menopause transition and the resulting health sequelae.

The gut microbiome offers a promising avenue for non-pharmacological treatments to enhance bone health. Foods rich in prebiotics, probiotics, fiber, and polyphenols such as anthocyanins (ACNs) have shown potential in modulating the microbiome in support of bone health. Dietary ACNs are potent antioxidants and anti-inflammatory compounds that have been shown to have therapeutic effects in various chronic diseases, including cardiovascular diseases, obesity, diabetes, neurodegenerative diseases, cancer, and bone loss (Mattioli et al., 2020; Salehi et al., 2020; Hair et al., 2021). Regarding bone health, ACNs have been shown to have beneficial roles in bone formation and the downregulation of osteoclastogenesis (Mao et al., 2021). In addition to the positive effects on bone, the health-promoting effects of ACNs have been suggested to be associated with the modulation of the gut microbiome (Tian et al., 2019). While dietary interventions with ACNs have presented promising findings, additional dietary components, such as fiber (Lucas et al., 2018), probiotics, and fermented foods (Rizzoli & Biver, 2020; He et al., 2024), offer their own benefits and may have synergistic effects.

Despite the potential benefits, designing effective gut-targeted dietary interventions can be challenging. Microbiome variability among individuals, influenced by factors such as genetics, diet, and health status, can impact the effectiveness of interventions. Additionally, adhering to dietary interventions can be challenging, particularly in aging populations with diverse dietary preferences and tolerances (Hu, 2024). Therefore, a better understanding of how dietary components interact with the gut microbiome and the development of personalized intervention strategies is needed to optimize bone health outcomes in postmenopausal women.

This chapter synthesizes current knowledge on menopause-related changes in the gut microbiome and examines how dietary components such as ACNs, fiber, and fermented foods may support bone health during and after menopausal transition. It further discusses potential synergistic effects, key challenges in intervention design, and the need for personalized, microbiome-informed strategies supported by longitudinal and multi-omics research.

8.2 Global Changes in the Gut Microbiome during the Menopause Transition

The composition of the gut microbiome shifts over the course of an individual's life. Research has implicated that sex hormones play an important role in shaping the gut microbiome structure during puberty and onward. Shifts towards sexual dimorphism of the gut microbiome become more apparent during puberty and adolescence, as observed in teenage male–female dizygotic twins, who exhibit greater dissimilarity in their gut microbiomes than same-sex dizygotic twins (Yatsunenko et al., 2012). Additionally, the gut microbiome has been shown to shift towards an adult-like state during puberty progression for girls (Korpela et al., 2021).

8.2.1 Hormonal Influences on Gut Microbiome Composition

Sex hormones appear to impact the gut microbiome, resulting in sexual dimorphism; however, gut microbiota are also involved in regulating circulating hormone levels, suggesting a bidirectional relationship (Peters et al., 2022a,b,c). Endogenous estrogens, including estradiol, estrone, and estriol, are metabolized through conjugation with glucuronide or sulfate groups in the liver, which allows for biliary excretion of estrogens via the intestinal tract (Peters et al., 2022). Some gut microbiota can deconjugate estrogens, releasing them for reabsorption into the enterohepatic circulation, thereby allowing estrogens to access systemic circulation and target tissues (Kwa et al., 2016) (Figure 8.1). Other hormones that are excreted in bile, including progesterone (Martin et al., 1975) and androgens (Colldén et al., 2019), are also deconjugated and recirculated by the gut microbiome, highlighting the potential of sex hormones to influence the gut by serving as substrates for microbial metabolism.

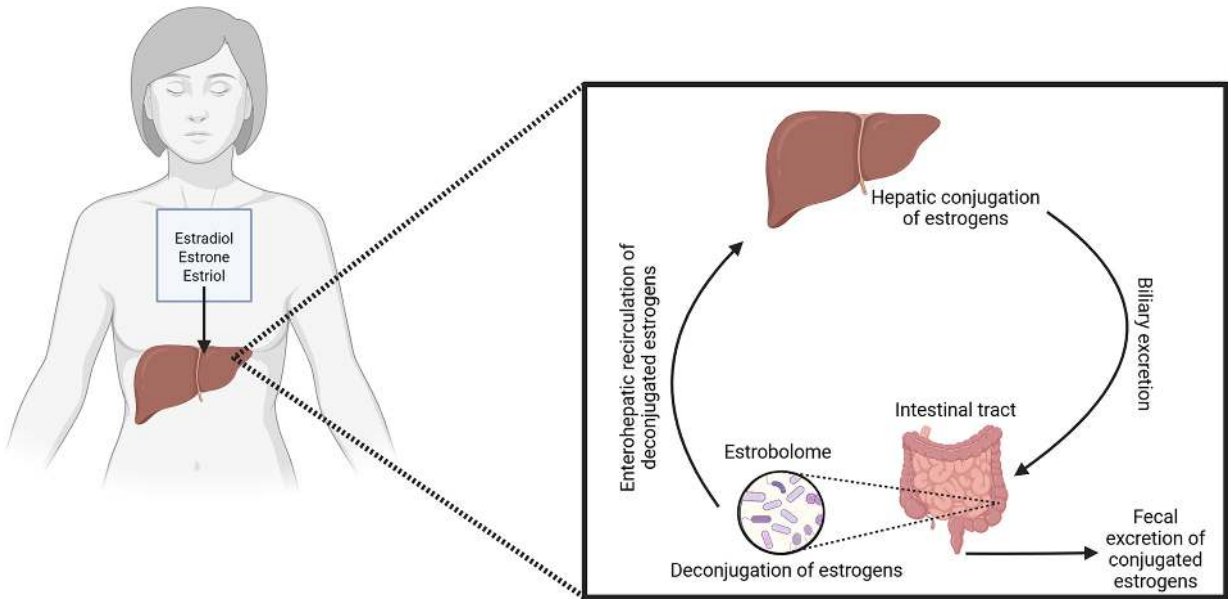

FIGURE 8.1 Enterohepatic re-circulation of estrogen via the gut microbiome. ⏎

In contrast, the gut influences sex hormone levels through the process of deconjugation. With the use of radioactively labeled estradiol and estrone, studies have found that while much of the estrogen is excreted in bile (Sandberg & Slaunwhite, 1957; Adlercreutz & Martin, 1980), smaller fractions occur in conjugated forms found in feces (Sandberg & Slaunwhite, 1957; Adlercreutz & Järvenpää, 1982), suggesting that proportions of estrogens are reabsorbed into circulation through deconjugation by the gut. Gut microbial β-glucuronidases are involved in the estrobolome; in particular, they have been shown to deconjugate estradiol and estrone glucuronides in vitro (Ervin et al., 2019). Major gut phyla, including Firmicutes and Bacteroidetes, possess β-glucuronidase (Dabek et al., 2008; Pollet et al., 2017) and sulfatase enzymes (Ervin et al., 2020), which are involved in deconjugating glucuronide and sulfate groups. This relationship is further supported by fecal microbiota transplantation (FMT). For example, FMT from young mice into aged mice improved ovarian function by reshaping the gut microbiota to a "younger-like phenotype," reducing systemic and ovarian inflammation, and modulating populations of immune cells (Xu et al., 2022). This finding highlights the potential for gut-targeted interventions to influence systemic hormonal changes related to menopause.

Some studies have examined the relationship between the gut microbiome and urinary estrogens, which have aided in determining the influence of menopause on gut microbiome changes. Flores et al. (2012) found a strong positive correlation

between urinary total estrogens and estrogen metabolites, as well as gut microbiome richness, diversity, and *Clostridia* abundance, in postmenopausal women and men. The functional activity of β-glucuronidase was inversely correlated with fecal total estrogens and positively correlated with urinary estrone, suggesting the involvement of β-glucuronidase in the recycling of estrogens (Flores et al., 2012). These associations found among postmenopausal women and men were not observed among premenopausal women in the same study. The authors suggested that this may be due to the variable levels of estrogens, given that the samples were collected irrespective of menstrual cycle timing (Flores et al., 2012). In another study with postmenopausal women, Fuhrman et al. (2014) saw a positive association between total urinary estrogens and *Ruminococcus*. *Ruminococcus* is an anaerobic gram-positive bacterial genus involved in the degradation and conversion of polysaccharides into various metabolites for hosts, including SCFAs (La Reau & Suen, 2018).

Other studies have investigated the relationship between blood estradiol and progesterone levels and the gut microbiome. In a study of pre- and postmenopausal women in Korea, serum estradiol levels were associated with increased microbial diversity and a lower abundance of *Slackia* and *Butyricimonas* (Shin et al., 2019). Additionally, among women with or without premature ovarian insufficiency in China, serum estradiol was associated with a higher abundance of *Firmicutes* and *Faecalibacterium* and a lower abundance of *Bacteroidetes* (Wu et al., 2021). Moreover, in pre- and postmenopausal women from Spain, serum estradiol was associated with a lower abundance of Prevotellaceae and a higher abundance of *Gammaproteobacteria* (Santos-Marcos et al., 2018). In a study of premenopausal women from Austria, while controlling for menstrual timing, the use of combined hormonal contraceptives, on average, decreased serum estradiol and progesterone and was associated with decreases in gut microbiome diversity with lower abundances of *Eubacterium*, *Haerophilus*, and unclassified *Firmicutes* as well as higher abundances of *Akkermansia* and *Barnesiella* (Mihajlovic et al., 2021). Another study of pre- and postmenopausal women from Spain found that gut microbiome composition could predict plasma progesterone levels (Mayneris-Perxachs et al., 2020). Lastly, in a study of Hispanic/Latino women in the United States, correlations were observed between serum progestin metabolites and gut microbial species in postmenopausal women, but not in premenopausal women (Peters et al., 2022). This study further reported that the estrobolome potential of the gut microbiome, related to orthologs of β-glucuronidase and aryl-sulfatase

genes, was positively associated with serum progestin metabolites in postmenopausal women, suggesting the recycling of progesterone by gut microbiota (Peters et al., 2022). Overall, these studies further support the bidirectional relationship between the gut microbiome and sex hormones, where higher levels of estrogens and progesterone serve as substrates for a range of species, promoting increased microbial diversity, while increased diversity and deconjugation activity aid in promoting hormone retention. In addition to recycling endogenous hormones, the gut microbiome can metabolize dietary compounds, such as plant lignans and isoflavones, into phytoestrogens (e.g., enterolignans and equol) with estrogenic activity, which can potentially contribute to systemic estrogen-like effects (Gaya et al., 2016; Baldi et al., 2023). Postmenopausal women have lower levels of estrogens and progesterone, and they lack ovarian hormone production; thus, the recycling of hormones by gut microbiota may play a crucial role in maintaining systemic estrogen and progesterone levels after menopause (Peters et al., 2022).

8.2.2 Microbial Translocation and Menopause

Microbial translocation is a process through which dysfunction of the gut epithelial barrier allows microbial products to translocate from the gut lumen to systemic circulation (Brenchley & Douek, 2012), another mechanism through which gut microbiota may influence human health. Under healthy milieus, the gut epithelial barrier is dynamic. It possesses several layers of defense, including a wide variety of gut microbes on the luminal side, with the basolateral side being essentially sterile (Nagpal & Yadav, 2017). However, under certain conditions, such as exposure to toxins, drugs, pathogenic invasion, or inflammation due to disease, the integrity of gut epithelia may be weakened or destabilized (Nagpal & Yadav, 2017). This disruption to gut integrity and permeability may lead to microbial translocation, allowing bacteria and/or their metabolites to leak into circulation, increasing susceptibility to various diseases by inducing chronic or acute inflammatory responses, which may be implicated in the pathogenesis of many diseases (Brenchley & Douek, 2012; Jia et al., 2021).

Estradiol and progesterone have been indicated to have a potential role in maintaining the gut barrier in addition to protecting the gut from injury. An ex vivo study evaluating whether the female gut is more resistant to injury and production of inflammatory responses found that when exposed to stresses, including hypoxia

and acidosis, female rat intestines are more resistant to injury than male rats, and upon administration of estradiol, this difference is attenuated (Homma et al., 2005). An in vitro model found that estradiol treatment protects mucus-producing epithelial cells from oxidative damage (Diebel et al., 2015). Furthermore, in vitro models have shown that estrogen receptor beta (ERβ) is involved in the maintenance of epithelial barrier function (Braniste et al., 2009; Looijer-van Langen et al., 2011) and that estradiol and progesterone upregulate tight junction proteins, which improve epithelial barrier function in gut epithelial cells (van der Giessen et al., 2019; Zhou et al., 2019). In mice, ovariectomy-induced estrogen deficiency can increase intestinal permeability through effects on tight junction protein occludin, which was also found to be significantly correlated with the expression of tumor necrosis factor-alpha (TNFα) and interleukin-1beta (IL-1β), suggesting a link between estrogen deficiency, intestinal permeability, and inflammatory gene expression (Collins et al., 2017). Together, this suggests that estradiol and progesterone are important in preventing microbial translocation and limiting intestinal permeability.

Although estrogens and progesterone have been shown to play a crucial role in preventing gut microbial translocation and enhancing gut barrier integrity, few studies have investigated the association between menopause and its potential impact on gut microbial translocation and gut barrier integrity. In an analysis of the Study of Women's Health Across the Nation (SWAN), markers of gut permeability, immune activation secondary to gut microbial translocation and inflammation, as well as BMD, were analyzed among 65 women (Shieh et al., 2020). Authors found that fatty acid binding protein 2 (FABP2; a marker of gut permeability), lipopolysaccharide-binding protein, and soluble CD14 (LBP and sCD14; markers of immune activation secondary to gut microbial translocation) increased from pre- to postmenopause (Figure 8.2) (Shieh et al., 2020). Additionally, lower plasma estradiol levels were associated with higher FABP2 and sCD14 levels. Greater LBP and sCD14 levels were associated with higher high-sensitivity C-reactive protein (hs-CRP) levels, and lower lumbar spine and total hip BMD were associated with FABP2, sCD14, and hs-CRP. These results suggest that gut permeability increases during the menopausal transition, and greater permeability is associated with increased inflammation and decreased BMD that may be related to sex hormone changes; however, more studies are needed to further confirm these findings. In one study of women with HIV, sCD14, but not intestinal fatty acid-binding protein (IFAB), was elevated in postmenopausal women compared to premenopausal

women (Peters et al., 2022). In a longer-term longitudinal study by the same authors, a comparison of women with and without HIV displayed a pattern of increased sCD14 during the menopausal transition, and a decrease thereafter was apparent in women with HIV, suggesting that ovarian aging may differ depending on HIV status (Peters et al., 2024). Similar to the first study, no significant changes were observed in IFAP or LBP. Researchers further found that free estradiol levels were inversely associated with sCD14 levels but not IFAB and LBP among women with HIV (Peters et al., 2024). However, in a smaller study, no differences in LPS or LBP were observed by menopause status (Santos-Marcos et al., 2018). Further research is needed to determine the influence of menopause on gut microbial translocation and gut barrier function in humans, as well as to delineate the differences in changes driven by aging alone versus those driven by menopause.

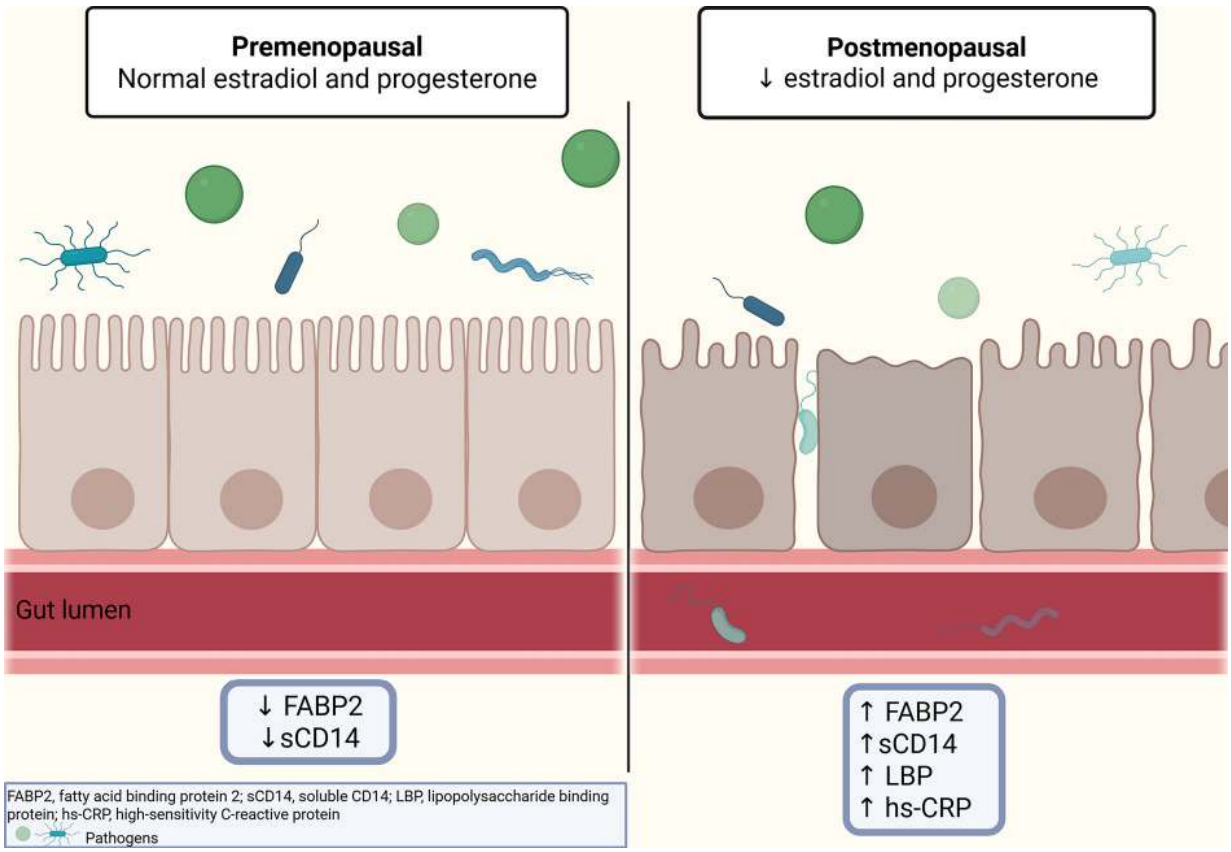

FIGURE 8.2 Proposed differences in gut barrier integrity between pre- and postmenopausal women. ⏎

8.2.3 Menopause and the Gut Microbiome: Human Studies

Several cross-sectional studies have compared the gut microbiome composition between pre-and postmenopausal women, often accounting for age. These studies are outlined in Table 8.1. However, it is of note that aging alone is associated with shifts in gut microbiome composition and function including reduced diversity, changes in abundances of SCFA producing taxa and corresponding metabolome changes (Sato et al., 2021). Zhao et al. (2019) reported decreased richness and alpha diversity at the genus level in postmenopausal women compared to age and BMI-matched premenopausal women. This study also identified a cluster containing the *Firmicutes* and *Proteobacteria* phyla, with some genera, including *Faecalibacterium*, *Alistipes*, *Eubacterium*, and *Roseburia*, being enriched in premenopausal women. A cluster was formed in postmenopausal women, including *Bacteroidetes* and *Verrucomicrobia* at the phylum level and *Bacteroides* and *Staphylococcus* at the genus level. Peters et al. (2021) compared microbial composition between pre- and postmenopausal women with and without HIV and found menopause to be associated with increased alpha diversity, the enrichment of Enterobacteriales, and depletion of abundant taxa within *Prevotella copri* among postmenopausal women with HIV compared to premenopausal women with HIV. Among postmenopausal women with HIV, HIV was also associated with the enrichment of metagenomic pathways related to Enterobacteriales, including the degradation of phenolic compounds and amino acids (Peters et al., 2021). In a comparison of postmenopausal and premenopausal women without HIV, a higher abundance of amplicon sequence variants (ASVs) from *Parabacteroides distasonis* and lower abundances of ASVs from *Roseburia, Shigella, inulinivorans*, and *Gemmiger formicilis* were observed in postmenopausal women compared to premenopausal (Peters et al., 2021).

Among studies that have evaluated the influence of menopause on the gut microbiome in humans, three studies found that the gut microbiome of postmenopausal women was more similar to that of men than that of premenopausal women compared to men (Table 8.1) (Santos-Marcos et al., 2018; Mayneris-Perxachs et al., 2020; Peters et al., 2022). Various gut bacterial taxa were identified to differ by menopausal status, although some consistent results have been reported across studies. This includes lower abundances of *Firmicutes* (Zhao et al., 2019; Wu et al., 2021) and *Ruminococcus* genus or species (Zhao et al., 2018; Mayneris-Perxachs et al., 2020) in addition to higher abundances of *Bacteroides* (Mayneris-Perxachs et al., 2020; Peters et al., 2022), *Prevotella* (Mayneris-Perxachs et al., 2020; Peters et al., 2022), *Dorea* (Mayneris-Perxachs et al., 2020;

Wu et al., 2021), and *Sutterella* (Wu et al., 2021; Peters et al., 2022) genera or species when comparing postmenopausal women to premenopausal women or among women with decreased estrogen. While these studies highlight specific taxon-level changes associated with menopause, they also point to broader patterns in microbial composition and function. In particular, global trends, including reductions in microbial diversity and the depletion of SCFA-producing bacteria such as *Faecalibacterium* and *Roseburia*, are commonly observed (Wu et al., 2021; Zhao et al., 2019). Additionally, enrichment of *Bacteroides* (Santos-Marcos et al., 2018; Mayneris-Perxachs et al., 2020; Peters et al., 2022) and a higher abundance of proinflammatory taxa, such as *Proteobacteria* and *Enterobacteriaceae*, have been reported (Santos-Marcos et al., 2018; Peters et al., 2022). These changes may reflect a shift in the gut microbiome composition toward a less stable, more inflammatory state with menopause.

Beyond taxonomic changes, some studies suggest a connection between menopausal status and gut microbiome profiles that may reflect enterotype-like patterns. For example, postmenopausal women are more likely to exhibit a *Bacteroides*-dominant microbiome, characterized by reduced microbial diversity and potential proinflammatory activity (Mayneris-Perxachs et al., 2020). In contrast, a *Prevotella*-dominant profile, associated with high fiber intake (Wu et al., 2011), appears to be less prevalent in this population (Mayneris-Perxachs et al., 2020). While these studies did not explicitly define these profiles as enterotypes, the enrichment of these genera observed in these studies aligns with enterotype-associated trends in gut microbiome composition. Notably, aging has also been linked to shifts in microbiome and metabolome profiles, including alterations to bile acid metabolism, amino acid degradation pathways, and SCFA production (Sato et al., 2021) all of which could further influence the enterotype-like patterns observed among postmenopausal women in these studies.

TABLE 8.1

Studies Evaluating Differences in Gut Microbiome Composition between Pre- and Postmenopausal Women

Subjects	Study Design	Results	Reference

Subjects	Study Design	Results	Reference
Premenopausal women (*n* = 24) Postmenopausal women (*n* = 24) matched to premenopausal women based on age and BMI	Cross-sectional (MWAS)	*Premenopausal Women:* Cluster containing the *Firmicutes* and Proteobacteria at the phylum level and *Faecalibacterium*, *Alistipes*, *Eubacterium*, and *Roseburia* at the genus level *Postmenopausal Women:* Large cluster, including *Bacteroidetes* and *Verrucomicrobia* at the phylum level and *Bacteroides* and *Staphylococcus* at the genus level ↓ α-diversity	Zhao et al. (2019)
Postmenopausal HIV+ (*n* = 182) Premenopausal HIV+ (*n* = 99) Postmenopausal HIV– (*n* = 80) Premenopausal HIV– (*n* = 71)	Cross-sectional	*Postmenopausal vs. Premenopausal (HIV+):* ↑ α-diversity ↑ Enterobacteriales ↓ *Prevotella copri* *Postmenopausal vs. Premenopausal (HIV–):* ↑ ASVs from *Parabacteroides distasonis* ↓ ASVs from *Shigella, Roseburia inulinivorans, Gemmiger formicilis*	Peters et al. (2021)
Premenopausal women (*n* = 295) Postmenopausal women (*n* = 1027) Men (*n* = 978) matched to pre- and postmenopausal women based on age, BMI, and ethnicity	Cross-sectional	*Postmenopausal Women:* ↓ α-diversity The microbiome is more similar to men than to premenopausal women ↑ *Bacteroides* sp. *Ga6A1, Prevotella marshii, Sutterella wadsworthensis* ↓ *Escherichia coli-Shigella* spp., *Oscillibacter* sp. *KLE1745, Akkermansia muciniphila, [Clostridium] lactatifermentans, Parabacteroides johnsonii, Veillonella seminalis* *Postmenopausal Women's Serum Progestin Metabolites:* ↑ *[Clostridium] lactatifermentans, Akkermansia muciniphila*, β-glucuronidase gene ortholog	Peters et al. (2022)

Subjects	Study Design	Results	Reference
Premenopausal women (*n* = 44) Postmenopausal women (*n* = 45) Men (*n* = 42)	Cross-sectional	*Postmenopausal Women:* The microbiome is more similar to men than premenopausal women compared to men Menopausal differences attenuated among obese participants ↑ *Bacteroides* sp. *CAG:661, Anaerovibrio* sp. *JC8, Prevotella* sp. *P6B1, Lactobacillus rhamnosus, Dorea* sp. *CAG:317, Veillonella dispar, Haemophilus parainfluenzae* ↓ *Ruminococcus* sp. *CAG:379, Clostridium neonatale, Acidiphilium* sp. *CAG: 727, Bifidobacterium angulatum, Alistipes* sp. *AL-1, Eubacterium* sp. *CAG:603, Enterococcus faecalis, Blautia* sp. *CAG:257*	Mayneris-Perxachs et al. (2020)
Premenopausal women (*n* = 17) Postmenopausal women (*n* = 20) Men (*n* = 39) matched to pre-and postmenopausal women based on age, BMI, and diet background	Cross-sectional	*Postmenopausal Women:* Microbiome composition is more similar to men than to premenopausal women ↑ Firmicutes, *Roseburia, Lachnospira* ↓ *Bilophila, Prevotella, Parabacteroides* *Plasma Estradiol Levels:* ↑ Gammaproteobacteria ↓ Prevotellaceae	Santos et al. (2018)
Premature ovarian insufficiency (POI) < 40 years (*n* = 35) Pre-menopausal with normal ovarian function <40 years (*n* = 18)	Case-control	*POI:* ↑ Bacteroidetes, *Butyricimonas, Dorea, Lachnobacterium, Sutterella* ↓ Firmicutes, *Bulleidia, Faecalibacterium* *Serum Estradiol:* ↑ Firmicutes, *Faecalibacterium* ↓ Bacteroidetes	Wu et al. (2021)

8.3 Dietary Strategies Targeting the Gut Microbiome for Bone Health

Emerging research studies a wide range of dietary compounds and dietary patterns that influence bone health via the gut-bone axis, including, but not limited to, dietary fibers and prebiotics, polyphenolic compounds, including ACNs and fermented foods/probiotics. Dietary factors such as diet patterns rich in protein, fat, micronutrients (e.g., vitamin D and calcium), and overall variety in the diet also shape gut microbiota composition and function with downstream effects on bone metabolism (Zhang et al., 2024). Given the strength of current evidence linking fiber/prebiotics, ACNs, and fermented foods/probiotics to bone health, this section focuses on these three dietary strategies and their influence on bone metabolism.

8.3.1 Dietary Fiber and Prebiotics

Dietary fibers can be classified by various parameters, including food source, chemical structure, water solubility, viscosity, and fermentability. They may also be subdivided into polysaccharides, including resistant starch and resistant oligosaccharides, as well as insoluble and soluble forms (Deehan et al., 2017). Insoluble forms typically have a bulking effect as they reach the colon and are slowly digested, or not digested at all, by gut bacteria. On the other hand, soluble fibers do not contribute to fecal bulking and are fermented by gut bacteria, resulting in the production of metabolites such as SCFAs (Makki et al., 2018). Some of these compounds are considered prebiotics due to their selective ability to support the growth and/or activity of beneficial bacterial populations that colonize the gut (Bindels et al., 2015). Essentially, all fibers induce shifts in gut microbiota composition as a result of competitive interactions. While the specific causal pathways mediating these changes remain to be fully elucidated, large-scale analyses link higher fiber intake with reduced risk of chronic disease, supporting the role of fiber-related microbiota shifts in promoting host health (Ecklu-Mensah et al., 2023). Mechanisms that have been shown to contribute to beneficial health outcomes display reliance on metabolic compounds (e.g., SCFAs) (Koh et al., 2016), physiological changes (e.g., pH lowering), or protection of the mucus layer (Schroeder et al., 2018; Zou et al., 2018).

SCFAs play key roles in regulating host metabolism, the immune system, and cell proliferation (Koh et al., 2016). In the cecum and proximal colon, SCFAs are found at high concentrations and serve as energy sources in colonocytes, particularly butyrate. However, they can also be transported to the peripheral circulation, where SCFA levels are typically low but may act as signaling

molecules to regulate various biological processes (Koh et al., 2016). Low fiber intake may lead to reduced microbial diversity and SCFA production, while also shifting gut microbial metabolism towards utilizing unfavorable substrates, such as dietary and endogenous proteins (Cummings & Macfarlane, 1991). In a human study where participants consumed a high-protein, low-carbohydrate diet, total SCFA and butyrate production were reduced, and increases in potentially harmful metabolites derived from amino acid fermentation, such as branched-chain fatty acids, ammonia, and amines, among others, were increased (Duncan et al., 2007). These metabolic changes are of concern as SCFAs are key regulators of epithelial barrier integrity and mucosal immunity and low levels of SCFAs in the intestines have been associated directly with susceptibility to inflammatory diseases (Mann et al., 2024). Diets low in fiber can also lead to alterations in gut microbiota, resulting in deterioration of the mucus layer and increased susceptibility to infections and chronic inflammatory diseases (Johansson et al., 2008; Desai et al., 2016; Schroeder et al., 2018; Zou et al., 2018).

Dietary fibers and SCFAs are beneficial for stimulating mucus production and secretion. *Bacteroides thetaiotaomicron*, a producer of acetate and propionate, promotes the differentiation of goblet cells and the expression of genes related to mucin production. In contrast, *Faecalibacterium prausnitzii*, an acetate consumer and butyrate producer, mitigates the effects of acetate on mucus, thereby preventing the overproduction of mucus that would disrupt the appropriate composition and structure of the gut epithelium (Wrzosek et al., 2013). Moreover, dietary fibers can mechanically stimulate mucus secretion by the intestinal epithelium (McRorie Jr & McKeown, 2017). Prolonged deficits of dietary fiber can damage the mucus barrier and are also associated with increases in the abundance of mucin-degrading bacteria, including *Akkermansia muciniphila* (Desai et al., 2016). Inulin, a prebiotic with bifidogenic potential, was shown to improve the penetrability of the mucus layer at low amounts, and at high levels, it enhanced gut health and attenuated low-grade inflammation in obese mice (Zou et al., 2018). The production of SCFAs from dietary fibers also contributes to maintaining gut microbial health by supporting the development and maturation of the immune system (Rescigno, 2014), which has implications for bone health. For instance, a fiber-rich diet has been shown to ameliorate bone destruction in rheumatoid arthritis (Müller et al., 2001). SCFAs are likely the primary drivers of the beneficial effects on bone after consuming fiber-rich diets, due to the prebiotic effects of fiber. In particular, inulin-type fructan supplementation has been shown to improve calcium absorption and

enhance bone mineralization during pubertal growth (Abrams et al., 2005). Additionally, galactooligosaccharides, nondigestible prebiotic oligosaccharides derived from lactose, increased volumetric BMD and benefited calcium utilization in rats via increased proportion of Bifidobacteria, decreased cecal pH, and increased cecal wall and content weight (Weaver et al., 2011).

8.3.2 Anthocyanins

ACNs are water-soluble polyphenolic compounds found in red, blue, and purple fruits and vegetables. Beyond their antioxidant and anti-inflammatory properties, ACNs influence bone metabolism by promoting osteoblast proliferation and inhibiting osteoclastogenesis. They also modulate the gut microbiome, which plays a key role in the metabolism of ACNs and produces bioactive metabolites that impact systemic health, including bone outcomes. ACNs have low bioavailability and reach the colon, where they are degraded by gut microbiota into phenolic acids such as protocatechuic, gallic, and vanillic acid. These microbial transformations can promote anti-inflammatory effects and beneficial immune responses. Notably, ACNs function as prebiotics, promoting the growth of commensal bacteria such as Bifidobacterium and Lactobacillus, which have been associated with improved gut barrier function and enhanced mineral absorption (Boto-Ordonez et al., 2014; Hidalgo et al., 2012; Sun et al., 2018; Zhu et al., 2018). These bacteria also contribute to the production of SCFAs, particularly butyrate, which exerts immunomodulatory effects and supports the induction of Treg cells, key players in reducing inflammatory cytokines involved in bone resorption. Animal studies have shown that ACNs from sources such as blackcurrant, bilberry, and black rice increase the populations of *Bifidobacterium* and *Lactobacillus*, potentially modulating immune responses relevant to bone health (Li et al., 2019; Paturi et al., 2018; Wang et al., 2020). Some of these microbial changes are linked to reduced inflammatory markers and enhanced SCFA production. SCFAs, such as butyrate and propionate, inhibit osteoclast differentiation, protect against bone resorption, and modulate gene expression through mechanisms including histone modification and cytokine regulation. Human studies are more limited but support the prebiotic potential of ACNs. For instance, dealcoholized red wine (rich in ACNs) increased fecal Bifidobacteria and correlated with higher urinary ACN metabolites, suggesting a link between ACN intake, microbiota shifts, and systemic metabolite production (Boto-Ordonez et al., 2014). However, results vary, with some studies

showing no effects of ACNs on microbial composition or inflammation. These inconsistencies may be related to differences in host status, dose, and dietary background, underscoring the need for further targeted research.

ACNs may also impact bone health through endocrine pathways. Estrogen deficiency in postmenopausal women contributes to increased inflammatory cytokine production and bone loss. ACNs and their metabolites may exhibit phytoestrogenic effects by interacting with ERs and stimulating estrogen-responsive genes. Blackcurrant-derived ACNs, such as cyanidin and delphinidin, have shown affinity for ERβ and modulate nitric oxide synthesis and other pathways relevant to bone and vascular health (Nanashima et al., 2017; Horie et al., 2019). In one study, blackcurrant supplementation increased enterolignan production, including enterodiol and enterolactone, which are gut-derived metabolites with estrogenic activity (Nosal et al., 2024). These compounds were linked to increased abundance of Ruminococcus 2, suggesting a microbial mechanism for phytoestrogenic action.

Collectively, ACNs may support bone health via multiple gut-mediated pathways, including immune modulation, SCFA production, and endocrine mimicry. Their role as prebiotics and sources of bioactive metabolites highlights their potential in dietary strategies targeting the gut-bone axis, particularly for postmenopausal women.

8.3.3 Fermented Foods and Probiotics

Fermented foods and probiotics have also been suggested to have beneficial effects on the gut microbiome, which may translate into beneficial effects on bone health. Taylor et al. (2020) assessed the influence of fermented food consumption on the gut microbiome and metabolome using a multi-omic approach with a subset of samples from the American Gut Project. Researchers found that gut microbiome profiles differed slightly between individuals who consumed fermented foods and those who did not, but not in overall microbial diversity, suggesting that the consumption of fermented foods may result in differences in composition.

Several studies have demonstrated the potential impact of fermented foods and probiotics on promoting beneficial bone health outcomes. *Lactobacilli*, also known as lactic acid bacteria, are widely used as a type of fermentation starter and have been shown to have beneficial effects on BMD (Cornes et al., 2022). The potential mechanisms include promoting calcium absorption and improving bone

metabolism by inhibiting proinflammatory cytokines and the Receptor Activator of Nuclear factor Kappa-B Ligand/ Osteoprotegerin (RANKL/OPG) pathways (He et al., 2024). Moreover, lactobacilli have been shown to shape gut metabolites, such as butyrate, which potentially influences BMD (He et al., 2024) either directly or through immune responses; however, further research is needed to establish these effects.

In humans, various intervention studies have been performed to evaluate the effects of fermented dairy products on parameters of bone health. One intervention, which included three servings of fortified milk and yogurt for 12 months, showed favorable changes in bone metabolism biomarkers and BMD compared to calcium supplementation alone in a sample of postmenopausal women (Manios et al., 2007). Milk and yogurt supplementation, ranging from 5 to 30 months in postmenopausal women, also displayed a benefit to BMD (Moschonis & Manios, 2006; Moschonis et al., 2010, 2011). Multiple studies have also reported that vitamin D and calcium-fortified yogurts or soft cheese have reduced serum markers of bone resorption and parathyroid hormone compared to usual diets (Manios et al., 2007, 2009; Bonjour et al., 2011, 2012; Tenta et al., 2011) or non-fortified fermented dairy products (Bonjour et al., 2013, 2015; Neyestani et al., 2015; Jafari et al., 2016). These studies suggest that fermented dairy products may improve BMD and positively influence bone metabolism and calcium balance.

8.3.4 Synergistic Effects

Combining ACNs with high-fiber and fermented foods may provide synergistic effects in their combined ability to modulate the gut microbiome, which holds implications for improving bone health. High-fiber diets have been shown to promote the growth of beneficial bacteria, which contribute to the increased production of SCFAs, including butyrate, propionate, and acetate, during fiber fermentation in the gut (Zeng et al., 2019). Moreover, these SCFAs generated from dietary prebiotic sources may directly affect calcium absorption (Xu et al., 2017) and reduce bone resorption (Liu et al., 2018; Lucas et al., 2018), which are two key factors involved in maintaining bone density (Table 8.2). ACNs have also demonstrated prebiotic effects, with the potential to enhance the growth of SCFA-producing bacteria (Hair et al., 2021). Probiotics and fermented foods may further complement this interaction, exerting beneficial effects on the gut microbiota, particularly by enhancing SCFA production, which can promote bone health (de

Sire et al., 2022). Together, these dietary components may mitigate bone loss related to inflammation. ACNs may enhance these effects through their antioxidant and prebiotic properties, promoting beneficial bacteria (Sun et al., 2018). In addition to their influence on SCFA-producing bacteria, ACNs may interact with gut microbiota to promote the production of other beneficial metabolites, such as secondary bile acids (Liang et al., 2024), which can indirectly affect bone metabolism through promoting the production of Glucagon-Like Peptide-1 (GLP-1), a hormone with established effects on bone health (Sandoval & D'Alessio, 2015). Ursodeoxycholic acid, specifically, has been shown to increase differentiation and mineralization of osteoblasts while neutralizing the detrimental effects of lithocholic acid (Dubreuil et al., 2013). In general, deoxycholic acids can simultaneously stimulate bone formation and suppress bone resorption, thereby preventing bone loss (Dubreuil et al., 2013; Ahn et al., 2020). These synergistic effects underscore the multifaceted ways in which ACNs can modulate microbiota-derived metabolites to enhance bone health outcomes.

While high-fiber and fermented foods primarily influence SCFA production, ACNs complement this effect by reducing gut and systemic inflammation, thereby further contributing to improved bone health outcomes (Hair et al., 2021). The combined effects address important elements of the gut-bone axis, including enhanced calcium absorption, modulation of immune responses, and direct effects on osteoblast and osteoclast activity. However, future clinical trials are essential to determine the efficacy of these dietary components in combination.

TABLE 8.2

Summary of Dietary Strategies Targeting the Gut Microbiome for Bone Health ⏎

Category	Source/Definition	Gut Microbiota Effects	Key Metabolites/Compounds	Mechanisms for Bone Health
ACNs	Polyphenolic compounds in red, blue, and purple fruits and vegetables	Promotes Bifidobacteria and Lactobacilli; modulates microbial metabolism	Phenolic acids, SCFAs (butyrate, propionate), enterolignans (e.g., enterodiol, enterolactone)	Anti-inflammatory, immunomodulatory (↑ Treg cells), phytoestrogenic via ERβ

Category	Source/Definition	Gut Microbiota Effects	Key Metabolites/Compounds	Mechanisms for Bone Health
Dietary fiber and prebiotics	Plant-derived polysaccharides (e.g., inulin); soluble vs. insoluble types	Increases SCFA-producing bacteria (e.g., Faecalibacterium); improves diversity	SCFAs (butyrate, acetate, propionate) decreased harmful protein-derived products	Enhances calcium absorption, reduces inflammation, and preserves the mucus barrier
Fermented foods and probiotics	Fermented dairy (e.g., yogurt, cheese), Lactobacilli, other live cultures	Modifies composition (e.g., Lactobacilli dominance); increases SCFA production	SCFAs (butyrate), possibly secondary bile acids (with fiber/ACN synergy)	Reduces inflammatory cytokines, supports calcium metabolism, ↑ and BMD markers

8.4 Considerations for Designing Gut Microbiome-Targeted Interventions

Developing interventions targeting the gut to improve bone health in postmenopausal women must account for influential factors, including microbiome variability and hormonal changes. Given that previous research suggests shifts in gut microbiota during menopause transition, interventions should also be designed to address these dynamic changes.

8.4.1 Microbiome Variability and Intervention Responses

Microbiome variability among postmenopausal women poses a challenge to dietary interventions. Factors such as genetics, diet, physical activity, and overall health status shape individual gut microbiome compositions, influencing responses to dietary, lifestyle, and pharmacological interventions (Gibbons et al., 2022). For instance, differences in baseline abundance of SCFA- producing bacteria may affect how individuals respond to prebiotic or probiotic supplementation.

Moreover, variability in the production of other metabolites, such as secondary bile acids and indole derivatives, may also impact intervention outcomes. For example, a higher baseline abundance of bile acid-modifying bacteria, such as *Clostridium* (Zhao et al., 2020), may enhance calcium absorption, while the

presence of indole-producing bacteria may mitigate inflammation (Wang et al., 2024), thereby supporting bone health. Additionally, global shifts observed in the postmenopausal gut microbiome, such as reduced diversity and enrichment of proinflammatory taxa, like *Proteobacteria*, may complicate intervention outcomes by creating a more inflammatory baseline state (Santos et al., 2018; Peters et al., 2022). These trends suggest that successful interventions should address not only individual variability but also broader community-level changes.

Intervention designs should consider these metabolites as part of a broader framework for understanding microbiome-mediated effects on bone health. For example, enterotype classifications may influence how individuals respond to dietary interventions. A *Bacteroides*-dominated enterotype may lead to less favorable outcomes from fiber-rich interventions due to reduced capacity for SCFA production. Conversely, a *Prevotella*-dominated enterotype, associated with higher fiber intake, might predict better responses to dietary interventions aimed at increasing SCFA production. Including enterotype analysis for baseline profiling may help tailor interventions to maximize their effectiveness.

Diet is another important component that may contribute to the effectiveness and responses of interventions. Individuals consuming diets high in fermentable carbohydrates may support the growth of *Bifidobacteria*, *Prevotella*, *Ruminococcus*, *Dorea*, and *Roseburia*, which are capable of degrading polysaccharides, oligosaccharides, and sugars (Dahl et al., 2020). On the other hand, high-fat diets can increase the prevalence of bile-resistant organisms, including *Bilophila* and *Bacteroides* (Dahl et al., 2020). Moreover, considering food forms (e.g., whole foods vs. ultra-processed) is an important consideration as this may alter macronutrient substrates for the colon due to differences in digestibility, which may further impact the microbiome and its metabolic activity (Dahl et al., 2020). Based on this, individuals may have differing responses to dietary interventions, given their baseline microbiome composition, which can be influenced by diet alone.

8.4.2 Considerations for Intervention Designs

Designing dietary interventions that are both safe and effective is the first critical component. Adherence to dietary interventions may be influenced by factors such as dietary preferences, tolerability, and lifestyle constraints. The duration of interventions is another critical consideration, as long-term changes in the gut

microbiome may require sustained dietary patterns rather than short-term supplementation. For instance, it has been suggested that microbiome composition can adapt quickly to certain dietary changes; however, some changes may take longer to manifest, depending on the individual microbiota (Kolodziejczyk et al., 2019). Therefore, intervention designs must account for short- and long-term outcomes.

In addition, microbiome function, rather than composition alone, should be prioritized when evaluating dietary interventions. Functional outputs such as SCFA production or bile acid modulation may provide more direct links to bone health outcomes than taxonomic shifts alone. For example, microbial species that increase SCFA production can vary widely across individuals (Quinn-Bohmann et al., 2024), emphasizing the importance of measuring functional markers over taxonomic profiles. Host-microbiome interactions are also critical, as the efficacy of interventions depends on the host's baseline microbiota, immune status, and metabolic capacity. For example, individuals with an inflammatory baseline state may need interventions targeting gut barrier function and proinflammatory taxa before in order for SCFA-enhancing strategies to be effective. Thus, personalized approaches that integrate host and microbiome factors are essential for optimizing outcomes.

Additionally, dietary intake should be considered in studies evaluating the association between the gut microbiome and health outcomes, as it may influence the gut microbiome through dietary habits. Differing dietary patterns, such as Western, Mediterranean, and vegetarian diets, play a role in modulating the gut microbiota, as well as more restrictive diets like the paleolithic and ketogenic diets (Dahl et al., 2020). Thus, it is important that these potential differences among participants are accounted for.

8.4.3 Importance of Longitudinal Studies and Multi-Omics Approaches

Longitudinal studies are essential to evaluate the effectiveness of gut-targeted interventions. These studies will enable monitoring of the dynamic changes in the gut microbiome and bone health biomarkers over time. Integrating multi-omics approaches, such as metabolomics and proteomics, can provide a comprehensive understanding of how dietary interventions impact microbial composition, SCFA production, and bone metabolism. This holistic approach can guide the

development of more precise and personalized strategies for improving bone health in this population.

Much of the current research focused on the gut-bone axis is randomized controlled trials or cross-sectional designs. However, recent work highlights the importance of multi-omics approaches and prospective, longitudinal observational studies due to bone health and the gut microbiome being subject to changes across the lifespan, and their relationship at critical periods of physiological change and development needs further investigation (Cronin et al., 2022).

A novel finding of associations between diet, gut microbiota composition, and serum metabolic signatures with low BMD was identified in postmenopausal women using a multi-omics approach, elucidating evidence of the underlying diet, microbiota, and metabolite-relevant mechanisms in the loss of BMD (Palacios-Gonzalez et al., 2020). This study highlights the potential for integrated approaches to uncover mechanisms and potential biomarkers in research related to the gut-bone axis. A recent metagenomic study revealed associations between BMD and multiple bacterial species, L-arginine biosynthesis, and butyrate production that was independent of age, diet, and lifestyle (Li et al., 2025). This study also identified more pronounced age-related declines in BMD among individuals with *Bacteroides*-dominant enterotypes versus *Prevotella*-dominant enterotypes. This study provides valuable insight into intervention design and highlights the importance of baseline microbial profiles in risk assessment for bone loss. Additionally, Mendelian randomization analysis has revealed a causal relationship between BMD across multiple sites and gut (Xue et al., 2024). The results of this study can be useful for mechanistic discoveries and emphasize the potential of microbiome-targeted strategies for preventing or mitigating osteoporosis. Together, these recent findings demonstrate how longitudinal designs and multi-omics frameworks can aid in biomarker discovery as well as inform microbiome-focused interventions for osteoporosis prevention tailored to individual characteristics to maximize effectiveness.

8.5 Conclusion

The menopausal transition is a critical period for bone health in women, typically marked by increased risk of osteoporosis and fractures due to hormonal changes. Research presented in this review has indicated that menopause may cause shifts in the gut microbiome, including decreased diversity and changes to microbial

composition, which may contribute to bone loss through increased inflammation and decreased SCFA production.

Dietary interventions targeting the gut microbiome offer a promising, non-pharmacological approach to mitigating bone loss in postmenopausal women. Global trends in the microbiome, such as reduced diversity and a shift toward proinflammatory taxa in postmenopausal women, highlight the need to target these changes through dietary strategies. Considering enterotype- specific responses may further optimize dietary interventions by enabling a more tailored approach for improving bone health outcomes.

In addition to SCFAs, other microbially derived metabolites, such as secondary bile acids, polyamines, and tryptophan-derived metabolites, may also play critical roles in maintaining bone health. These compounds have been linked to improved calcium absorption, anti-inflammatory signaling, and reduced osteoclast activity, providing additional mechanisms through which dietary strategies may mitigate bone loss.

However, individual microbiome variability can influence the effectiveness of these interventions, making personalized dietary strategies a crucial component for optimizing outcomes. Additionally, the implementation of gut-targeted dietary interventions requires consideration of factors such as dietary adherence, safety, and feasibility, particularly in older populations with diverse dietary preferences and lifestyles. To develop effective interventions, the integration of multi-omics approaches is necessary to provide a more comprehensive understanding of microbiome and bone interactions, as well as more personalized approaches.

References

Abrams, S. A., I. J. Griffin, K. M. Hawthorne, L. Liang, S. K. Gunn, G. Darlington and K. J. Ellis (2005). “A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents.” *Am J Clin Nutr* **82**(2): 471–476. ⏎

Adlercreutz, H. and P. Järvenpää (1982). “Assay of estrogens in human feces.” *J Steroid Biochem* **17**(6): 639–645. ⏎

Adlercreutz, H. and F. Martin (1980). “Biliary excretion and intestinal metabolism of progesterone and estrogens in man.” *J Steroid Biochem* **13**(2): 231–244. ⏎

Ahn, T.-K., K.-T. Kim, H. P. Joshi, K. H. Park, J. W. Kyung, U.-Y. Choi, S. Sohn, S.-H. Sheen, D.-E. Shin and S.-H. Lee (2020). "Therapeutic potential of tauroursodeoxycholic acid for the treatment of osteoporosis." *Int J Mol Sci* **21**(12): 4274.

Baldi, S., M. Tristán Asensi, M. Pallecchi, F. Sofi, G. Bartolucci and A. Amedei (2023). "Interplay between lignans and gut microbiota: nutritional, functional and methodological aspects." *Molecules* **28**(1): 343. ⏎

Bindels, L. B., N. M. Delzenne, P. D. Cani and J. Walter (2015). "Towards a more comprehensive concept for prebiotics." *Nat Rev Gastroenterol Hepatol* **12**(5): 303–310. ⏎

Boto-Ordóñez, M., Urpi-Sarda M, Queipo-Ortuño MI, Tulipani S, Tinahones FJ, Andres-Lacueva C. (2014). "High levels of Bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: a randomized clinical trial." *Food Funct* **5**(8): 1932–1938. doi: 10.1039/c4fo00029c. ⏎

Bonjour, J. P., V. Benoit, S. Atkin and S. Walrand (2015). "Fortification of yogurts with vitamin D and calcium enhances the inhibition of serum parathyroid hormone and bone resorption markers: a double blind randomized controlled trial in women over 60 living in a community dwelling home." *J Nutr Health Aging* **19**(5): 563–569. ⏎

Bonjour, J. P., V. Benoit, F. Payen and M. Kraenzlin (2013). "Consumption of yogurts fortified in vitamin D and calcium reduces serum parathyroid hormone and markers of bone resorption: a double-blind randomized controlled trial in institutionalized elderly women." *J Clin Endocrinol Metab* **98**(7): 2915–2921. ⏎

Bonjour, J. P., V. Benoit, O. Pourchaire, B. Rousseau and J. C. Souberbielle (2011). "Nutritional approach for inhibiting bone resorption in institutionalized elderly women with vitamin D insufficiency and high prevalence of fracture." *J Nutr Health Aging* **15**(5): 404–409. ⏎

Bonjour, J. P., V. Benoit, B. Rousseau and J. C. Souberbielle (2012). "Consumption of vitamin D- and calcium-fortified soft white cheese lowers the biochemical marker of bone resorption TRAP 5b in postmenopausal women at moderate risk of osteoporosis fracture." *J Nutr* **142**(4): 698–703.

Braniste, V., M. Leveque, C. Buisson-Brenac, L. Bueno, J. Fioramonti and E. Houdeau (2009). "Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells." *J Physiol* **587**(Pt 13): 3317–3328. ⏎

Brenchley, J. M. and D. C. Douek (2012). “Microbial translocation across the GI tract.” *Annu Rev Immunol* **30**: 149–173. ⏎

Colldén, H., A. Landin, V. Wallenius, E. Elebring, L. Fändriks, M. E. Nilsson, H. Ryberg, M. Poutanen, K. Sjögren, L. Vandenput and C. Ohlsson (2019). “The gut microbiota is a major regulator of androgen metabolism in intestinal contents.” *Am J Physiol Endocrinol Metab* **317**(6): E1182–E1192. ⏎

Collins, F. L., N. D. Rios-Arce, S. Atkinson, H. Bierhalter, D. Schoenherr, J. N. Bazil, L. R. McCabe and N. Parameswaran (2017). “Temporal and regional intestinal changes in permeability, tight junction, and cytokine gene expression following ovariectomy-induced estrogen deficiency.” *Physiol Rep* **5**(9): e13263. ⏎

Cornes, R., C. Sintes, A. Peña, S. Albin, K. O. O’Brien, S. A. Abrams and C. M. Donangelo (2022). “Daily intake of a functional synbiotic yogurt increases calcium absorption in young adult women.” *J Nutr* **152**(7): 1647–1654. ⏎

Cronin, O., S. A. Lanham-New, B. M. Corfe, C. L. Gregson, A. L. Darling, K. R. Ahmadi, P. S. Gibson, J. H. Tobias, K. A. Ward, M. H. Traka, M. Rossi, C. Williams, N. C. Harvey, C. Cooper, K. Whelan, A. G. Uitterlinden, P. W. O’Toole, C. Ohlsson, J. E. Compston and S. H. Ralston (2022). “Role of the microbiome in regulating bone metabolism and susceptibility to osteoporosis.” *Calcif Tissue Int* **110**(3): 273–284. ⏎

Cummings, J. and G. Macfarlane (1991). “The control and consequences of bacterial fermentation in the human colon.” *J Appl Bacteriol* **70**(6): 443–459. ⏎

Dabek, M., S. I. McCrae, V. J. Stevens, S. H. Duncan and P. Louis (2008). “Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria.” *FEMS Microbiol Ecol* **66**(3): 487–495. ⏎

Dahl, W. J., D. Rivero Mendoza and J. M. Lambert (2020). “Diet, nutrients and the microbiome.” *Prog Mol Biol Transl Sci* **171**: 237–263. ⏎

de Sire, A., R. de Sire, C. Curci, F. Castiglione and W. Wahli (2022). “Role of dietary supplements and probiotics in modulating microbiota and bone health: the gut-bone axis.” *Cells* **11**(4): 743. ⏎

Deehan Edward, C., M. Duar Rebbeca, M. Armet Anissa, E. Perez-Muñoz Maria, M. Jin and J. Walter (2017). “Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health.” *Microbiol Spectr* **5**(5): 10.1128/microbiolspec.bad-0019–2017. ⏎

Desai, M. S., A. M. Seekatz, N. M. Koropatkin, N. Kamada, C. A. Hickey, M. Wolter, N. A. Pudlo, S. Kitamoto, N. Terrapon and A. Müller (2016). "A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility." *Cell* **167**(5): 1339–1353.e1321. ⏎

Diebel, M. E., L. N. Diebel, C. W. Manke and D. M. Liberati (2015). "Estrogen modulates intestinal mucus physiochemical properties and protects against oxidant injury." *J Trauma Acute Care Surg* **78**(1): 94–99. ⏎

Dubreuil, M., S. Ruiz-Gaspà, N. Guañabens, P. Peris, L. Álvarez, A. Monegal, A. Combalia and A. Parés (2013). "Ursodeoxycholic acid increases differentiation and mineralization and neutralizes the damaging effects of bilirubin on osteoblastic cells." *Liver Int* **33**(7): 1029–1038.

Duncan, S. H., A. Belenguer, G. Holtrop, A. M. Johnstone, H. J. Flint and G. E. Lobley (2007). "Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces." *Appl Environ Microbiol* **73**(4): 1073–1078. ⏎

Ecklu-Mensah, G., C. Choo-Kang, M. G. Maseng, S. Donato, P. Bovet, B. Viswanathan, K. Bedu-Addo, J. Plange-Rhule, P. Oti Boateng, T. E. Forrester, M. Williams, E. V. Lambert, D. Rae, N. Sinyanya, A. Luke, B. T. Layden, S. O'Keefe, J. A. Gilbert and L. R. Dugas (2023). "Gut microbiota and fecal short chain fatty acids differ with adiposity and country of origin: the METS-microbiome study." *Nat Commun* **14**(1): 5160. ⏎

Ervin, S. M., H. Li, L. Lim, L. R. Roberts, X. Liang, S. Mani and M. R. Redinbo (2019). "Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens." *J Biol Chem* **294**(49): 18586–18599. ⏎

Ervin, S. M., J. B. Simpson, M. E. Gibbs, B. C. Creekmore, L. Lim, W. G. Walton, R. Z. Gharaibeh and M. R. Redinbo (2020). "Structural insights into endobiotic reactivation by human gut microbiome-encoded sulfatases." *Biochemistry* **59**(40): 3939–3950. ⏎

Flores, R., J. Shi, B. Fuhrman, X. Xu, T. D. Veenstra, M. H. Gail, P. Gajer, J. Ravel and J. J. Goedert (2012). "Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: a cross-sectional study." *J Transl Med* **10**: 253. ⏎

Fu Y, Guzior DV, Okros M, Bridges C, Rosset SL, González CT, Martin C, Karunarathne H, Watson VE, Quinn RA (2025). Balance between bile acid

conjugation and hydrolysis activity can alter outcomes of gut inflammation. *Nat Commun*. 16(1):3434. doi: 10.1038/s41467-025-58649-x.

Fuhrman, B. J., H. S. Feigelson, R. Flores, M. H. Gail, X. Xu, J. Ravel and J. J. Goedert (2014). "Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women." *J Clin Endocrinol Metab* **99**(12): 4632–4640. ⏎

Furusawa, Y., Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, N. N. Fukuda, S. Murakami, E. Miyauchi, S. Hino, K. Atarashi, S. Onawa, Y. Fujimura, T. Lockett, J. M. Clarke, D. L. Topping, M. Tomita, S. Hori, O. Ohara, T. Morita, H. Koseki, J. Kikuchi, K. Honda, K. Hase and H. Ohno (2013). "Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells." *Nature* **504**(7480): 446–450. ⏎

Gaya, P., M. Medina, A. Sánchez-Jiménez and J. M. Landete (2016). "Phytoestrogen metabolism by adult human gut microbiota." *Molecules* **21**(8): 1034. ⏎

Gibbons, S. M., T. Gurry, J. W. Lampe, A. Chakrabarti, V. Dam, A. Everard, A. Goas, G. Gross, M. Kleerebezem, J. Lane, J. Maukonen, A. L. B. Penna, B. Pot, A. M. Valdes, G. Walton, A. Weiss, Y. C. Zanzer, N. V. Venlet and M. Miani (2022). "Perspective: leveraging the gut microbiota to predict personalized responses to dietary, prebiotic, and probiotic interventions." *Adv Nutr* **13**(5): 1450–1461. ⏎

Hair, R., J. R. Sakaki and O. K. Chun (2021). "ACNs, microbiome and health benefits in aging." *Molecules* **26**(3): 2358–2374. ⏎

Han, D., W. Wang, J. Gong, Y. Ma and Y. Li (2024). "Microbiota metabolites in bone: shaping health and confronting disease." *Heliyon* **10**(7): e28435. ⏎

He, W., H. C. Bertram, J. Y. Yin and S. P. Nie (2024). "Lactobacilli and their fermented foods as a promising strategy for enhancing bone mineral density: a review." *J Agric Food Chem* **72**(32): 17730–17745. ⏎

Hidalgo, M., M. J. Oruna-Concha, S. Kolida, G. E. Walton, S. Kallithraka, J. P. Spencer and S. de Pascual-Teresa (2012). "Metabolism of ACNs by human gut microflora and their influence on gut bacterial growth." *J Agric Food Chem* **60**(15): 3882–3890. ⏎

Homma, H., E. Hoy, D. Z. Xu, Q. Lu, R. Feinman and E. A. Deitch (2005). "The female intestine is more resistant than the male intestine to gut injury and

inflammation when subjected to conditions associated with shock states." *Am J Physiol Gastrointest Liver Physiol* **288**(3): G466–472. ⏎

Horie, K., N. Nanashima and H. Maeda (2019). "Phytoestrogenic effects of blackcurrant ACNs increased endothelial nitric oxide synthase (eNOS) expression in human endothelial cells and ovariectomized rats." *Molecules* **24**(7): 1259. ⏎

Hu, F. B. (2024). "Diet strategies for promoting healthy aging and longevity: An epidemiological perspective." *J Intern Med* **295**(4): 508–531. ⏎

Jafari, T., E. Faghihimani, A. Feizi, B. Iraj, S. H. Javanmard, A. Esmaillzadeh, A. A. Fallah and G. Askari (2016). "Effects of vitamin D-fortified low-fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women: a randomised controlled clinical trial." *Clin Nutr* **35**(1): 67–76. ⏎

Jia, X., R. Yang, J. Li, L. Zhao, X. Zhou and X. Xu (2021). "Gut-bone axis: a non-negligible contributor to periodontitis." *Front Cell Infect Microbiol* **11**: 752708. ⏎

Johansson, M. E., M. Phillipson, J. Petersson, A. Velcich, L. Holm and G. C. Hansson (2008). "The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria." *Proc Natl Acad Sci* **105**(39): 15064–15069. ⏎

Karlamangla, A. S., S. M. Burnett-Bowie and C. J. Crandall (2018). "Bone health during the menopause transition and beyond." *Obstet Gynecol Clin North Am* **45**(4): 695–708. ⏎

Koh, A., F. De Vadder, P. Kovatcheva-Datchary and F. Bäckhed (2016). "From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites." *Cell* **165**(6): 1332–1345. ⏎

Kolodziejczyk, A. A., D. Zheng and E. Elinav (2019). "Diet–microbiota interactions and personalized nutrition." *Nat Rev Microbiol* **17**(12): 742–753. ⏎

Korpela, K., S. Kallio, A. Salonen, M. Hero, A. K. Kukkonen, P. J. Miettinen, E. Savilahti, E. Kohva, L. Kariola, M. Suutela, A. Tarkkanen, W. M. de Vos, T. Raivio and M. Kuitunen (2021). "Gut microbiota develop towards an adult profile in a sex- specific manner during puberty." *Sci Rep* **11**(1): 23297. ⏎

Kwa, M., C. S. Plottel, M. J. Blaser and S. Adams (2016). "The intestinal microbiome and estrogen receptor-positive female breast cancer." *J Natl Cancer Inst* **108**(8): djw029. ⏎

La Reau, A. J. and G. Suen (2018). “The Ruminococci: key symbionts of the gut ecosystem.” *J Microbiol* **56**(3): 199–208. ⏎

Li, J., *et al*. (2019). “Bilberry anthocyanin extract promotes intestinal barrier function and inhibits digestive enzyme activity by regulating the gut microbiota in aging rats.” *Food Funct* **10**(1): 333–343. ⏎

Li, Y., Z. Shi, X. Zhang, H. Ren, H. Ji, F. Yang, Z. Fu, X. Kong, X. Cheng, J. Li, K. Wu, Y. Zhong, H. Zhong and L. Ji (2025). “Metagenomic analysis revealing links between age, gut microbiota and bone loss in Chinese adults.” *NPJ Metab Health Dis* **3**(1): 18. ⏎

Liang, A., W. Leonard, J. T. Beasley, Z. Fang, P. Zhang and C. S. Ranadheera (2024). “ACNs-gut microbiota-health axis: a review.” *Crit Rev Food Sci Nutr* **64**(21): 7563–7588. ⏎

Liu, H., J. Wang, T. He, S. Becker, G. Zhang, D. Li and X. Ma (2018). “Butyrate: a double-edged sword for health?” *Adv Nutr* **9**(1): 21–29. ⏎

Looijer-van Langen, M., N. Hotte, L. A. Dieleman, E. Albert, C. Mulder and K. L. Madsen (2011). “Estrogen receptor-β signaling modulates epithelial barrier function.” *Am J Physiol Gastrointest Liver Physiol* **300**(4): G621–626. ⏎

Lu, L., X. Chen, Y. Liu and X. Yu (2021). “Gut microbiota and bone metabolism.” *FASEB J* **35**(7): e21740. ⏎

Lucas, S., Y. Omata, J. Hofmann, M. Böttcher, A. Iljazovic, K. Sarter, O. Albrecht, O. Schulz, B. Krishnacoumar, G. Krönke, M. Herrmann, D. Mougiakakos, T. Strowig, G. Schett and M. M. Zaiss (2018). “Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss.” *Nat Commun* **9**(1): 55. ⏎

Makki, K., E. C. Deehan, J. Walter and F. Bäckhed (2018). “The impact of dietary fiber on gut microbiota in host health and disease.” *Cell Host Microbe* **23**(6): 705–715. ⏎

Manios, Y., G. Moschonis, K. Koutsikas, S. Papoutsou, I. Petraki, E. Bellou, A. Naoumi, S. Kostea and S. Tanagra (2009). “Changes in body composition following a dietary and lifestyle intervention trial: the postmenopausal health study.” *Maturitas* **62**(1): 58–65. ⏎

Manios, Y., G. Moschonis, G. Trovas and G. P. Lyritis (2007). “Changes in biochemical indexes of bone metabolism and bone mineral density after a 12-month dietary intervention program: the Postmenopausal Health Study.” *Am J Clin Nutr* **86**(3): 781–789. ⏎

Mann, E. R., Y. K. Lam and H. H. Uhlig (2024). “Short-chain fatty acids: linking diet, the microbiome and immunity.” *Nat Rev Immunol* **24**(8): 577–595. ⏎

Mao, W., G. Huang, H. Chen, L. Xu, S. Qin and A. Li (2021). “Research progress of the role of ACNs on bone regeneration.” *Front Pharmacol* **12**: 773660. ⏎

Mariño, E., J. L. Richards, K. H. McLeod, D. Stanley, Y. A. Yap, J. Knight, C. McKenzie, J. Kranich, A. C. Oliveira, F. J. Rossello, B. Krishnamurthy, C. M. Nefzger, L. Macia, A. Thorburn, A. G. Baxter, G. Morahan, L. H. Wong, J. M. Polo, R. J. Moore, T. J. Lockett, J. M. Clarke, D. L. Topping, L. C. Harrison and C. R. Mackay (2017). “Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes.” *Nat Immunol* **18**(5): 552–562. ⏎

Martin, F., J. Peltonen, T. Laatikainen, M. Pulkkinen and H. Adlercreutz (1975). “Excretion of progesterone metabolites and estriol in faeces from pregnant women during ampicillin administration.” *J Steroid Biochem* **6**(9): 1339–1346. ⏎

Mattioli, R., A. Francioso, L. Mosca and P. Silva (2020). “ACNs: a comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases.” *Molecules* **25**(17): 3809. ⏎

Mayneris-Perxachs, J., M. Arnoriaga-Rodríguez, D. Luque-Córdoba, F. Priego-Capote, V. Pérez-Brocal, A. Moya, A. Burokas, R. Maldonado and J. M. Fernández-Real (2020). “Gut microbiota steroid sexual dimorphism and its impact on gonadal steroids: influences of obesity and menopausal status.” *Microbiome* **8**(1): 136. ⏎

McRorie Jr, J. W. and N. M. McKeown (2017). “Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber.” *J Acad Nutr Diet* **117**(2): 251–264. ⏎

Mihajlovic, J., M. Leutner, B. Hausmann, G. Kohl, J. Schwarz, H. Röver, N. Stimakovits, P. Wolf, K. Maruszczak, M. Bastian, A. Kautzky-Willer and D. Berry (2021). “Combined hormonal contraceptives are associated with minor changes in composition and diversity in gut microbiota of healthy women.” *Environ Microbiol* **23**(6): 3037–3047. ⏎

Moschonis, G., S. Kanellakis, N. Papaioannou, A. Schaafsma and Y. Manios (2011). “Possible site-specific effect of an intervention combining nutrition and

lifestyle counselling with consumption of fortified dairy products on bone mass: the Postmenopausal Health Study II." *J Bone Miner Metab* **29**(4): 501–506. ⏎

Moschonis, G., I. Katsaroli, G. P. Lyritis and Y. Manios (2010). "The effects of a 30-month dietary intervention on bone mineral density: the Postmenopausal Health Study." *Br J Nutr* **104**(1): 100–107. ⏎

Moschonis, G. and Y. Manios (2006). "Skeletal site-dependent response of bone mineral density and quantitative ultrasound parameters following a 12-month dietary intervention using dairy products fortified with calcium and vitamin D: the Postmenopausal Health Study." *Br J Nutr* **96**(6): 1140–1148. ⏎

Müller, H., F. W. de Toledo and K. L. Resch (2001). "Fasting followed by vegetarian diet in patients with rheumatoid arthritis: a systematic review." *Scand J Rheumatol* **30**(1): 1–10. ⏎

Nagpal, R. and H. Yadav (2017). "Bacterial Translocation from the gut to the distant organs: an overview." *Ann Nutr Metab* **71**(Suppl 1): 11–16. ⏎

Nanashima, N., K. Horie and H. Maeda (2017). "Phytoestrogenic activity of blackcurrant ACNs is partially mediated through estrogen receptor beta." *Molecules* **23**(1): 74. ⏎

Neyestani, T. R., B. Nikooyeh, A. Kalayi, M. Zahedirad and N. Shariatzadeh (2015). "A Vitamin D-calcium-fortified yogurt drink decreased serum PTH but did not affect osteocalcin in subjects with Type 2 diabetes." *Int J Vitam Nutr Res* **85**(1–2): 61–69. ⏎

Nosal, B. M., S. N. Thornton, A. V. Melnik, A. Lotfi, M. D. Mofrad, A. Aksenov, E. C. Lee and O. K. Chun (2024). "Blackcurrant ACNs attenuate estrogen-deficiency-induced bone loss through modulating microbial-derived short-chain carboxylic acids and phytoestrogen metabolites in peri- and early postmenopausal women." *Metabolites* **14**(10): 541. ⏎

Palacios-González, B., E. G. Ramírez-Salazar, B. Rivera-Paredez, M. Quiterio, Y. N. Flores, L. Macias-Kauffer, S. Moran-Ramos, E. Denova-Gutiérrez, I. Ibarra-González, M. Vela-Amieva, S. Canizales-Quinteros, J. Salmerón and R. Velázquez-Cruz (2020). "A multi-omic analysis for low bone mineral density in postmenopausal women suggests a RELATIONSHIP between diet, metabolites, and microbiota." *Microorganisms* **8**(11): 1630. ⏎

Paturi, G., C. A. Butts, J. A. Monro and D. Hedderley (2018). "Effects of blackcurrant and dietary fibers on large intestinal health biomarkers in rats." *Plant Foods Hum Nutr* **73**(1): 54–60. ⏎

Peters, B. A., D. B. Hanna, X. Xue, K. Weber, A. A. Appleton, S. G. Kassaye, E. Topper, R. P. Tracy, C. Guillemette, P. Caron, P. C. Tien, Q. Qi, R. D. Burk, A. Sharma, K. Anastos and R. C. Kaplan (2024). "Menopause and estrogen associations with gut barrier, microbial translocation, and immune activation biomarkers in women with and without HIV." *J Acquir Immune Defic Syndr* **96**(3): 214–222. ⏎

Peters, B. A., J. Lin, Q. Qi, M. Usyk, C. R. Isasi, Y. Mossavar-Rahmani, C. A. Derby, N. Santoro, K. M. Perreira, M. L. Daviglus, M. A. Kominiarek, J. Cai, R. Knight, R. D. Burk and R. C. Kaplan (2022a). "Menopause is associated with an altered gut microbiome and estrobolome, with implications for adverse cardiometabolic risk in the Hispanic Community Health Study/Study of Latinos." *mSystems* **7**(3): e0027322.

Peters, B. A., N. Santoro, R. C. Kaplan and Q. Qi (2022b). "Spotlight on the gut microbiome in menopause: current insights." *Int J Womens Health* **14**: 1059–1072.

Peters, B. A., X. Xue, L. A. Sheira, Q. Qi, A. Sharma, N. Santoro, M. L. Alcaide, I. Ofotokun, A. A. Adimora, H. S. McKay, P. C. Tien, K. G. Michel, D. Gustafson, B. Turan, A. L. Landay, R. C. Kaplan and S. D. Weiser (2022c). "Menopause is associated with immune activation in women with HIV." *J Infect Dis* **225**(2): 295–305. ⏎

Peters, B. A., X. Xue, Z. Wang, M. Usyk, N. Santoro, A. Sharma, K. Anastos, P. C. Tien, E. T. Golub, K. M. Weber, D. Gustafson, R. C. Kaplan, R. Burk and Q. Qi (2021). "Menopausal status and observed differences in the gut microbiome in women with and without HIV infection." *Menopause* **28**(5): 491–501. ⏎

Pollet, R. M., E. H. D'Agostino, W. G. Walton, Y. Xu, M. S. Little, K. A. Biernat, S. J. Pellock, L. M. Patterson, B. C. Creekmore, H. N. Isenberg, R. R. Bahethi, A. P. Bhatt, J. Liu, R. Z. Gharaibeh and M. R. Redinbo (2017). "An atlas of β-glucuronidases in the human intestinal microbiome." *Structure* **25**(7): 967–977.e965. ⏎

Quinn-Bohmann, N., T. Wilmanski, K. R. Sarmiento, L. Levy, J. W. Lampe, T. Gurry, N. Rappaport, E. M. Ostrem, O. S. Venturelli, C. Diener and S. M. Gibbons (2024). "Microbial community-scale metabolic modelling predicts personalized short-chain fatty acid production profiles in the human gut." *Nat Microbiol* **9**(7): 1700–1712. ⏎

Rescigno, M. (2014). “Intestinal microbiota and its effects on the immune system.” *Cell Microbiol* **16**(7): 1004–1013. ⏎

Riis, B. J., M. A. Hansen, A. M. Jensen, K. Overgaard and C. Christiansen (1996). “Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study.” *Bone* **19**(1): 9–12. ⏎

Rizzoli, R. and E. Biver (2020). “Are probiotics the new calcium and vitamin D for bone health?” *Curr Osteoporos Rep* **18**(3): 273–284. ⏎

Ruiz-Gaspà, S., N. Guañabens, A. Enjuanes, P. Peris, A. Martinez-Ferrer, M. J. de Osaba, B. Gonzalez, L. Alvarez, A. Monegal, A. Combalia and A. Parés (2010). “Lithocholic acid downregulates vitamin D effects in human osteoblasts.” *Eur J Clin Invest* **40**(1): 25–34. ⏎

Salehi, B., J. Sharifi-Rad, F. Cappellini, Ž. Reiner, D. Zorzan, M. Imran, B. Sener, M. Kilic, M. El-Shazly, N. M. Fahmy, E. Al-Sayed, M. Martorell, C. Tonelli, K. Petroni, A. O. Docea, D. Calina and A. Maroyi (2020). “The therapeutic potential of ACNs: current approaches based on their molecular mechanism of action.” *Front Pharmacol* **11**: 1300. ⏎

Sandberg, A. A. and W. R. Slaunwhite, Jr. (1957). “Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliary-enteric circulation of C14-estrone and C14-estradiol in women.” *J Clin Invest* **36**(8): 1266–1278. ⏎

Sandoval, D. A. and D. A. D’Alessio (2015). “Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease.” *Physiol Rev* **95**(2): 513–548. ⏎

Santos-Marcos, J. A., O. A. Rangel-Zuñiga, R. Jimenez-Lucena, G. M. Quintana-Navarro, S. Garcia-Carpintero, M. M. Malagon, B. B. Landa, M. Tena-Sempere, P. Perez-Martinez, J. Lopez-Miranda, F. Perez-Jimenez and A. Camargo (2018). “Influence of gender and menopausal status on gut microbiota.” *Maturitas* **116**: 43–53. ⏎

Sato, Y., K. Atarashi, D. R. Plichta, Y. Arai, S. Sasajima, S. M. Kearney, W. Suda, K. Takeshita, T. Sasaki, S. Okamoto, A. N. Skelly, Y. Okamura, H. Vlamakis, Y. Li, T. Tanoue, H. Takei, H. Nittono, S. Narushima, J. Irie, H. Itoh, K. Moriya, Y. Sugiura, M. Suematsu, N. Moritoki, S. Shibata, D. R. Littman, M. A. Fischbach, Y. Uwamino, T. Inoue, A. Honda, M. Hattori, T. Murai, R. J. Xavier, N. Hirose and K. Honda (2021). “Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians.” *Nature* **599**(7885): 458–464. ⏎

Schroeder, B. O., G. M. Birchenough, M. Ståhlman, L. Arike, M. E. Johansson, G. C. Hansson and F. Bäckhed (2018). "Bifidobacteria or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration." *Cell Host Microbe* **23**(1): 27–40.e27. ⏎

Shieh, A., M. Epeldegui, A. S. Karlamangla and G. A. Greendale (2020). "Gut permeability, inflammation, and bone density across the menopause transition." *JCI Insight* **5**(2): e134092. ⏎

Shin, J. H., Y. H. Park, M. Sim, S. A. Kim, H. Joung and D. M. Shin (2019). "Serum level of sex steroid hormone is associated with diversity and profiles of human gut microbiome." *Res Microbiol* **170**(4–5): 192–201. ⏎

Sindel, D. (2023). "Osteoporosis: spotlight on current approaches to pharmacological treatment." *Turk J Phys Med Rehabil* **69**(2): 140–152. ⏎

Sun, H., P. Zhang, Y. Zhu, Q. Lou and S. He (2018). "Antioxidant and prebiotic activity of five peonidin-based ACNs extracted from purple sweet potato (*Ipomoea batatas* (L.) *Lam*.)." *Sci Rep* **8**(1): 5018. ⏎

Taylor, B. C., F. Lejzerowicz, M. Poirel, J. P. Shaffer, L. Jiang, A. Aksenov, N. Litwin, G. Humphrey, C. Martino, S. Miller-Montgomery, P. C. Dorrestein, P. Veiga, S. J. Song, D. McDonald, M. Derrien and R. Knight (2020). "Consumption of fermented foods is associated with systematic differences in the gut microbiome and metabolome." *mSystems* **5**(2): e00901-19. ⏎

Tenta, R., G. Moschonis, M. Koutsilieris and Y. Manios (2011). "Calcium and vitamin D supplementation through fortified dairy products counterbalances seasonal variations of bone metabolism indices: the Postmenopausal Health Study." *Eur J Nutr* **50**(5): 341–349. ⏎

Tian, L., Y. Tan, G. Chen, G. Wang, J. Sun, S. Ou, W. Chen and W. Bai (2019). "Metabolism of ACNs and consequent effects on the gut microbiota." *Crit Rev Food Sci Nutr* **59**(6): 982–991. ⏎

van der Giessen, J., C. J. van der Woude, M. P. Peppelenbosch and G. M. Fuhler (2019). "A direct effect of sex hormones on epithelial barrier function in inflammatory bowel disease models." *Cells* **8**(3): 261. ⏎

Wang, G., Y. Fan, G. Zhang, S. Cai, Y. Ma, L. Yang, Y. Wang, H. Yu, S. Qiao and X. Zeng (2024). "Microbiota-derived indoles alleviate intestinal inflammation and modulate microbiome by microbial cross-feeding." *Microbiome* **12**(1): 59. ⏎

Wang, H., *et al*. (2020). "Dietary supplementation of black rice anthocyanin extract regulates cholesterol metabolism and improves gut microbiota dysbiosis in

C57BL/6J mice fed a high-fat and cholesterol diet." *Mol Nutr Food Res* **64**(8): e1900876. ⏎

Weaver, C. M., B. R. Martin, C. H. Nakatsu, A. P. Armstrong, A. Clavijo, L. D. McCabe, G. P. McCabe, S. Duignan, M. H. Schoterman and E. G. van den Heuvel (2011). "Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation." *J Agric Food Chem* **59**(12): 6501–6510. ⏎

Wrzosek, L., S. Miquel, M.-L. Noordine, S. Bouet, M. J. Chevalier-Curt, V. Robert, C. Philippe, C. Bridonneau, C. Cherbuy and C. Robbe-Masselot (2013). "*Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent." *BMC Biol* **11**: 1–13. ⏎

Wu, G. D., J. Chen, C. Hoffmann, K. Bittinger, Y.-Y. Chen, S. A. Keilbaugh, M. Bewtra, D. Knights, W. A. Walters, R. Knight, R. Sinha, E. Gilroy, K. Gupta, R. Baldassano, L. Nessel, H. Li, F. D. Bushman and J. D. Lewis (2011). "Linking long-term dietary patterns with gut microbial enterotypes." *Science* **334**(6052): 105–108. ⏎

Wu, J., Y. Zhuo, Y. Liu, Y. Chen, Y. Ning and J. Yao (2021). "Association between premature ovarian insufficiency and gut microbiota." *BMC Pregnancy Childbirth* **21**(1): 418. ⏎

Xu, L., Q. Zhang, X. Dou, Y. Wang, J. Wang, Y. Zhou, X. Liu and J. Li (2022). "Fecal microbiota transplantation from young donor mice improves ovarian function in aged mice." *J Genet Genomics* **49**(11): 1042–1052. ⏎

Xu, X., X. Jia, L. Mo, C. Liu, L. Zheng, Q. Yuan and X. Zhou (2017). "Intestinal microbiota: a potential target for the treatment of postmenopausal osteoporosis." *Bone Res* **5**(1): 17046. ⏎

Xue, Y., X. Wang, H. Liu, J. Kang, X. Liang, A. Yao and Z. Dou (2024). "Assessment of the relationship between gut microbiota and bone mineral density: a two-sample Mendelian randomization study." *Front Microbiol* **15**: 1298838. ⏎

Yamamoto, T., E. Hinoi, H. Fujita, T. Iezaki, Y. Takahata, M. Takamori and Y. Yoneda (2012). "The natural polyamines spermidine and spermine prevent bone loss through preferential disruption of osteoclastic activation in ovariectomized mice." *Br J Pharmacol* **166**(3): 1084–1096. ⏎

Yatsunenko, T., F. E. Rey, M. J. Manary, I. Trehan, M. G. Dominguez-Bello, M. Contreras, M. Magris, G. Hidalgo, R. N. Baldassano, A. P. Anokhin, A. C. Heath, B. Warner, J. Reeder, J. Kuczynski, J. G. Caporaso, C. A. Lozupone, C. Lauber, J. C. Clemente, D. Knights, R. Knight and J. I. Gordon (2012). "Human gut microbiome viewed across age and geography." *Nature* **486**(7402): 222–227. ⏎

Zaiss, M. M., R. M. Jones, G. Schett and R. Pacifici (2019). "The gut-bone axis: how bacterial metabolites bridge the distance." *J Clin Invest* **129**(8): 3018–3028. ⏎

Zeng, H., S. Umar, B. Rust, D. Lazarova and M. Bordonaro (2019). "Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer." *Int J Mol Sci* **20**(5). ⏎

Zhang, Y. W., P. R. Song, S. C. Wang, H. Liu, Z. M. Shi and J. C. Su (2024). "Diets intervene osteoporosis via gut-bone axis." *Gut Microbes* **16**(1): 2295432. ⏎

Zhao, D., E. Guallar, P. Ouyang, V. Subramanya, D. Vaidya, C. E. Ndumele, J. A. Lima, M. A. Allison, S. J. Shah, A. G. Bertoni, M. J. Budoff, W. S. Post and E. D. Michos (2018). "Endogenous sex hormones and incident cardiovascular disease in postmenopausal women." *J Am Coll Cardiol* **71**(22): 2555–2566. ⏎

Zhao, H., J. Chen, X. Li, Q. Sun, P. Qin and Q. Wang (2019). "Compositional and functional features of the female premenopausal and postmenopausal gut microbiota." *FEBS Lett* **593**(18): 2655–2664. ⏎

Zhao, L., W. Yang, Y. Chen, F. Huang, L. Lu, C. Lin, T. Huang, Z. Ning, L. Zhai, L. L. Zhong, W. Lam, Z. Yang, X. Zhang, C. Cheng, L. Han, Q. Qiu, X. Shang, R. Huang, H. Xiao, Z. Ren, D. Chen, S. Sun, H. El-Nezami, Z. Cai, A. Lu, X. Fang, W. Jia and Z. Bian (2020). "A *Clostridia*-rich microbiota enhances bile acid excretion in diarrhea-predominant irritable bowel syndrome." *J Clin Invest* **130**(1): 438–450. ⏎

Zhou, Z., C. Bian, Z. Luo, C. Guille, E. Ogunrinde, J. Wu, M. Zhao, S. Fitting, D. L. Kamen, J. C. Oates, G. Gilkeson, and W. Jiang (2019). "Progesterone decreases gut permeability through upregulating occludin expression in primary human gut tissues and Caco-2 cells." *Sci Rep* **9**(1): 8367. ⏎

Zhu, Y., H. Sun, S. He, Q. Lou, M. Yu, M. Tang and L. Tu (2018). "Metabolism and prebiotics activity of ACNs from black rice (*Oryza sativa* L.) in vitro." *PLoS One* **13**(4): e0195754. ⏎

Zou, J., B. Chassaing, V. Singh, M. Pellizzon, M. Ricci, M. D. Fythe, M. V. Kumar and A. T. Gewirtz (2018). "Fiber-mediated nourishment of gut microbiota protects against diet-induced obesity by restoring IL-22-mediated colonic health." *Cell Host Microbe* **23**(1): 41–53. e44. ⏎

9

Gut Microbiome and Eating Disorders: From the Body–Mind Connection to the Therapeutic Perspectives

Simone Baldi, Francesca Cuffaro, Amal Tahri, and Amedeo Amedei

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9.1 Introduction

Over the past two decades, our understanding of the human body has shifted: the gut is no longer seen solely as a digestive organ but as a central node in a complex, bidirectional communication network linking the gastrointestinal (GI) tract, the brain, and the rest of the body. This gut–brain axis (GBA) operates through neural, immune, endocrine, and metabolic pathways. At the axis's core lies the gut microbiome (GM), a diverse community of microorganisms whose genetic and metabolic activities exert wide-ranging effects on host physiology, including metabolism, immune function, energy balance, and brain processes.

Eating disorders (EDs) remain among the most complex and challenging conditions in psychiatry and medicine. Their etiology is multifactorial,

involving psychological, sociocultural, genetic, and environmental contributors, and, despite substantial research, long-term outcomes are often unsatisfactory, with high relapse rates.

In this scenario, the GM offers a compelling and still underexplored link between body and mind. Emerging evidence indicates that GM dysbiosis can affect digestion and metabolism as well as neuroendocrine regulation, immune signaling, appetite control, mood, and behavior—processes that are intimately connected to ED development, maintenance, and severity.

In this chapter, we focus on the intersection of GM research and EDs, synthesizing current mechanistic and clinical evidence while highlighting relevant therapeutic implications. By integrating findings from microbiology, neuroscience, immunology, and clinical psychiatry, this chapter identifies promising avenues for more effective and personalized interventions.

9.2 Mechanisms Linking Gut Microbiota and Eating Behavior

The GBA is a complex, bidirectional communication network integrating the enteric and central nervous systems (CNSs). Beyond a simple anatomical connection, it operates through neural, endocrine, metabolic, and immune pathways that enable dynamic interactions between the gut and the brain. Key GBA components include the autonomic nervous system, the hypothalamic– pituitary–adrenal (HPA) axis, and the neuronal circuits of the GI tract, primarily regulated by the vagus nerve (Cryan & Dinan, 2012). These systems allow the brain to control essential GI functions such as motility, secretion, and immune activity. Conversely, gut-derived signals can influence brain function, shaping mood, cognition, and mental health (Carabotti et al., 2015).

The communication within the GBA occurs across four main levels—neuroanatomical, neuroendocrine, immunological, and biochemical—with the latter involving neurotransmitters, neuropeptides, and GM-derived metabolites such as short-chain fatty acids (SCFAs), tryptophan and indole

derivatives, and bile acids (Li et al., 2023). Among these metabolites, SCFAs are the primary products of microbial fermentation, and evidence from both human and animal studies demonstrates their role in modulating food-related decision-making and reward processing (Choe, 2025; den Besten et al., 2013). In addition, appetite-regulating hormones, including leptin, ghrelin, peptide YY (PYY), and neuropeptide Y (NPY), play a central role in eating behavior and appear to be modulated by the GM. Under physiological conditions, leptin acts as a satiety signal, whereas ghrelin stimulates hunger. PYY exerts anorexigenic effects proportional to caloric intake, while NPY, similar to ghrelin, promotes feeding (Lucka & Wysokiński, 2019). Rodent studies show that leptin levels correlate positively with *Bifidobacterium* spp. and *Lactobacillus* spp., but inversely with *Clostridium* spp., *Bacteroides* spp., and *Prevotella* spp. Ghrelin levels, in contrast, show a negative relationship with *Lactobacillus* spp. and a positive association with *Bacteroides* spp. (Queipo-Ortuño et al., 2013). Beyond its orexigenic role, ghrelin also acts on mesolimbic reward pathways, influencing hedonic eating and sensitivity to food cues (Plassmann et al., 2022). The GM contributes to peripheral ghrelin regulation: a 12-week oligofructose intervention reduced circulating ghrelin (Parnell & Reimer, 2009), and antibiotic-induced microbial depletion produced similar decreases (Yanagi et al., 2017). Finally, elevated ghrelin has been associated with heightened reward sensitivity (Ralevski et al., 2018).

The immune system plays an additional role in linking gut microbes to appetite-regulating hormones. Human serum contains immunoglobulin G (IgG) and immunoglobulin A (IgA) autoantibodies against leptin, ghrelin, PYY, and NPY, which can cross the blood–brain barrier (BBB) and interact with hunger-regulating regions such as the arcuate nucleus (Fetissov et al., 2008a,b). Notably, sequence homology between these peptides and microbial antigens from *Lactobacillus* spp., *Bacteroides* spp., *Helicobacter pylori*, *Escherichia coli*, and *Candida* spp. suggests a molecular mimicry mechanism altering feeding behavior (Smitka et al., 2021). The vagus nerve also relays nutrient-related signals such as glucagon-like peptide-1 (GLP-1),

cholecystokinin (CCK), and PYY from the gut to the brain, modulating neural and endocrine responses that regulate food intake (Browning et al., 2017; Howland, 2014; Waise et al., 2018). These hormone-driven, appetite-suppressing effects vary by nutrient type, and vagal signaling further influences reward processing in humans (Moris et al., 2022).

Moreover, the GM modulates neurotransmitter systems central to appetite and reward. Microbes synthesize or alter the availability of serotonin, dopamine, glutamate, and γ-aminobutyric acid (GABA) (García-Cabrerizo et al., 2021). Approximately 90% of peripheral serotonin is produced in the gut, and its levels are influenced by microbial tryptophan metabolism (Yano et al., 2015). Altered serotonergic signaling has been linked to changes in food preference and dietary decisions (Feng et al., 2025; van Galen et al., 2021).

Dopaminergic pathways are equally critical for food reward: germ-free rodents exhibit abnormal dopamine turnover and reduced dopaminergic metabolites in reward circuits (Wang et al., 2023). Antibiotic-induced microbial depletion alters tyrosine hydroxylase and dopamine receptor expression, while supplementation with *Lactobacillus plantarum* PS128 restores dopaminergic activity (W. H. Liu et al., 2016; Lotan et al., 2014). Finally, colonization with *Clostridium difficile* has been shown to modify dopamine levels (Vinithakumari et al., 2022).

Together, these findings indicate that microbial modulation of neurotransmitters, mainly serotonin and dopamine, is a key mechanism by which gut microbes shape food reward and eating behavior.

It is known that the GM can activate the innate immune system, leading to the release of both pro- and anti-inflammatory cytokines (Zheng et al., 2020). These immune mediators may trigger neuroinflammation and alter serotonergic and dopaminergic signaling (Felger & Treadway, 2017), thereby influencing reward processing and eating-related decision-making (Treadway et al., 2019). In mice, lipopolysaccharide-induced inflammation reduces effort expenditure despite preserved reward sensitivity (Vichaya et al., 2014), while in humans, inflammation similarly decreases incentive motivation and willingness to exert effort for rewards (Berridge et al., 2009;

Lasselin et al., 2017). Thus, microbially driven systemic inflammation may modulate eating behavior through immune-related pathways.

In addition, the GM shapes feeding behavior by interacting with central neural circuits. The hypothalamus, a major GBA hub, integrates hormonal, sensory, environmental, and GI signals to maintain energy homeostasis (Gupta et al., 2020; Moura-Assis et al., 2021). Its arcuate nucleus contains anorexigenic pro-opiomelanocortin neurons and orexigenic agouti-related peptide/NPY neurons (Liu & Kanoski, 2018). Germ-free mice show altered expression of appetite-regulating neuropeptides compared with conventionally colonized mice, including decreased hypothalamic brain-derived neurotrophic factor (BDNF). Moreover, microbial status influences the balance of anorexigenic (*Pomc*, *Cart*) and orexigenic (*Npy*, *Agrp*) genes and markers of leptin resistance such as Socs-3 (Schéle et al., 2013; Yao et al., 2020).

Proteomic analyses further reveal differences in hypothalamic neuropeptide and synaptic proteins (R. Liu et al., 2020). Colonization of germ-free mice enhances the expression of genes linked to extracellular matrix remodeling, highlighting a role for extracellular pathways in microbiota–hypothalamus interactions (Qi et al., 2020); nonetheless, the exact mechanisms remain incompletely understood.

The brainstem is another crucial GBA hub. It integrates peripheral and central signals to regulate feeding, processing vagal inputs, circulating metabolites, hormones, and signals from forebrain regions (Grill & Hayes, 2012). The nucleus of the solitary tract (NTS) receives vagal sensory input and projects to both homeostatic and higher-order brain areas and, through its vagal connections, the GM is hypothesized to influence NTS activity (Yu & Hsiao, 2021). Conventional mice show decreased anorexigenic neuropeptides such as GLP-1 and BDNF in the NTS compared with germ-free mice (Schéle et al., 2013), consistent with GM-dependent regulation. The dorsal raphe nucleus (DRN), a serotonergic center involved in appetite control, also appears sensitive to microbial status: germ-free mice exhibit heightened neuronal activity and long-term plasticity within the DRN (Campos et al., 2016). Cortical and limbic circuits contribute further to the

regulation of eating behavior by integrating motivational, cognitive, and emotional signals. The prefrontal cortex, orbitofrontal cortex, and insula regulate food reward and motivation (Azevedo et al., 2022). In rodents, the GM modulates prefrontal cortex function, influencing social and stress-related behaviors (Ntranos & Casaccia, 2018). Mechanisms include the regulation of myelination, microRNAs involved in myelin translation (Hoban et al., 2016), and broader metabolic and synaptic pathways (Gacias et al., 2016; Hoban et al., 2016). In humans, because cortical circuits involved in social behaviors overlap with those governing feeding, GM-dependent cortical modulation likely influences eating (Jennings et al., 2019). Moreover, evidence shows that the GM modulates dopaminergic transmission through amino acid metabolism, neurotransmitter availability, and receptor expression (Kawase et al., 2017); in humans, increased microbial phenylalanine synthesis correlates with reduced ventral striatal activity during reward anticipation (Aarts et al., 2017). GM-dependent variations in impulsivity and dopamine receptor expression have also been described (Jadhav et al., 2018). Although the mechanisms remain incompletely defined, tryptophan and indole metabolites appear to influence mesolimbic circuits through GI or vagal pathways (Byrne et al., 2016; Osadchiy et al., 2018).

Finally, the hippocampus serves as an additional node linking the GM to feeding regulation. As a region that integrates energy status, the memory of previous meals, environmental cues, and learned behavior, the hippocampus plays a key role in shaping eating patterns (Azevedo et al., 2019). In detail, the GM influences hippocampal neurogenesis, neurochemistry, structural plasticity, and gene expression (Tang et al., 2020), as well as microglial function essential for brain development (Erny et al., 2015).

9.3 GM Alterations in EDs

Disruptions at any level of the GBA may contribute to the onset and progression of neuropsychiatric disorders, including EDs (Lam et al., 2017).

The GM is a critical component of the GBA, exerting anxiolytic and anti-stress effects via the modulation of vagal activity and the synthesis of neuroactive compounds (e.g., GABA, serotonin, and SCFAs) (Breit et al., 2018; Silva et al., 2020). In addition, the GM influences CNS function by altering the production and bioavailability of dopamine, serotonin, and melatonin, substances critical for emotional regulation, mood stability, and cognition, and it contributes directly to digestion, hunger/satiety signaling, and food intake regulation (Fetissov & Hökfelt, 2019; Strandwitz, 2018). Notably, psychiatric and GI conditions are highly comorbid: approximately 40–60% of individuals with functional GI disorders show psychiatric symptoms, and up to 50% of psychiatric patients meet the criteria for irritable bowel syndrome (Wang et al., 2025).

These observations underscore the strong GBA influence on both gut and mental health and motivate growing interest in the GM role in EDs, but the real GM contribution to ED pathophysiology remains incompletely understood and requires further investigation.

9.3.1 Anorexia Nervosa

Accumulating evidence indicates that GM composition is markedly altered during the phases of dietary restriction, refeeding, and weight recovery in anorexia nervosa (AN). Compared with healthy controls, AN subjects consistently exhibit differences in microbial diversity, taxonomic composition, and relative abundance. A recurring finding is decreased representation of Bacillota, including genera such as *Roseburia*, *Lactobacillus*, *Streptococcus*, and *Clostridium* (Burton Murray et al., 2022a, b); Morita et al., 2015). In detail, *Roseburia* spp., a major SCFA producer, has been repeatedly reported as diminished in AN (Hanachi et al., 2019; Mack et al., 2016). Three genera, *Alistipes*, *Parabacteroides*, and *Roseburia*, have been proposed as potential discriminative markers between AN and healthy controls (Di Lodovico et al., 2021). These compositional shifts are often associated with lower alpha diversity, which can impair nutrient absorption and energy harvest and thereby contribute to the

persistence of the malnourished state (Mörkl et al., 2017). Caloric restriction and macronutrient imbalances that characterize AN further exacerbate microbial shifts, with increased abundances of *Escherichia coli* and the methanogen *Methanobrevibacter smithii*, with the latter being associated with enhanced energy extraction under nutrient-deprived conditions (Armougom et al., 2009). Mechanistic animal studies reinforce a contributory role for the GM: fecal microbiota transplantation (FMT) from AN patients into germ-free mice reproduces metabolic and behavioral features relevant to AN (Maschek et al., 2025).

Additionally, microbial dysbiosis in AN is associated with immune perturbations and systemic inflammation (Roubalová et al., 2020). Reduced microbial richness may impair mucosal immunity and compromise barrier integrity, producing downstream effects on the GBA that could reinforce AN psychopathology. Although nutritional rehabilitation remains the cornerstone of AN treatment and dietary interventions strongly influence GM composition (Beam et al., 2021), restoration of a healthy GM is not always achieved (Kleiman et al., 2015). Some refeeding diets, often high in fat, have been linked to declines in Bacillota, increases in *Bacteroides* spp. and mucin-degrading bacteria, and decreases in butyrate-producing taxa.

Of note, longitudinal studies indicate that GM alterations may persist despite clinical improvement or body mass index (BMI) normalization: in adolescents, GM disturbances have been found to be unresolved one year after hospital admission (Kleiman et al., 2015). Interestingly, higher abundance of *Sutterella*, a genus implicated in immune regulation, has been associated with more favorable weight gain, suggesting potential prognostic microbial value (Andreani et al., 2024). Host factors such as BMI and physical activity also shape microbial ecology; for example, increased Coriobacteriaceae, observed in both AN patients and individuals engaging in intense exercise, may mirror shared signatures of low energy availability (Mörkl et al., 2017).

Metagenomic sequencing has further expanded our understanding, revealing eleven previously uncharacterized bacterial species and four novel human-associated microeukaryotic communities in AN patients,

thereby opening new avenues for functional GM research (Gouba et al., 2014).

In addition, fecal SCFA abundances are often reduced in AN patients, especially in restricting subtypes, mirroring the loss of butyrate producers like *Roseburia* spp., which are key components of the microbiome (Borgo et al., 2017; Speranza et al., 2018). During nutritional rehabilitation, fecal acetate and butyrate typically increase alongside shifts in GM composition and a post-refeeding rise in Bacillota (Kleiman et al., 2015).

In sum, these collective findings suggest that GM alterations in AN both mirror and potentially contribute to the disorder, and that targeted interventions (e.g., dietary modulation, prebiotics, probiotics, and FMT) need evaluation as adjuncts to support long-term recovery.

9.3.2 Bulimia Nervosa

Research on GM alterations in bulimia nervosa (BN) is comparatively limited. A recent Mendelian randomization study reported nine microbial taxa with putative causal links to BN, with six (including Clostridiales) associated with increased risk and three (including Oxalobacteraceae) appearing protective (Yu et al., 2024).

One mechanistic pathway of interest involves microbial mimicry of host neuropeptides: bacterial proteins such as caseinolytic protease B (ClpB), produced by *Escherichia coli*, can mimic the anorexigenic peptide α-melanocyte-stimulating hormone (α-MSH) and trigger autoimmune responses (Fetissov et al., 2005, 2008) . ClpB is detectable in healthy individuals and ED patients but is elevated in the latter; concentrations are significantly higher in the latter; IgG autoantibodies against α-MSH have been found in both AN and BN patients, with distinct binding patterns that influence feeding behavior (Breton et al., 2016; Lucas et al., 2019). In BN, IgG/α-MSH complexes preferentially bind the C-terminal region of α-MSH (a configuration associated with increased hunger), whereas AN patients frequently exhibit N-terminal binding linked to satiety (Fetissov & Hökfelt, 2019). This “molecular switch” may contribute to diagnostic crossover

among AN, BN, and binge eating disorder (BED) via modulation of melanocortin signaling. Compensatory behaviors common in BN, like excessive exercise, self-induced vomiting, and laxative or diuretic use, also alter the GM and should be considered confounders in microbiome research. In detail, laxative use has been strongly associated with reduced microbial diversity, impacting both commensals and potential pathobionts (Igudesman et al., 2023). Some studies report decreased *Faecalibacterium prausnitzii* in BN patients, and supplementation with this species in murine models improved binge-like behavior (Fan et al., 2023), suggesting therapeutic potential. Overall, BN appears to involve a complex interplay between microbial, immune-mediated neuroendocrine pathways, and behavioral dynamics; however, existing evidence is fragmented, and prospective studies with larger, well-characterized cohorts are urgently needed.

9.3.3 Binge Eating Disorder

Growing evidence supports a bidirectional relationship between GM alterations and BED. Unstable eating patterns characteristic of BED can disrupt microbial communities, and dysbiosis in turn may strengthen binge eating via effects on appetite, food preferences, mood, and reward processing through metabolic, immune, hormonal, and neural pathways (Herman & Bajaka, 2021).

BED is frequently comorbid with obesity, a state marked by metabolic disturbances and chronic low-grade inflammation (Caroleo et al., 2019; Succurro et al., 2015).

Patterns observed in obesity (e.g., reduced alpha diversity and altered Bacillota/Bacteroidota ratios) are also reported in BED (Carbone et al., 2020), and high-fat diets further exacerbate these shifts by increasing Bacillota and Pseudomonadota while decreasing Bacteroidota, thereby promoting metabolic inflammation (Murphy et al., 2015). Lower alpha diversity is observed across BMI extremes and correlates with higher

depression scores, pointing to a microbiota–mood–behavior nexus (Mörkl et al., 2017).

Recent studies indicate that obese patients with BED may harbor distinct GM profiles compared with obese individuals without BED: reductions in *Akkermansia* spp. and *Intestinimonas* spp., alongside increases in *Bifidobacterium* spp., *Roseburia* spp., and *Anaerostipes* spp. have been documented (Leyrolle et al., 2021); these shifts are associated with reduced diversity and links to metabolic dysfunction (Barone et al., 2022).

Finally, animal models show enrichment of Bacteroidaceae and Lachnospiraceae and declines in Lactobacillaceae and Ruminococcaceae in binge-like eating, aligning with a transdiagnostic model in which dysbiosis, neuroinflammation, and neurotransmitter imbalances (notably serotonin) drive shifts across the ED spectrum (Fan et al., 2023).

From a neurobiological standpoint, BED is strongly influenced by stress hormones and neurotransmitters: HPA activation and cortisol release modulate appetite and energy intake, and BED subjects exhibit a higher propensity to binge after stress (Nasser et al., 2004). Neuroimaging studies demonstrate hyperactivation of reward circuitry to food cues in BED patients compared with non-BED patients (Schag et al., 2013).

The GM contributes an additional modulatory layer by producing or modulating serotonin, dopamine, noradrenaline, and acetylcholine (Mitra et al., 2023); with serotonin dysregulation being most consistently implicated in BN and BED (Dalley & Roiser, 2012; Kaye & Bailer, 2011). Fasting-induced reductions in serotonin are more pronounced in BN patients than in controls, potentially contributing to irritability and binge episodes (Steiger et al., 2001); furthermore, serotonergic abnormalities often persist in remission, suggesting trait-like dysfunction (Kaye, 2008). Supporting this, acute tryptophan depletion worsens mood and increases binge urges in BN patients (Bruce et al., 2009), and activation of serotonin receptor 5-HT2CR decreases binge behavior in animal models (Bruce et al., 2009). Given the strong GM role in serotonin biosynthesis, gut dysbiosis may reduce serotonin bioavailability in BED and BN (Rantala et al., 2019), reinforcing

maladaptive feedback loops between gut health, mood, and disordered eating.

Despite promising leads, the BED literature is heterogeneous due to methodological variability, small samples, and cross- sectional designs. Larger, longitudinal, and controlled studies are needed to rightly clarify causal relationships and to evaluate the use of the GM use as a diagnostic marker or therapeutic target.

9.3.4 Avoidant/Restrictive Food Intake Disorder

Avoidant/restrictive food intake disorder (ARFID) is characterized by restrictive eating that is not driven by body image concerns but by sensory sensitivities, low appetite, limited interest in food, or fear of adverse consequences (e.g., choking, vomiting). ARFID patients often consume diets low in fruits, vegetables, and protein, while being relatively enriched in sugars and refined carbohydrates (Harshman et al., 2019). Protein deficiency may limit tryptophan availability, constraining serotonin biosynthesis and thereby impacting both feeding behavior and mood regulation (Zhu et al., 2024).

Moreover, ARFID is usually associated with micronutrient deficiencies, especially of B vitamins, vitamin K, iron, and zinc, which are essential for both host physiology and microbial metabolism (Schmidt et al., 2021). Many commensals require B vitamins for SCFA production, creating a feedback loop in which insufficient SCFAs impair mineral absorption and perpetuate dysbiosis (Alexander et al., 2019).

Clinical features in ARFID, like early satiety and GI fullness, may mirror disrupted GBA signaling. Altered levels of appetite hormones (leptin, ghrelin, and CCK) have been observed in ARFID (Aulinas et al., 2020; Burton Murray et al., 2022a, b), and both SCFAs and tryptophan influence their secretion (Boscaini et al., 2022). Microbial tryptophan metabolism has been linked to hedonic food intake and reward-related brain activity, implicating GM-dependent pathways in both the homeostatic and hedonic components of ARFID (Osadchiy et al., 2018; Trevelline & Kohl,

2022). Sensory sensitivities commonly overlap with autism spectrum disorder (ASD), which frequently co-occurs in patients with ARFID (Sader et al., 2025); studies in children with ASD show that restrictive diets diminish microbial diversity and impair gut function, potentially contributing to overlapping behavioral phenotypes through Gut Microbiota (GM)–brain interactions (Hung & Margolis, 2024; Restrepo et al., 2025; Yap et al., 2021). In the ARFID subtype dominated by a fear of adverse consequences, maladaptive fear-learning mechanisms appear central: experimental models indicate that gut dysbiosis impairs fear responses, and human data suggest butyrate and other SCFAs modulate stress and fear regulation (Dalile et al., 2024; Hoban et al., 2018). These findings raise the prospect that GM-mediated regulation of emotional learning contributes to ARFID pathophysiology.

9.4 Clinical Treatment Approaches and Emerging Microbiome-Informed Perspectives

Current national and international guidelines consistently emphasize that the cornerstone of ED treatment is specialized, evidence-based psycho-behavioral therapy, most often delivered in outpatient settings (Hilbert et al., 2017; National Guideline, 2017). In more severe cases, or when outpatient care proves insufficient, higher levels of care, such as partial hospitalization or inpatient treatment within specialist services, may be required. Psychotherapeutic interventions delivered by clinicians with specific ED expertise show the strongest efficacy, being associated with lower hospitalization rates and greater cost-effectiveness (Hay et al., 2019). Given the complex nutritional, psychological, and medical dimensions of EDs, effective treatment requires a multidisciplinary approach; and the core teams typically include psychotherapists, psychiatrists, dietitians, and primary care physicians, while more complex cases may require additional collaborations (e.g., with pediatricians, specialist physicians, nurses, occupational therapists, social workers, and family therapists). Illness

severity and clinical complexity guide the care level and referral urgency (Monteleone et al., 2019).

Among psychological treatments, cognitive behavioral therapy-enhanced (CBT-E) is the most established and effective transdiagnostic intervention and is generally delivered over several months (Zipfel et al., 2014). Despite growing interest in the role of the GBA and GM in ED pathophysiology, current clinical guidelines do not yet incorporate GM-targeted strategies. Nutritional rehabilitation therefore remains a central treatment pillar, principally in restrictive EDs such as AN. Primary goals include reversing malnutrition, restoring hunger and satiety cues, normalizing eating behaviors, and re-establishing physiological functioning. Early and rapid weight gain is one of the strongest predictors of recovery in AN, supporting the use of assertive refeeding protocols that often rely on high-calorie, high-fat diets due to their caloric density (Peebles et al., 2017; Smith et al., 2016).

As widely documented, diet composition strongly influences GM structure. Diets high in saturated fat and low in fiber are associated with reduced microbial diversity and dysbiosis, whereas diets rich in unsaturated fats (mainly those from olive oil and fish) are linked to more favorable microbial profiles (De Filippo et al., 2010; Fava et al., 2013). Refeeding in AN tends to increase microbial diversity and partially restores GM composition; however, post-treatment microbial signatures often remain distinct from those of healthy controls (Kleiman et al., 2015; Mack et al., 2016). This persistence suggests that nutritional rehabilitation may benefit from integrating GM-supportive strategies, such as optimizing fiber intake, or incorporating prebiotics, or making targeted dietary adjustments.

ARFID may similarly be conceptualized as a GBA interaction disorder, potentially influenced by some of the same GM-behavior mechanisms observed in AN (Burton Murray et al., 2022a, b); however, no studies have specifically examined GM modulation in ARFID.

Of note, two longitudinal studies in human AN provide insight into GM changes during refeeding. Both observed increases in microbial diversity, yet post-treatment communities remained significantly different from those

of healthy controls. Increases in *Ruminococcus* spp. were noted, likely reflecting higher fiber intake (Ze et al., 2012), while one study showed a shift toward Bacillota and a decrease in Bacteroidota, a pattern often associated with high-fat diets (Murphy et al., 2015). Nutritional rehabilitation can be provided through tailored meals, oral supplements, or nasogastric feeding (Kells & Kelly-Weeder, 2016). Enteral formulas, however, may negatively affect the GM due to their low fiber content and the presence of emulsifiers (Krezalek et al., 2017), although probiotics may help reduce associated inflammation (Xie et al., 2018). Parenteral nutrition appears even more disruptive to the GM, although its effects have not been studied specifically in AN patients (Pierre, 2017).

Regarding symptomatology, up to 90% of ED patients report GI discomfort (Schalla & Stengel, 2019). While weight gain tends to improve lower GI symptoms such as constipation, upper GI symptoms, like bloating and early satiety, often persist. Diets higher in fiber and unsaturated fats may support beneficial microbial populations and improve GI symptoms, but they must be introduced gradually to avoid exacerbating satiety or discomfort (Mack et al., 2016).

Although no refeeding protocols explicitly aim to modulate the GM, evidence suggests that GM-supportive diets should emphasize diverse fibers, nondigestible carbohydrates, and healthy fats while limiting saturated fats (Singh et al., 2017).

At the same time, therapeutic exposure to “feared” or trigger foods remains a core component of ED treatment and must be preserved. The gradual, low-volume introduction of prebiotic-rich foods may promote GM restoration without compromising caloric adequacy, satiety tolerance, or treatment adherence. Given the substantial interindividual variability in GM composition, personalized refeeding strategies may become increasingly relevant.

More specifically, integrating GM–supportive strategies into nutritional rehabilitation may ultimately provide a valuable supplement to existing ED interventions (Yang et al., 2021).

Prebiotic administration has been shown to improve GM diversity and quality of life in tube-fed individuals, suggesting potential relevance for ED refeeding contexts (Wierdsma et al., 2009). Psychobiotics, which are probiotics with documented mental health benefits, may also offer therapeutic value by decreasing anxiety and depressive symptoms, although more research is needed before their incorporation into standard ED care (Huang et al., 2016; Pirbaglou et al., 2016).

In adolescents with AN, supplementation with *Lactobacillus reuteri* DSM17938 improved BMI, weight gain trajectories, and stool regularity (Žaja et al., 2021). Case-based evidence also indicates the potential efficacy of FMT: in one patient with recurrent AN, FMT from a healthy donor led to increased body weight, fat mass, and microbial diversity over 36 weeks, potentially via enhanced SCFA production (de Clercq et al., 2019). Ongoing trials are currently evaluating whether FMT can restore GM composition and improve clinical outcomes in AN (Wilson et al., 2023). Other adjuncts, such as PUFA supplementation, may also modulate the GM and support improvements in weight and BMI (Keller et al., 2022). SCFA supplementation is being explored as well, based on the hypothesis that these molecules may promote less restrictive eating patterns.

Given known alterations in reward processing in patients with AN, GM-targeted interventions that influence dopaminergic and serotonergic pathways represent another promising, yet insufficiently validated, therapeutic direction.

Beyond AN, preclinical data suggest that GM modulation may offer therapeutic opportunities in BN and BED, mainly by influencing reward-related behaviors. For example, *Bacteroides uniformis* administration reduced binge-like eating in a rodent BED model, accompanied by the normalization of dopamine signaling in key reward regions (Agustí et al., 2021). Similarly, *Faecalibacterium prausnitzii* supplementation has been shown to diminish the intake of hyperpalatable foods, and *Lactobacillus johnsonii* reduced sucrose bingeing in antibiotic-treated mice (Ousey et al., 2023).

Finally, the limited human evidence is also encouraging: in a randomized controlled trial involving individuals with obesity undergoing gastric bypass surgery, supplementation with *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 led to significant reductions in both binge-eating and food-addiction scores one year post-surgery (Carlos et al., 2022).

In summary, these findings point toward the GM as a promising adjunctive target for addressing maladaptive eating behaviors and reward dysregulation in EDs; however, major challenges remain, including establishing causality in human populations, identifying specific strains and mechanisms, scaling interventions for clinical use, and accounting for the GM variability that may shape treatment response.

Given the expanding knowledge surrounding GM-informed care in EDs, dietitians with specialized training are uniquely positioned to integrate GM-supportive strategies into individualized treatment plans. These approaches may aim to enhance microbial diversity, reduce GI distress, and help restore appetite-related GBA signaling. Incorporating such treatments not only reinforces the core objectives of nutritional rehabilitation but may also improve tolerance to refeeding protocols, reduce GI-related barriers to treatment adherence, and support long-term recovery trajectories. In this context, the dietitian's role extends beyond ensuring nutritional adequacy to delivering personalized, GM-informed interventions within a coordinated multidisciplinary care framework (Heruc et al., 2020; Yang et al., 2021).

9.5 Conclusions

Overall, EDs exhibit both disorder-specific and transdiagnostic alterations in GM composition and function. Research in AN is the most extensive and consistently demonstrates reduced microbial diversity and the depletion of SCFA-producing bacterial taxa, whereas evidence for BN, BED, and ARFID is emerging but remains heterogeneous. An overall and adequate evaluation across studies is complicated by methodological variability,

including differences in diagnostic criteria, small and cross-sectional samples, dietary confounders, medication effects, and compensatory behaviors.

To advance the field, several priorities are critical: standardized sampling and analytic pipelines; well-powered longitudinal cohorts; integrated multi-omics approaches (metagenomics, metatranscriptomics, metabolomics); rigorous control for dietary and behavioral factors; and mechanistic translational studies using germ-free models, FMT, and targeted microbial interventions. These steps will be essential for determining whether GM-directed strategies can meaningfully support recovery and improve long-term outcomes in EDs.

In sum, the GM represents a promising avenue for augmenting conventional ED treatments by addressing biological, metabolic, and gut–brain pathways that are not fully restored through refeeding and psychotherapy alone. However, its translation into routine clinical care will require high-quality mechanistic and clinical research to clarify causal relationships, identify effective interventions, and determine which patients are most likely to benefit. If these challenges are met, GM-targeted approaches may become valuable components of personalized, integrative treatment paradigms that enhance both short-term recovery and the long-term prognosis for individuals with EDs.

References

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., Timmerman, H. M., … Arias Vasquez, A. (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One*, *12*(9), e0183509. doi:10.1371/journal.pone.0183509 ⏎

Agustí, A., Campillo, I., Balzano, T., Benítez-Páez, A., López-Almela, I., Romaní-Pérez, M., … Sanz, Y. (2021). Bacteroides uniformis CECT 7771 modulates the brain reward response to reduce binge eating and

anxiety-like behavior in rat. *Mol Neurobiol*, *58*(10), 4959–4979. doi:10.1007/s12035-021-02462-2 ⏎

Alexander, C., Swanson, K. S., Fahey, G. C., & Garleb, K. A. (2019). Perspective: physiologic importance of short-chain fatty acids from nondigestible carbohydrate fermentation. *Adv Nutr*, *10*(4), 576–589. doi:10.1093/advances/nmz004 ⏎

Andreani, N. A., Sharma, A., Dahmen, B., Specht, H. E., Mannig, N., Ruan, V., … Seitz, J. (2024). Longitudinal analysis of the gut microbiome in adolescent patients with anorexia nervosa: microbiome-related factors associated with clinical outcome. *Gut Microbes*, *16*(1), 2304158. doi:10.1080/19490976.2024.2304158 ⏎

Armougom, F., Henry, M., Vialettes, B., Raccah, D., & Raoult, D. (2009). Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and Methanogens in anorexic patients. *PLoS One*, *4*(9), e7125. doi:10.1371/journal.pone.0007125 ⏎

Aulinas, A., Marengi, D. A., Galbiati, F., Asanza, E., Slattery, M., Mancuso, C. J., … Lawson, E. A. (2020). Medical comorbidities and endocrine dysfunction in low-weight females with avoidant/restrictive food intake disorder compared to anorexia nervosa and healthy controls. *Int J Eat Disord*, *53*(4), 631–636. doi:10.1002/eat.23261 ⏎

Azevedo, E. P., Ivan, V. J., Friedman, J. M., & Stern, S. A. (2022). Higher-order inputs involved in appetite control. *Biol Psychiatry*, *91*(10), 869–878. doi:10.1016/j.biopsych.2021.07.015 ⏎

Azevedo, E. P., Pomeranz, L., Cheng, J., Schneeberger, M., Vaughan, R., Stern, S. A., … Friedman, J. M. (2019). A role of Drd2 hippocampal neurons in context-dependent food intake. *Neuron*, *102*(4), 873–886.e875. doi:10.1016/j.neuron.2019.03.011 ⏎

Barone, M., Garelli, S., Rampelli, S., Agostini, A., Matysik, S., D'Amico, F., … Turroni, S. (2022). Multi-omics gut microbiome signatures in obese women: role of diet and uncontrolled eating behavior. *BMC Med*, *20*(1), 500. doi:10.1186/s12916-022-02689-3 ⏎

Beam, A., Clinger, E., & Hao, L. (2021). Effect of diet and dietary components on the composition of the gut microbiota. *Nutrients*, *13*(8), 2795. doi:10.3390/nu13082795 ⏎

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol*, *9*(1), 65–73. doi:10.1016/j.coph.2008.12.014 ⏎

Borgo, F., Riva, A., Benetti, A., Casiraghi, M. C., Bertelli, S., Garbossa, S., … Borghi, E. (2017). Microbiota in anorexia nervosa: the triangle between bacterial species, metabolites and psychological tests. *PLoS One*, *12*(6), e0179739. doi:10.1371/journal.pone.0179739 ⏎

Boscaini, S., Leigh, S. J., Lavelle, A., García-Cabrerizo, R., Lipuma, T., Clarke, G., … Cryan, J. F. (2022). Microbiota and body weight control: weight watchers within? *Mol Metab*, *57*, 101427. doi:10.1016/j.molmet.2021.101427 ⏎

Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus nerve as modulator of the brain–gut axis in psychiatric and inflammatory disorders. *Front Psychiatry*, *9*, 44. doi:10.3389/fpsyt.2018.00044 ⏎

Breton, J., Legrand, R., Akkermann, K., Järv, A., Harro, J., Déchelotte, P., & Fetissov, S. O. (2016). Elevated plasma concentrations of bacterial ClpB protein in patients with eating disorders. *Int J Eat Disord*, *49*(8), 805–808. doi:10.1002/eat.22531 ⏎

Browning, K. N., Verheijden, S., & Boeckxstaens, G. E. (2017). The vagus nerve in appetite regulation, mood, and intestinal inflammation. *Gastroenterology*, *152*(4), 730–744. doi:10.1053/j.gastro.2016.10.046 ⏎

Bruce, K. R., Steiger, H., Young, S. N., Kin, N. M., Israël, M., & Lévesque, M. (2009). Impact of acute tryptophan depletion on mood and eating-related urges in bulimic and nonbulimic women. *J Psychiatry Neurosci*, *34*(5), 376–382. ⏎

Burton Murray, H., Becker, K. R., Harshman, S., Breithaupt, L., Kuhnle, M., Dreier, M. J., … Lawson, E. A. (2022a). Elevated fasting satiety-promoting cholecystokinin (CCK) in avoidant/restrictive food intake

disorder compared to healthy controls. *J Clin Psychiatry*, *83*(5), 21m14111. doi:10.4088/JCP.21m14111 ⏎

Burton Murray, H., Riddle, M., Rao, F., McCann, B., Staller, K., Heitkemper, M., & Zia, J. (2022b). Eating disorder symptoms, including avoidant/restrictive food intake disorder, in patients with disorders of gut–brain interaction. *Neurogastroenterol Motil*, *34*(8), e14258. doi:10.1111/nmo.14258

Byrne, C. S., Chambers, E. S., Alhabeeb, H., Chhina, N., Morrison, D. J., Preston, T., … Frost, G. S. (2016). Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *Am J Clin Nutr*, *104*(1), 5–14. doi:10.3945/ajcn.115.126706 ⏎

Campos, A. C., Rocha, N. P., Nicoli, J. R., Vieira, L. Q., Teixeira, M. M., & Teixeira, A. L. (2016). Absence of gut microbiota influences lipopolysaccharide-induced behavioral changes in mice. *Behav Brain Res*, *312*, 186–194. doi:10.1016/j.bbr.2016.06.027 ⏎

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut–brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol*, *28*(2), 203–209. ⏎

Carbone, E. A., D'Amato, P., Vicchio, G., De Fazio, P., & Segura-Garcia, C. (2020). A systematic review on the role of microbiota in the pathogenesis and treatment of eating disorders. *Eur Psychiatry*, *64*(1), e2. doi:10.1192/j.eurpsy.2020.109 ⏎

Carlos, L. O., Ramos, M. R. Z., Wagner, N. R. F., Freitas, L. A. C., Felicidade, I., & Campos, A. C. L. (2022). Probiotic supplementation attenuates binge eating and food addiction 1 year after roux-en-y gastric bypass: a randomized, double-blind, placebo-controlled trial. *Arq Bras Cir Dig*, *35*, e1659. doi:10.1590/0102-672020210002e1659 ⏎

Caroleo, M., Carbone, E. A., Greco, M., Corigliano, D. M., Arcidiacono, B., Fazia, G., … Brunetti, A. (2019). Brain–behavior–immune interaction: serum cytokines and growth factors in patients with eating disorders at extremes of the body mass index (BMI) spectrum. *Nutrients*, *11*(9), 1995. doi:10.3390/nu11091995 ⏎

Choe, U. (2025). Role of dietary fiber and short-chain fatty acids in preventing neurodegenerative diseases through the gut–brain axis. *J Funct Foods*, *129*, 106870. doi:https://doi.org/10.1016/j.jff.2025.106870 ⏎

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*, *13*(10), 701–712. doi:10.1038/nrn3346 ⏎

Dalile, B., Fuchs, A., La Torre, D., Vervliet, B., Van Oudenhove, L., & Verbeke, K. (2024). Colonic butyrate administration modulates fear memory but not the acute stress response in men: a randomized, triple-blind, placebo-controlled trial. *Prog Neuropsychopharmacol Biol Psychiatry*, *131*, 110939. doi:10.1016/j.pnpbp.2024.110939 ⏎

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience*, *215*, 42–58. doi:10.1016/j.neuroscience.2012.03.065 ⏎

de Clercq, N. C., Frissen, M. N., Davids, M., Groen, A. K., & Nieuwdorp, M. (2019). Weight gain after fecal microbiota transplantation in a patient with recurrent underweight following clinical recovery from anorexia nervosa. *Psychother Psychosom*, *88*(1), 58–60. doi:10.1159/000495044 ⏎

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., … Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A*, *107*(33), 14691–14696. doi:10.1073/pnas.1005963107 ⏎

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*, *54*(9), 2325–2340. doi:10.1194/jlr.R036012 ⏎

Di Lodovico, L., Mondot, S., Doré, J., Mack, I., Hanachi, M., & Gorwood, P. (2021). Anorexia nervosa and gut microbiota: a systematic review and quantitative synthesis of pooled microbiological data. *Prog*

Neuropsychopharmacol Biol Psychiatry, *106*, 110114. doi:10.1016/j.pnpbp.2020.110114 ⏎

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci*, *18*(7), 965–977. doi:10.1038/nn.4030 ⏎

Fan, S., Guo, W., Xiao, D., Guan, M., Liao, T., Peng, S., … Xiong, W. (2023). Microbiota–gut–brain axis drives overeating disorders. *Cell Metab*, *35*(11), 2011–2027.e2017. doi:10.1016/j.cmet.2023.09.005 ⏎

Fava, F., Gitau, R., Griffin, B. A., Gibson, G. R., Tuohy, K. M., & Lovegrove, J. A. (2013). The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. *Int J Obes (Lond)*, *37*(2), 216–223. doi:10.1038/ijo.2012.33 ⏎

Felger, J. C., & Treadway, M. T. (2017). Inflammation effects on motivation and motor activity: role of dopamine. *Neuropsychopharmacology*, *42*(1), 216–241. doi:10.1038/npp.2016.143 ⏎

Feng, L., Marquina-Solis, J., Yue, L., Harnagel, A., Greenfeld, Y., & Bargmann, C. I. (2025). Context-dependent serotonin signaling links dietary quality to foraging decisions. *Nat Commun*, *16*(1), 10479. doi:10.1038/s41467-025-65491-8 ⏎

Fetissov, S. O., Hamze Sinno, M., Coëffier, M., Bole-Feysot, C., Ducrotté, P., Hökfelt, T., & Déchelotte, P. (2008a). Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora. *Nutrition*, *24*(4), 348–359. doi:10.1016/j.nut.2007.12.006 ⏎

Fetissov, S. O., Hamze Sinno, M., Coquerel, Q., Do Rego, J. C., Coëffier, M., Gilbert, D., … Déchelotte, P. (2008b). Emerging role of autoantibodies against appetite-regulating neuropeptides in eating disorders. *Nutrition*, *24*(9), 854–859. doi:10.1016/j.nut.2008.06.021

Fetissov, S. O., Harro, J., Jaanisk, M., Järv, A., Podar, I., Allik, J., … Hökfelt, T. (2005). Autoantibodies against neuropeptides are associated with psychological traits in eating disorders. *Proc Natl Acad Sci U S A*, *102*(41), 14865–14870. doi:10.1073/pnas.0507204102

Fetissov, S. O., & Hökfelt, T. (2019). On the origin of eating disorders: altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. *Curr Opin Pharmacol*, *48*, 82–91. doi:10.1016/j.coph.2019.07.004 ⏎

Gacias, M., Gaspari, S., Santos, P. M., Tamburini, S., Andrade, M., Zhang, F., … Casaccia, P. (2016). Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *Elife*, *5*, e13442. doi:10.7554/eLife.13442 ⏎

García-Cabrerizo, R., Carbia, C., KJ, O. R., Schellekens, H., & Cryan, J. F. (2021). Microbiota–gut–brain axis as a regulator of reward processes. *J Neurochem*, *157*(5), 1495–1524. doi:10.1111/jnc.15284 ⏎

Gouba, N., Raoult, D., & Drancourt, M. (2014). Gut microeukaryotes during anorexia nervosa: a case report. *BMC Research Notes*, *7*(1), 33. doi:10.1186/1756-0500-7-33 ⏎

Grill, H. J., & Hayes, M. R. (2012). Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. *Cell Metab*, *16*(3), 296–309. doi:10.1016/j.cmet.2012.06.015 ⏎

Gupta, A., Osadchiy, V., & Mayer, E. A. (2020). Brain–gut–microbiome interactions in obesity and food addiction. *Nat Rev Gastroenterol Hepatol*, *17*(11), 655–672. doi:10.1038/s41575-020-0341-5 ⏎

Hanachi, M., Manichanh, C., Schoenenberger, A., Pascal, V., Levenez, F., Cournède, N., … Melchior, J. C. (2019). Altered host-gut microbes symbiosis in severely malnourished anorexia nervosa (AN) patients undergoing enteral nutrition: An explicative factor of functional intestinal disorders? *Clin Nutr*, *38*(5), 2304–2310. doi:10.1016/j.clnu.2018.10.004 ⏎

Harshman, S. G., Wons, O., Rogers, M. S., Izquierdo, A. M., Holmes, T. M., Pulumo, R. L., … Thomas, J. J. (2019). A diet high in processed

foods, total carbohydrates and added sugars, and low in vegetables and protein is characteristic of youth with avoidant/restrictive food intake disorder. *Nutrients*, *11*(9), 2013. doi:10.3390/nu11092013 ⏎

Hay, P. J., Touyz, S., Claudino, A. M., Lujic, S., Smith, C. A., & Madden, S. (2019). Inpatient versus outpatient care, partial hospitalisation and waiting list for people with eating disorders. *Cochrane Database Syst Rev*, *1*(1), Cd010827. doi:10.1002/14651858.CD010827.pub2 ⏎

Herman, A., & Bajaka, A. (2021). The role of the intestinal microbiota in eating disorders—bulimia nervosa and binge eating disorder. *Psychiatry Res*, *300*, 113923. doi:10.1016/j.psychres.2021.113923 ⏎

Heruc, G., Hart, S., Stiles, G., Fleming, K., Casey, A., Sutherland, F., … Hurst, K. (2020). ANZAED practice and training standards for dietitians providing eating disorder treatment. *J Eat Disord*, *8*(1), 77. doi:10.1186/s40337-020-00334-z ⏎

Hilbert, A., Hoek, H. W., & Schmidt, R. (2017). Evidence-based clinical guidelines for eating disorders: international comparison. *Curr Opin Psychiatry*, *30*(6), 423–437. doi:10.1097/yco.0000000000000360 ⏎

Hoban, A. E., Stilling, R. M., Moloney, G., Shanahan, F., Dinan, T. G., Clarke, G., & Cryan, J. F. (2018). The microbiome regulates amygdala-dependent fear recall. *Mol Psychiatry*, *23*(5), 1134–1144. doi:10.1038/mp.2017.100 ⏎

Hoban, A. E., Stilling, R. M., Ryan, F. J., Shanahan, F., Dinan, T. G., Claesson, M. J., … Cryan, J. F. (2016). Regulation of prefrontal cortex myelination by the microbiota. *Transl Psychiatry*, *6*(4), e774. doi:10.1038/tp.2016.42 ⏎

Howland, R. H. (2014). Vagus nerve stimulation. *Curr Behav Neurosci Rep*, *1*(2), 64–73. doi:10.1007/s40473-014-0010-5 ⏎

Huang, R., Wang, K., & Hu, J. (2016). Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. *Nutrients*, *8*(8), 483. doi:10.3390/nu8080483 ⏎

Hung, L. Y., & Margolis, K. G. (2024). Autism spectrum disorders and the gastrointestinal tract: insights into mechanisms and clinical relevance.

Nat Rev Gastroenterol Hepatol, *21*(3), 142–163. doi:10.1038/s41575-023-00857-1 ⏎

Igudesman, D., Abbaspour, A., Reed, K. K., Flatt, R. E., Becken, B., Thornton, L. M., … Carroll, I. M. (2023). Laxative abuse is associated with a depleted gut microbial community structure among women and men with binge-eating disorder or bulimia nervosa: the binge eating genetics initiative. *Psychosom Med*, *85*(8), 727–735. doi:10.1097/psy.0000000000001226 ⏎

Jadhav, K. S., Peterson, V. L., Halfon, O., Ahern, G., Fouhy, F., Stanton, C., … Boutrel, B. (2018). Gut microbiome correlates with altered striatal dopamine receptor expression in a model of compulsive alcohol seeking. *Neuropharmacology*, *141*, 249–259. doi:10.1016/j.neuropharm.2018.08.026 ⏎

Jennings, J. H., Kim, C. K., Marshel, J. H., Raffiee, M., Ye, L., Quirin, S., … Deisseroth, K. (2019). Interacting neural ensembles in orbitofrontal cortex for social and feeding behaviour. *Nature*, *565*(7741), 645–649. doi:10.1038/s41586-018-0866-8 ⏎

Kawase, T., Nagasawa, M., Ikeda, H., Yasuo, S., Koga, Y., & Furuse, M. (2017). Gut microbiota of mice putatively modifies amino acid metabolism in the host brain. *Br J Nutr*, *117*(6), 775–783. doi:10.1017/s0007114517000678 ⏎

Kaye, W. (2008). Neurobiology of anorexia and bulimia nervosa. *Physiol Behav*, *94*(1), 121–135. doi:10.1016/j.physbeh.2007.11.037 ⏎

Kaye, W. H., & Bailer, U. F. (2011). Understanding the neural circuitry of appetitive regulation in eating disorders. *Biol Psychiatry*, *70*(8), 704–705. doi:10.1016/j.biopsych.2011.08.018 ⏎

Keller, L., Dempfle, A., Dahmen, B., Schreiber, S., Adan, R. A. H., Andreani, N. A., … Herpertz-Dahlmann, B. (2022). The effects of polyunsaturated fatty acid (PUFA) administration on the microbiome-gut–brain axis in adolescents with anorexia nervosa (the MiGBAN study): study protocol for a longitudinal, double-blind, randomized,

placebo-controlled trial. *Trials*, *23*(1), 545. doi:10.1186/s13063-022-06413-7 ⏎

Kells, M., & Kelly-Weeder, S. (2016). Nasogastric tube feeding for individuals with anorexia nervosa: an integrative review. *J Am Psychiatr Nurses Assoc*, *22*(6), 449–468. doi:10.1177/1078390316657872 ⏎

Kleiman, S. C., Watson, H. J., Bulik-Sullivan, E. C., Huh, E. Y., Tarantino, L. M., Bulik, C. M., & Carroll, I. M. (2015). The intestinal microbiota in acute anorexia nervosa and during renourishment: relationship to depression, anxiety, and eating disorder psychopathology. *Psychosom Med*, *77*(9), 969–981. doi:10.1097/psy.0000000000000247 ⏎

Krezalek, M. A., Yeh, A., Alverdy, J. C., & Morowitz, M. (2017). Influence of nutrition therapy on the intestinal microbiome. *Curr Opin Clin Nutr Metab Care*, *20*(2), 131–137. doi:10.1097/mco.0000000000000348 ⏎

Lam, Y. Y., Maguire, S., Palacios, T., & Caterson, I. D. (2017). Are the gut bacteria telling us to eat or not to eat? Reviewing the role of gut microbiota in the etiology, disease progression and treatment of eating disorders. *Nutrients*, *9*(6), 602. doi:10.3390/nu9060602 ⏎

Lasselin, J., Treadway, M. T., Lacourt, T. E., Soop, A., Olsson, M. J., Karshikoff, B., … Lekander, M. (2017). Lipopolysaccharide alters motivated behavior in a monetary reward task: a randomized trial. *Neuropsychopharmacology*, *42*(4), 801–810. doi:10.1038/npp.2016.191 ⏎

Leyrolle, Q., Cserjesi, R., Mulders, M., Zamariola, G., Hiel, S., Gianfrancesco, M. A., … Delzenne, N. M. (2021). Specific gut microbial, biological, and psychiatric profiling related to binge eating disorders: a cross-sectional study in obese patients. *Clin Nutr*, *40*(4), 2035–2044. doi:10.1016/j.clnu.2020.09.025 ⏎

Li, S., Liu, M., Cao, S., Liu, B., Li, D., Wang, Z., … Shi, Y. (2023). The mechanism of the gut–brain axis in regulating food intake. *Nutrients*, *15*(17), 3728. doi:10.3390/nu15173728 ⏎

Liu, C. M., & Kanoski, S. E. (2018). Homeostatic and non- homeostatic controls of feeding behavior: distinct vs. common neural systems.

Physiol Behav, *193*(Pt B), 223–231. doi:10.1016/j.physbeh.2018.02.011 ⏎

Liu, R., Wei, P., Keller, C., Orefice, N. S., Shi, Y., Li, Z., … Li, L. (2020). Integrated label-free and 10-Plex DiLeu isobaric tag quantitative methods for profiling changes in the mouse hypothalamic neuropeptidome and proteome: assessment of the impact of the gut microbiome. *Anal Chem*, *92*(20), 14021–14030. doi:10.1021/acs.analchem.0c02939 ⏎

Liu, W. H., Chuang, H. L., Huang, Y. T., Wu, C. C., Chou, G. T., Wang, S., & Tsai, Y. C. (2016). Alteration of behavior and monoamine levels attributable to *Lactobacillus plantarum* PS128 in germ-free mice. *Behav Brain Res*, *298*(Pt B), 202–209. doi:10.1016/j.bbr.2015.10.046 ⏎

Lotan, D., Cunningham, M., & Joel, D. (2014). Antibiotic treatment attenuates behavioral and neurochemical changes induced by exposure of rats to group a streptococcal antigen. *PLoS One*, *9*(6), e101257. doi:10.1371/journal.pone.0101257 ⏎

Lucas, N., Legrand, R., Bôle-Feysot, C., Breton, J., Coëffier, M., Akkermann, K., … Fetissov, S. O. (2019). Immunoglobulin G modulation of the melanocortin 4 receptor signaling in obesity and eating disorders. *Transl Psychiatry*, *9*(1), 87. doi:10.1038/s41398-019-0422-9 ⏎

Lucka, A., & Wysokiński, A. (2019). Association between adiposity and fasting serum levels of appetite-regulating peptides: leptin, neuropeptide Y, desacyl ghrelin, peptide YY(1-36), obestatin, cocaine and amphetamine-regulated transcript, and agouti-related protein in nonobese participants. *Chin J Physiol*, *62*(5), 217–225. doi:10.4103/cjp.Cjp_29_19 ⏎

Mack, I., Cuntz, U., Grämer, C., Niedermaier, S., Pohl, C., Schwiertz, A., … Penders, J. (2016). Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints. *Sci Rep*, *6*, 26752. doi:10.1038/srep26752 ⏎

Maschek, S., Østergaard, T. H., Krych, L., Zachariassen, L. F., Sørensen, D. B., Junker Mentzel, C. M., … Barfod, K. K. (2025). Investigating fecal

microbiota transplants from individuals with anorexia nervosa in antibiotic-treated mice using a cross-over study design. *J Eat Disord*, *13*(1), 82. doi:10.1186/s40337-025-01276-0 ⏎

Mitra, S., Dash, R., Nishan, A. A., Habiba, S. U., & Moon, I. S. (2023). Brain modulation by the gut microbiota: from disease to therapy. *J Adv Res*, *53*, 153–173. doi:10.1016/j.jare.2022.12.001 ⏎

Monteleone, A. M., Fernandez-Aranda, F., & Voderholzer, U. (2019). Evidence and perspectives in eating disorders: a paradigm for a multidisciplinary approach. *World Psychiatry*, *18*(3), 369–370. doi:10.1002/wps.20687 ⏎

Moris, J. M., Heinold, C., Blades, A., & Koh, Y. (2022). Nutrient-based appetite regulation. *J Obes Metab Syndr*, *31*(2), 161–168. doi:10.7570/jomes22031 ⏎

Morita, C., Tsuji, H., Hata, T., Gondo, M., Takakura, S., Kawai, K., … Sudo, N. (2015). Gut dysbiosis in patients with anorexia nervosa. *PLoS One*, *10*(12), e0145274. doi:10.1371/journal.pone.0145274 ⏎

Mörkl, S., Lackner, S., Müller, W., Gorkiewicz, G., Kashofer, K., Oberascher, A., … Holasek, S. (2017). Gut microbiota and body composition in anorexia nervosa inpatients in comparison to athletes, overweight, obese, and normal weight controls. *Int J Eat Disord*, *50*(12), 1421–1431. doi:10.1002/eat.22801 ⏎

Moura-Assis, A., Friedman, J. M., & Velloso, L. A. (2021). Gut-to-brain signals in feeding control. *Am J Physiol Endocrinol Metab*, *320*(2), E326–e332. doi:10.1152/ajpendo.00388.2020 ⏎

Murphy, E. A., Velazquez, K. T., & Herbert, K. M. (2015). Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care*, *18*(5), 515–520. doi:10.1097/mco.0000000000000209 ⏎

Nasser, J. A., Gluck, M. E., & Geliebter, A. (2004). Impulsivity and test meal intake in obese binge eating women. *Appetite*, *43*(3), 303–307. doi:10.1016/j.appet.2004.04.006 ⏎

National Guideline, A. (2017). National Institute for Health and Care Excellence: Guidelines. In *Eating Disorders: Recognition and Treatment*. London: National Institute for Health and Care Excellence (NICE) Copyright © National Institute for Health and Care Excellence. ⏎

Ntranos, A., & Casaccia, P. (2018). The microbiome-gut-behavior axis: crosstalk between the gut microbiome and oligodendrocytes modulates behavioral responses. *Neurotherapeutics*, *15*(1), 31–35. doi:10.1007/s13311-017-0597-9 ⏎

Osadchiy, V., Labus, J. S., Gupta, A., Jacobs, J., Ashe-McNalley, C., Hsiao, E. Y., & Mayer, E. A. (2018). Correlation of tryptophan metabolites with connectivity of extended central reward network in healthy subjects. *PLoS One*, *13*(8), e0201772. doi:10.1371/journal.pone.0201772 ⏎

Ousey, J., Boktor, J. C., & Mazmanian, S. K. (2023). Gut microbiota suppress feeding induced by palatable foods. *Curr Biol*, *33*(1), 147–157.e147. doi:10.1016/j.cub.2022.10.066 ⏎

Parnell, J. A., & Reimer, R. A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr*, *89*(6), 1751–1759. doi:10.3945/ajcn.2009.27465 ⏎

Peebles, R., Lesser, A., Park, C. C., Heckert, K., Timko, C. A., Lantzouni, E., … Weaver, L. (2017). Outcomes of an inpatient medical nutritional rehabilitation protocol in children and adolescents with eating disorders. *J Eat Disord*, *5*, 7. doi:10.1186/s40337-017-0134-6 ⏎

Pierre, J. F. (2017). Gastrointestinal immune and microbiome changes during parenteral nutrition. *Am J Physiol Gastrointest Liver Physiol*, *312*(3), G246–g256. doi:10.1152/ajpgi.00321.2016 ⏎

Pirbaglou, M., Katz, J., de Souza, R. J., Stearns, J. C., Motamed, M., & Ritvo, P. (2016). Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. *Nutr Res*, *36*(9), 889–898. doi:10.1016/j.nutres.2016.06.009 ⏎

Plassmann, H., Schelski, D. S., Simon, M. C., & Koban, L. (2022). How we decide what to eat: toward an interdisciplinary model of gut–brain

interactions. *Wiley Interdiscip Rev Cogn Sci*, *13*(1), e1562. doi:10.1002/wcs.1562 ⏎

Qi, X., Zhong, X., Xu, S., Zeng, B., Chen, J., Zang, G., … Xie, P. (2020). Extracellular matrix and oxidative phosphorylation: important role in the regulation of hypothalamic function by gut microbiota. *Front Genet*, *11*, 520. doi:10.3389/fgene.2020.00520 ⏎

Queipo-Ortuño, M. I., Seoane, L. M., Murri, M., Pardo, M., Gomez-Zumaquero, J. M., Cardona, F., … Tinahones, F. J. (2013). Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One*, *8*(5), e65465. doi:10.1371/journal.pone.0065465 ⏎

Ralevski, E., Shanabrough, M., Newcomb, J., Gandelman, E., Hayden, R., Horvath, T. L., & Petrakis, I. (2018). Ghrelin is related to personality differences in reward sensitivity and impulsivity. *Alcohol Alcohol*, *53*(1), 52–56. doi:10.1093/alcalc/agx082 ⏎

Rantala, M. J., Luoto, S., Krama, T., & Krams, I. (2019). Eating disorders: an evolutionary psychoneuroimmunological approach. *Front Psychol*, *10*, 2200. doi:10.3389/fpsyg.2019.02200 ⏎

Restrepo, B., Taylor, S. L., Dominic Ponzini, M., Angkustsiri, K., Solomon, M., Rogers, S. J., … Wu Nordahl, C. (2025). A longitudinal evaluation of gastrointestinal symptoms in children with autism spectrum disorder. *Autism*, *29*(11), 2832–2845. doi:10.1177/13623613251362349 ⏎

Roubalová, R., Procházková, P., Papežová, H., Smitka, K., Bilej, M., & Tlaskalová-Hogenová, H. (2020). Anorexia nervosa: gut microbiota–immune–brain interactions. *Clin Nutr*, *39*(3), 676–684. doi:10.1016/j.clnu.2019.03.023 ⏎

Sader, M., Weston, A., Buchan, K., Kerr-Gaffney, J., Gillespie-Smith, K., Sharpe, H., & Duffy, F. (2025). The co-occurrence of autism and avoidant/restrictive food intake disorder (ARFID): a prevalence-based meta-analysis. *Int J Eat Disord*, *58*(3), 473–488. doi:10.1002/eat.24369 ⏎

Schag, K., Teufel, M., Junne, F., Preissl, H., Hautzinger, M., Zipfel, S., & Giel, K. E. (2013). Impulsivity in binge eating disorder: food cues elicit increased reward responses and disinhibition. *PLoS One*, *8*(10), e76542. doi:10.1371/journal.pone.0076542 ⏎

Schalla, M. A., & Stengel, A. (2019). Gastrointestinal alterations in anorexia nervosa—a systematic review. *Eur Eat Disord Rev*, *27*(5), 447–461. doi:10.1002/erv.2679 ⏎

Schéle, E., Grahnemo, L., Anesten, F., Hallén, A., Bäckhed, F., & Jansson, J. O. (2013). The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. *Endocrinology*, *154*(10), 3643–3651. doi:10.1210/en.2012-2151 ⏎

Schmidt, R., Hiemisch, A., Kiess, W., von Klitzing, K., Schlensog-Schuster, F., & Hilbert, A. (2021). Macro- and micronutrient intake in children with avoidant/restrictive food intake disorder. *Nutrients*, *13*(2), 400. doi:10.3390/nu13020400 ⏎

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut–brain communication. *Front Endocrinol (Lausanne)*, *11*, 25. doi:10.3389/fendo.2020.00025 ⏎

Singh, R. K., Chang, H. W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., … Liao, W. (2017). Influence of diet on the gut microbiome and implications for human health. *J Transl Med*, *15*(1), 73. doi:10.1186/s12967-017-1175-y ⏎

Smith, K., Lesser, J., Brandenburg, B., Lesser, A., Cici, J., Juenneman, R., … Le Grange, D. (2016). Outcomes of an inpatient refeeding protocol in youth with Anorexia Nervosa and atypical Anorexia Nervosa at Children's Hospitals and Clinics of Minnesota. *J Eat Disord*, *4*, 35. doi:10.1186/s40337-016-0124-0 ⏎

Smitka, K., Prochazkova, P., Roubalova, R., Dvorak, J., Papezova, H., Hill, M., … Tlaskalova-Hogenova, H. (2021). Current aspects of the role of autoantibodies directed against appetite- regulating hormones and the gut

microbiome in eating disorders. *Front Endocrinol (Lausanne)*, *12*, 613983. doi:10.3389/fendo.2021.613983 ⏎

Speranza, E., Cioffi, I., Santarpia, L., Del Piano, C., De Caprio, C., Naccarato, M., … Pasanisi, F. (2018). Fecal short chain fatty acids and dietary intake in Italian women with restrictive anorexia nervosa: a pilot study. *Front Nutr*, *5*, 119. doi:10.3389/fnut.2018.00119 ⏎

Steiger, H., Koerner, N., Engelberg, M. J., Israël, M., Ng Ying Kin, N. M., & Young, S. N. (2001). Self-destructiveness and serotonin function in bulimia nervosa. *Psychiatry Res*, *103*(1), 15–26. doi:10.1016/s0165-1781(01)00264-5 ⏎

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res*, *1693*(Pt B), 128–133. doi:10.1016/j.brainres.2018.03.015 ⏎

Succurro, E., Segura-Garcia, C., Ruffo, M., Caroleo, M., Rania, M., Aloi, M., … Arturi, F. (2015). Obese patients with a binge eating disorder have an unfavorable metabolic and inflammatory profile. *Medicine (Baltimore)*, *94*(52), e2098. doi:10.1097/md.0000000000002098 ⏎

Tang, W., Meng, Z., Li, N., Liu, Y., Li, L., Chen, D., & Yang, Y. (2020). Roles of gut microbiota in the regulation of hippocampal plasticity, inflammation, and hippocampus-dependent behaviors. *Front Cell Infect Microbiol*, *10*, 611014. doi:10.3389/fcimb.2020.611014 ⏎

Treadway, M. T., Cooper, J. A., & Miller, A. H. (2019). Can't or won't? Immunometabolic constraints on dopaminergic drive. *Trends Cogn Sci*, *23*(5), 435–448. doi:10.1016/j.tics.2019.03.003 ⏎

Trevelline, B. K., & Kohl, K. D. (2022). The gut microbiome influences host diet selection behavior. *Proc Natl Acad Sci U S A*, *119*(17), e2117537119. doi:10.1073/pnas.2117537119 ⏎

van Galen, K. A., Ter Horst, K. W., & Serlie, M. J. (2021). Serotonin, food intake, and obesity. *Obes Rev*, *22*(7), e13210. doi:10.1111/obr.13210 ⏎

Vichaya, E. G., Hunt, S. C., & Dantzer, R. (2014). Lipopolysaccharide reduces incentive motivation while boosting preference for high reward in mice. *Neuropsychopharmacology*, *39*(12), 2884–2890. doi:10.1038/npp.2014.141 ⏎

Vinithakumari, A. A., Padhi, P., Hernandez, B., Lin, S. J., Dunkerson-Kurzhumov, A., Showman, L., … Mooyottu, S. (2022). *Clostridioides difficile* infection dysregulates brain dopamine metabolism. *Microbiol Spectr*, *10*(2), e0007322. doi:10.1128/spectrum.00073-22 ⏎

Waise, T. M. Z., Dranse, H. J., & Lam, T. K. T. (2018). The metabolic role of vagal afferent innervation. *Nat Rev Gastroenterol Hepatol*, *15*(10), 625–636. doi:10.1038/s41575-018-0062-1 ⏎

Wang, F., Liu, Y. L., Jiang, C. H., Wu, H. Y., Jin, J., Sun, Y. W., … Fang, X. (2025). Association between psychiatric disorders and irritable bowel syndrome: a bidirectional Mendelian randomization study. *J Affect Disord*, *368*, 865–871. doi:10.1016/j.jad.2024.09.030 ⏎

Wang, Y., Qiao, H., & Zhang, Y. (2023). Changes of dopamine and tyrosine hydroxylase levels in the brain of germ-free mice. *Iran J Biotechnol*, *21*(1), e2798. doi:10.30498/ijb.2022.236732.2798 ⏎

Wierdsma, N. J., van Bodegraven, A. A., Uitdehaag, B. M., Arjaans, W., Savelkoul, P. H., Kruizenga, H. M., & van Bokhorst-de van der Schueren, M. A. (2009). Fructo-oligosaccharides and fibre in enteral nutrition has a beneficial influence on microbiota and gastrointestinal quality of life. *Scand J Gastroenterol*, *44*(7), 804–812. doi:10.1080/00365520902839675 ⏎

Wilson, B. C., Derraik, J. G. B., Albert, B. B., Leong, K. S. W., Tweedie-Cullen, R. Y., Creagh, C., … O'Sullivan, J. M. (2023). An open-label pilot trial of faecal microbiome transfer to restore the gut microbiome in anorexia nervosa: protocol. *BMJ Open*, *13*(7), e070616. doi:10.1136/bmjopen-2022-070616 ⏎

Xie, H., Lu, Q., Wang, H., Zhu, X., & Guan, Z. (2018). Effects of probiotics combined with enteral nutrition on immune function and inflammatory response in postoperative patients with gastric cancer. *J Buon*, *23*(3), 678–683. ⏎

Yanagi, H., Tsuda, A., Matsushima, M., Takahashi, S., Ozawa, G., Koga, Y., & Takagi, A. (2017). Changes in the gut microbiota composition and the plasma ghrelin level in patients with *Helicobacter pylori*-infected

patients with eradication therapy. *BMJ Open Gastroenterol*, *4*(1), e000182. doi:10.1136/bmjgast-2017-000182 ⏎

Yang, Y., Conti, J., McMaster, C. M., & Hay, P. (2021). Beyond refeeding: the effect of including a dietitian in eating disorder treatment. A systematic review. *Nutrients 13*(12), 4490. doi:10.3390/nu13124490 ⏎

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., … Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. doi:10.1016/j.cell.2015.02.047 ⏎

Yao, H., Fan, C., Fan, X., Lu, Y., Wang, Y., Wang, R., … Qi, K. (2020). Effects of gut microbiota on leptin expression and body weight are lessened by high-fat diet in mice. *Br J Nutr*, *124*(4), 396–406. doi:10.1017/s0007114520001117 ⏎

Yap, C. X., Henders, A. K., Alvares, G. A., Wood, D. L. A., Krause, L., Tyson, G. W., … Gratten, J. (2021). Autism-related dietary preferences mediate autism-gut microbiome associations. *Cell*, *184*(24), 5916–5931.e5917. doi:https://doi.org/10.1016/j.cell.2021.10.015 ⏎

Yu, K. B., & Hsiao, E. Y. (2021). Roles for the gut microbiota in regulating neuronal feeding circuits. *J Clin Invest*, *131*(10), e143772. doi:10.1172/jci143772 ⏎

Yu, Z., Guo, M., Yu, B., Wang, Y., Yan, Z., & Gao, R. (2024). Anorexia nervosa and bulimia nervosa: a Mendelian randomization study of gut microbiota. *Front Microbiol*, *15*, 1396932. doi:10.3389/fmicb.2024.1396932 ⏎

Žaja, O., Fiolić, M., Ćuk, M. C., & Tiljak, M. K. (2021). The role of *L. reuteri* DSM17938 in nutritional recovery and treatment of constipation in children and adolescents with anorexia nervosa—a randomized, double blind, placebo controlled study. *Clin Nutr ESPEN*, *46*, 47–53. doi:10.1016/j.clnesp.2021.08.016 ⏎

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *Isme J*, *6*(8), 1535–1543. doi:10.1038/ismej.2012.4 ⏎

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res*, *30*(6), 492–506. doi:10.1038/s41422-020-0332-7 ⏎

Zhu, Y., Yin, L., Liu, Q., Guan, Y., Nie, S., Zhu, Y., & Mo, F. (2024). Tryptophan metabolic pathway plays a key role in the stress-induced emotional eating. *Curr Res Food Sci*, *8*, 100754. doi:10.1016/j.crfs.2024.100754 ⏎

Zipfel, S., Wild, B., Groß, G., Friederich, H. C., Teufel, M., Schellberg, D., … Herzog, W. (2014). Focal psychodynamic therapy, cognitive behaviour therapy, and optimised treatment as usual in outpatients with anorexia nervosa (ANTOP study): randomised controlled trial. *Lancet*, *383*(9912), 127–137. doi:10.1016/s0140-6736(13)61746-8 ⏎

10

The Viruses in Our Gut: A Key Parameter for Understanding Gut Health

Giulia Ospite, Camilla Anna Kristina Berg, and Torben Sølbeck Rasmussen

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10.1 Introduction to the Gut Virome

Viruses are the most abundant biological entities on Earth, with an estimated 10^{31} virus-like particles (VLPs) globally. Among these, the human gut virome represents one of the densest and most complex viral ecosystems, with an estimated 109–10^{10} VLPs per gram of feces (Hoyles *et al.* 2014). However, opinions on the real quantity remain divided (Liang and Bushman 2021, Shkoporov *et al.* 2022, Pinto *et al.* 2024, Lopez *et al.* 2025), where methodological biases may partly explain the different observations (Soria-Villalba *et al.* 2024, Li *et al.* 2025). The gut virome develops soon after birth and forms a dynamic relationship with both the host and the gut microbiome, contributing to gut homeostasis (i.e., a balanced and healthy state) and immune function (Carding *et al.* 2017, Seo and Kweon 2019). The gut virome is highly individualized, stable over

time, and shaped by factors such as diet, geography, antibiotic exposure, and lifestyle (Hoyles *et al*. 2014, Shkoporov *et al*. 2019, Cao *et al*. 2022). While the gut virome includes all major nucleic acid types (single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA)) (Reyes *et al*. 2010, Minot *et al*. 2013, Zuo, Sun, *et al*. 2020, Pinto *et al*. 2024), it is dominated by bacteriophages (phages), which account for more than 90% of viral sequences detected in fecal samples (Shkoporov *et al*. 2018, Nayfach *et al*. 2021). Phages are viruses that specifically infect and replicate within bacterial hosts (Barrangou *et al*. 2002, Holmfeldt *et al*. 2007). Their morphological characteristics include a capsid head containing the genetic material (Kellenberger 1968), a tail that provides structural support and enables penetration of the bacterial cell wall (Kanamaru *et al*. 2002, Xiang *et al*. 2008), and tail fibers that determine host specificity by recognizing and attaching to distinct receptors on the bacterial surface (Le *et al*. 2013, Hyman and van Raaij 2018) (Figure 10.1).

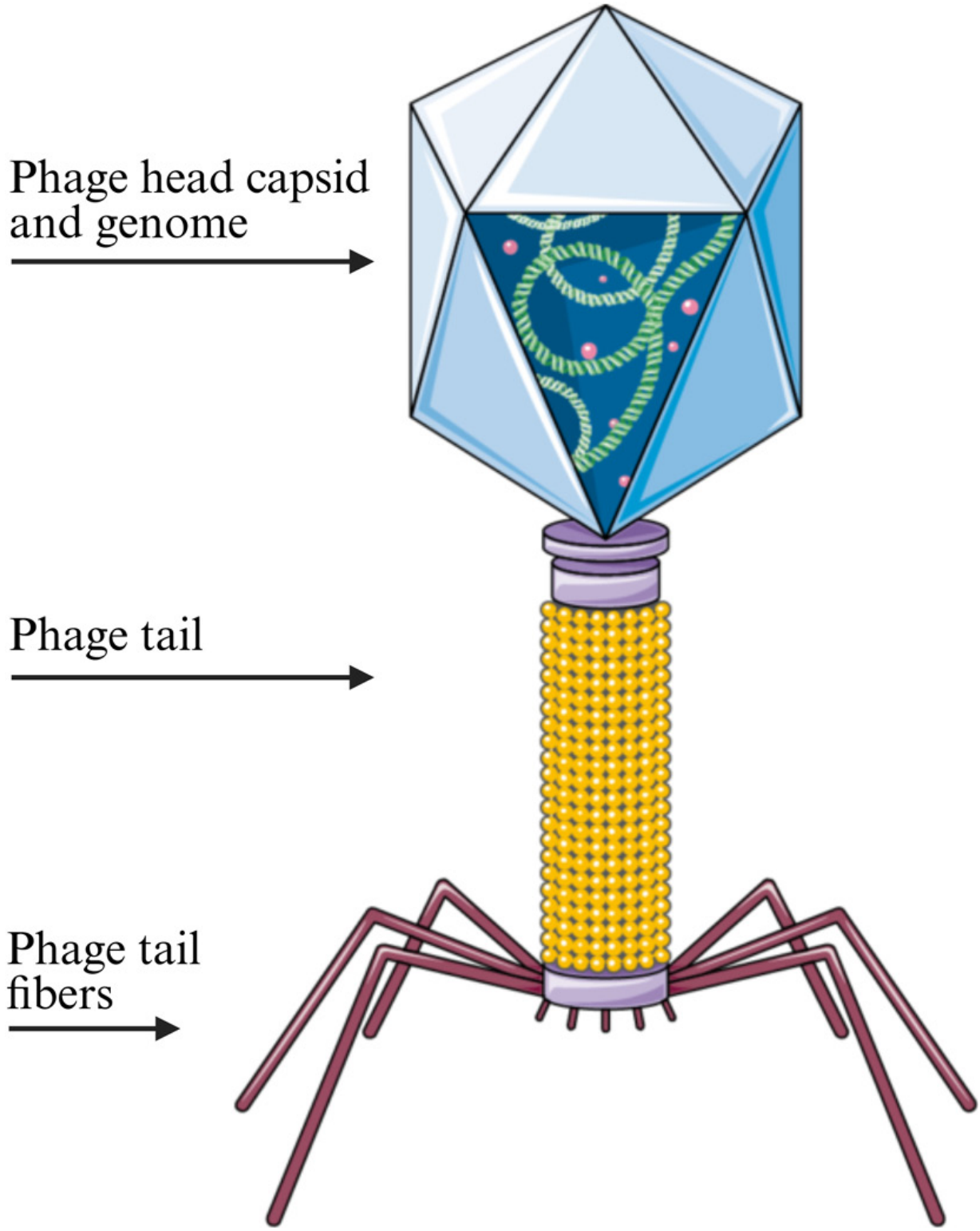

FIGURE 10.1 One example on phage morphology. A T4-like phage used to show the main components of phages, head capsid containing the genome, phage tail used as a structural component and to penetrate bacterial cell

walls, and phage fibers used to recognize the bacterial hosts. (Graphical icon were downloaded from Smart Servier Medical Art (https://smart.servier.com/) under the CC BY 3.0 license. Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/heou95n.) ⏎

Phages regulate bacterial populations through predation on susceptible host strains primarily via either a lytic or lysogenic life cycle (Lopez *et al.* 2025) (Figure 10.2). In the lytic cycle, phages inject their genetic material into a bacterial cell, hijack the host's enzymatic machinery to replicate, and eventually cause cell lysis, thereby releasing new phage particles that can infect additional hosts. Phages that exclusively follow the lytic cycle are called virulent phages. In contrast, the lysogenic cycle involves phages integrating their genome into the bacterial host's DNA, to form a prophage. This allows the phage genome to replicate along with the bacterial genome. Under certain environmental conditions, the prophage can be induced to exit the lysogenic state and enter the lytic cycle (Sutcliffe *et al.* 2023). These inducible prophages, known as temperate phages, often carry genes that can alter bacterial traits, including virulence, antibiotic resistance, and metabolic functions (Mathieu *et al.* 2020, Tisza and Buck 2021, Shah *et al.* 2023). As an exception, filamentous phages, such as M13 do not lyse their host cells, but instead are released continuously while the infected bacterial cells continue to grow (Pratt *et al.* 1969, Smeal *et al.* 2017).

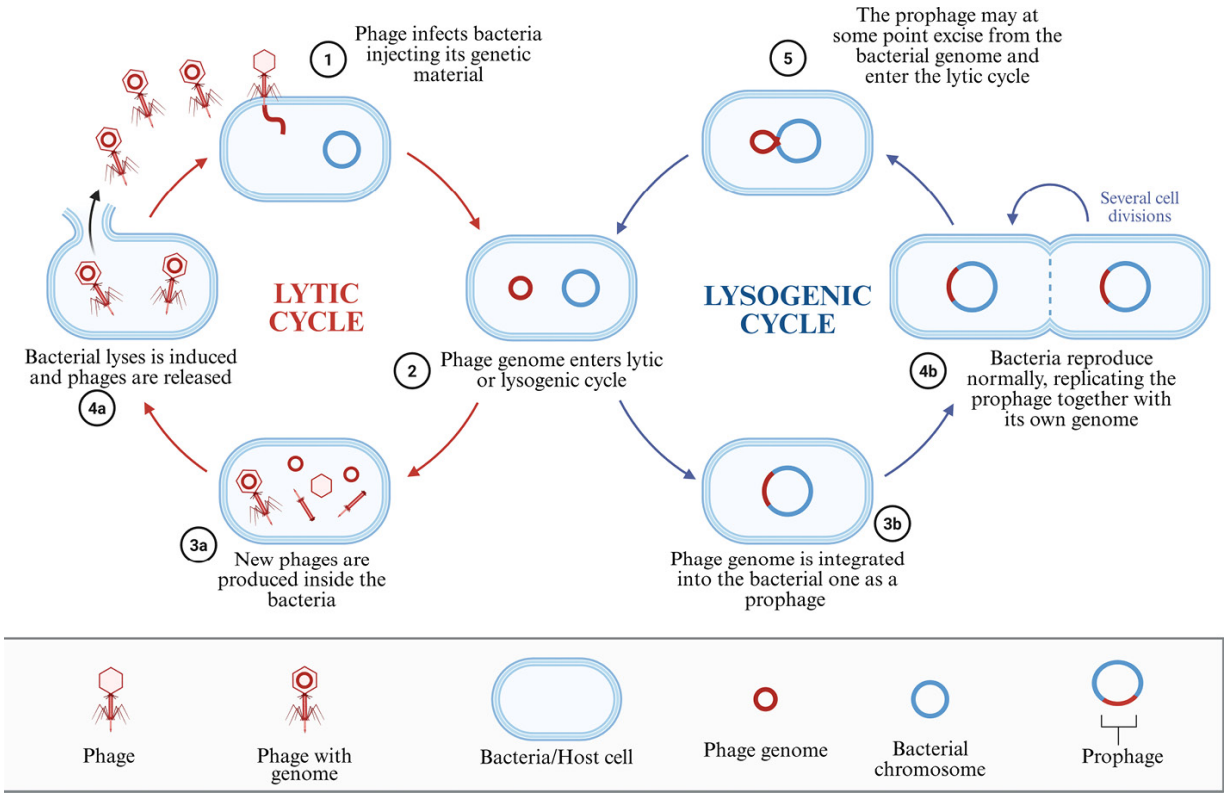

FIGURE 10.2 Lytic and lysogenic life cycles of bacteriophages. In the lytic cycle, (1) the phage infects a bacterium by injecting its genome, which then (3a) replicates and directs the assembly of new virions, (4a) subsequently released through bacterial lysis. In the lysogenic cycle, (3b) the phage genome integrates into the bacterial chromosome as a prophage and (4b) is passively replicated as the host cell divides. Under specific inducing conditions, (5) the prophage may detach from the bacterial chromosome, thereby initiating the lytic cycle that convert the prophage to a temperate phage. (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/pyzo7iq.) ⏎

Ecological models illustrate phage–host dynamics. One example comes from the ocean, where phage–host interactions generally follow the "kill-the-winner" model (Breitbart *et al.* 2018, Marantos *et al.* 2022), in which dominant bacterial populations are suppressed by lytic phage predation, maintaining diversity. Unlike the open ocean, the gut is a densely populated and spatially structured environment wherein bacteria form biofilms and adhere to mucus layers (Barr *et al.* 2013). In the gut, there is a larger ratio

of phage genomes to bacterial genomes (~4:1), implying that most gut phages are effectively integrated as prophages (Lopez *et al*. 2025). Instead, the "piggyback-the-winner" model better explains gut ecology: at high bacterial densities, phages favor lysogeny, conferring benefits such as immunity against superinfection (i.e., prophages protection of the bacterial host against infection of identical temperate phages), enabling coexistence and persistence (Shkoporov and Hill 2019, Shkoporov *et al*. 2019, Tisza and Buck 2021, Shah *et al*. 2023, Sutcliffe *et al*. 2023). An interesting perspective of host–phage interactions in the gut is to consider the size of phages relative to the length of the journey throughout the gastrointestinal tract and their actual chance of finding a host (Figure 10.3).

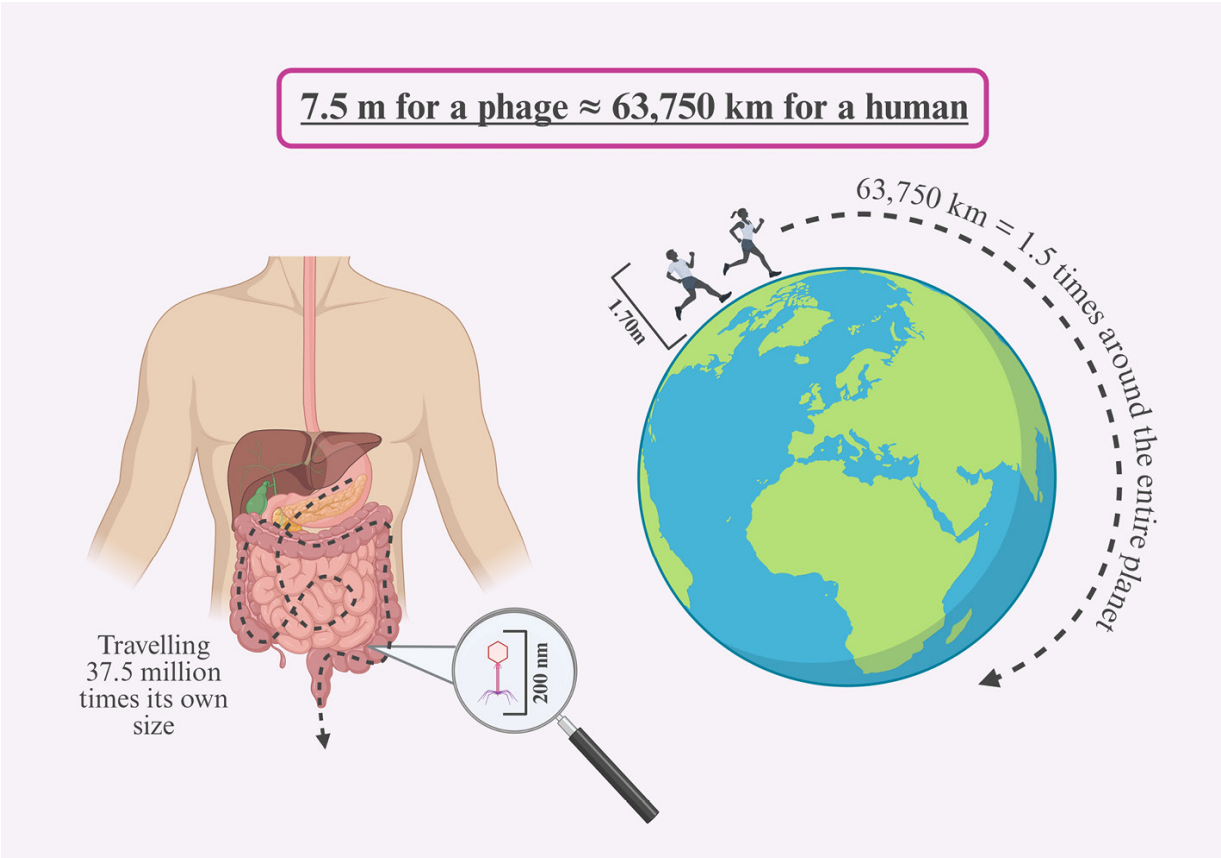

FIGURE 10.3 Comparing the journey of a phage in the gut to a human traveling around the earth. The scale of the physical challenge phages face in the gastrointestinal tract becomes clear when the distance they travel is expressed relatively to their size. A phage moving through the human gut covers approximately 37.5 million times its own length. For comparison, a person 1.70 m tall would need to run roughly 1.5 times around the planet (≈ 63,750 km) to cover an equivalent relative distance. (Figure made by Willemijn J.C. Brandwijk. Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/2ex04g2.) ⏎

Beyond phages, the gut virome also contains eukaryotic viruses that infect human or plant cells and, to a lesser extent, archaeal viruses (Cao *et al.* 2022). Although plant viruses are typically transient, their presence reflects dietary and environmental exposure (David *et al.* 2014). It is critical to understand how the gut viral community interacts with its bacterial hosts and the human immune system to maintain gut homeostasis. These

interactions form a delicate equilibrium that supports health from early life and onward.

10.2 The Gut Virome: A Delicate Balance between Bacteria, Phages, and the Host

10.2.1 Establishment of the Early-Life Gut Virome

The gut microbiome established during the first years of life is critical for maintaining homeostasis and supporting immune system maturation (Duerkop and Hooper 2013, Lim *et al*. 2015, Lynn *et al*. 2021, Zeng *et al.* 2024, Zhang *et al*. 2025). Perturbations in this early-life microbiome have been associated with conditions such as necrotizing enterocolitis (NEC), inflammatory bowel disease (IBD), autoimmune diseases, metabolic syndrome, and malnutrition (Sarkar *et al*. 2021, Kaelin *et al*. 2022, Wilson *et al*. 2023). The infant gut virome develops in distinct stages beginning immediately after birth. Initial colonization by bacteria from maternal and environmental sources introduces temperate phages that are integrated within bacterial chromosomes (Lim *et al*. 2015, Mathieu *et al*. 2020, Shamash and Maurice 2022, Dahlman *et al*. 2025). By approximately 4 months of age, viruses capable of replicating in human cells (eukaryotic viruses) become more prevalent, with notable differences between breastfed and formula-fed infants (Liang, Zhao, *et al*. 2020). As infants grow, the initially diverse, phage-rich virome gradually declines in richness (i.e., the number of unique viruses), reaching an adult-like composition by approximately 3 years of age (Shamash and Maurice 2022). Across multiple studies, there is strong agreement that temperate phages constitute the dominant viral fraction of the human early-life gut virome (ELGV) (Mathieu *et al*. 2020, Shah *et al*. 2023, Dahlman *et al*. 2025, Lopez *et al.* 2025). Although temperate phages prevail numerically, virulent phages demonstrate higher infectivity, suggesting that gut virome ecology is shaped

by a dynamic interplay between lysogenic persistence and lytic activity (Mathieu *et al*. 2020).

Characterizing the infant gut virome requires extensive viral sequence databases. For example, the ELGV database compiles over 160,000 non-redundant viral sequences and 82,000 candidate species from 8,130 infant fecal samples (Zeng *et al*. 2024). These sequences represent numerous viral families (99.6% are phages), mainly *Caudoviricetes*, *Peduoviridae*, and *Microviridae*, while a small fraction comprises eukaryotic viruses such as *Caliciviridae*, *Picornaviridae*, and *Astroviridae* (Gregory *et al*. 2020, Liang and Bushman 2021, Zeng *et al*. 2024). Another minor but notable component of the ELGV is the Anelloviridae family, which infects humans via multiple routes and is frequently detected in viral hepatitis coinfections (Najafimemar *et al*. 2018, Sabbaghian *et al*. 2024). *Anelloviruses* have been associated with conditions including IBD and asthma (Norman *et al*. 2015, Tokarz *et al*. 2019, Sabbaghian *et al*. 2024, Boulund *et al*. 2025), although their direct role in early-life is not clear. Their abundance in infant blood and feces is typically low during the first months of life, likely due to protective factors in breast milk, but increases markedly between 6 and 15 months (Lim *et al*. 2015, Tyschik *et al*. 2018, Liang, Feng, *et al*. 2020, Boulund *et al*. 2025). Another recent study also revealed more than 10,000 new phage species, which constituted 70% of the data, highlighting the uniqueness of the infant gut as a specialized viral niche distinct from the adult gut (Shah *et al*. 2023).

10.2.2 Composition of the Adult Gut Virome

Since the first fecal metagenome was sequenced in 2003 with a focus on the virome (Breitbart *et al*. 2003), new technologies have revealed that the human adult gut virome is also dominated by phages, particularly dsDNA tailed phages such as *Caudoviricetes*, along with families like Ackermannviridae, Herelleviridae, and Microviridae. Furthermore, crAss-like (*Crassvirales*) phages represent a major clade across datasets (Gregory *et al*. 2020, Camarillo-Guerrero *et al*. 2021, Nayfach *et al*. 2021, Tisza and

Buck 2021). As in the infant gut, eukaryotic viruses form a minor fraction, including ssDNA families (Anelloviridae, Circoviridae, Genomoviridae) and RNA viruses such as Caliciviridae and Picornaviridae, with the occasional detection of plant and fungal viruses. Archaeal viruses are rare (<0.1%) (Gregory *et al*. 2020, Tisza and Buck 2021), but recent evidence suggests that their abundance may be underestimated (Li *et al*. 2022).

Collectively, several databases (Gregory *et al*. 2020, Camarillo-Guerrero *et al*. 2021, Nayfach *et al*. 2021, Tisza and Buck 2021) have cataloged hundreds of thousands of viral genomes, highlighting the extensive viral diversity within highly individualized adult gut viromes. Most sequences represent previously uncharacterized phages, emphasizing the complexity of the gut virome and its strong phage-dominated composition.

Understanding how gut bacteria and their phages adapt to persist within the gastrointestinal tract requires consideration of the selective pressures shaped by the environment. The gut fosters diverse and dynamic microbial niches that drive bacterial adaptation (De Sordi *et al*. 2019). One key strategy involves the formation of gut mucus and biofilms, which not only support microbial diversity but also directly influence phage diversity, mobility, and infection dynamics within the gut ecosystem (Hansen *et al*. 2019, Meneses *et al*. 2023).

10.2.3 The Mucus Layer as a Reservoir for Gut Phages

The gut mucus layer forms a dynamic barrier composed of mucin glycoproteins that protect epithelial surfaces. Phages can adhere to mucins via immunoglobulin-like domains on their capsids, a feature that may evolve to enhance persistence within the mucosal environment by slowing down their movements, thereby allowing them to utilize the gut mucus as a phage reservoir (Barr *et al*. 2013, Chin *et al*. 2022). The strength of this adherence is balanced by mucus turnover, forming the basis of the bacteriophage adherence to mucus (BAM) model, which is suggested to provide a non–host-derived defense against pathogenic bacterial invasion by reducing their chances to colonize and thereby protecting epithelial

integrity (Barr *et al*. 2013). It has even been demonstrated that phages can evolve mutations specifically enhancing their persistence in the mucus layer (Chin *et al*. 2022). These mechanisms therefore have potential therapeutic applications for mucosal infections (Almeida *et al*. 2024), although extensive advances are needed in our understanding of the interactions between gut mucus, the human host, bacteria, and phages (Rothschild-Rodriguez *et al*. 2023). For example, none of the current models are considering the impact of the bacteria-derived biofilm on phage dynamics within the gut mucus (Palestrant *et al*. 2004), despite biofilm is well-known to act as a protective layer against phage infections in medical, industrial, and environmental contexts (Simmons *et al*. 2018). A recent commentary raises the question of whether the bacteria– biofilm–phage dynamics observed in other microbial ecosystems can be applied to understand the combination of gut mucus and bacterial biofilms (Mortensen *et al*. 2025). To address this issue, continuous mucus secretion (Deplancke and Gaskins 2001) combined with biofilm-forming bacteria (Palestrant *et al*. 2004), was therefore suggested to constitute a hybrid matrix termed "mucofilm," with the purpose to integrate host-derived mucus and bacterial biofilms as one unity in the terminology (Mortensen *et al*. 2025). The mucofilm was hypothesized to promote bacterial resilience while limiting phage movement, acting as both a protective shield against pathogens and a dynamic microbial ecosystem. Suggested phage barriers include biofilm molecules that impede phage diffusion, low bacterial metabolic activity, and the emergence of phage-resistant phenotypes (Mortensen *et al*. 2025); however, this hypothesis remain to be experimentally validated.

While the gut mucus layer serves as a critical interface for phage reservoir and bacterial control, the epithelial cells beneath it represent another key point of interaction. These cells not only form the physical barrier separating microbes from host tissues but also engage in molecular cross-talk with phages, influencing immune signaling and gut barrier integrity (Vancamelbeke and Vermeire 2017).

10.2.4 Phage Interactions with Host Cells and the Immune System

Phages cannot replicate within human cells, but they can interact with them through several mechanisms (Dabrowska *et al*. 2005, Kan and Barr 2023). Typically, phages first encounter mammalian cells at the glycocalyx, the sugar-rich layer coating the cellular surface. Because phages are adapted to recognize bacterial glycans, they can also interact with similar glycan structures on mammalian cells. Subsequent interactions may involve cell membranes, receptors, and other surface structures, often through nonspecific binding (Dabrowska *et al*. 2005, Lehti *et al*. 2017, Nguyen *et al*. 2017, Bichet *et al*. 2021, Kan and Barr 2023).

Three different hypotheses explain phage translocation through the epithelial barrier and subsequent cell uptake (Figure 10.4):

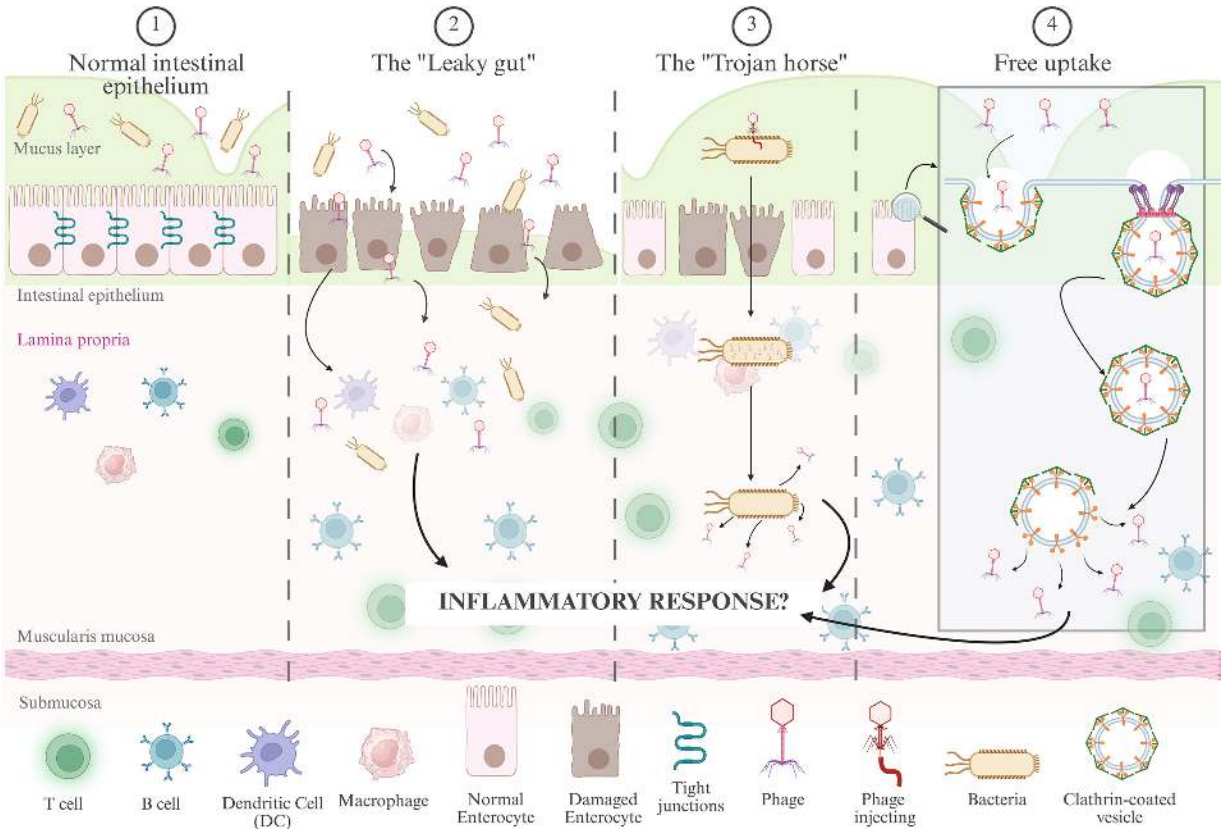

FIGURE 10.4 Three different theories have been proposed to explain how phages translocate through the epithelial barrier and cell uptake. (1) Normal intestinal epithelium. Cells are connected by tight junctions and form a selective barrier that maintain both immune and microbial balance; phages and bacteria stay in the lumen and mucus layer. No inflammatory response is established. (2) The "Leaky gut" theory: due to different causes (by, e.g., inflammation) the epithelium is disrupted and phages, as well as other bacteria, toxins, and macromolecules, can passively translocate through the barrier. Once in the lamina propria, phages may cause an inflammatory cascade. (3) The "Trojan horse" theory: a phage-infected bacteria translocate through the disrupted epithelium; phages replicated inside the bacteria are hereafter released and spread after bacterial lysis. These may also cause an inflammatory response. (4) The "Free uptake" theory: through a non-specific process, cells internalize extracellular material, which might include phages. Once in the cell, phages may cause inflammation. (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/2jo6475.) ⏎

1. *The Leaky Gut Hypothesis:* Increased intestinal permeability allows macromolecules, bacteria, and phages to cross into the lamina propria, potentially contributing to inflammation (Tetz and Tetz 2016).
2. *The Trojan Horse Hypothesis:* Phage-infected bacteria translocate through the epithelial barrier and later lyse, releasing phages extracellularly (Hsia *et al*. 2000, Nguyen *et al*. 2017).
3. *The Free Uptake Hypothesis:* Human cells engulf extracellular material, including phages, via actin-mediated ruffles (Nguyen *et al.* 2017), enabling phage accumulation and triggering anti-inflammatory (e.g., phages T4 and T7) or pro-inflammatory effects (e.g., filamentous phage Pf) (Bichet *et al*. 2023).

Some mammalian cells, but not all, can internalize phages and even use them as a resource (Figure 10.5). For example, phage T4 can trigger protein production and phosphorylation in cell signaling pathways. By activating the AKT pathway—a serine/threonine-specific protein kinase—T4 phages can promote cell growth, survival, and macropinocytosis (the uptake of larger volumes of extracellular fluid and molecules). At the same time, T4 phages have been shown to inhibit the cyclin-dependent kinase 1 (CDK1) pathway, thereby slowing down the cell's division cycle while prolonging the G_1 phase, which increases cellular growth (Bichet *et al*. 2023). After internalization, phages may be destined for diverse intracellular fates (Figure 10.5).

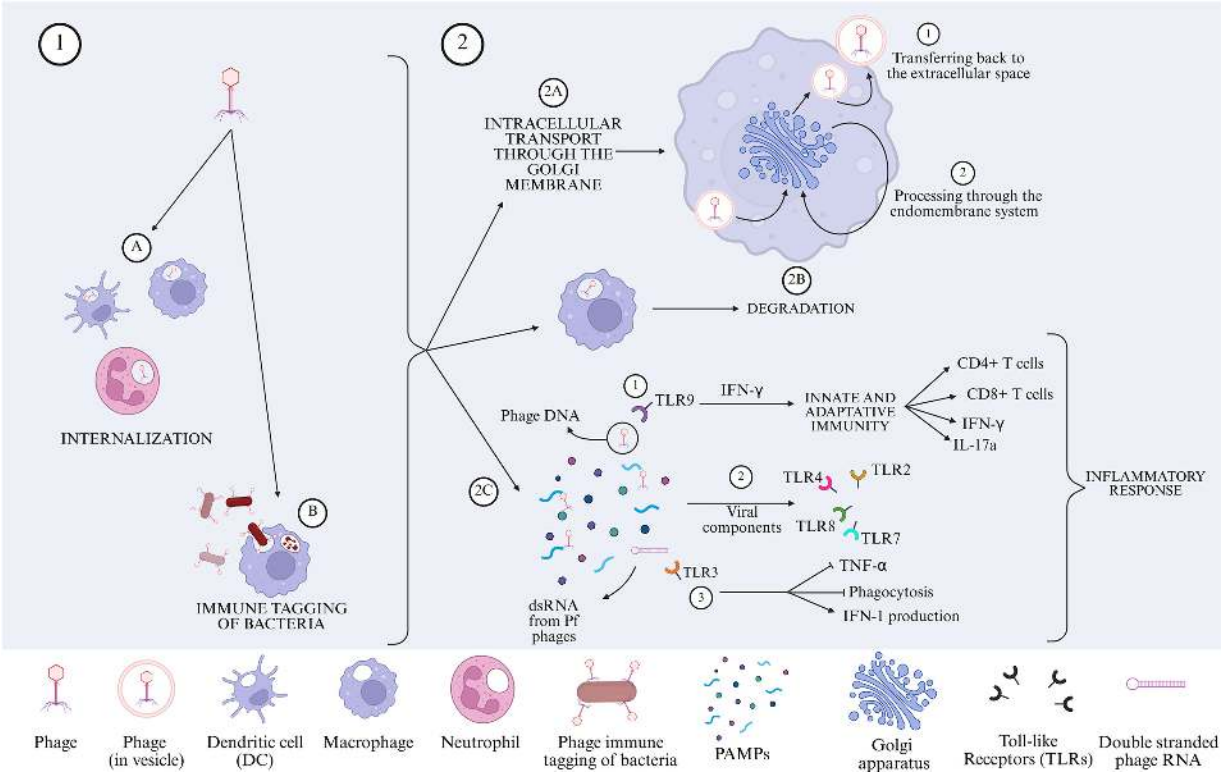

FIGURE 10.5 Phages interactions with the host immune system. In the cells, phages may be either (1A) internalized by phagocytic cells of the innate immune system such as macrophages, neutrophils, and DCs, or (1B) adhere to bacteria, immune tagging them and making it easier for immune cells to recognize and eliminate them. Phages may now undergo various pathways. They may (2A) undergo intracellular transport through the Golgi apparatus, and be either (2A.1) recycled back to the membrane or (2A.2) proceed further through the endomembrane system. (2B) Phages may be simply degraded. (2C) An inflammatory response may be started through immune recognition of PAMPs via TLRs. (2C.1) TLR9 can bind phage DNA, stimulating IFN-γ mediated response and increasing both innate and adaptive immunity, CD4+ and CD8+ T cells, IFN-γ, and IL-17a. (2C.2) TLR2, TLR4, TLR7, TLR8 can be stimulated by viral components. (2C.3) TLR3 can recognize dsRNA from Pf phages, enhancing IFN-I, and inhibiting TNF-α and phagocytosis. (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/q807g23.) ⏎

Growing evidence links phage–mammalian interactions to both health and disease processes. In some cases, phages can trigger inflammatory

responses (Bichet *et al.* 2023), whereas other scenarios suggest anti-inflammatory effects (Van Belleghem *et al.* 2017). They may trigger diverse cellular pathways when in contact with varied mammalian cell types. Phagocytic cells such as macrophages, neutrophils, and dendritic cells (DCs) can internalize phages. Phages can also induce bacterial phagocytosis by macrophages (Sweere *et al.* 2019) by tagging bacteria cells for immune recognition—similar to opsonization—which aids the immune response. Phages and the immune system are therefore suggested to work synergistically in certain cases to clear bacterial infections (Górski *et al.* 2012, Kaur *et al.* 2014, Jończyk-Matysiak *et al.* 2015, Jariah and Hakim 2019).

Immune cells recognize phage components as pathogen-associated molecular patterns (PAMPs) via Toll-like receptors (TLRs), initiating inflammatory cascades. For instance, phage DNA can stimulate interferon-mediated responses via TLR9, increasing CD4+ and CD8+ T cells and cytokine production (Gogokhia *et al.* 2019), while ssDNA from Pf phages is transcribed to dsRNA that activates TLR3, enhancing type I interferon (IFN-I) production but inhibiting tumor necrosis factor alpha (TNF-α) and phagocytosis (Sweere *et al.* 2019). Other potential phage-sensing receptors include TLR2, TLR4, TLR7, and TLR8, which are known for their ability to recognize viral components; however, they have not yet been directly been reported to react on phage components (Kan and Barr 2023).

10.3 Alterations in the Gut Virome Are Associated with Many Diseases

The gut virome plays a critical role in health and disease. Alterations in its composition and diversity have been linked to multiple conditions (Cao *et al.* 2022), which can broadly be grouped into three categories: gut-associated diseases, metabolic diseases, and autoimmune diseases (Figure 10.6).

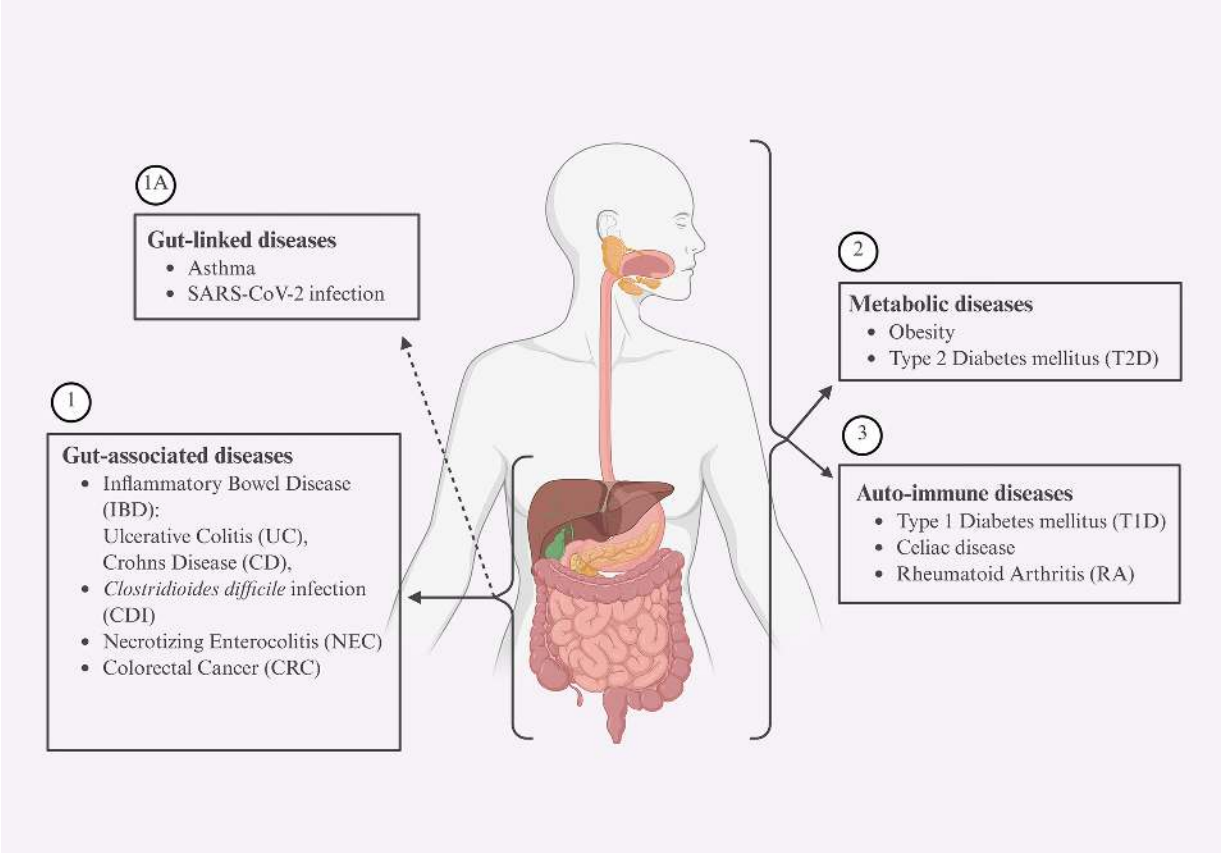

FIGURE 10.6 Various diseases are associated with perturbations in the gut virome. These can overall be divided into three main categories. (1) Gut-associated diseases: IBD (Tao 2018, Zuo *et al.* 2019, Horst *et al.* 2025), CDI (Ott *et al.* 2017, Zuo *et al.* 2018), NEC (Yi *et al.* 2024, Spiegelhauer *et al.* 2025), colorectal cancer (CRC) (Gogokhia *et al.* 2019, Zuo *et al.* 2022, Luo *et al.* 2023), (2) Metabolic diseases: obesity and type 2 diabetes mellitus (T2D) (Yang *et al.* 2021, Zhang *et al.* 2021), and (3) Auto-immune diseases: type 1 diabetes mellitus (T1D) (Santiago-Rodriguez and Hollister 2019, Tetz *et al.* 2019), celiac disease (Lindfors *et al.* 2020, El Mouzan *et al.* 2022), and rheumatoid arthritis (RA) (Mangalea *et al.* 2021, Tomofuji *et al.* 2022). Some might be defined as (1A) Gut-linked diseases, as they are strongly influenced and influence the gut themselves: asthma (Johnson and Ownby 2017, Leal Rodríguez *et al.* 2024), and SARS-CoV-2 infection (Liang, Feng, *et al.* 2020). (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/fo6l7bs.) ⏎

10.3.1 Gut-Associated Diseases

IBD, including ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic intestinal inflammation and alternating periods of flare and remission. Although its exact cause remains unclear, an altered gut bacteriome and virome appear to be implicated in the pathogenesis (Norman *et al.* 2015, Clooney *et al.* 2019, Federici *et al.* 2022, Tian *et al.* 2024). One indication of IBD is a bloom of temperate phages coupled with a decline in their bacterial hosts, suggesting prophage induction during disease flares (Norman *et al.* 2015, Godsil *et al.* 2025). A study on the gut mucosal virome found a pronounced expansion of *Escherichia* and *Enterobacteria* phages in the inflamed mucosa of UC patients (Zuo *et al.* 2019), while another study observed that the transplantation of phages from UC patient feces into human-microbiota-associated mice aggravated colitis and altered bacterial taxa associated with IBD (Sinha *et al.* 2022). Primary sclerosing cholangitis (PSC) is a chronic biliary disease often associated with IBD, and is characterized by chronic inflammation and fibrosis that progressively destroys the bile ducts (Verschluss der Gallenwege durch Verdickung der Wandungen 1867, Williamson and Chapman 2015). Patients with concurrent PSC and IBD (PSC-IBD) exhibit distinct microbial profiles compared to patients with IBD alone, including an increased abundance of *Veillonella* species (Bajer *et al.* 2017, Kummen *et al.* 2017, Horst *et al.* 2025). However, no studies have yet looked into the role of the gut virome in PSC-IBD patients.

Clostridioides difficile infection (CDI) is another condition linked to virome dysbiosis, which typically manifests as reduced viral diversity (Zuo *et al.* 2018). It is caused by a Gram-positive, anaerobic, toxin- and spore-forming bacterium that colonizes the lower gastrointestinal tract (Zuo *et al.* 2018); the organism was first described in newborn infants in 1935 (Hall 1935). CDI is one of the most common hospital-acquired infections and accounts for 15–20% of antibiotic-associated diarrhea, ranging from mild diarrhea to severe inflammatory conditions such as pseudomembranous colitis (Gerding *et al.* 1995, Bartlett and Gerding 2008, Khanna *et al.* 2012,

Liu *et al.* 2023). It remains a major healthcare challenge affecting approximately half a million people annually in the USA alone (Zhang *et al.* 2016) and is widespread across European hospitals (European Centre for Disease Prevention and Control 2022). Antibiotics are the first-line treatment, but they fail in up to one-third of patients (Musher *et al.* 2005, Pépin *et al.* 2006, Louie *et al.* 2011) and may lead to recurrent CDI (rCDI) due to antibiotic-induced microbiome damage and reducing secondary bile acids that normally inhibit *C. difficile* spore germination (Giel *et al.* 2010, Theriot *et al.* 2016). Fecal microbiota transplantation (FMT) has proven highly effective for treating rCDI, with cure rates exceeding 85–90%, and its success may partly depend on restoring phage diversity (Ott *et al.* 2017, Zuo *et al.* 2018, Fujimoto *et al.* 2021).

NEC, a severe inflammatory disease in preterm infants (gestational age < 37 weeks and/or birthweight < 1500 g) (Niño *et al.* 2016, Brunse *et al.* 2022), is associated with an immature immune system and gut microbiome imbalance characterized by a loss of bacterial diversity and the overgrowth of single species (Tarracchini *et al.* 2021). Decreased viral beta diversity has been noted up to 10 days prior to NEC onset, with specific viral signatures and viral-bacterial interactions (Kaelin *et al.* 2022). Several viruses known to cause gastroenteritis, such as *echovirus*, *coronavirus*, *norovirus*, and *rotavirus*, are also thought to be linked to NEC (Coggins *et al.* 2015). In addition, other viruses have been sporadically detected, including *astrovirus*, *adenovirus*, *torovirus*, and *cytomegalovirus* (Shimamura 2016, Cheng *et al.* 2021). However, findings vary among studies, and in some cases, no specific viral pathogens have been detected.

Growing evidence suggests that the gut virome contributes to cancer development, particularly gastrointestinal cancers. Significant alterations of the intestinal phageome have been observed in colorectal cancer (CRC) (Nakatsu *et al.* 2018, Marongiu *et al.* 2021), though whether these alterations drive malignancy or result from it remains unclear. Phages may have a dual effect on both the initiation and progression of CRC by reshaping the bacterial community (Gogokhia *et al.* 2019) and increasing

intestinal permeability, thereby promoting chronic inflammation (Tetz and Tetz 2016).

10.3.2 Gut-Linked Diseases

Asthma and allergic diseases have increased sharply in developed countries over the past four decades. While initially attributed to the hygiene hypothesis, current evidence supports the microbiome hypothesis, which highlights the importance of early-life microbial exposure (Penders *et al.* 2007, Johnson and Ownby 2017). Factors such as cesarean delivery (Sevelsted *et al.* 2016), antibiotic use (Duong *et al.* 2022), breastfeeding, and environmental exposures influence gut microbiome composition and are linked to asthma risk (Leal Rodríguez *et al.* 2024, Boulund *et al.* 2025). Recent work extends this connection to the virome: in the COPSAC2010 cohort (n = 647), children who developed asthma showed altered virome profiles, including an increased abundance of temperate phages and fewer phages predicted to infect bacteria within the classes *Clostridiales*, *Oscillospirales*, and *Bacteroidales* (Leal Rodríguez *et al.* 2024). These associations were partly independent of bacterial changes and were modulated by host TLR9 genotype, suggesting a direct role for intestinal phages in asthma development (Leal Rodríguez *et al.* 2024).

COVID-19, caused by SARS-CoV-2, primarily affects the respiratory tract but also seems to involve the gastrointestinal system. Between 5% and 33% of patients experience symptoms such as diarrhea, nausea, and vomiting (Liang, Feng, *et al.* 2020). Transcriptionally active SARS-CoV-2 has been detected in feces and may persist after respiratory clearance (Lamers *et al.* 2020, Mao *et al.* 2020, Zuo, Zhan, *et al.* 2020, Triana *et al.* 2021). Patients with high viral infectivity exhibit distinct gut microbiome signatures, including an enrichment of *Collinsella aerofaciens*, *Collinsella tanakaei*, *Streptococcus infantis*, and *Morganella morganii*, alongside increased biosynthetic activity. Importantly, dysbiosis of both the bacteriome and virome can persist long after hospital discharge, indicating

possible lasting effects on gut ecology and host physiology (Zuo, Zhan, *et al*. 2020, Zuo *et al*. 2021).

10.3.3 Metabolic Diseases

Metabolic syndrome, which includes obesity and type 2 diabetes (T2D), is globally prevalent and characterized by disrupted metabolism and changes in gut microbial and viral communities compared to normal-weight control groups (Bikel *et al*. 2021, Yang *et al*. 2021, de Jonge *et al*. 2022, Cervantes-Echeverría *et al*. 2023). A human study on T2D patients found an increased relative abundance of *Caudoviricetes* (Ma *et al*. 2018), along with synergistic alterations in both gut bacteria and phages (Chen *et al*. 2021), while another study identified the widespread phage family Candidatus *Heliusviridae* as associated with metabolic syndrome (de Jonge *et al*. 2022). Gut virome changes associated with metabolic syndrome have also been confirmed in a preclinical study demonstrating that high-fat diet (HFD)-induced obese mice exhibiting a marked decrease in lysogenic *Caudoviricetes* family and an increase in *Microviridae*, *Phycodnaviridae*, and *Mimiviridae* within the fecal virome (Schulfer *et al*. 2020).

10.3.4 Autoimmune Diseases

Type 1 diabetes (T1D) is a chronic autoimmune disease of considerable complexity. its pathogenesis is characterized by a T-cell-driven loss of beta cells within the pancreatic islets of Langerhans and commonly involves circulating autoantibodies directed against beta-cell antigens (Morahan 2012, Santiago-Rodriguez and Hollister 2019); moreover, it is linked to *enterovirus* infections and distinct virome signatures (Roivainen 2006). Children with T1D exhibit a higher prevalence of *Circoviridae* and *Picornaviridae* sequences compared to healthy controls (Zhao *et al*. 2017).

Celiac disease is an autoimmune enteropathy of the small intestine triggered by dietary gluten and associated with autoantibodies targeting tissue transglutaminase. Among other environmental factors, viral exposure

has been investigated as a potential contributor to disease development (Mårild *et al.* 2015, Kemppainen *et al.* 2017). Early-life infections with certain enteric viruses, particularly *enterovirus*, have been linked to the later onset of autoimmunity (Lindfors *et al.* 2020). Interestingly, minimal differences have been observed between the gut eukaryotic virome of diagnosed celiac disease patients and that of healthy children (El Mouzan *et al.* 2022).

Rheumatoid arthritis (RA) is another autoimmune disease characterized by the presence of serum anti-cyclic citrullinated protein (CCP) antibodies (Mangalea *et al.* 2021). Its etiology is multifactorial, and the interaction between gut bacteria, their phages, and the host immune system appears to play an important role. Individuals at risk for RA harbor distinct phage communities that may play a role in altering the intestinal metabolism, either by reducing the metabolic activity of essential bacterial species or through phage-mediated introduction of specific auxiliary metabolic genes (Mangalea *et al.* 2021). An expansion of *Bacteroidaceae*-targeting phages was observed in both CCP- positive and CCP-negative groups, although *Bacteroidaceae* bacteria levels remained unchanged. Notably, *Bacteroidaceae* expansion has been previously reported in mouse models of induced arthritis (Liu *et al.* 2016).

10.4 Restoring a Stable Gut Microbiome—From FMT to Phage-Based Therapies

A balanced gut microbiome, including its virome component, is essential for health (Shkoporov *et al.* 2019, Fassarella *et al.* 2021), whereas an imbalanced gut microbiome has been associated with a wide range of diseases (Clooney *et al.* 2019, Cao *et al.* 2022, Huang *et al.* 2024, Ritz *et al.* 2024). Whether these associations reflect causal mechanisms originating in the gut or are secondary to other factors remains largely unresolved (Rasmussen, Koefoed, *et al.* 2020, Cao *et al.* 2022). Nevertheless, this body of evidence raises a fundamental question: *How can we restore a healthy*

and stable gut microbiome, and thereby directly or indirectly treat gut-related diseases?

Over the past two decades, the primary investigated therapeutic approach has been FMT. FMT has demonstrated clinical potential in various contexts, including enhancing drug efficacy in cancer treatment (Routy *et al.* 2018), increasing remission rates in UC (Imdad *et al.* 2023), altering gut microbiome composition in obesity (Allegretti *et al.* 2020), improving insulin sensitivity in metabolic syndrome (Kootte *et al.* 2017, Wortelboer *et al.* 2023), and protecting against NEC in a preterm piglet model (Brunse *et al.* 2019). However, FMT has shown the most consistent and high efficacy in treating recurrent *Clostridioides difficile* infection (rCDI), with success rates exceeding 90% (Gupta *et al.* 2016). This has led to its adoption as a standard therapeutic practice, with formal guidelines established in both the European Union and the US for cases where antibiotic treatment (e.g., vancomycin) proves ineffective (McDonald *et al.* 2018, Mullish *et al.* 2018, Baunwall *et al.* 2021, van Prehn *et al.* 2021).

The mechanisms underlying the curative effects of FMT in rCDI remain unclear; however, several studies have reported consistent engraftment of phages from the donor microbiome into the recipient following successful treatment (Broecker *et al.* 2017, Ott *et al.* 2017, Draper *et al.* 2018, Zuo *et al.* 2018, Fujimoto *et al.* 2021), and an increased richness of the phage order *Caudoviricetes* has also been associated with positive outcomes (Zuo *et al.* 2018). Despite these promising observations, FMT harbors several challenges, including the cost and complexity of donor screening (Kassam *et al.* 2019), donor variability in terms of treatment efficacy and the reproducibility of similar donor gut microbiomes (Kassam *et al.* 2019, Wilson *et al.* 2019), and safety concerns. No screening method can definitively exclude the transfer of pathogenic microorganisms. The significance of this risk was highlighted by two cases in the United States where patients developed severe bacterial infections following FMT, one of which was fatal (US Food & Drug Administration 2019). Furthermore, vulnerable populations like immunocompromised patients are of particular concern. This was emphasized in a NEC model using preterm piglets,

where FMT caused increased mortality, likely due to bacterial overgrowth (Brunse *et al*. 2019).

These concerns have led to growing interest in fecal virome transplantation (FVT; also referred to as fecal filtrate transplantation, FFT), which involves transferring only the viral fraction of donor feces, from which most metabolites are typically removed using ultrafiltration (Deng *et al*. 2019, Larsen, Offersen, *et al*. 2023) (Figure 10.7). By excluding bacteria, FVT offers a potentially safer alternative with a reduced risk of bacterial infections. Two small pilot studies have reported promising results: one successfully treated all five patients with rCDI using non-lyophilized FVT (Ott *et al*. 2017), while another achieved similar outcomes using a lyophilized sterile filtrate (Kao *et al*. 2019). FVT has also shown high efficacy in a preclinical mouse model of rCDI (Rasmussen *et al*. 2024). However, a recent multicenter, randomized, double-blinded clinical trial found that lyophilized FVT was inferior to lyophilized donor stool in treating rCDI (Kao *et al*. 2025). While this study represents an important contribution to the field, several questions remain. Notably, the concentration of phages in the treatment preparations was not reported (Kao *et al*. 2025), despite evidence that phage dosage is a critical factor for therapeutic efficacy (Sarker *et al*. 2016, Jault *et al*. 2019, Rasmussen, Koefoed, *et al*. 2020). Furthermore, the potential impact of lyophilization on phage activity (i.e., capsid integrity and infectivity) was not addressed, raising concerns about whether this preservation method is suitable for FVT.

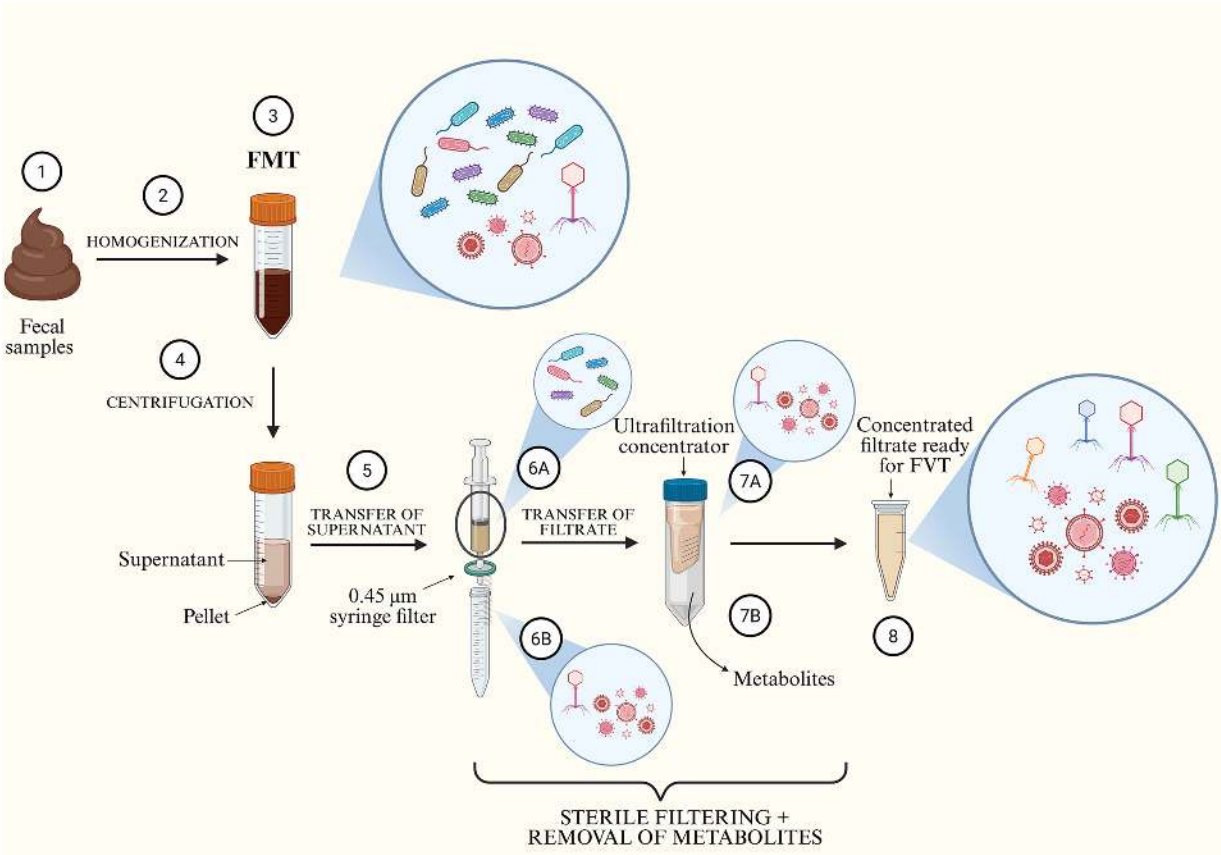

FIGURE 10.7 Preparation of FMT versus FVT. (1) Fecal samples are thawed on ice, (2) homogenized in filter stomacher bags with saline buffer (3) which then constitute the FMT that contains bacteria, phages, eukaryotic viruses, and fecal metabolites. (4) To prepare FVT the homogenized stool proceed to centrifugation in order to separate the pellet and the supernatant. The supernatant is processed through two steps of filtering: (5) first it is transferred to a syringe for sterile filtration (0.45 µm), which allows to obtain a filtrate without (6A) bacteria, but only (6B) phages, viruses, and other small particles like metabolites. The filtrate is then transferred to an ultrafiltration concentrator that separates (7A) phages and viruses from (7B) metabolites, based on molecule size measured in kDa. (9) The remaining concentrated filtrate, which mainly contains phages and viruses are now ready for FVT. (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/ksrbgry.) ⏎

Despite these mixed results observed in rCDI studies, the findings overall challenge the assumption that bacterial transfer is the primary

therapeutic agent in FMT and suggest that the enteric virome also plays a central role in gut microbiome restoration. This is supported by multiple preclinical studies reporting phenotypic changes following FVT. For example, FVT has been shown to protect against NEC development in preterm piglets (Brunse *et al*. 2022, Offersen *et al*. 2024, Spiegelhauer *et al*. 2025), reduce diarrhea onset in NEC (Offersen *et al*. 2025) and post-weaning diarrhea in pigs (Larsen, Andersen, *et al*. 2023), enhance intestinal barrier integrity in broiler chickens (Feng *et al*. 2024), promote proliferation of the commensal gut bacterium *Akkermansia muciniphila* in mice (Rasmussen *et al*. 2023), and impact the restoration of the gut microbiome of mice after antibiotic treatment (Draper *et al*. 2020), and that autochthonously (i.e., treated with virome isolated from its own feces) transferred FVT is protecting against stress-associated behavior in mice (Ritz *et al*. 2024). Notably, four independent preclinical studies have demonstrated that FVT can alleviate symptoms of metabolic syndrome (Rasmussen, Mentzel, *et al*. 2020, Borin *et al*. 2023, Cervantes-Echeverría *et al*. 2024, Mao *et al*. 2024), and a clinical study confirmed that FVT can be safely administered to patients with metabolic syndrome, resulting in transient alterations to their gut microbiome (Wortelboer *et al*. 2023).

10.4.1 Limitations of FVT and the Development of Pure Phage-Based Therapies

FVT offers a distinct advantage over FMT by excluding bacteria and thereby reducing the risk of bacterial infections. However, three major challenges must be addressed before FVT can be considered a broadly applicable therapeutic strategy (Figure 10.8):

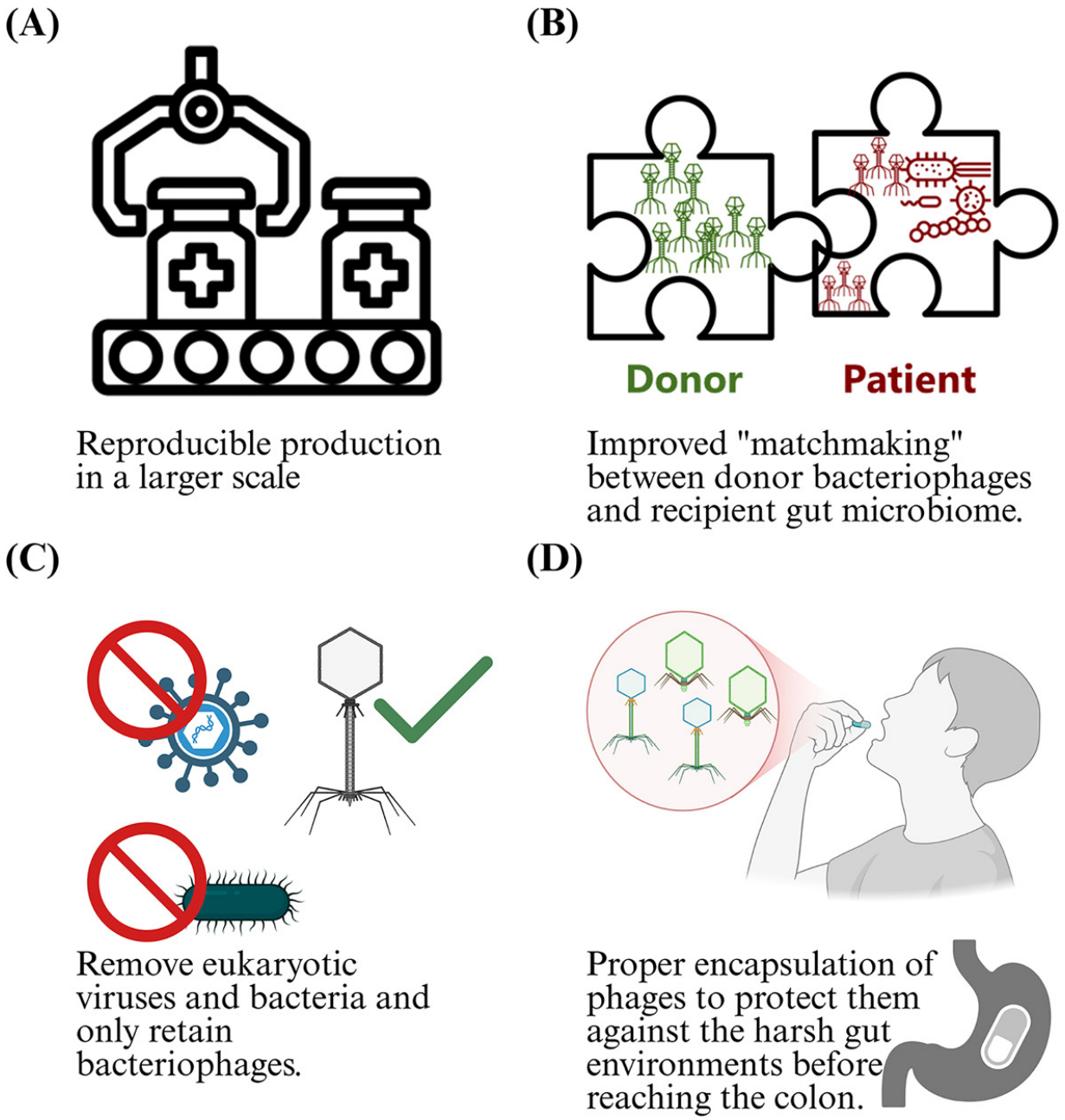

FIGURE 10.8 Illustrating the main limitations of FVT. (A) Reproducibility and scalability of phage production, (B) improved match between the highly individual gut microbiome of the donor and the recipient patient, (C) retaining only phages, (D) developing suitable drug delivery strategies for phages. (Created in BioRender. Rasmussen, T. (2026) https://BioRender.com/is3lca5.) ⏎

1. There is a need to procure and reproduce sufficient quantities of enteric phage solutions (Kassam *et al*. 2019) that are compatible with the highly individualized gut microbiome profiles of donors and recipients (Zhernakova *et al*. 2016).
2. The sterile filtration used in FVT preparation does not eliminate the risk of transferring human eukaryotic viruses due to their small size. Although donor feces can be screened for known pathogenic viruses, the human gastrointestinal tract harbors hundreds of eukaryotic viruses with unknown functions (Lim *et al*. 2015, Cao *et al*. 2022, Shah *et al*. 2023, Boulund *et al*. 2025). While many of these viruses are likely harmless, it cannot be ruled out that some may contribute to disease development later in life, as exemplified by human papillomavirus, which is a known risk factor for cervical cancer years after infection (Doorbar *et al*. 2015).
3. Phages must be able to survive the acidic environment of the stomach and reach the intestinal mucus (Barr *et al*. 2013) in therapeutically relevant concentrations.

To address these limitations, it has recently been suggested to develop a continuous fermentation reactor system designed to increase reproducibility, scale production, and eliminate eukaryotic viruses from phage preparations by dilution (Adamberg *et al*. 2024, Offersen *et al*. 2025). This system is inoculated with donor feces and allows only enteric bacteria and phages to replicate, resulting in a reproducible phageome depleted of eukaryotic viruses (Adamberg *et al*. 2024, Offersen *et al*. 2025). This approach has shown therapeutic potential in three preclinical models, including the treatment of *C. difficile* infections (Rasmussen *et al*. 2024), diet-induced obesity in mice (Mao *et al*. 2024), and NEC (Offersen *et al*. 2025). There is also a growing interest in developing encapsulation strategies that protect phages as they transit the gastrointestinal tract and enable their targeted release in the colon through pH-responsive triggers or mucus-interacting coatings (Yang *et al*. 2023), including the use of hydrogels (Yang *et al*. 2025).

However, because fermentation methodologies remain in the early stages of development, hence, further studies are needed to validate their efficacy and safety. If successful, this system could serve as a foundation for the development of phage-based therapies targeting gut-related diseases.

It is important to note that phage therapy is not a novel concept. The therapeutic use of phages dates back to the early 20th century, shortly after their discovery (Twort 1915), when Felix d'Herelle used phages to treat dysentery (D'Herelle 1917). Although phage products were commercially available in several Western countries until the 1940s, enthusiasm declined due to inconsistent results and the emergence of antibiotics. Nevertheless, phage therapy continued in Eastern Europe, particularly in Georgia and Poland (Myelnikov 2018). Today, the rise of multidrug-resistant bacteria has led to a renewed interest in phage therapy. Despite ongoing regulatory challenges, especially regarding the characterization and approval of phage therapy, significant progress has been made in recent years. Advances include improved regulatory frameworks, the application of artificial intelligence in phage discovery and design, and increased investment in phage therapeutics (Cui *et al*. 2024, Rivera-Lopez et al. 2025, Wang and Yu 2025). These developments may also benefit the emerging field of phage-based therapies targeting the gut microbiome.

10.4.2 Potential Mechanisms behind Phage-Mediated Modulation of the Gut Microbiome

Despite growing evidence supporting the use of FVT and other phage-based therapies to modulate the gut microbiome and influence host phenotype, the underlying mechanisms remain poorly understood. The therapeutic effects observed following FVT in conditions such as rCDI suggest that the virome plays a systemic role in shaping the gut microbial ecosystem. While phages with broad host ranges may directly target pathogens such as *C. difficile* (Souza *et al*. 1972, Hamdi *et al*. 2017, Zhao *et al*. 2019), the evidence increasingly points toward a more global restoration of the gut microbial ecosystem. In rCDI, the introduction of a donor virome may promote the

proliferation of beneficial bacterial populations while suppressing opportunistic pathogens through competition, thereby shifting the gut microbiome toward a more stable state in which *C. difficile* cannot thrive (Ott *et al*. 2017, Kao *et al*. 2019, Rasmussen *et al*. 2024). Similarly, the prevention of NEC in preterm piglets following FVT may be attributed to the stabilizing influence of a healthy virome, which mitigates bacterial overgrowth and the associated immune activation (Brunse *et al*. 2022).

Recent findings by Borodovich *et al*. (2024) may offer some mechanistic insight into how such systemic effects could arise. Using classical phage biology methodologies to ensure the complete elimination of bacterial cells and only retain only VLPs from human feces, the authors were able to sequence viral genomes via a long-read sequencing platform. The study revealed the extensive presence of bacterial DNA inside phage capsids as well as gene transfer agent (GTA)-like elements. This capsid-mediated DNA transfer was not confined to prophage regions but included large segments of bacterial genomes, indicating active horizontal gene transfer within the gut. Furthermore, the study identified both generalized and lateral transduction events across multiple taxa, including lateral transduction in *Bacteroides* and widespread GTA-like and generalized transduction-associated packaging in *Faecalibacterium* and *Gemmiger*, suggesting that phage-mediated gene flow is a widespread and taxonomically diverse phenomenon in the human gut (Borodovich *et al*. 2024).

These findings support the hypothesis that phage transfer via FVT can initiate cascading ecological interactions (Hsu *et al*. 2019, Rasmussen, Koefoed, *et al*. 2020). Phages may influence bacterial populations indirectly, as demonstrated in two independently studies using gnotobiotic mouse models where phage infections altered microbial composition without directly lysing dominant strains (Reyes *et al*. 2013, Hsu *et al*. 2019). Thus, even a limited number of phages may influence non-targeted microbial populations (Reyes *et al*. 2013, Cieplak *et al*. 2018, Fazzino *et al*. 2019, Febvre *et al*. 2019, Hsu *et al*. 2019). The ability of phages to interact with distantly related hosts (Hwang *et al*. 2023), possibly aided by phage

satellites (Eppley *et al.* 2022, Barcia-Cruz *et al.* 2024), and to transfer metabolic genes (Heyerhoff *et al.* 2022, Wittmers *et al.* 2022), further expands their ecological impact. The persistence of donor viromes in recipients for extended periods following FMT or FVT (Broecker *et al.* 2017, Ott *et al.* 2017, Draper *et al.* 2018, Zuo *et al.* 2018, Fujimoto *et al.* 2021) suggests that phages can establish long-term niches in the gut that may be facilitated by adsorption to mucus (Barr *et al.* 2013) and epithelial surfaces (Shan *et al.* 2018). These virome–host interactions may result in broader shifts in the microbiome and metabolome, thus potentially influencing host phenotype.

10.5 Future Perspectives of Phage-Based Therapy Targeting Gut-Related Diseases

Phage-based therapy is gaining momentum as a promising strategy to modulate the gut microbiome and treat diseases linked to microbial dysbiosis. FVT, in particular, has shown therapeutic potential in conditions such as rCDI, NEC, and metabolic syndrome, with evidence suggesting that its effects are not limited to direct pathogen targeting but involve a broader ecological restoration of the gut microbiome. Compared to FMT, FVT may offer a safer and more refined approach, though direct comparative studies are still lacking.

The field is still in its very early stages, and key challenges remain, including the identification of specific phages responsible for therapeutic effects, the development of scalable production methods, ensuring safety by excluding eukaryotic viruses, standardized phage quality control, and meeting the demands from the regulatory authorities. Advances in sequencing and fermentation technologies have begun to address these issues, enabling the generation of reproducible phage preparations with reduced risk profiles. Continued research is essential to define optimal phage consortia and delivery strategies to ensure effective and targeted microbiome modulation.

References

Adamberg, S., Rasmussen, T.S., Larsen, S.B., Mao, X., Nielsen, D.S., and Adamberg, K., 2024. Reproducible chemostat cultures to minimize eukaryotic viruses from fecal transplant material. *iScience*, 27 (8), 110460. ⏎

Allegretti, J.R., Kassam, Z., Mullish, B.H., Chiang, A., Carrellas, M., Hurtado, J., Marchesi, J.R., McDonald, J.A.K., Pechlivanis, A., Barker, G.F., Miguéns Blanco, J., Garcia-Perez, I., Wong, W.F., Gerardin, Y., Silverstein, M., Kennedy, K., and Thompson, C., 2020. Effects of fecal microbiota transplantation with oral capsules in obese patients. *Clinical Gastroenterology and Hepatology*, 18 (4), 855–863.e2. ⏎

Almeida, G.M. de F., Ravantti, J., Grdzelishvili, N., Kakabadze, E., Bakuradze, N., Javakhishvili, E., Megremis, S., Chanishvili, N., Papadopoulos, N., and Sundberg, L.-R., 2024. Relevance of the bacteriophage adherence to mucus model for *Pseudomonas aeruginosa* phages. *Microbiology Spectrum*, 12 (8), e0352023. ⏎

Bajer, L., Kverka, M., Kostovcik, M., Macinga, P., Dvorak, J., Stehlikova, Z., Brezina, J., Wohl, P., Spicak, J., and Drastich, P., 2017. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. *World Journal of Gastroenterology*, 23 (25), 4548. ⏎

Barcia-Cruz, R., Goudenège, D., Moura de Sousa, J.A., Piel, D., Marbouty, M., Rocha, E.P.C., and Le Roux, F., 2024. Phage-inducible chromosomal minimalist islands (PICMIs), a novel family of small marine satellites of virulent phages. *Nature Communications*, 15 (1), 664. ⏎

Barr, J.J., Auro, R., Furlan, M., Whiteson, K.L., Erb, M.L., Pogliano, J., Stotland, A., Wolkowicz, R., Cutting, A.S., Doran, K.S., Salamon, P., Youle, M., and Rohwer, F., 2013. Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proceedings of the National Academy of Sciences*, 110 (26), 10771–10776. ⏎

Barrangou, R., Yoon, S.-S., Breidt, F., Fleming, H.P., and Klaenhammer, T.R., 2002. Characterization of six *Leuconostoc fallax* bacteriophages

isolated from an industrial sauerkraut fermentation. *Applied and Environmental Microbiology*, 68 (11), 5452–8. ⏎

Bartlett, J.G. and Gerding, D.N., 2008. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clinical Infectious Diseases*, 46 (Supplement_1), S12–S18. ⏎

Baunwall, S.M.D., Dahlerup, J.F., Engberg, J.H., Erikstrup, C., Helms, M., Juel, M.A., Kjeldsen, J., Nielsen, H.L., Nilsson, A.C., Rode, A.A., Vinter-Jensen, L., and Hvas, C.L., 2021. Danish national guideline for the treatment of *Clostridioides difficile* infection and use of faecal microbiota transplantation (FMT). *Scandinavian Journal of Gastroenterology*, 56 (9), 1056–1077. ⏎

Bichet, M.C., Adderley, J., Avellaneda-Franco, L., Magnin-Bougma, I., Torriero-Smith, N., Gearing, L.J., Deffrasnes, C., David, C., Pepin, G., Gantier, M.P., Lin, R.C., Patwa, R., Moseley, G.W., Doerig, C., and Barr, J.J., 2023. Mammalian cells internalize bacteriophages and use them as a resource to enhance cellular growth and survival. *PLoS Biology*, 21 (10), e3002341. ⏎

Bichet, M.C., Chin, W.H., Richards, W., Lin, Y.-W., Avellaneda-Franco, L., Hernandez, C.A., Oddo, A., Chernyavskiy, O., Hilsenstein, V., Neild, A., Li, J., Voelcker, N.H., Patwa, R., and Barr, J.J., 2021. Bacteriophage uptake by mammalian cell layers represents a potential sink that may impact phage therapy. *iScience*, 24 (4), 102287. ⏎

Bikel, S., López-Leal, G., Cornejo-Granados, F., Gallardo-Becerra, L., García-López, R., Sánchez, F., Equihua-Medina, E., Ochoa-Romo, J.P., López-Contreras, B.E., Canizales-Quinteros, S., Hernández-Reyna, A., Mendoza-Vargas, A., and Ochoa-Leyva, A., 2021. Gut dsDNA virome shows diversity and richness alterations associated with childhood obesity and metabolic syndrome. *iScience*, 24 (8), 102900. ⏎

Borin, J.M., Liu, R., Wang, Y., Wu, T.-C., Chopyk, J., Huang, L., Kuo, P., Ghose, C., Meyer, J.R., Tu, X.M., Schnabl, B., and Pride, D.T., 2023. Fecal virome transplantation is sufficient to alter fecal microbiota and

drive lean and obese body phenotypes in mice. *Gut Microbes*, 15 (1), 2236750. ⏎

Borodovich, T., Wilson, J.S., Bardy, P., Smith, M., Hill, C., Khokhlova, E. V., Govi, B., Fogg, P.C.M., Hill, C., and Shkoporov, A.N., 2026. Large scale capsid-mediated mobilisation of bacterial genomic DNA in the gut microbiome. *Nature Communications*, 17 (1), 2046. ⏎

Boulund, U., Thorsen, J., Trivedi, U., Tranæs, K., Jiang, J., Shah, S.A., and Stokholm, J., 2025. The role of the early-life gut microbiome in childhood asthma. *Gut Microbes*, 17 (1), 2457489. ⏎

Breitbart, M., Bonnain, C., Malki, K., and Sawaya, N.A., 2018. Phage puppet masters of the marine microbial realm. *Nature Microbiology*, 3 (7), 754–766. ⏎

Breitbart, M., Hewson, I., Felts, B., Mahaffy, J.M., Nulton, J., Salamon, P., and Rohwer, F., 2003. Metagenomic analyses of an uncultured viral community from human feces. *Journal of Bacteriology*, 185 (20), 6220–6223. ⏎

Broecker, F., Russo, G., Klumpp, J., and Moelling, K., 2017. Stable core virome despite variable microbiome after fecal transfer. *Gut Microbes*, 8 (3), 214–220. ⏎

Brunse, A., Deng, L., Pan, X., Hui, Y., Castro-Mejía, J.L., Kot, W., Nguyen, D.N., Secher, J.B.-M., Nielsen, D.S., and Thymann, T., 2022. Fecal filtrate transplantation protects against necrotizing enterocolitis. *ISME Journal*, 16 (3), 686–694. ⏎

Brunse, A., Martin, L., Rasmussen, T.S., Christensen, L., Skovsted Cilieborg, M., Wiese, M., Khakimov, B., Pieper, R., Nielsen, D.S., Sangild, P.T., and Thymann, T., 2019. Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. *ISME Journal*, 13 (3), 720–733. ⏎

Camarillo-Guerrero, L.F., Almeida, A., Rangel-Pineros, G., Finn, R.D., and Lawley, T.D., 2021. Massive expansion of human gut bacteriophage diversity. *Cell*, 184 (4), 1098–1109.e9. ⏎

Cao, Z., Sugimura, N., Burgermeister, E., Ebert, M.P., Zuo, T., and Lan, P., 2022. The gut virome: a new microbiome component in health and disease. *eBioMedicine*, 81, 104113. ⏎

Carding, S.R., Davis, N., and Hoyles, L., 2017. Review article: the human intestinal virome in health and disease. *Alimentary Pharmacology & Therapeutics*, 46 (9), 800–815. ⏎

Cervantes-Echeverría, M., Gallardo-Becerra, L., Cornejo-Granados, F., and Ochoa-Leyva, A., 2023. The two-faced role of crAssphage subfamilies in obesity and metabolic syndrome: between good and evil. *Genes*, 14 (1), 139. ⏎

Cervantes-Echeverría, M.J., Jimenez-Rico, M.A., Manzo, R., Hernández-Reyna, A., Cornejo-Granados, F., Sánchez-López, F., Salazar-León, J., Pedraza-Alva, G., Perez-Martinez, L., and Leyva, A.O., 2024. Fecal virome transplantation (FVT) from healthy humans remodels the gut bacteriome and virome and reduces metabolic syndrome in mice. *bioRxiv*, 2024.02.17.580809. ⏎

Chen, Q., Ma, X., Li, C., Shen, Y., Zhu, W., Zhang, Y., Guo, X., Zhou, J., and Liu, C., 2021. Enteric phageome alterations in patients with type 2 diabetes. *Frontiers in Cellular and Infection Microbiology*, 10, 575084. ⏎

Cheng, C., He, Y., Xiao, S., Ai, Q., and Yu, J., 2021. The association between enteric viruses and necrotizing enterocolitis. *European Journal of Pediatrics*, 180 (1), 225–232. ⏎

Chin, W.H., Kett, C., Cooper, O., Müseler, D., Zhang, Y., Bamert, R.S., Patwa, R., Woods, L.C., Devendran, C., Korneev, D., Tiralongo, J., Lithgow, T., McDonald, M.J., Neild, A., and Barr, J.J., 2022. Bacteriophages evolve enhanced persistence to a mucosal surface. *Proceedings of the National Academy of Sciences*, 119 (27), e2116197119. ⏎

Cieplak, T., Soffer, N., Sulakvelidze, A., and Nielsen, D.S., 2018. A bacteriophage cocktail targeting *Escherichia coli* reduces *E. coli* in

simulated gut conditions, while preserving a non-targeted representative commensal normal microbiota. *Gut Microbes*, 9 (5), 391–399. ⏎

Clooney, A.G., Sutton, T.D.S., Shkoporov, A.N., Holohan, R.K., Daly, K.M., O'Regan, O., Ryan, F.J., Draper, L.A., Plevy, S.E., Ross, R.P., and Hill, C., 2019. Whole-virome analysis sheds light on viral dark matter in inflammatory bowel disease. *Cell Host & Microbe*, 26 (6), 764–778.e5. ⏎

Coggins, S.A., Wynn, J.L., and Weitkamp, J.-H., 2015. Infectious causes of necrotizing enterocolitis. *Clinics in Perinatology*, 42 (1), 133–154. ⏎

Cui, L., Kiga, K., Kondabagil, K., and Węgrzyn, A., 2024. Current and future directions in bacteriophage research for developing therapeutic innovations. *Scientific Reports*, 14 (1), 24404. ⏎

Dabrowska, K., Switala-Jelen, K., Opolski, A., Weber-Dabrowska, B., and Gorski, A., 2005. Bacteriophage penetration in vertebrates. *Journal of Applied Microbiology*, 98 (1), 7–13. ⏎

Dahlman, S., Avellaneda-Franco, L., Rutten, E.L., Gulliver, E.L., Solari, S., Chonwerawong, M., Kett, C., Subedi, D., Young, R.B., Campbell, N., Gould, J.A., Bell, J.D., Docherty, C.A.H., Turkington, C.J.R., Nezam-Abadi, N., Grasis, J.A., Lyras, D., Edwards, R.A., Forster, S.C., and Barr, J.J., 2025. Isolation, engineering and ecology of temperate phages from the human gut. *Nature*, 647 (8090), 698–705. ⏎

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A. V, Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J., and Turnbaugh, P.J., 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505 (7484), 559–563. ⏎

de Jonge, P.A., Wortelboer, K., Scheithauer, T.P.M., van den Born, B.-J.H., Zwinderman, A.H., Nobrega, F.L., Dutilh, B.E., Nieuwdorp, M., and Herrema, H., 2022. Gut virome profiling identifies a widespread bacteriophage family associated with metabolic syndrome. *Nature Communications*, 13 (1), 3594.

Deng, L., Silins, R., Castro-Mejía, J.L., Kot, W., Jessen, L., Thorsen, J., Shah, S., Stokholm, J., Bisgaard, H., Moineau, S., and Nielsen, D.S., 2019. A protocol for extraction of infective viromes suitable for metagenomics sequencing from low volume fecal samples. *Viruses*, 11 (7), 667. ⏎

Deplancke, B. and Gaskins, H.R., 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *American Journal of Clinical Nutrition*, 73 (6), 1131S–1141S. ⏎

De Sordi, L., Lourenço, M., and Debarbieux, L., 2019. "I will survive": a tale of bacteriophage–bacteria coevolution in the gut. *Gut Microbes*, 10 (1), 92–99.

D'Herelle, F., 1917. On an invisible microbe antagonistic toward dysenteric bacilli: brief note by Mr. F. D'Herelle, presented by Mr. Roux. *Research in Microbiology*, 158 (7), 553–554. ⏎

Doorbar, J., Egawa, N., Griffin, H., Kranjec, C., and Murakami, I., 2015. Human papillomavirus molecular biology and disease association. *Reviews in Medical Virology*, 25 (S1), 2–23. ⏎

Draper, L.A., Ryan, F.J., Dalmasso, M., Casey, P.G., McCann, A., Velayudhan, V., Ross, R.P., and Hill, C., 2020. Autochthonous faecal viral transfer (FVT) impacts the murine microbiome after antibiotic perturbation. *BMC Biology*, 18 (1), 173. ⏎

Draper, L.A., Ryan, F.J., Smith, M.K., Jalanka, J., Mattila, E., Arkkila, P.A., Ross, R.P., Satokari, R., and Hill, C., 2018. Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. *Microbiome*, 6 (1), 1–9. ⏎

Duerkop, B. A. and Hooper, L. V., 2013. Resident viruses and their interactions with the immune system. *Nature Immunology*, 14 (7), 654–659. ⏎

Duong, Q.A., Pittet, L.F., Curtis, N., and Zimmermann, P., 2022. Antibiotic exposure and adverse long-term health outcomes in children: a systematic review and meta-analysis. *Journal of Infection*, 85 (3), 213–300. ⏎

El Mouzan, M., Assiri, A., Al Sarkhy, A., Alasmi, M., Saeed, A., Al-Hussaini, A., AlSaleem, B., and Al Mofarreh, M., 2022. Viral dysbiosis in children with new-onset celiac disease. *PLoS One*, 17 (1), e0262108.

Eppley, J.M., Biller, S.J., Luo, E., Burger, A., and DeLong, E.F., 2022. Marine viral particles reveal an expansive repertoire of phage-parasitizing mobile elements. *Proceedings of the National Academy of Sciences U S A*, 119 (43), e2212722119. ⏎

European Centre for Disease Prevention and Control, 2022. *Clostridioides (Clostridium) difficile infections—Annual Epidemiological Report for 2016–2017 [online]*. European Centre for Disease Prevention and Control. ⏎

Fassarella, M., Blaak, E.E., Penders, J., Nauta, A., Smidt, H., and Zoetendal, E.G., 2021. Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. *Gut*, 70 (3), 595–605. ⏎

Fazzino, L., Anisman, J., Chacón, J.M., Heineman, R.H., and Harcombe, W.R., 2019. Lytic bacteriophage have diverse indirect effects in a synthetic cross-feeding community. *ISME Journal*, 14 (1), 123–134. ⏎

Febvre, H.P., Rao, S., Gindin, M., Goodwin, N.D.M., Finer, E., Vivanco, J.S., Lu, S., Manter, D.K., Wallace, T.C., and Weir, T.L., 2019. PHAGE study: effects of supplemental bacteriophage intake on inflammation and gut microbiota in healthy adults. *Nutrients*, 11 (3), 1–12. ⏎

Federici, S., Kredo-Russo, S., Valdés-Mas, R., Kviatcovsky, D., Weinstock, E., Matiuhin, Y., Silberberg, Y., Atarashi, K., Furuichi, M., Oka, A., Liu, B., Fibelman, M., Weiner, I.N., Khabra, E., Cullin, N., Ben-Yishai, N., Inbar, D., Ben-David, H., Nicenboim, J., Kowalsman, N., Lieb, W., Kario, E., Cohen, T., Geffen, Y.F., Zelcbuch, L., Cohen, A., Rappo, U., Gahali-Sass, I., Golembo, M., Lev, V., Dori-Bachash, M., Shapiro, H., Moresi, C., Cuevas-Sierra, A., Mohapatra, G., Kern, L., Zheng, D., Nobs, S.P., Suez, J., Stettner, N., Harmelin, A., Zak, N., Puttagunta, S., Bassan, M., Honda, K., Sokol, H., Bang, C., Franke, A., Schramm, C., Maharshak, N., Sartor, R.B., Sorek, R., and Elinav, E., 2022. Targeted

suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*, 185 (16), 2879–2898. ⏎

Feng, H., Xiong, J., Liang, S., Wang, Y., Zhu, Y., Hou, Q., Yang, X., and Yang, X., 2024. Fecal virus transplantation has more moderate effect than fecal microbiota transplantation on changing gut microbial structure in broiler chickens. *Poultry Science*, 103 (2), 103282. ⏎

Fujimoto, K., Kimura, Y., Allegretti, J.R., Yamamoto, M., Zhang, Y., Katayama, K., Tremmel, G., Kawaguchi, Y., Shimohigoshi, M., Hayashi, T., Uematsu, M., Yamaguchi, K., Furukawa, Y., Akiyama, Y., Yamaguchi, R., Crowe, S.E., Ernst, P.B., Miyano, S., Kiyono, H., Imoto, S., and Uematsu, S., 2021. Functional restoration of bacteriomes and viromes by fecal microbiota transplantation. *Gastroenterology*, 160 (6), 2089–2102.e12. ⏎

Gerding, D.N., Johnson, S., Peterson, L.R., Mulligan, M.E., and Silva Jr., J. 1995. *Clostridium difficile*-associated diarrhea and colitis. *Infection Control & Hospital Epidemiology*, 16 (8), 459–477. ⏎

Giel, J.L., Sorg, J.A., Sonenshein, A.L., and Zhu, J., 2010. Metabolism of bile salts in mice influences spore germination in *Clostridium difficile*. *PLoS One*, 5 (1), e8740. ⏎

Godsil, M., Ritz, N.L., Venkatesh, S., and Meeske, A.J., 2025. Gut phages and their interactions with bacterial and mammalian hosts. *Journal of Bacteriology*, 207 (2), e0042824. ⏎

Gogokhia, L., Buhrke, K., Bell, R., Hoffman, B., Brown, D.G., Hanke-Gogokhia, C., Ajami, N.J., Wong, M.C., Ghazaryan, A., Valentine, J.F., Porter, N., Martens, E., O'Connell, R., Jacob, V., Scherl, E., Crawford, C., Stephens, W.Z., Casjens, S.R., Longman, R.S., and Round, J.L., 2019. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. *Cell Host & Microbe*, 25 (2), 285–299.e8. ⏎

Górski, A., Miedzybrodzki, R., Borysowski, J., Dabrowska, K., Wierzbicki, P., Ohams, M., Korczak-Kowalska, G., Olszowska-Zaremba, N., Łusiak-Szelachowska, M., Kłak, M., Jończyk, E., Kaniuga, E., Gołaś, A.,

Purchla, S., Weber-Dabrowska, B., Letkiewicz, S., Fortuna, W., Szufnarowski, K., Pawełczyk, Z., Rogóz, P., and Kłosowska, D., 2012. Phage as a modulator of immune responses: practical implications for phage therapy. *Advances in Virus Research*, 83, 41–71. ⏎

Gregory, A.C., Zablocki, O., Zayed, A.A., Howell, A., Bolduc, B., and Sullivan, M.B., 2020. The gut virome database reveals age-dependent patterns of virome diversity in the human gut. *Cell Host and Microbe*, 28 (5), 724–740.e8. ⏎

Gupta, S., Allen-Vercoe, E., and Petrof, E.O., 2016. Fecal microbiota transplantation: in perspective. *Therapeutic Advances in Gastroenterology*, 9 (2), 229–239. ⏎

Hall, I.C., 1935. Intestinal flora in new-born infants. *American Journal of Diseases of Children*, 49 (2), 390. ⏎

Hamdi, S., Rousseau, G.M., Labrie, S.J., Tremblay, D.M., Kourda, R.S., Slama, K. Ben, and Moineau, S., 2017. Characterization of two polyvalent phages infecting Enterobacteriaceae. *Scientific Reports*, 7 (December 2016), 1–12. ⏎

Hansen, M.F., Svenningsen, S. Lo, Røder, H.L., Middelboe, M., and Burmølle, M., 2019. Big impact of the tiny: bacteriophage–bacteria interactions in biofilms. *Trends in Microbiology*, 27 (9), 739–752. ⏎

Heyerhoff, B., Engelen, B., and Bunse, C., 2022. Auxiliary metabolic gene functions in pelagic and benthic viruses of the Baltic Sea. *Frontiers in Microbiology*, 13, 863620. ⏎

Holmfeldt, K., Middelboe, M., Nybroe, O., and Riemann, L., 2007. Large variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their Flavobacterium hosts. *Applied and Environmental Microbiology*, 73 (21), 6730–6739. ⏎

Horst, L.J., Kempski, J., Walmsley, M., Huber, S., and Schramm, C., 2025. PSC and colitis: a complex relationship. *Hepatology*, 82 (4), 960–984. ⏎

Hoyles, L., McCartney, A.L., Neve, H., Gibson, G.R., Sanderson, J.D., Heller, K.J., and van Sinderen, D., 2014. Characterization of virus-like

particles associated with the human faecal and caecal microbiota. *Research in Microbiology*, 165 (10), 803–812. ⏎

Hsia, R., Ohayon, H., Gounon, P., Dautry-Varsat, A., and Bavoil, P.M., 2000. Phage infection of the obligate intracellular bacterium, strain Guinea Pig Inclusion Conjunctivitis. *Microbes and Infection*, 2 (7), 761–772. ⏎

Hsu, B.B., Gibson, T.E., Yeliseyev, V., Liu, Q., Lyon, L., Bry, L., Silver, P.A., and Gerber, G.K., 2019. Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host & Microbe*, 25 (6), 803–814.e5. ⏎

Huang, H., Yang, Y., Wang, X., Wen, B., Yang, X., Zhong, W., Wang, Q., He, F., and Li, J., 2024. Gut virome dysbiosis impairs antitumor immunity and reduces 5-fluorouracil treatment efficacy for colorectal cancer. *Frontiers in Oncology*, 14, 1501981. ⏎

Hwang, Y., Roux, S., Coclet, C., Krause, S.J.E., and Girguis, P.R., 2023. Viruses interact with hosts that span distantly related microbial domains in dense hydrothermal mats. *Nature Microbiology*, 8 (5), 946–957. ⏎

Hyman, P. and van Raaij, M., 2018. Bacteriophage T4 long tail fiber domains. *Biophysical Reviews*, 10 (2), 463–471. ⏎

Imdad, A., Pandit, N.G., Zaman, M., Minkoff, N.Z., Tanner-Smith, E.E., Gomez-Duarte, O.G., Acra, S., and Nicholson, M.R., 2023. Fecal transplantation for treatment of inflammatory bowel disease. *Cochrane Database of Systematic Reviews*, 2023 (4), CD012774. ⏎

Jariah, R.O.A. and Hakim, M.S., 2019. Interaction of phages, bacteria, and the human immune system: evolutionary changes in phage therapy. *Reviews in Medical Virology*, 29 (5), e2055. ⏎

Jault, P., Leclerc, T., Jennes, S., Pirnay, J.P., Que, Y.-A., Resch, G., Rousseau, A.F., Ravat, F., Carsin, H., Le Floch, R., Schaal, J.V., Soler, C., Fevre, C., Arnaud, I., Bretaudeau, L., and Gabard, J., 2019. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised,

controlled, double-blind phase 1/2 trial. *Lancet Infectious Diseases*, 19 (1), 35–45. ⏎

Johnson, C.C. and Ownby, D.R., 2017. The infant gut bacterial microbiota and risk of pediatric asthma and allergic diseases. *Translational Research*, 179, 60–70. ⏎

Jończyk-Matysiak, E., Łusiak-Szelachowska, M., Kłak, M., Bubak, B., Międzybrodzki, R., Weber-Dąbrowska, B., Zaczek, M., Fortuna, W., Rogóz, P., Letkiewicz, S., Szufnarowski, K., and Górski, A., 2015. The effect of bacteriophage preparations on intracellular killing of bacteria by phagocytes. *Journal of Immunology Research*, 2015, 482863. ⏎

Kaelin, E.A., Rodriguez, C., Hall-Moore, C., Hoffmann, J.A., Linneman, L.A., Ndao, I.M., Warner, B.B., Tarr, P.I., Holtz, L.R., and Lim, E.S., 2022. Longitudinal gut virome analysis identifies specific viral signatures that precede necrotizing enterocolitis onset in preterm infants. *Nature Microbiology*, 7 (5), 653–662. ⏎

Kan, L. and Barr, J.J., 2023. A mammalian cell's guide on how to process a bacteriophage. *Annual Review of Virology*, 10 (1), 183–198. ⏎

Kanamaru, S., Leiman, P.G., Kostyuchenko, V.A., Chipman, P.R., Mesyanzhinov, V. V., Arisaka, F., and Rossmann, M.G., 2002. Structure of the cell-puncturing device of bacteriophage T4. *Nature*, 415 (6871), 553–557. ⏎

Kao, D., Wong, K., Lee, C., Steiner, T., Franz, R., McDougall, C., Silva, M., Schmidt, T.S.B., Walter, J., Loebenberg, R., Monaghan, T.M., Giebelhaus, R.T., Harynuk, J.J., Xu, H., Yaskina, M., MacDonald, K. V, Marshall, D.A., and Louie, T., 2025. Effects of lyophilised faecal filtrate compared with lyophilised donor stool on *Clostridioides difficile* recurrence: a multicentre, randomised, double-blinded, non-inferiority trial. *Lancet Gastroenterology & Hepatology*, 10 (11), 986–997. ⏎

Kao, D.H., Roach, B., Walter, J., Lobenberg, R., and Wong, K., 2019. Effect of lyophilized sterile fecal filtrate vs lyophilized donor stool on recurrent *Clostridium difficile* infection (rCDI): prelimenary results from

a randomized, double-blind pilot study. *Journal of the Canadian Association of Gastroenterology*, 2 (Supplement_2), 101–102. ⏎

Kassam, Z., Dubois, N., Ramakrishna, B., Ling, K., Qazi, T., Smith, M., Kelly, C.R., Fischer, M., Allegretti, J.R., Budree, S., Panchal, P., Kelly, C.P., and Osman, M., 2019. Donor screening for fecal microbiota transplantation. *New England Journal of Medicine*, 381 (21), 2070–2072. ⏎

Kaur, S., Harjai, K., and Chhibber, S., 2014. Bacteriophage-aided intracellular killing of engulfed methicillin-resistant *Staphylococcus aureus* (MRSA) by murine macrophages. *Applied Microbiology and Biotechnology*, 98 (10), 4653–4661. ⏎

Kellenberger, E., 1968. Studies on the morphopoiesis of the head of phage T-even. *Virology*, 34 (3), 549–561. ⏎

Kemppainen, K.M., Lynch, K.F., Liu, E., Lönnrot, M., Simell, V., Briese, T., Koletzko, S., Hagopian, W., Rewers, M., She, J.X., Simell, O., Toppari, J., Ziegler, A.G., Akolkar, B., Krischer, J.P., Lernmark, Å., Hyöty, H., Triplett, E.W., and Agardh, D., 2017. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. *Clinical Gastroenterology and Hepatology*, 15 (5), 694–702.e5. ⏎

Khanna, S., Pardi, D.S., Aronson, S.L., Kammer, P.P., Orenstein, R., St Sauver, J.L., Harmsen, W.S., and Zinsmeister, A.R., 2012. The epidemiology of community-acquired clostridium difficile infection: a population-based study. *American Journal of Gastroenterology*, 107 (1), 89–95. ⏎

Kootte, R.S., Levin, E., Salojärvi, J., Smits, L.P., Hartstra, A. V., Udayappan, S.D., Hermes, G., Bouter, K.E., Koopen, A.M., Holst, J.J., Knop, F.K., Blaak, E.E., Zhao, J., Smidt, H., Harms, A.C., Hankemeijer, T., Bergman, J.J.G.H.M.G.H.M., Romijn, H.A., Schaap, F.G., Olde Damink, S.W.M.M., Ackermans, M.T., Dallinga-Thie, G.M., Zoetendal, E., de Vos, W.M., Serlie, M.J., Stroes, E.S.G.G., Groen, A.K., and Nieuwdorp, M., 2017. Improvement of insulin sensitivity after lean

donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metabolism*, 26 (4), 611–619.e6. ⏎

Kummen, M., Holm, K., Anmarkrud, J.A., Nygård, S., Vesterhus, M., Høivik, M.L., Trøseid, M., Marschall, H.U., Schrumpf, E., Moum, B., Røsjø, H., Aukrust, P., Karlsen, T.H., and Hov, J.R., 2017. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. *Gut*, 66 (4), 611–619. ⏎

Lamers, M.M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T.I., Ravelli, R.B.G., Paul van Schayck, J., Mykytyn, A.Z., Duimel, H.Q., van Donselaar, E., Riesebosch, S., Kuijpers, H.J.H., Schipper, D., van de Wetering, W.J., de Graaf, M., Koopmans, M., Cuppen, E., Peters, P.J., Haagmans, B.L., and Clevers, H., 2020. SARS-CoV-2 productively infects human gut enterocytes. *Science (New York, N.Y.)*, 369 (6499), 50–54. ⏎

Larsen, C., Andersen, A.B., Sato, H., Brunse, A., and Thymann, T., 2023. Transplantation of fecal filtrate to neonatal pigs reduces post-weaning diarrhea: a pilot study. *Frontiers in Veterinary Science*, 10, 1110128. ⏎

Larsen, F., Offersen, S.M., Li, V.R., Deng, L., Nielsen, D.S., and Rasmussen, T.S., 2023. Choice of ultrafilter affects recovery rate of bacteriophages. *Viruses*, 15 (10), 2051. ⏎

Le, S., He, X., Tan, Y., Huang, G., Zhang, L., Lux, R., Shi, W., and Hu, F., 2013. Mapping the tail fiber as the receptor binding protein responsible for differential host specificity of pseudomonas aeruginosa bacteriophages PaP1 and JG004. *PLoS One*, 8 (7), e68562. ⏎

Leal Rodríguez, C., Shah, S.A., Rasmussen, M.A., Thorsen, J., Boulund, U., Pedersen, C.E.T., Castro-Mejía, J.L., Poulsen, C.E., Poulsen, C.S., Deng, L., Larsen, F.A.N., Widdowson, M., Zhang, Y., Sørensen, S.J., Moineau, S., Petit, M.A., Chawes, B., Bønnelykke, K., Nielsen, D.S., and Stokholm, J., 2024. The infant gut virome is associated with preschool asthma risk independently of bacteria. *Nature Medicine*, 30 (1), 138–148. ⏎

Lehti, T.A., Pajunen, M.I., Skog, M.S., and Finne, J., 2017. Internalization of a polysialic acid-binding *Escherichia coli* bacteriophage into eukaryotic neuroblastoma cells. *Nature Communications*, 8 (1), 1915. ⏎

Li, R., Wang, Y., Hu, H., Tan, Y., and Ma, Y., 2022. Metagenomic analysis reveals unexplored diversity of archaeal virome in the human gut. *Nature Communications*, 13 (1), 7978. ⏎

Li, Y., Sun, C., Zhu, J., Geng, M., Li, M., Zhao, X.-M., and Chen, W.-H., 2025. Biases and complementarity in gut viromes obtained from bulk and virus-like particle-enriched metagenomic sequencing. *Microbiology Spectrum*, 13 (8), e0001325. ⏎

Liang, G. and Bushman, F.D., 2021. The human virome: assembly, composition and host interactions. *Nature Reviews Microbiology*, 19 (8), 514–527. ⏎

Liang, G., Zhao, C., Zhang, H., Mattei, L., Sherrill-Mix, S., Bittinger, K., Kessler, L.R., Wu, G.D., Baldassano, R.N., DeRusso, P., Ford, E., Elovitz, M.A., Kelly, M.S., Patel, M.Z., Mazhani, T., Gerber, J.S., Kelly, A., Zemel, B.S., and Bushman, F.D., 2020. The stepwise assembly of the neonatal virome is modulated by breastfeeding. *Nature*, 581 (7809), 470–474. ⏎

Liang, W., Feng, Z., Rao, S., Xiao, C., Xue, X., Lin, Z., Zhang, Q., and Qi, W., 2020. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut*, 69 (6), 1141–1143. ⏎

Lim, E.S., Zhou, Y., Zhao, G., Bauer, I.K., Droit, L., Ndao, I.M., Warner, B.B., Tarr, P.I., Wang, D., and Holtz, L.R., 2015. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nature Medicine*, 21 (10), 1228–1234. ⏎

Lindfors, K., Lin, J., Lee, H.S., Hyöty, H., Nykter, M., Kurppa, K., Liu, E., Koletzko, S., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A.G., Akolkar, B., Krischer, J.P., Petrosino, J.F., Lloyd, R.E., and Agardh, D., 2020. Metagenomics of the faecal virome indicate a cumulative effect of enterovirus and gluten amount on the risk of coeliac disease

autoimmunity in genetically at risk children: the TEDDY study. *Gut*, 69 (8), 1416–1422. ⏎

Liu, C., Monaghan, T., Yadegar, A., Louie, T., and Kao, D., 2023. Insights into the evolving epidemiology of *Clostridioides difficile* infection and treatment: a global perspective. *Antibiotics*, 12 (7), 1141. ⏎

Liu, X., Zeng, B., Zhang, J., Li, W., Mou, F., Wang, H., Zou, Q., Zhong, B., Wu, L., Wei, H., and Fang, Y., 2016. Role of the gut microbiome in modulating arthritis progression in mice. *Scientific Reports*, 6 (1), 30594. ⏎

Lopez, J.A., McKeithen-Mead, S., Shi, H., Nguyen, T.H., Huang, K.C., and Good, B.H., 2025. Abundance measurements reveal the balance between lysis and lysogeny in the human gut microbiome. *Current Biology*, 35 (10), 2282–2294.e11. ⏎

Louie, T.J., Miller, M.A., Mullane, K.M., Weiss, K., Lentnek, A., Golan, Y., Gorbach, S., Sears, P., and Shue, Y.-K., 2011. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *New England Journal of Medicine*, 364 (5), 422–431. ⏎

Luo, S., Ru, J., Mirzaei, M.K., Xue, J., Peng, X., Ralser, A., Mejías-Luque, R., Gerhard, M., and Deng, L., 2023. Gut virome profiling identifies an association between temperate phages and colorectal cancer promoted by *Helicobacter pylori* infection. *Gut Microbes*, 15 (2), 2257291. ⏎

Lynn, M.A., Eden, G., Ryan, F.J., Bensalem, J., Wang, X., Blake, S.J., Choo, J.M., Chern, Y.T., Sribnaia, A., James, J., Benson, S.C., Sandeman, L., Xie, J., Hassiotis, S., Sun, E.W., Martin, A.M., Keller, M.D., Keating, D.J., Sargeant, T.J., Proud, C.G., Wesselingh, S.L., Rogers, G.B., and Lynn, D.J., 2021. The composition of the gut microbiota following early-life antibiotic exposure affects host health and longevity in later life. *Cell Reports*, 36 (8), 109564. ⏎

Ma, Y., You, X., Mai, G., Tokuyasu, T., and Liu, C., 2018. A human gut phage catalog correlates the gut phageome with type 2 diabetes. *Microbiome*, 6 (1), 24. ⏎

Mangalea, M.R., Paez-Espino, D., Kieft, K., Chatterjee, A., Chriswell, M.E., Seifert, J.A., Feser, M.L., Demoruelle, M.K., Sakatos, A., Anantharaman, K., Deane, K.D., Kuhn, K.A., Holers, V.M., and Duerkop, B.A., 2021. Individuals at risk for rheumatoid arthritis harbor differential intestinal bacteriophage communities with distinct metabolic potential. *Cell Host & Microbe*, 29 (5), 726–739.e5. ⏎

Mao, R., Qiu, Y., He, J.S., Tan, J.Y., Li, X.H., Liang, J., Shen, J., Zhu, L.R., Chen, Y., Iacucci, M., Ng, S.C., Ghosh, S., and Chen, M.H., 2020. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterology and Hepatology*, 5 (7), 667–678. ⏎

Mao, X., Larsen, S.B., Zachariassen, L.S.F., Brunse, A., Adamberg, S., Mejia, J.L.C., Larsen, F., Adamberg, K., Nielsen, D.S., Hansen, A.K., Hansen, C.H.F., and Rasmussen, T.S., 2024. Transfer of modified gut viromes improves symptoms associated with metabolic syndrome in obese male mice. *Nature Communications*, 15 (1), 1–19. ⏎

Marantos, A., Mitarai, N., and Sneppen, K., 2022. From kill the winner to eliminate the winner in open phage-bacteria systems. *PLOS Computational Biology*, 18 (8), e1010400. ⏎

Mårild, K., Kahrs, C.R., Tapia, G., Stene, L.C., and Størdal, K., 2015. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. *American Journal of Gastroenterology*, 110 (10), 1475–1484. ⏎

Marongiu, L., Landry, J.J.M., Rausch, T., Abba, M.L., Delecluse, S., Delecluse, H., and Allgayer, H., 2021. Metagenomic analysis of primary colorectal carcinomas and their metastases identifies potential microbial risk factors. *Molecular Oncology*, 15 (12), 3363–3384. ⏎

Mathieu, A., Dion, M., Deng, L., Tremblay, D., Moncaut, E., Shah, S.A., Stokholm, J., Krogfelt, K.A., Schjørring, S., Bisgaard, H., Nielsen, D.S., Moineau, S., and Petit, M.A., 2020. Virulent coliphages in 1-year-old children fecal samples are fewer, but more infectious than temperate coliphages. *Nature Communications*, 11 (1), 378. ⏎

McDonald, L.C., Gerding, D.N., Johnson, S., Bakken, J.S., Carroll, K.C., Coffin, S.E., Dubberke, E.R., Garey, K.W., Gould, C. V., Kelly, C., Loo, V., Shaklee Sammons, J., Sandora, T.J., and Wilcox, M.H., 2018. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clinical Infectious Diseases*, 66 (7), e1–e48. ⏎

Meneses, L., Brandão, A.C., Coenye, T., Braga, A.C., Pires, D.P., and Azeredo, J., 2023. A systematic review of the use of bacteriophages for in vitro biofilm control. *European Journal of Clinical Microbiology & Infectious Diseases*, 42 (8), 919–928. ⏎

Minot, S., Bryson, A., Chehoud, C., Wu, G.D., Lewis, J.D., and Bushman, F.D., 2013. Rapid evolution of the human gut virome. *Proceedings of the National Academy of Sciences U S A*, 110 (30), 12450–12455. ⏎

Morahan, G., 2012. Insights into type 1 diabetes provided by genetic analyses. *Current Opinion in Endocrinology, Diabetes and Obesity*, 19 (4), 263–270. ⏎

Mortensen, A.S., Nielsen, D.S., Røder, H.L., and Sølbeck Rasmussen, T., 2025. Mucofilm: a nexus for phage–microbiome interactions in gut ecology. *Applied and Environmental Microbiology*, 91 (11), e0126925. ⏎

Mullish, B.H., Quraishi, M.N., Segal, J.P., McCune, V.L., Baxter, M., Marsden, G.L., Moore, D.J., Colville, A., Bhala, N., Iqbal, T.H., Settle, C., Kontkowski, G., Hart, A.L., Hawkey, P.M., Goldenberg, S.D., and Williams, H.R.T., 2018. The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: Joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut*, 67 (11), 1920–1941. ⏎

Musher, D.M., Aslam, S., Logan, N., Nallacheru, S., Bhaila, I., Borchert, F., and Hamill, R.J., 2005. Relatively poor outcome after treatment of *Clostridium difficile* colitis with metronidazole. *Clinical Infectious Diseases*, 40 (11), 1586–1590. ⏎

Myelnikov, D., 2018. An alternative cure: the adoption and survival of bacteriophage therapy in the USSR, 1922–1955. *Journal of the History of Medicine and Allied Sciences*, 73 (4), 385–411. ⏎

Najafimemar, Z., Tabarraei, A., Talei, G., and Moradi, A., 2018. Prevalence and genotyping of torque teno virus in HBV/HIV and chronic HBV patients in Iran. *Iranian Biomedical Journal*, 22 (5), 338. ⏎

Nakatsu, G., Zhou, H., Wu, W.K.K., Wong, S.H., Coker, O.O., Dai, Z., Li, X., Szeto, C.H., Sugimura, N., Lam, T.Y.T., Yu, A.C.S., Wang, X., Chen, Z., Wong, M.C.S., Ng, S.C., Chan, M.T.V., Chan, P.K.S., Chan, F.K.L., Sung, J.J.Y., and Yu, J., 2018. Alterations in enteric virome are associated with colorectal cancer and survival outcomes. *Gastroenterology*, 155 (2), 529–541.e5. ⏎

Nayfach, S., Páez-Espino, D., Call, L., Low, S.J., Sberro, H., Ivanova, N.N., Proal, A.D., Fischbach, M.A., Bhatt, A.S., Hugenholtz, P., and Kyrpides, N.C., 2021. Metagenomic compendium of 189,680 DNA viruses from the human gut microbiome. *Nature Microbiology*, 6 (7), 960–970. ⏎

Nguyen, S., Baker, K., Padman, B.S., Patwa, R., Dunstan, R.A., Weston, T.A., Schlosser, K., Bailey, B., Lithgow, T., Lazarou, M., Luque, A., Rohwer, F., Blumberg, R.S., and Barr, J.J., 2017. Bacteriophage transcytosis provides a mechanism to cross epithelial cell layers. *mBio*, 8 (6), e01874-17. ⏎

Niño, D.F., Sodhi, C.P., and Hackam, D.J., 2016. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nature Reviews Gastroenterology and Hepatology*, 13 (10), 590–600. ⏎

Norman, J.M., Handley, S.A., Baldridge, M.T., Droit, L., Liu, C.Y., Keller, B.C., Kambal, A., Monaco, C.L., Zhao, G., Fleshner, P., Stappenbeck, T.S., McGovern, D.P.B.B., Keshavarzian, A., Mutlu, E.A., Sauk, J., Gevers, D., Xavier, R.J., Wang, D., Parkes, M., and Virgin, H.W., 2015. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell*, 160 (3), 447–460. ⏎

Offersen, S.M., Adamberg, S., Spiegelhauer, M.R., Mao, X., Rasmussen, T.S., Larsen, F., Zhong, J., Nguyen, D.N., Nielsen, D.S., Aunsholt, L., Thymann, T., Adamberg, K., and Brunse, A., 2025. Chemostat culturing reduces fecal eukaryotic virus load and delays diarrhea after virome transplantation. *eLife*, 14:RP105991. ⏎

Offersen, S.M., Mao, X., Spiegelhauer, M.R., Larsen, F., Li, V.R., Sandris Nielsen, D., Aunsholt, L., Thymann, T., and Brunse, A., 2024. Fecal virus-like particles are sufficient to reduce necrotizing enterocolitis. *Gut Microbes*, 16 (1). ⏎

Ott, S.J., Waetzig, G.H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J.A., Cassidy, L., Tholey, A., Fickenscher, H., Seegert, D., Rosenstiel, P., and Schreiber, S., 2017. Efficacy of sterile fecal filtrate transfer for treating patients with *Clostridium difficile* infection. *Gastroenterology*, 152 (4), 799–811.e7. ⏎

Palestrant, D., Holzknecht, Z.E., Collins, B.H., Parker, W., Miller, S.E., and Bollinger, R.R., 2004. Microbial biofilms in the gut: visualization by electron microscopy and by acridine orange staining. *Ultrastructural Pathology*, 28 (1), 23–27. ⏎

Penders, J., Stobberingh, E.E., van den Brandt, P.A., and Thijs, C., 2007. The role of the intestinal microbiota in the development of atopic disorders. *Allergy*, 62 (11), 1223–1236. ⏎

Pépin, J., Routhier, S., Gagnon, S., and Brazeau, I., 2006. Management and outcomes of a first recurrence of *Clostridium difficile*-associated disease in Quebec, Canada. *Clinical Infectious Diseases*, 42 (6), 758–764. ⏎

Pinto, Y., Chakraborty, M., Jain, N., and Bhatt, A.S., 2024. Phage-inclusive profiling of human gut microbiomes with Phanta. *Nature Biotechnology*, 42 (4), 651–662. ⏎

Pratt, D., Tzagoloff, H., and Beaudoin, J., 1969. Conditional lethal mutants of the small filamentous coliphage M13. II. Two genes for coat proteins. *Virology*, 39 (1), 42–53. ⏎

Rasmussen, T.S., Koefoed, A.K., Jakobsen, R.R., Deng, L., Castro-Mejía, J.L., Brunse, A., Neve, H., Vogensen, F.K., and Nielsen, D.S., 2020.

Bacteriophage-mediated manipulation of the gut microbiome—promises and presents limitations. *FEMS Microbiology Reviews*, 44 (4), 507–521. ⏎

Rasmussen, T.S., Mao, X., Forster, S., Larsen, S.B., Von Münchow, A., Tranæs, K.D., Brunse, A., Larsen, F., Mejia, J.L.C., Adamberg, S., Hansen, A.K., Adamberg, K., Hansen, C.H.F., and Nielsen, D.S., 2024. Overcoming donor variability and risks associated with fecal microbiota transplants through bacteriophage-mediated treatments. *Microbiome*, 12 (1), 119. ⏎

Rasmussen, T.S., Mentzel, C.M.J., Danielsen, M.R., Jakobsen, R.R., Zachariassen, L.S.F., Castro Mejia, J.L., Brunse, A., Hansen, L.H., Hansen, C.H.F., Hansen, A.K., and Nielsen, D.S., 2023. Fecal virome transfer improves proliferation of commensal gut *Akkermansia muciniphila* and unexpectedly enhances the fertility rate in laboratory mice. *Gut Microbes*, 15 (1), 2208504. ⏎

Rasmussen, T.S., Mentzel, C.M.J., Kot, W., Castro-Mejía, J.L., Zuffa, S., Swann, J.R., Hansen, L.H., Vogensen, F.K., Hansen, A.K., and Nielsen, D.S., 2020. Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. *Gut*, 69 (12), 2122–2130. ⏎

Reyes, A., Haynes, M., Hanson, N., Angly, F.E., Heath, A.C., Rohwer, F., and Gordon, J.I., 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature*, 466 (7304), 334–8. ⏎

Reyes, A., Wu, M., McNulty, N.P., Rohwer, F.L., and Gordon, J.I., 2013. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. *Proceedings of the National Academy of Sciences U S A*, 110 (50), 20236–20241. ⏎

Ritz, N.L., Draper, L.A., Bastiaanssen, T.F.S., Turkington, C.J.R., Peterson, V.L., van de Wouw, M., Vlckova, K., Fülling, C., Guzzetta, K.E., Burokas, A., Harris, H., Dalmasso, M., Crispie, F., Cotter, P.D., Shkoporov, A.N., Moloney, G.M., Dinan, T.G., Hill, C., and Cryan, J.F., 2024. The gut virome is associated with stress-induced changes in

behaviour and immune responses in mice. *Nature Microbiology*, 9 (2), 359–376. ⏎

Rivera-Lopez, E.O., Tirko, N.N., and Dudley, E.G., 2025. Regulatory landscape and the potential of bacteriophage applications in the United States' Food Industry. *Journal of Food Protection*, 88 (6), 100510. ⏎

Roivainen, M., 2006. Enteroviruses: new findings on the role of enteroviruses in type 1 diabetes. *International Journal of Biochemistry & Cell Biology*, 38 (5–6), 721–725. ⏎

Rothschild-Rodriguez, D., Hedges, M., Kaplan, M., Karav, S., and Nobrega, F.L., 2023. Phage-encoded carbohydrate-interacting proteins in the human gut. *Frontiers in Microbiology*, 13, 1083208. ⏎

Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M.P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragón, L., Jacquelot, N., Qu, B., Ferrere, G., Clémenson, C., Mezquita, L., Masip, J.R., Naltet, C., Brosseau, S., Kaderbhai, C., Richard, C., Rizvi, H., Levenez, F., Galleron, N., Quinquis, B., Pons, N., Ryffel, B., Minard-Colin, V., Gonin, P., Soria, J.-C., Deutsch, E., Loriot, Y., Ghiringhelli, F., Zalcman, G., Goldwasser, F., Escudier, B., Hellmann, M.D., Eggermont, A., Raoult, D., Albiges, L., Kroemer, G., and Zitvogel, L., 2018. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science*, 359 (6371), 91–97. ⏎

Sabbaghian, M., Gheitasi, H., Shekarchi, A.A., Tavakoli, A., and Poortahmasebi, V., 2024. The mysterious anelloviruses: investigating its role in human diseases. *BMC Microbiology*, 24 (1), 40. ⏎

Santiago-Rodriguez, T.M. and Hollister, E.B., 2019. Human virome and disease: high-throughput sequencing for virus discovery, identification of phage-bacteria dysbiosis and development of therapeutic approaches with emphasis on the human gut. *Viruses*, 11 (7), 656. ⏎

Sarkar, A., Yoo, J.Y., Dutra, S.V.O., Morgan, K.H., and Groer, M., 2021. The association between early-life gut microbiota and long-term health

and diseases. *Journal of Clinical Medicine*, 10 (3), 1–24. ⏎

Sarker, S.A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F., Bourdin, G., McCallin, S., Ngom-Bru, C., Neville, T., Akter, M., Huq, S., Qadri, F., Talukdar, K., Kassam, M., Delley, M., Loiseau, C., Deng, Y., El Aidy, S., Berger, B., and Brüssow, H., 2016. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. *EBioMedicine*, 4, 124–137. ⏎

Schulfer, A., Santiago-Rodriguez, T.M., Ly, M., Borin, J.M., Chopyk, J., Blaser, M.J., and Pride, D.T., 2020. Fecal viral community responses to high-fat diet in mice. *mSphere*, 5 (1), 1–16. ⏎

Seo, S.-U. and Kweon, M.-N., 2019. Virome–host interactions in intestinal health and disease. *Current Opinion in Virology*, 37, 63–71. ⏎

Sevelsted, A., Stokholm, J., and Bisgaard, H., 2016. Risk of asthma from cesarean delivery depends on membrane rupture. *Journal of Pediatrics*, 171, 38–42.e4. ⏎

Shah, S.A., Deng, L., Thorsen, J., Pedersen, A.G., Dion, M.B., Castro-Mejía, J.L., Silins, R., Romme, F.O., Sausset, R., Jessen, L.E., Ndela, E.O., Hjelmsø, M., Rasmussen, M.A., Redgwell, T.A., Leal Rodríguez, C., Vestergaard, G., Zhang, Y., Chawes, B., Bønnelykke, K., Sørensen, S.J., Bisgaard, H., Enault, F., Stokholm, J., Moineau, S., Petit, M.-A., and Nielsen, D.S., 2023. Expanding known viral diversity in the healthy infant gut. *Nature Microbiology*, 8 (5), 986–998. ⏎

Shamash, M. and Maurice, C.F., 2022. Phages in the infant gut: a framework for virome development during early life. *ISME Journal*, 16 (2), 323–330. ⏎

Shan, J., Ramachandran, A., Thanki, A.M., Vukusic, F.B.I., Barylski, J., and Clokie, M.R.J., 2018. Bacteriophages are more virulent to bacteria with human cells than they are in bacterial culture; insights from HT-29 cells. *Scientific Reports*, 8 (1), 5091. ⏎

Shimamura, M., 2016. Is there a link between viruses and necrotising enterocolitis? *Acta Paediatrica*, 105 (1), 15–16. ⏎

Shkoporov, A.N., Clooney, A.G., Sutton, T.D.S., Ryan, F.J., Daly, K.M., Nolan, J.A., McDonnell, S.A., Khokhlova, E. V., Draper, L.A., Forde, A., Guerin, E., Velayudhan, V., Ross, R.P., and Hill, C., 2019. The human gut virome is highly diverse, stable, and individual specific. *Cell Host & Microbe*, 26 (4), 527–541.e5. ⏎

Shkoporov, A.N. and Hill, C., 2019. Bacteriophages of the human gut: the "known unknown" of the microbiome. *Cell Host and Microbe*, 25 (2), 195–209. ⏎

Shkoporov, A.N., Khokhlova, E. V., Fitzgerald, C.B., Stockdale, S.R., Draper, L.A., Ross, R.P., and Hill, C., 2018. ΦCrAss001 represents the most abundant bacteriophage family in the human gut and infects *Bacteroides intestinalis*. *Nature Communications*, 9 (1), 1–8. ⏎

Shkoporov, A.N., Stockdale, S.R., Lavelle, A., Kondova, I., Heuston, C., Upadrasta, A., Khokhlova, E. V., van der Kamp, I., Ouwerling, B., Draper, L.A., Langermans, J.A.M., Paul Ross, R., and Hill, C., 2022. Viral biogeography of the mammalian gut and parenchymal organs. *Nature Microbiology*, 7 (8), 1301–1311. ⏎

Simmons, E.L., Drescher, K., Nadell, C.D., and Bucci, V., 2018. Phage mobility is a core determinant of phage–bacteria coexistence in biofilms. *ISME Journal*, 12 (2), 532–543. ⏎

Sinha, A., Li, Y., Mirzaei, M.K., Shamash, M., Samadfam, R., King, I.L., and Maurice, C.F., 2022. Transplantation of bacteriophages from ulcerative colitis patients shifts the gut bacteriome and exacerbates the severity of DSS colitis. *Microbiome*, 10 (1), 105. ⏎

Smeal, S.W., Schmitt, M.A., Pereira, R.R., Prasad, A., and Fisk, J.D., 2017. Simulation of the M13 life cycle I: assembly of a genetically-structured deterministic chemical kinetic simulation. *Virology*, 500, 259–274. ⏎

Soria-Villalba, A., Pesantes, N., Jiménez-Hernández, N., Pons, J., Moya, A., and Pérez-Brocal, V., 2024. Comparison of experimental methodologies based on bulk-metagenome and virus-like particle enrichment: pros and cons for representativeness and reproducibility in the study of the fecal human virome. *Microorganisms*, 12 (1), 162. ⏎

Souza, K.A., Ginoza, H.S., and Haight, R.D., 1972. Isolation of a polyvalent bacteriophage for *Escherichia coli*, *Klebsiella pneumoniae*, and *Aerobacter aerogenes*. *Journal of Virology*, 9 (5), 851–856. ⏎

Spiegelhauer, M.R., Offersen, S.M., Mao, X., Gambino, M., Sandris Nielsen, D., Nguyen, D.N., and Brunse, A., 2025. Protection against experimental necrotizing enterocolitis by fecal filtrate transfer requires an active donor virome. *Gut Microbes*, 17 (1), 2486517. ⏎

Sutcliffe, S.G., Reyes, A., and Maurice, C.F., 2023. Bacteriophages playing nice: lysogenic bacteriophage replication stable in the human gut microbiota. *iScience*, 26 (2), 106007. ⏎

Sweere, J.M., Van Belleghem, J.D., Ishak, H., Bach, M.S., Popescu, M., Sunkari, V., Kaber, G., Manasherob, R., Suh, G.A., Cao, X., de Vries, C.R., Lam, D.N., Marshall, P.L., Birukova, M., Katznelson, E., Lazzareschi, D. V, Balaji, S., Keswani, S.G., Hawn, T.R., Secor, P.R., and Bollyky, P.L., 2019. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science (New York, N.Y.)*, 363 (6434), eaat9691. ⏎

Tao, Z., 2018. Unveiling the gut virome in human health and diseases. *International Journal of Clinical Virology*, 2 (1), 001–003. ⏎

Tarracchini, C., Milani, C., Longhi, G., Fontana, F., Mancabelli, L., Pintus, R., Lugli, G.A., Alessandri, G., Anzalone, R., Viappiani, A., Turroni, F., Mussap, M., Dessì, A., Marincola, F.C., Noto, A., Magistris, A. De, Vincent, M., Bernasconi, S., Picaud, J.-C., Fanos, V., and Ventura, M., 2021. Unraveling the microbiome of necrotizing enterocolitis: insights in novel microbial and metabolomic biomarkers. *Microbiology Spectrum*, 9 (2), e01176–21. ⏎

Tetz, G., Brown, S.M., Hao, Y., and Tetz, V., 2019. Type 1 diabetes: an association between autoimmunity, the dynamics of gut amyloid-producing *E. coli* and their phages. *Scientific Reports*, 9 (1), 9685. ⏎

Tetz, G. and Tetz, V., 2016. Bacteriophage infections of microbiota can lead to leaky gut in an experimental rodent model. *Gut Pathogens*, 8 (1), 33. ⏎

Theriot, C.M., Bowman, A.A., and Young, V.B., 2016. Antibiotic-induced alterations of the gut microbiota alter secondary bile acid production and allow for *Clostridium difficile* spore germination and outgrowth in the large intestine. *mSphere*, 1 (1), e00045-15. ⏎

Tian, X., Li, S., Wang, C., Zhang, Y., Feng, X., Yan, Q., Guo, R., Wu, F., Wu, C., Wang, Y., Huo, X., and Ma, X., 2024. Gut virome-wide association analysis identifies cross-population viral signatures for inflammatory bowel disease. *Microbiome*, 12 (1), 130. ⏎

Tisza, M.J. and Buck, C.B., 2021. A catalog of tens of thousands of viruses from human metagenomes reveals hidden associations with chronic diseases. *Proceedings of the National Academy of Sciences of the United States of America U S A*, 118 (23), 1–11. ⏎

Tokarz, R., Hyams, J.S., Mack, D.R., Boyle, B., Griffiths, A.M., Leleiko, N.S., Sauer, C.G., Shah, S., Markowitz, J., Baker, S.S., Rosh, J., Baldassano, R.N., Kugathasan, S., Walters, T., Tagliafierro, T., Sameroff, S., Lee, B., Che, X., Oleynik, A., Denson, L.A., and Lipkin, W.I., 2019. Characterization of stool virome in children newly diagnosed with moderate to severe ulcerative colitis. *Inflammatory Bowel Diseases*, 25 (10), 1656. ⏎

Tomofuji, Y., Kishikawa, T., Maeda, Y., Ogawa, K., Nii, T., Okuno, T., Oguro-Igashira, E., Kinoshita, M., Yamamoto, K., Sonehara, K., Yagita, M., Hosokawa, A., Motooka, D., Matsumoto, Y., Matsuoka, H., Yoshimura, M., Ohshima, S., Nakamura, S., Inohara, H., Mochizuki, H., Takeda, K., Kumanogoh, A., and Okada, Y., 2022. Whole gut virome analysis of 476 Japanese revealed a link between phage and autoimmune disease. *Annals of the Rheumatic Diseases*, 81 (2), 278–288. ⏎

Triana, S., Metz-Zumaran, C., Ramirez, C., Kee, C., Doldan, P., Shahraz, M., Schraivogel, D., Gschwind, A.R., Sharma, A.K., Steinmetz, L.M., Herrmann, C., Alexandrov, T., Boulant, S., and Stanifer, M.L., 2021. Single-cell analyses reveal SARS-CoV-2 interference with intrinsic immune response in the human gut. *Molecular Systems Biology*, 17 (4), e10232. ⏎

Twort, F.W., 1915. An investigation on the nature of ultra- microscopic viruses. *Lancet*, 186 (4814), 1241–1243. ⏎

Tyschik, E.A., Rasskazova, A.S., Degtyareva, A. V., Rebrikov, D. V., and Sukhikh, G.T., 2018. Torque teno virus dynamics during the first year of life. *Virology Journal*, 15 (1), 96. ⏎

U.S. Food & Drug Administration, 2019. Important safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse reactions due to transmission of multi-drug resistant organisms [online]. *Available from:* www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse [Accessed 13 Jun 2023]. ⏎

Van Belleghem, J.D., Clement, F., Merabishvili, M., Lavigne, R., and Vaneechoutte, M., 2017. Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages. *Scientific Reports*, 7 (1), 8004.

Vancamelbeke, M. and Vermeire, S., 2017. The intestinal barrier: a fundamental role in health and disease. *Expert Review of Gastroenterology & Hepatology*, 11 (9), 821–834. ⏎

van Prehn, J., Reigadas, E., Vogelzang, E.H., Bouza, E., Hristea, A., Guery, B., Krutova, M., Norén, T., Allerberger, F., Coia, J.E., Goorhuis, A., van Rossen, T.M., Ooijevaar, R.E., Burns, K., Scharvik Olesen, B.R., Tschudin-Sutter, S., Wilcox, M.H., Vehreschild, M.J.G.T., Fitzpatrick, F., and Kuijper, E.J., 2021. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults. *Clinical Microbiology and Infection*, 27, S1–S21.

Verschluss der Gallenwege durch Verdickung der Wandungen, 1867. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin*, 39 (2), 206–215. ⏎

Wang, Y. and Yu, Y., 2025. Phage therapy as a revitalized weapon for treating clinical diseases. *Microbiome Research Reports*, 4 (3), 35. ⏎

Williamson, K.D. and Chapman, R.W., 2015. Primary sclerosing cholangitis: a clinical update. *British Medical Bulletin*, 114 (1), 53–64. ⏎

Wilson, A., Bogie, B., Chaaban, H., and Burge, K., 2023. The nonbacterial microbiome: fungal and viral contributions to the preterm infant gut in health and disease. *Microorganisms*, 11 (4), 909. ⏎

Wilson, B.C., Vatanen, T., Cutfield, W.S., and O'Sullivan, J.M., 2019. The super-donor phenomenon in fecal microbiota transplantation. *Frontiers in Cellular and Infection Microbiology*, 9 (January), 1–11. ⏎

Wittmers, F., Needham, D.M., Hehenberger, E., Giovannoni, S.J., and Worden, A.Z., 2022. Genomes from uncultivated Pelagiphages reveal multiple phylogenetic clades exhibiting extensive auxiliary metabolic genes and cross-family multigene transfers. *mSystems*, 7 (5), e0152221. ⏎

Wortelboer, K., de Jonge, P.A., Scheithauer, T.P.M., Attaye, I., Kemper, E.M., Nieuwdorp, M., and Herrema, H., 2023. Phage-microbe dynamics after sterile faecal filtrate transplantation in individuals with metabolic syndrome: a double-blind, randomised, placebo-controlled clinical trial assessing efficacy and safety. *Nature Communications*, 14 (1), 5600. ⏎

Xiang, Y., Morais, M.C., Cohen, D.N., Bowman, V.D., Anderson, D.L., and Rossmann, M.G., 2008. Crystal and cryoEM structural studies of a cell wall degrading enzyme in the bacteriophage φ29 tail. *Proceedings of the National Academy of Sciences U S A*, 105 (28), 9552–9557. ⏎

Yang, K., Niu, J., Zuo, T., Sun, Y., Xu, Z., Tang, W., Liu, Q., Zhang, J., Ng, E.K.W., Wong, S.K.H., Yeoh, Y.K., Chan, P.K.S., Chan, F.K.L., Miao, Y., and Ng, S.C., 2021. Alterations in the gut virome in obesity and type 2 diabetes mellitus. *Gastroenterology*, 161 (4), 1257–1269.e13. ⏎

Yang, Y., Du, H., Zou, G., Song, Z., Zhou, Y., Li, H., Tan, C., Chen, H., Fischetti, V.A., and Li, J., 2023. Encapsulation and delivery of phage as a novel method for gut flora manipulation in situ: a review. *Journal of Controlled Release*, 353, 634–649. ⏎

Yang, Y., Li, R., Zhong, Q., Guo, Y., Wu, R., Chen, H., Zhou, R., Ye, R., Dąbrowska, K., Prajsnar, T.K., Stafford, G.P., Zou, G., Zhou, Y., Li, J.,

and Song, Z., 2025. In situ gut microbiota editing: enhancing therapeutic efficacy for bacterial colitis by compatible oral hydrogel microspheres with phages. *Nature Communications*, 16 (1), 9785. ⏎

Yi, C., Chen, J., and She, X., 2024. The emerging role of the gut virome in necrotizing enterocolitis. *Heliyon*, 10 (9), e30496. ⏎

Zeng, S., Almeida, A., Li, S., Ying, J., Wang, H., Qu, Y., Paul Ross, R., Stanton, C., Zhou, Z., Niu, X., Mu, D., and Wang, S., 2024. A metagenomic catalog of the early-life human gut virome. *Nature Communications*, 15 (1), 1864. ⏎

Zhang, L., Chu, J., Hao, W., Zhang, J., Li, H., Yang, C., Yang, J., Chen, X., and Wang, H., 2021. Gut microbiota and type 2 diabetes mellitus: association, mechanism, and translational applications. *Mediators of Inflammation*, 2021 (1), 5110276. ⏎

Zhang, S., Palazuelos-Munoz, S., Balsells, E.M., Nair, H., Chit, A., and Kyaw, M.H., 2016. Cost of hospital management of *Clostridium difficile* infection in United States—a meta- analysis and modelling study. *BMC Infectious Diseases*, 16 (1), 447. ⏎

Zhang, Y., Castro-Mejía, J.L., Deng, L., Shah, S.A., Thorsen, J., Leal Rodríguez, C., Jessen, L.E., Dion, M.B., Chawes, B., Bønnelykke, K., Sørensen, S.J., Bisgaard, H., Moineau, S., Petit, M.-A., Stokholm, J., and Nielsen, D.S., 2025. The influence of early life exposures on the infant gut virome. *Gut Microbes*, 17 (1), 2501194. ⏎

Zhao, G., Vatanen, T., Droit, L., Park, A., Kostic, A.D., Poon, T.W., Vlamakis, H., Siljander, H., Härkönen, T., Hämäläinen, A.M., Peet, A., Tillmann, V., Ilonen, J., Wang, D., Knip, M., Xavier, R.J., and Virgin, H.W., 2017. Intestinal virome changes precede autoimmunity in type I diabetes-susceptible children. *Proceedings of the National Academy of Sciences of the United States of America U S A*, 114 (30), E6166–E6175. ⏎

Zhao, Y., Ye, M., Zhang, X., Sun, M., Zhang, Z., Chao, H., Huang, D., Wan, J., Zhang, S., Jiang, X., Sun, D., Yuan, Y., and Hu, F., 2019. Comparing polyvalent bacteriophage and bacteriophage cocktails for

controlling antibiotic-resistant bacteria in soil-plant system. *Science of the Total Environment*, 657, 918–925. ⏎

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., Wang, J., Imhann, F., Brandsma, E., Jankipersadsing, S.A., Joossens, M., Cenit, M.C., Deelen, P., Swertz, M.A., LifeLines cohort study, Weersma, R.K., Feskens, E.J.M., Netea, M.G., Gevers, D., Jonkers, D., Franke, L., Aulchenko, Y.S., Huttenhower, C., Raes, J., Hofker, M.H., Xavier, R.J., Wijmenga, C., and Fu, J., 2016. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science (New York, N.Y.)*, 352 (6285), 565–9. ⏎

Zuo, T., Liu, Q., Zhang, F., Yeoh, Y.K., Wan, Y., Zhan, H., Lui, G.C.Y., Chen, Z., Li, A.Y.L., Cheung, C.P., Chen, N., Lv, W., Ng, R.W.Y., Tso, E.Y.K., Fung, K.S.C., Chan, V., Ling, L., Joynt, G., Hui, D.S.C., Chan, F.K.L., Chan, P.K.S., and Ng, S.C., 2021. Temporal landscape of human gut RNA and DNA virome in SARS-CoV-2 infection and severity. *Microbiome*, 9 (1), 91. ⏎

Zuo, T., Lu, X.J., Zhang, Y., Cheung, C.P., Lam, S., Zhang, F., Tang, W., Ching, J.Y.L., Zhao, R., Chan, P.K.S., Sung, J.J.Y., Yu, J., Chan, F.K.L., Cao, Q., Sheng, J.Q., and Ng, S.C., 2019. Gut mucosal virome alterations in ulcerative colitis. *Gut*, 68 (7), 1169–1179. ⏎

Zuo, T., Sun, Y., Wan, Y., Yeoh, Y.K., Zhang, F., Cheung, C.P., Chen, N., Luo, J., Wang, W., Sung, J.J.Y., Chan, P.K.S., Wang, K., Chan, F.K.L., Miao, Y., and Ng, S.C., 2020. Human-gut-DNA virome variations across geography, ethnicity, and urbanization. *Cell Host and Microbe*, 28 (5), 741–751.e4. ⏎

Zuo, T., Wong, S.H., Lam, K., Lui, R., Cheung, K., Tang, W., Ching, J.Y.L., Chan, P.K.S., Chan, M.C.W., Wu, J.C.Y., Chan, F.K.L., Yu, J., Sung, J.J.Y., and Ng, S.C., 2018. Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut*, 67 (4), 634–643. ⏎

Zuo, T., Zhan, H., Zhang, F., Liu, Q., Tso, E.Y.K., Lui, G.C.Y., Chen, N., Li, A., Lu, W., Chan, F.K.L., Chan, P.K.S., and Ng, S.C., 2020. Alterations in fecal fungal microbiome of patients with COVID-19 during time of hospitalization until discharge. *Gastroenterology*, 159 (4), 1302–1310.e5. ⏎

Zuo, W., Michail, S., and Sun, F., 2022. Metagenomic analyses of multiple gut datasets revealed the association of phage signatures in colorectal cancer. *Frontiers in Cellular and Infection Microbiology*, 12, 918010. ⏎

Section III

Disease-Associated Microbiomes

11

Human Skin Microbiome in Inflammatory Cutaneous Diseases of the Face

Caroline T. Cheung and Brigitte Dreno

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11.1 Introduction

The commensal microorganisms residing on and within human skin, including bacteria and bacteriophages, eukaryotic fungi, human viruses, and Demodex mites, are collectively referred to as the skin microbiome. These microorganisms form a complex symbiotic ecosystem that interacts with host cells to maintain skin homeostasis. They contribute to immune education, epidermal barrier integrity, modulation of the skin microenvironment, resistance to pathogens, regulation of odor, vitamin metabolism, ultraviolet (UV) protection, neuroimmune signaling, and metabolic activity.

Initial colonization of the skin microbiome begins at birth and is influenced by the mode of delivery. Vaginally delivered infants acquire microbial communities resembling the maternal vaginal microbiota, dominated primarily by *Lactobacillus* spp. and *Prevotella* (Casterline & Paller, 2021). In contrast, infants delivered by Cesarean section harbor microbial communities similar to those found on adult skin, enriched in *Staphylococcus*, *Corynebacterium*, and *Cutibacterium* (formerly *Propionibacterium*) species (Dominguez-Bello et al.,

2010). It has been proposed that early skin contact and exposure to environmental microbes, including those present in hospital environments, contribute to the establishment of the neonatal skin microbiome in cesarean-delivered infants (Foessleitner et al., 2024; Shin et al., 2015).

The skin microbiome is highly personalized and varies substantially between individuals and across different body sites within the same individual. Microbial composition is influenced by genetic background (Moitinho-Silva et al., 2022), lifestyle (Kortekaas Krohn et al., 2024), anatomical location (Perez Perez et al., 2016), age (Howard et al., 2022), gender (Robert et al., 2022), medication use (Jo et al., 2021), environmental exposure (Selway et al., 2020), climate (Isler et al., 2023), urbanization (Ying et al., 2015), and animal contact (Peng & Biswas, 2020). More than 90% of cutaneous microorganisms are bacterial and belong predominantly to the phyla *Actinomycetota* (formerly *Actinobacteria*), *Bacillota* (formerly *Firmicutes*), *Pseudomonadota* (formerly *Proteobacteria*), and *Bacteroidota* (formerly *Bacteroidetes*) (Grice & Segre, 2011). It is estimated that approximately 10^4–10^6 bacterial cells inhabit each square centimeter of skin (Cundell, 2018). However, microbial composition at the strain level differs markedly according to local microenvironmental conditions, including moisture, lipid content, and oxygen availability (Roux et al., 2022).

The skin is continuously exposed to external stressors such as mechanical forces, UV radiation, oxidative stress, pollution, and climatic variation, as well as internal factors including aging, psychological stress, and genetic predisposition (Khmaladze et al., 2020). It represents the first line of immunological defense. Many inflammatory skin disorders, including acne, atopic dermatitis, and psoriasis, arise from the combined effects of environmental triggers and intrinsic host factors that activate inflammatory pathways regulated by the skin-associated lymphoid tissue (SALT) (Quaresma, 2019). It is now well established that the skin microbiome plays a critical role in the development, maturation, and regulation of the cutaneous immune system.

Dysbiosis refers to a disruption of the microbiome characterized by alterations in microbial composition, functional activity, metabolic output, or spatial organization, leading to a pathogenic or unhealthy state (Moskovicz et

al., 2020). Dysbiosis has been associated with impaired barrier function, increased pathogen colonization, metabolic imbalance, and enhanced inflammatory responses in the skin.

This chapter discusses methodologies for studying the skin microbiome, the composition of commensal microorganisms in healthy facial skin, physiological modulators of the facial microbiome, interactions between the skin ecosystem, immunity, and the exposome, the contribution of the gut–skin axis to inflammatory facial dermatoses, and emerging microbiome-targeted therapeutic strategies.

11.2 Methods for Studying Skin Microbiome

11.2.1 Sampling Techniques

Several approaches are used to collect skin samples for microbiome analysis, including swabbing, tape stripping, skin scraping, and skin biopsy. Swabbing, performed using either conventional cotton swabs or flocked swabs, is the most commonly applied method (Van Horn et al., 2008). Flocked swabs are coated with short nylon fibers arranged perpendicularly, which improves sample collection and release (Wise et al., 2021). Swabbing is standardized, inexpensive, and reproducible; however, it primarily captures microorganisms residing on the skin surface.

Tape stripping is another noninvasive technique that enables the collection of stratum corneum material using adhesive films. Compared with swabbing, tape stripping can recover higher numbers of viable bacteria, but it may induce mild skin irritation (Ogai et al., 2018). Skin scraping using a sterile blade allows sampling of deeper epidermal layers and generally yields higher microbial biomass than swabbing, although it requires greater technical expertise to avoid patient injury (Smith et al., 2024).

Skin punch biopsies provide access to both epidermal and dermal compartments, enabling comprehensive sampling of follicular and dermal microbial populations (Prast-Nielsen et al., 2019). However, these procedures are invasive, painful, and associated with a risk of scarring, which limits their routine use in clinical and research settings.

Emerging sampling technologies include adhesive patch–based biopsy devices (Yao et al., 2017), microprojection arrays (Liang et al., 2022), and microneedle patches (S. H. Kim et al., 2022). Adhesive patch devices enable minimally invasive transepidermal sampling and capture microbes from deeper skin layers. Microprojection arrays and microneedle systems allow spatially resolved sampling across different skin compartments and may support high-resolution mapping of microbial distribution in the future.

11.2.2 Culture-Dependent and Culture-Independent Approaches

Early studies of the skin microbiome relied primarily on culture-based techniques to obtain sufficient microbial biomass for downstream analysis. Culture conditions were optimized using diverse nutrient media under aerobic and anaerobic environments to maximize microbial recovery. More recently, culturomics approaches have been developed, combining high-throughput microbial cultivation under diverse conditions with rapid identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) and 16S ribosomal RNA (rRNA) gene sequencing, followed by functional characterization of isolated strains (Ndiaye et al., 2024; Prieto, 2023).

Advances in microfluidics have further enhanced microbial isolation. Droplet-based microfluidic platforms encapsulate individual microbial cells in water-in-oil droplets, enabling controlled single-cell cultivation based on Poisson statistics and facilitating high-throughput isolation and characterization (Yu et al., 2022).

Despite these advances, culture-dependent methods remain labor-intensive, time-consuming, and inherently biased, as many skin microorganisms are fastidious or uncultivable under standard laboratory conditions. Rapidly growing species are often overrepresented, whereas slow-growing or nutritionally demanding taxa are under-detected.

Culture-independent approaches have therefore become central to skin microbiome research. Amplicon sequencing of bacterial 16S rRNA genes (Jo, Kennedy, et al., 2016) and fungal internal transcribed spacer (ITS) regions (Leung et al., 2016) remains the most cost-effective and widely applied

strategy. The bacterial 16S rRNA gene contains conserved regions interspersed with hypervariable regions that enable taxonomic discrimination. Universal primers targeting conserved regions allow amplification of hypervariable segments and facilitate broad bacterial detection. However, primer bias, limited taxonomic resolution, and the inability to detect viruses, mites, and many archaea represent important limitations (Jo, Kennedy, et al., 2016).

Full-length 16S rRNA sequencing overcomes some of these constraints by improving species-level resolution (Zhang et al., 2024). Earlier approaches relied on overlapping amplicons assembled by Sanger sequencing, whereas current long-read sequencing technologies enable direct high-throughput full-length sequencing, albeit with higher error rates.

Shotgun metagenomic sequencing analyzes all DNA present in a sample, allowing simultaneous characterization of bacterial, fungal, viral, and archaeal communities with strain-level resolution and functional annotation (Garcia-Garcerà et al., 2013; Meisel et al., 2016). Sequenced fragments are assembled into contigs and clustered into draft genomes based on sequence composition and coverage depth. Integration of multilocus strain typing further enhances taxonomic precision (Bangayan et al., 2020). However, high host DNA content, assembly errors, contamination between closely related genomes, and higher costs remain important technical limitations.

11.2.3 Systems Biology Approaches to Studying the Skin Microbiome

Comprehensive characterization of the skin microbiome requires integrative multi-omics strategies that extend beyond taxonomic profiling to capture functional activity and host–microbe interactions. Metagenomics, metatranscriptomics, metaproteomics, metabolomics, and lipidomics collectively enable systems-level analysis of microbial ecosystems (Fernández-Carro et al., 2025).

Metagenomics provides an unbiased assessment of microbial genetic potential and supports high-resolution taxonomic and functional profiling (Kim et al., 2024). Metatranscriptomics characterizes actively expressed genes by sequencing microbial RNA, thereby reflecting metabolically active

populations and dynamic responses to environmental or host-derived stimuli (Chia et al., 2025). This approach enables direct linkage between microbial gene expression and physiological function.

Metaproteomics identifies and quantifies proteins using mass spectrometry–based shotgun proteomics, providing direct insight into enzymatic activity, signaling pathways, and post-translational modifications (X. Zhang et al., 2025). Metabolomics measures small-molecule metabolites produced by both microbes and host cells, enabling functional interpretation of metabolic interactions that influence barrier function, inflammation, odor production, and disease severity (Bianchi et al., 2025). Given the lipid-rich environment of the skin, lipidomics is particularly informative for characterizing sebum-derived lipids and microbially modified fatty acids that regulate microbial colonization and barrier integrity (Chudzik et al., 2024; Li et al., 2024).

Together, these complementary methodologies enable holistic interrogation of microbial composition, functional output, and biochemical interactions within the skin ecosystem.

11.2.4 Bioinformatics and Data Interpretation

Bioinformatic pipelines and curated databases are essential for integrating, analyzing, and interpreting large-scale microbiome datasets. Multi-omics platforms facilitate the integration of genomic, transcriptomic, proteomic, and metabolomic data to reconstruct molecular networks and identify functional pathways involved in host–microbe interactions.

Reference databases such as SILVA support accurate taxonomic classification through curated ribosomal RNA sequence libraries (Quast et al., 2013). The Human Microbial Metabolome Database provides detailed annotations linking microbial metabolites to biological functions (Wishart et al., 2023). Skin-focused repositories, including the SKIOME Project (Agostinetto et al., 2022) and the Human Skin Microbiome Database (http://bioinfodermatome.com/), enable standardized cross-study comparisons through harmonized metadata and curated microbial annotations. Broader resources such as MicrobiomeDB (Oliveira et al., 2018) and BodyMeta (D.

Zhang et al., 2025) support integrative analyses across multiple human body sites.

Robust bioinformatic workflows, standardized metadata reporting, and reproducible analytical pipelines remain critical to ensure data comparability, minimize batch effects, and enable meaningful biological interpretation.

11.3 Human Commensal Microbes in Normal Skin of the Face

The microbial community residing on healthy human skin is dominated by bacteria, which account for more than 90% of the total microbial population, followed by fungi, archaea, viruses, and microscopic parasites (Grice et al., 2009). The bacterial component is largely composed of four major phyla: *Actinomycetota*, *Bacillota*, *Pseudomonadota*, and *Bacteroidota* (Grice & Segre, 2011). Among eukaryotic microorganisms, the cutaneous mycobiome is primarily dominated by *Malassezia* species (Gupta & Kohli, 2004). Additional residents include skin-associated archaea and arthropods, with *Demodex* mites representing common inhabitants of sebaceous follicles on facial skin **(**Table 11.1**)** (El-Moamly & El-Swify, 2025; Umbach et al., 2021).

On the face, the most abundant bacterial genera include *Cutibacterium*, *Staphylococcus*, *Streptococcus*, and *Corynebacterium*, all of which are predominantly Gram-positive (Cosseau et al., 2016). Across diverse populations, *Cutibacterium acnes* and *Staphylococcus epidermidis* consistently emerge as dominant core commensals (Yu et al., 2024). Although their overall predominance is conserved, comparative studies have identified population-specific differences in relative abundance and in the representation of less dominant taxa, particularly between Asian and European cohorts (Mukherjee et al., 2016; Seong & Quince, 2025). These variations likely reflect differences in host genetics, environmental exposure, climate, lifestyle, and skin physiology.

Fungi represent an important eukaryotic component of the skin microbiome and exhibit strong site specificity across the body. *Malassezia* species dominate most anatomical locations; however, other genera, including *Aspergillus*, *Cladosporium*, *Epicoccum*, *Phoma*, *Cryptococcus*, *Rhodotorula*, *Saccharomyces*, and *Candida*, as well as dermatophytes such as *Microsporum*,

Trichophyton, and *Epidermophyton*, may be detected at low abundance (Findley et al., 2013; Gao et al., 2010; Jo, Deming, et al., 2016; Leung et al., 2016; Nguyen & Kalan, 2022). Among *Malassezia*, *M. restricta*, *Malassezia globosa*, and *Malassezia sympodialis* predominate in sebaceous regions due to their inability to synthesize fatty acids de novo, making them dependent on host-derived sebum as a nutrient source (Schmid et al., 2022).

Shotgun metagenomic studies indicate that bacteriophages dominate the skin virome, although viral sequences typically account for only 0.1–2% of total metagenomic reads (Hannigan et al., 2015). Phage distribution closely mirrors that of their bacterial hosts, suggesting strong ecological coupling and a regulatory role in controlling bacterial population dynamics (Haddock et al., 2023). Consequently, bacteriophages are increasingly considered potential endogenous biocontrol agents within the skin ecosystem.

The skin virome is composed primarily of bacteriophages belonging to the order *Caudoviricetes*, which infect common cutaneous commensals. Phages associated with *Actinomyces Corynebacterium*, *Cutibacterium*, *Pseudomonas*, and *Streptococcus* have been identified (Paul et al., 2025). In addition to bacteriophages, eukaryotic viruses including molluscum contagiosum virus, Merkel cell polyomavirus, alphapapillomaviruses, polyomaviruses (e.g., human polyomavirus 7 (HPyV7)), herpesviruses, and poxviruses can be detected on healthy skin (Che et al., 2025; Tirosh et al., 2018). These viruses tend to be less site-specific and more transient than bacteriophages but may dominate under specific physiological or pathological conditions.

Although archaea have been detected on human skin, they remain relatively rare and inconsistently present across individuals (Umbach et al., 2021). On average, archaeal signatures represent approximately 0.7% of detected genes, corresponding to roughly 1–2% of total microbial cells. Detected taxa belong primarily to the phyla *Thaumarchaeota* and *Euryarchaeota*, with minor representation of methanogenic lineages (Koskinen et al., 2017; Moissl-Eichinger et al., 2017). Archaeal abundance may be underestimated due to limited primer coverage in commonly used sequencing protocols.

Demodex mites are obligate ectoparasites and established members of the skin ecosystem, with two human-associated species: *Demodex folliculorum* and *Demodex brevis* (El-Moamly & El-Swify, 2025; Kahraman et al., 2025).

These mites preferentially inhabit sebaceous-rich areas of the face, including the eyebrows, eyelashes, nose, and cheeks, while also colonizing the scalp, ears, neck, chest, and back. *D. folliculorum* is more prevalent on facial skin, whereas *D. brevis* is more frequently detected on the trunk. *Demodex* mites harbor internal bacteria, express lipases that modify sebum, and may transport bacteria on their surface, contributing indirectly to microbial interactions and inflammatory responses (Clanner-Engelshofen et al., 2020; Kubiak et al., 2018).

TABLE 11.1
Dominant Commensal Micro-Organisms of the Human Skin

Bacteria Genera (Phyla)	Fungi	Viruses	Archaea	Arthropods/ Parasites
Cutibacterium (Actinomycetota)	*Malassezia*	*Bacteriophages* (*Caudoviricetes*)	*Thaumarchaeota*	*Demodex*
Staphylococcus (Bacillota)	*Candida*	Eukaryotic viruses	*Euryarchaeota*	
***Streptococcus* (Bacillota)**	*Aspergillus*			
***Corynebacterium* (Actinomycetota)**	*Cladosporium*			
***Pseudomonas* (Pseudomonadota)**				

11.4 Physiological Modulating Factors of the Skin Microbiome of the Face

11.4.1 Facial Site Specificity

Profiling of multiple facial skin sites demonstrates marked spatial heterogeneity in microbial composition while also identifying representative sampling locations. Comparative analyses indicate that the cheek most closely

reflects the microbial composition of the entire face, whereas the forehead is the least representative site (Lee et al., 2021). Detailed mapping across the cheek, chin, eyelid, forehead, perioral skin, oral cavity, nose, nostril, and philtrum further reveals pronounced differences in community structure driven by local microenvironmental conditions (Brandwein et al., 2021). These findings are consistent with the concept that facial microbial diversity is primarily governed by skin physiology and habitat preference across dry, moist, and sebaceous regions.

Sebaceous-associated taxa dominate most facial sites. Members of the family Propionibacteriaceae are particularly abundant on the forehead, cheek, chin, nose, upper lip, and lips, reflecting elevated sebum production in these areas (Brandwein et al., 2021). In contrast, eyelids and nostrils harbor higher proportions of Streptococcaceae, whereas Staphylococcaceae are relatively enriched in the oral cavity and nostrils (Brandwein et al., 2021). The lips, eyelids, and oral region exhibit the greatest microbial diversity, likely due to increased environmental exposure, variable hydration, and frequent mechanical perturbation.

At the species level, *C. acnes* and *S. epidermidis* consistently dominate facial microbial communities (Garlet et al., 2024; Li et al., 2025; Saheb Kashaf et al., 2022). However, secondary taxa vary substantially between individuals and may include *Corynebacterium*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, and *Delftia* species. There also appears to be sex-related differences; *Bacillota* influenced mainly the male skin microbiota, while *Pseudomonadota* influenced the female microbiota (Robert et al., 2022). Alpha diversity measurements further support the cheek as a representative facial sampling site (Brandwein et al., 2021). Microbial communities often cluster more strongly within individuals than across anatomical sites, reflecting a pronounced host-specific microbial signature, while corresponding facial sites across individuals share partial similarity driven by shared microenvironmental constraints (Perez Perez et al., 2016).

Figure 11.1 shows an overview of the factors that influence the human skin microbiome of the face and the roles that it is involved in.

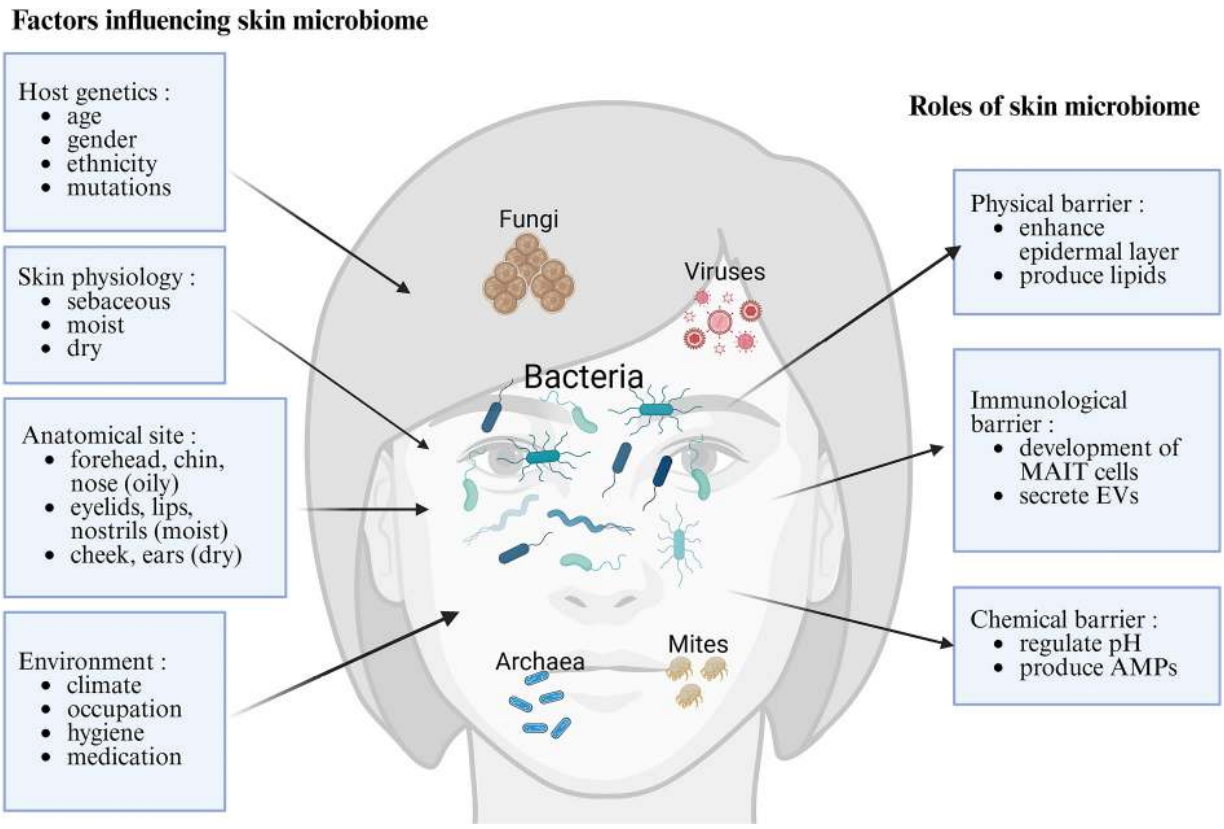

FIGURE 11.1 Overview of the factors that influence the human skin microbiome of the face, and the roles that it is involved in. ⏎

11.4.2 Age

Age is a major determinant of skin microbiome composition, diversity, and function, reflecting progressive changes in skin physiology across the lifespan (Garlet et al., 2024; Somboonna et al., 2017). Studies in adult populations demonstrate higher overall microbial diversity in middle-aged adults compared with adolescents and elderly individuals, whereas interindividual variability increases markedly with aging (Howard et al., 2022; Wu et al., 2020; Ying et al., 2015). In centenarians, microbial communities display substantial heterogeneity, with an increased relative abundance of potentially pathogenic *Staphylococcus* and *Streptococcus* species (Wu et al., 2020).

Longitudinal analyses indicate that the skin microbiome is dynamic rather than static, with year-to-year shifts in composition and abundance closely linked to changes in stratum corneum barrier properties, follicular porphyrin

production, and sebaceous gland activity. An age-related reduction in sebaceous gland size correlates positively with *Cutibacterium* and *Lactobacillus* abundance and negatively with *Corynebacterium* and *Enhydrobacter* (Garlet et al., 2024). Concurrent changes in skin lipid composition, including reduced monounsaturated fatty acids and increased long-chain fatty acids, ceramides, cholesterol, and sphingolipids, further shape microbial selection (Howard et al., 2022; Pagac et al., 2025).

Natural moisturizing factors (NMFs), derived primarily from filaggrin degradation, undergo significant age-related modulation (Howard et al., 2022). Although total NMF content may increase in elderly skin, barrier function declines, with reduced hydration, elevated transepidermal water loss (TEWL), and increased surface pH (Tsurumachi et al., 2023). Correlations between NMF composition and microbial abundance suggest selective effects on bacterial adherence and proliferation.

Fungal diversity tends to increase with age in several anatomical regions, while *Malassezia* abundance may decline in elderly populations when assessed using sequencing-based approaches (Howard et al., 2022; Jo, Deming, et al., 2016). In contrast, infants and young children exhibit reduced abundance of *Cutibacterium*, *Corynebacterium*, and *Malassezia*, with increased *Staphylococcus* and *Streptococcus* dominance and a mycobiome often enriched in *Candida* species (H.-J. Kim et al., 2022; Townsend & Kalan, 2023). Archaea display a triphasic age-related pattern, with higher prevalence in childhood and older age and reduced abundance during adolescence and adulthood (Moissl-Eichinger et al., 2017).

Collectively, aging is associated with coordinated alterations in host physiology, microbial diversity, community structure, and metabolic capacity, resulting in increasing individualization of the skin microbiome over time.

11.4.3 Ethnicity and Host Genetics

Ethnicity influences skin microbiome composition across multiple anatomical sites. Comparative studies demonstrate distinct microbial profiles among individuals of African, East Asian, and South Asian ancestry (Mukherjee et al., 2016; Perez Perez et al., 2016). One proposed genetic contributor is

polymorphism in the Adenosine triphosphate (ATP)-binding cassette transporter gene *ABCC11*, which regulates apocrine secretion composition (Stevens & Roesch, 2024). The A/A genotype, highly prevalent in East Asian populations, is associated with altered sweat composition and reduced odor production, potentially shaping microbial colonization patterns compared with populations of African or European ancestry (Amari et al., 2025; Harker et al., 2014).

Environmental factors, cultural practices, hygiene behaviors, climate, and cosmetic usage likely interact with host genetics to further modulate ethnic differences in microbial community structure (Kim et al., 2021; Wang et al., 2021). Although phototype-specific microbiome data remain limited, pigmentation-associated differences in barrier function, UV response, and lipid composition may indirectly influence microbial selection.

11.4.4 Skin Biophysical Parameters: pH, Sebum, and Sweat

Skin surface pH, sebaceous lipid production, and eccrine sweat secretion exert strong selective pressure on microbial community composition and metabolic activity (Swaney et al., 2023). The acidic pH of healthy skin restricts pathogen proliferation while favoring acid-tolerant commensals. Sebum provides both nutrients and antimicrobial free fatty acids following microbial lipase activity, promoting lipophilic taxa such as *Cutibacterium* and *Malassezia*. Sweat contributes water, electrolytes, lactate, urea, and antimicrobial peptides (AMPs), shaping osmotic balance and microbial metabolism.

Physiological or pathological alterations in these parameters during puberty, aging, endocrine disorders, or pharmacological interventions significantly remodel microbial communities and inflammatory susceptibility.

11.4.5 Sex, Body Mass Index, and Host Physiology

Sex-related differences in skin microbiome structure reflect underlying anatomical and hormonal differences. Men generally exhibit reduced alpha diversity and a higher relative abundance of anaerobic taxa, driven largely by increased sebum production and reduced oxygen tension (Robert et al., 2022).

Higher levels of free fatty acids and lactic acid contribute to surface acidification, further shaping microbial selection.

Women typically display higher microbial diversity, potentially related to a thinner epidermis, lower sebum output, higher hydration, and behavioral factors such as cosmetic use and hygiene practices (Ross et al., 2017). These behaviors may increase temporal variability in microbial composition.

Body mass index (BMI) is increasingly recognized as a systemic determinant of skin physiology and microbial composition (Ma et al., 2024). Elevated BMI is associated with impaired barrier function, altered hydration, and modified lipid composition, which in turn influence microbial diversity and community structure.

11.5 Skin Ecosystem and Microbiome of the Face

11.5.1 Role of the Skin Barrier

The skin barrier provides essential protection against external aggressors, including pathogenic microorganisms, chemical agents, allergens, environmental toxins, oxidative stress, and UV radiation (Bouwstra et al., 2023). Barrier function is mediated through three integrated components: mechanical, chemical, and immunological defenses (Baker et al., 2023). The mechanical barrier resides primarily within the epidermis, particularly the stratum corneum, which consists of terminally differentiated keratinocytes forming flattened corneocytes embedded in a lipid-rich extracellular matrix. These corneocytes are surrounded by a highly cross-linked cornified envelope that confers mechanical strength and resilience.

The chemical barrier is largely governed by the acidic surface pH generated by sebum-derived fatty acids, sweat lactate, and microbial metabolites (Baker et al., 2023). This acidic environment optimizes lipid-processing enzymes, including β-glucocerebrosidase and acid sphingomyelinase, which regulate ceramide production and epidermal permeability. Acidic pH also suppresses kallikrein protease activity, thereby preserving corneodesmosomal cohesion and limiting excessive desquamation (Jang et al., 2016).

The immunological barrier is mediated by specialized immune cells and the intrinsic immune competence of keratinocytes and sebocytes. Together, these components regulate inflammatory responses and microbial surveillance.

Barrier integrity is reinforced by the unique lipid architecture of the stratum corneum, which restricts TEWL and limits penetration of exogenous substances (Bouwstra et al., 2023). Molecules traverse the epidermis via intercellular lipid diffusion or transcellular pathways. Skin appendages further contribute to barrier function. Hair provides mechanical and UV protection, sebaceous glands secrete antimicrobial lipids, and sweat glands regulate hydration, thermoregulation, and surface pH.

Melanin pigmentation also contributes to barrier defense by protecting keratinocyte DNA from UV-induced damage (Baker et al., 2023; Bouwstra et al., 2023). Adequate micronutrient availability, including vitamins A, B3, B7, C, and D, is essential for epidermal differentiation, lipid synthesis, immune competence, and barrier repair.

11.5.2 Role of the Microbiome

The skin microbiome actively contributes to barrier maintenance and immune modulation (Grice & Segre, 2011). Microbial metabolites activate the aryl hydrocarbon receptor in keratinocytes, promoting epidermal differentiation and strengthening barrier integrity. Commensal bacteria such as *S. epidermidis* regulate lipid homeostasis through sphingomyelinase production, indirectly supporting ceramide synthesis. *Cutibacterium* and *Corynebacterium* species hydrolyze sebum triglycerides into free fatty acids, contributing to acidification, pathogen inhibition, and immune activation.

Short-chain fatty acids produced by *C. acnes* modulate immune signaling through inhibition of histone deacetylases and activation of fatty acid receptors, leading to cytokine induction via Toll-like receptor pathways (Shu et al., 2013). Unconventional T-cell populations, including mucosal-associated invariant T (MAIT) cells and invariant natural killer T cells, recognize conserved microbial antigens, promoting immune surveillance and tolerance (Constantinides et al., 2019).

Skin lipids such as sapienic acid exhibit intrinsic antimicrobial activity (Li et al., 2024). Positive correlations between commensal taxa and essential ceramides underscore cooperative interactions between microbes and lipid-mediated barrier protection. Regional differences in lipid composition contribute to site-specific microbial specialization.

11.5.3 Antimicrobial Peptides

AMPs constitute a central component of innate cutaneous defense. Major AMPs include β-defensins (hBD-1, hBD-2, and hBD-3), cathelicidin (LL-37), psoriasin, RNase 7, dermcidin, and lysozyme (Adnan et al., 2025). These peptides exhibit broad-spectrum activity against bacteria, fungi, and viruses while also exerting immunomodulatory functions. They regulate immune cell recruitment, cytokine production, oxidative responses, angiogenesis, and wound repair.

AMP expression is regulated by microbial signals, pH, lipid composition, and inflammatory mediators. Commensal microorganisms stimulate AMP production by keratinocytes, whereas AMPs shape microbial community structure, creating a dynamic feedback loop that stabilizes microbial homeostasis (Alexander et al., 2024; Belizário, 2025).

Coagulase-negative staphylococci (CoNS) play a prominent protective role. Certain strains of *Staphylococcus hominis* produce AMPs that inhibit *Staphylococcus aureus* colonization, while phenol-soluble modulins antagonize competing pathogens and enhance host AMP activity (Bier & Schittek, 2021). Some *S. epidermidis* strains secrete serine proteases that impair pathogen biofilm formation. *Cutibacterium acnes*–derived short-chain fatty acids further reinforce barrier integrity through the activation of PPAR-α signaling (Almoughrabie et al., 2023).

Fungal commensals also modulate barrier function. *Malassezia* species produce indole derivatives that activate aryl hydrocarbon receptor signaling, promoting epidermal differentiation while limiting excessive inflammation (Nguyen & Kalan, 2022).

11.5.4 Extracellular Vesicles

Extracellular vesicles (EVs) produced by skin-resident microorganisms represent a critical mode of interkingdom communication. These nanoscale bilayered particles transport bioactive cargo, including lipids, proteins, enzymes, metabolites, nucleic acids, and immunostimulatory molecules (Brown et al., 2015).

Bacterial EVs differ in biogenesis between Gram-negative and Gram-positive organisms (Toyofuku et al., 2019). Gram-negative vesicles can contain only the outer or inner membrane, or both, and carry lipopolysaccharides, whereas Gram-positive vesicles derive from the cytoplasmic membrane and present peptidoglycan and lipoteichoic acid.

EVs mediate microbial competition, biofilm formation, antimicrobial resistance, phage neutralization, and horizontal gene transfer (Toyofuku et al., 2023). They also deliver virulence factors and immunomodulatory molecules to host cells.

Pathogenic EVs may disrupt epidermal integrity and amplify inflammation (Dagnelie et al., 2020). *S. aureus* EVs enriched in α-hemolysin induce keratinocyte cytotoxicity, while *C. acnes* EVs activate inflammatory signaling pathways and contribute to acne pathophysiology. Fungal EVs from *M. sympodialis* contain allergenic components capable of inducing cytokine production in atopic skin (Johansson et al., 2018).

Conversely, EVs derived from commensal bacteria may support immune tolerance and barrier homeostasis (Kawagishi et al., 2025). Altered vesicle profiles have been observed in inflammatory dermatoses, suggesting that a loss of protective vesicle signaling may contribute to disease susceptibility.

11.5.5 Innate Immunity

The skin microbiome plays a foundational role in shaping innate immune development and function. Early microbial colonization promotes regulatory T-cell expansion and long-term immune tolerance toward commensal organisms (Coates et al., 2019). Germ-free animal models demonstrate impaired MAIT-cell development and defective wound healing, underscoring the importance of early microbial exposure (Jabeen & Hinks, 2023).

Throughout life, microbial signals regulate keratinocyte immunity and immune cell activation, maintaining barrier resilience and antimicrobial defense. Fungal commensals further modulate cytokine production and prostaglandin signaling (Nguyen & Kalan, 2022).

Microbiome-derived ligands activate aryl hydrocarbon receptor pathways in keratinocytes, enhancing differentiation and accelerating barrier repair. Collectively, these interactions establish a tightly integrated network linking microbial ecology, immune regulation, and epidermal integrity.

11.5.6 Climate, UV Radiation, and Urbanization

UV radiation exerts profound effects on skin biology, influencing cellular homeostasis, immune regulation, and oxidative stress (Burns et al., 2019). The skin microbiome modulates these responses by shaping local inflammatory tone and metabolic activity. Microbial metabolites can contribute to UV-induced immunomodulation and may provide partial protection against photo-induced damage. Specific commensals, including *Staphylococcus epidermidis*, attenuate UV-associated inflammation and oxidative stress by inhibiting NF-κB translocation, reducing sunburn responses, modulating antimicrobial peptide expression, and limiting melanosome transfer (Keshari et al., 2019).

Seasonal variation in microbial composition has also been reported. An increased abundance of *Sphingomonas* during summer months correlates with higher UV exposure, and metabolites produced by this genus exhibit antioxidant properties capable of neutralizing UV-induced oxidative stress (Harel et al., 2023). *Cutibacterium acnes* produces porphyrins within sebaceous follicles, which absorb visible light and generate reactive oxygen species upon photoexcitation, exerting bactericidal effects that regulate microbial population density under UV exposure (Ashkenazi et al., 2003; Johnson et al., 2016).

Experimental models further demonstrate microbiome-dependent immune modulation following UV exposure. Germ-free mice exposed to UV-B radiation exhibit enhanced immunosuppressive responses, characterized by increased interleukin (IL)-10 expression, whereas conventionally colonized mice display pro-inflammatory cytokine induction (Patra et al., 2019). These

findings suggest that the skin microbiome acts as a mediator of UV-driven immune signaling.

Urbanization also shapes microbial exposure patterns. Urban residents exhibit an increased relative abundance of human-associated microbes and indoor-derived taxa, whereas individuals with greater environmental contact, such as rural residents or outdoor workers, harbor microbial communities influenced by soil, water, and plant-associated organisms (Leung et al., 2016; Selway et al., 2020; Ying et al., 2015). These differences may influence microbial diversity, resilience, and susceptibility to inflammatory disease.

11.5.7 Hygiene and Cosmetic Practices

Cosmetic products exert complex and context-dependent effects on the skin microbiome. Certain ingredients may serve as nutrient substrates for microbial growth, whereas preservatives and surfactants may suppress specific taxa (Mim et al., 2024). Cosmetic use has been associated with transient reductions in microbial load and increased bacterial diversity, reflecting a perturbation of surface chemistry, hydration, and lipid composition.

In highly hydrated skin, cosmetic-induced increases in hydration and reductions in sebum may decrease *Cutibacterium* abundance (Wojciechowska & Dos Santos Szewczyk, 2025). Preservative combinations exhibit variable antimicrobial selectivity and may disrupt commensal balance. Some cosmetic ingredients may generate genotoxic byproducts under UV exposure, highlighting formulation safety considerations.

Overall, cosmetic practices represent a major modifiable determinant of skin microbial ecology and require careful formulation strategies to preserve microbial homeostasis.

11.5.8 Occupation and Environmental Exposure

Occupational environments influence microbial exposure and skin colonization patterns. Agricultural and livestock workers frequently exhibit an increased abundance of environmental *Pseudomonadota*, including *Pseudomonas* and *Acinetobacter*, with a reduced representation of typical skin-

associated *Actinomycetota* (Peng & Biswas, 2020). Repeated animal contact is associated with decreased *Staphylococcus* and *Streptococcus* abundance, consistent with biodiversity-driven microbial reshaping.

Healthcare workers experience frequent mechanical and chemical stress due to repetitive hand hygiene practices, which reduce microbial diversity and alter community structure, often enriching CoNS (Rosenthal et al., 2013). In swine and agricultural settings, dust-derived microbial transfer facilitates the acquisition of opportunistic pathogens and antibiotic resistance genes, representing potential occupational health risks (Slizovskiy et al., 2025).

11.5.9 Topical and Systemic Medications

Medical therapies, particularly oncologic treatments, exert profound effects on skin physiology and microbial ecology. Chemotherapy, radiotherapy, and targeted therapies frequently induce cutaneous toxicities, including dermatitis, hand–foot syndrome, and alopecia, which are accompanied by shifts in microbial diversity and composition (Bai et al., 2025; Bratborska et al., 2025).

Radiation-induced dermatitis is associated with reduced microbial richness and altered community structure, including an enrichment of *Pseudomonas*, *Staphylococcus*, and *Stenotrophomonas* (Bratborska et al., 2025). Disease severity correlates with microbial shifts, suggesting the potential prognostic value of microbiome profiling. Localized microbial alterations have been observed in cancer-affected versus contralateral healthy skin.

Systemic therapies may also disrupt the microbial–immune equilibrium in mechanically stressed regions, further amplifying barrier dysfunction and inflammation.

11.5.10 Psychological Stress

Psychological stress influences skin physiology through neuroendocrine signaling pathways and alters microbial ecology (Duarte et al., 2024). Stress-induced modulation of cutaneous hormones, neuropeptides, and cytokines can directly affect microbial growth and metabolic activity.

Chronic stress is associated with surface acidification and altered barrier function, which may favor acid-tolerant taxa and contribute to dysbiosis (Duarte et al., 2024). These changes may indirectly exacerbate inflammatory susceptibility and impair barrier resilience.

11.6 Specificities of Skin Microbiome in Main Inflammatory Diseases of the Face

11.6.1 Acne Vulgaris

Acne vulgaris is a multifactorial inflammatory disorder driven by follicular hyperkeratinization, increased sebum production, microbial dysbiosis, and immune activation (Dagnelie, Corvec, et al., 2019; Dagnelie, Montassier, et al., 2019). Although *C. acnes* has long been implicated in acne pathogenesis, accumulating evidence indicates that disease severity is more closely associated with a loss of strain diversity and the dominance of pro-inflammatory phylotypes rather than absolute bacterial abundance (Dagnelie et al., 2018). Acne lesions are frequently enriched in *C. acnes* phylotype IA1, which exhibits enhanced inflammatory potential (Cheung et al., 2024).

The interaction between *C. acnes* and *Staphylococcus epidermidis* plays a critical role in maintaining follicular homeostasis (Dagnelie et al., 2022). Certain *C. acnes* strains produce porphyrins that generate reactive oxygen species, triggering keratinocyte and monocyte activation. In contrast, *S. epidermidis* produces succinic acid, which inhibits *C. acnes* proliferation and attenuates inflammatory cytokine release. Balanced interspecies interactions therefore contribute to immune equilibrium.

Additional pathogenic mechanisms include biofilm formation, which enhances bacterial persistence and antimicrobial tolerance, and EV–mediated signaling, which modulates keratinocyte proliferation and differentiation (Ruffier d'Epenoux et al., 2024). Dysregulated microbial interactions amplify inflammatory mediators including IL-6, IL-10, complement factors, and vascular endothelial growth factor (Kurokawa & Nakase, 2020). Collectively, acne reflects an ecological imbalance rather than simple bacterial overgrowth.

11.6.2 Seborrheic Dermatitis

Seborrheic dermatitis is a chronic, relapsing inflammatory disorder affecting sebaceous-rich regions of the face and scalp (Galizia et al., 2024). Psychological stress and neuroimmune dysregulation are recognized triggers. A consistent feature of seborrheic dermatitis is reduced bacterial diversity and altered fungal composition.

Malassezia species play a central pathogenic role through lipid metabolism and immune activation (Kim, 2009; Vijaya Chandra et al., 2021). Lipase-mediated hydrolysis of sebum triglycerides generates free fatty acids that disrupt keratinocyte integrity, promote abnormal desquamation, and activate inflammatory signaling pathways, including MAPK and NF-κB. *Malassezia*-derived metabolites activate aryl hydrocarbon receptor signaling and contribute to barrier dysregulation.

Seborrheic dermatitis is also associated with an increased abundance of *Staphylococcus* and *Cutibacterium* species and the enrichment of additional fungal genera such as *Candida*, *Aspergillus*, and *Filobasidium* (Galizia et al., 2024). These shifts reflect a transition toward pro-inflammatory microbial communities that perpetuate barrier dysfunction.

11.6.3 Atopic Dermatitis

Atopic dermatitis is characterized by impaired epidermal barrier function, altered lipid composition, filaggrin deficiency, and increased TEWL (Koh et al., 2022). These changes facilitate allergen penetration and microbial colonization, promoting dysbiosis.

Colonization by *Staphylococcus aureus* represents a hallmark of atopic dermatitis (Huang et al., 2025). Elevated skin pH, reduced antimicrobial peptide production, and enhanced bacterial adhesion promote *S. aureus* overgrowth. Secreted proteases degrade structural proteins, disrupt tight junctions, and amplify inflammatory cascades. Toxins including α-toxin and δ-toxin induce keratinocyte injury and mast cell activation, whereas phenol-soluble modulins stimulate cytokine production.

Commensal *S. epidermidis* exhibits strain-dependent effects, with some isolates producing cytotoxic peptides, whereas others inhibit *S. aureus*

virulence (Bier & Schittek, 2021). Fungal dysbiosis, particularly involving *Malassezia* species, contributes to Th17-driven inflammation and protease-mediated barrier degradation (Huang et al., 2025; Koh et al., 2022). Increased oxygen tension in inflamed skin favors aerobic bacterial expansion and alters bacteriophage dynamics, further destabilizing microbial equilibrium.

11.6.4 Psoriasis

Psoriasis is a chronic immune-mediated disease characterized by keratinocyte hyperproliferation and aberrant differentiation (Celoria et al., 2023). Dysbiosis in psoriatic lesions includes reduced microbial diversity, a decreased abundance of *Cutibacterium* and *Actinomycetota*, and the enrichment of *Staphylococcus*, *Pseudomonas*, and *Streptococcus* species.

Microbial antigens may promote Th17 polarization and molecular mimicry, contributing to autoimmune amplification (Pereira et al., 2022). Dysregulated cytokine networks involving TNFα, IL-23, IL-17α, and IL-36γ drive keratinocyte activation, antimicrobial peptide overproduction, and neutrophil recruitment (Liu et al., 2020; Park & Heo, 2024; Sachen et al., 2022). These immune–microbial interactions generate self-sustaining inflammatory loops that perpetuate disease activity.

Fungal alterations, including changes in *Malassezia* composition and *Candida* colonization, further contribute to immune dysregulation (Celoria et al., 2023; Radaschin et al., 2024).

11.6.5 Rosacea

Rosacea is characterized by neurovascular dysregulation and innate immune hyperreactivity. An increased abundance of *S. epidermidis* has been observed in inflamed facial skin (Smith et al., 2025; Suri et al., 2025). A prominent feature is an elevated density of *Demodex* mites, which proliferate in warmer cutaneous microenvironments (Kahraman et al., 2025).

Mechanical follicular obstruction, barrier disruption, and activation of Toll-like receptor pathways by mite-associated antigens promote inflammatory

cascades (Lacey et al., 2007). Secondary bacterial carriage by *Demodex* may further amplify immune activation.

11.6.6 Actinic Keratosis and Photoaging

Actinic keratosis arises from chronic UV exposure and oxidative stress, leading to DNA damage, extracellular matrix degradation, and chronic inflammation (George et al., 2024; Wang et al., 2024). UV radiation alters microbial composition, favoring opportunistic pathogens such as *Staphylococcus aureus* (Kehrmann et al., 2023). Microbial toxins and inflammatory mediators may exacerbate oxidative damage and tumor-promoting microenvironments.

In contrast, *Cutibacterium* and *Malassezia* species are more prevalent in nonlesional photodamaged skin (Wood et al., 2018). Microbiome composition may influence therapeutic responsiveness to physical and topical interventions, supporting microbiome-informed precision strategies.

11.7 Gut–Skin Axis

11.7.1 Interactions Skin and Gut Microbiome in Inflammatory Skin Diseases of the Face

The gut–skin axis describes the bidirectional communication between the intestinal microbiota and the cutaneous ecosystem, whereby microbial metabolites, immune mediators, and neuroendocrine signals influence skin physiology and inflammation (Ellis et al., 2019). The gut microbiome, dominated primarily by the phyla *Bacillota* and *Bacteroidota*, plays essential roles in nutrient metabolism, xenobiotic processing, immune education, and the maintenance of epithelial barrier integrity.

Gut-derived metabolites, particularly short-chain fatty acids, enter the systemic circulation and modulate keratinocyte differentiation, immune tolerance, and inflammatory signaling pathways in the skin (Szántó et al., 2019). Dysbiosis of the gut microbiota disrupts intestinal barrier function, increases permeability, and facilitates the translocation of microbial products

into circulation, promoting chronic low-grade inflammation that can exacerbate cutaneous disease.

In atopic dermatitis, gut dysbiosis is characterized by reduced microbial diversity and the enrichment of potentially pathogenic taxa, which correlate with *Staphylococcus aureus* overgrowth on the skin and increased disease severity (Blicharz et al., 2025). In psoriasis, alterations in gut microbial composition, including a reduced abundance of anti-inflammatory, short-chain fatty acid–producing bacteria, contribute to systemic immune activation and Th17 polarization (Chen et al., 2024). The detection of gut-derived bacterial DNA in plasma supports the concept of microbial translocation under conditions of barrier impairment.

In rosacea, emerging evidence suggests associations with gastrointestinal dysbiosis, including *Helicobacter pylori* infection, although the mechanistic pathways remain incompletely defined (Szántó et al., 2019). Experimental models demonstrate that oral probiotic administration can attenuate rosacea-like inflammation, supporting a functional gut–skin connection (Szántó et al., 2019).

Overall, the modulation of the gut microbiome through dietary intervention, probiotics, synbiotics, and microbiota-targeted therapies represents a promising adjunctive strategy for managing inflammatory facial dermatoses.

11.8 Therapeutic Approach: Bacteriotherapy

11.8.1 Cutaneous Prebiotics, Probiotics, and Postbiotics

Topical microbiome-based interventions aim to restore ecological balance rather than eradicate microorganisms (França, 2021). Prebiotics selectively promote beneficial commensals by providing metabolic substrates or modulating surface pH (Wojciechowska & Dos Santos Szewczyk, 2025). Probiotic strategies involve the application of live beneficial strains, including selected CoNS or nonpathogenic *Cutibacterium* phylotypes capable of inhibiting pathogens, stimulating antimicrobial peptide production, and reinforcing barrier integrity (França, 2021). Postbiotics, encompassing microbial metabolites, peptides, and EVs, offer improved safety, stability, and

regulatory tractability while preserving biological activity (Prajapati et al., 2025).

11.8.2 Oral Prebiotics, Probiotics, and Postbiotics

Oral microbiome modulation influences systemic immune tone and metabolite availability through the gut–skin axis. Dietary fiber supplementation, fermented foods, and probiotic formulations may enhance short-chain fatty acid production and regulatory immune pathways, indirectly improving inflammatory skin conditions (Munteanu et al., 2025).

11.8.3 Phage Therapy and Endolysins

Bacteriophages and phage-derived endolysins enable the highly selective targeting of pathogenic strains while preserving commensal diversity (Natarelli et al., 2023). Phage-based approaches targeting *S. aureus* and pathogenic *C. acnes* lineages represent promising precision antimicrobial strategies with a reduced risk of resistance development and minimal ecological disruption.

11.8.4 Vaccination and Immune Modulation

Vaccination strategies targeting microbial virulence factors or host inflammatory mediators aim to reduce pathogenicity without disrupting microbial ecology (Bousbaine, 2025). Immunomodulatory approaches may complement microbiome-targeted therapies in refractory inflammatory dermatoses.

11.8.5 Clinical Evidence for Microbiome-Based Therapies in Facial Dermatoses (Table 11.2)

Several microbiome-targeted interventions have progressed from preclinical development to early-stage clinical evaluation, particularly in atopic dermatitis and acne vulgaris (Jacobson et al., 2024; Myles et al., 2020; Notay et al., 2020; Silverberg et al., 2023).

In atopic dermatitis, the topical administration of *Roseomonas mucosa* demonstrated significant reductions in disease severity scores, pruritus, and *Staphylococcus aureus* colonization in phase I/II clinical studies, with favorable safety profiles (Myles et al., 2018, 2020). The autologous transplantation of selected CoNS strains producing AMPs has also shown the targeted suppression of *S. aureus* and the improvement of barrier function in proof-of-concept human trials (Nakatsuji et al., 2021).

In acne vulgaris, the topical application of ammonia-oxidizing bacteria such as *Nitrosomonas eutropha* reduced inflammatory lesion counts and improved skin tolerance in early clinical investigations, potentially through nitric oxide production and the modulation of skin pH (AOBiome, 2017). Postbiotic formulations and bacteriophage-based approaches targeting pathogenic *C. acnes* strains are currently under clinical development (França, 2021; Kim & Kim, 2024).

Oral probiotic supplementation has demonstrated modest, but reproducible, clinical benefits in reducing disease severity and inflammatory biomarkers in atopic dermatitis, acne, and rosacea, supporting the relevance of systemic microbiome modulation through the gut–skin axis (Micu et al., 2026; Szántó et al., 2019).

Despite encouraging early signals, most studies remain limited by small sample sizes, heterogeneous endpoints, short follow-up durations, and variability in formulation and dosing strategies. Long-term safety, durability of microbial engraftment, regulatory classification of live biotherapeutic products, and manufacturing scalability remain critical translational challenges.

TABLE 11.2

Clinical Studies of Bacteriotherapy in Facial Inflammatory Disorders

Indication	Intervention	Study Type/Phase	Key Outcomes	Comments	References

Indication	Intervention	Study Type/Phase	Key Outcomes	Comments	References
Atopic dermatitis	Topical *Roseomonas mucosa*	Phase I/II clinical trials	Reduced Eczema Area and Severity Index (EASI) score, pruritus, and *S. aureus* colonization; good tolerability	Demonstrated safety and biological activity	Jacobson et al. (2024), Myles et al. (2018, 2020)
Atopic dermatitis	Autologous coagulase-negative staphylococci transplantation	Proof-of-concept human study	Targeted suppression of *S. aureus*, barrier improvement	Personalized approach; scalability challenges	Nakatsuji et al. (2021)
Acne vulgaris	Topical *Nitrosomonas eutropha*	Early clinical studies	Reduction of inflammatory lesions; improved skin tolerance	Mechanism involves nitric oxide and pH modulation	AOBiome (2017)
Acne vulgaris	Phage-based therapy targeting *C. acnes*	Early-stage clinical development	Strain-selective antimicrobial activity	Regulatory pathway still evolving	França (2021), Kim and Kim (2024)
Multiple dermatoses (AD, acne, rosacea)	Oral probiotics	Meta-analyses of randomized trials	Modest but significant clinical improvement	Heterogeneous strains and dosing	Micu et al. (2026), Szántó et al. (2019)

11.9 Conclusion

The facial skin microbiome represents a highly specialized and dynamic ecosystem integrating microbial diversity, barrier function, immune regulation, and environmental exposure. Dysbiosis contributes directly to inflammatory dermatoses through altered microbial signaling, impaired barrier integrity, and immune dysregulation. Advances in multi-omics technologies, systems biology, and microbiome-targeted therapeutics are enabling a transition toward mechanism-driven precision dermatology. Continued interdisciplinary research will be essential to translate microbiome science into clinically effective, safe, and personalized therapeutic strategies.

References

Adnan, S. B., Maarof, M., Fauzi, M. B., & Fadilah, N. I. M. (2025). Antimicrobial peptides in wound healing and skin regeneration: Dual roles in immunity and microbial defense. *International Journal of Molecular Sciences*, *26*(13), 5920. https://doi.org/10.3390/ijms26135920 ⏎

Agostinetto, G., Bozzi, D., Porro, D., Casiraghi, M., Labra, M., & Bruno, A. (2022). SKIOME Project: A curated collection of skin microbiome datasets enriched with study-related metadata. *Database: The Journal of Biological Databases and Curation*, *2022*, baac033. https://doi.org/10.1093/database/baac033 ⏎

Alexander, P. J., Oyama, L. B., Olleik, H., Godoy Santos, F., O'Brien, S., Cookson, A., Cochrane, S. A., Gilmore, B. F., Maresca, M., & Huws, S. A. (2024). Microbiome-derived antimicrobial peptides show therapeutic activity against the critically important priority pathogen, *Acinetobacter baumannii*. *npj Biofilms and Microbiomes*, *10*(1), 92. https://doi.org/10.1038/s41522-024-00560-2 ⏎

Almoughrabie, S., Cau, L., Cavagnero, K., O'Neill, A. M., Li, F., Roso-Mares, A., Mainzer, C., Closs, B., Kolar, M. J., Williams, K. J., Bensinger, S. J., & Gallo, R. L. (2023). Commensal *Cutibacterium acnes* induce epidermal

lipid synthesis important for skin barrier function. *Science Advances*, *9*(33), eadg6262. https://doi.org/10.1126/sciadv.adg6262 ⏎

Amari, Y., Hosonuma, M., Mizukami, T., Isobe, J., Azetsu, Y., Funayama, E., Maruyama, Y., Tsurui, T., Tajima, K., Sasaki, A., Yamazaki, Y., Nakano, R., Sano, Y., Ishida, A., Nakanishi, T., Mochizuki, S., Yoshizawa, Y., Kumagai, S., Yasuhara, S., … Uchida, N. (2025). Association between the ABCC11 gene polymorphism-determined earwax properties and external auditory canal microbiota in healthy adults. *Microbiology Spectrum*, *13*(2), e01698-24. https://doi.org/10.1128/spectrum.01698-24 ⏎

AOBiome. (2017). AOBiome therapeutics reports positive efficacy results from phase 2b clinical trial of ammonia oxidizing bacteria (AOB) for the treatment of acne vulgaris. *AOBiome*. www.aobiome.com/pressreleases/aobiome-therapeutics-reports-positive-efficacy-results-from-phase-2b-clinical-trial-of-ammonia-oxidizing-bacteria-aob-for-the-treatment-of-acne-vulgaris/ ⏎

Ashkenazi, H., Malik, Z., Harth, Y., & Nitzan, Y. (2003). Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunology & Medical Microbiology*, *35*(1), 17–24. https://doi.org/10.1111/j.1574-695X.2003.tb00644.x ⏎

Bai, J., Gong, C., Hu, Y.-J., Bruner, D. W., Torres, M. A., Buchwald, Z. S., & Lin, J. Y. (2025). Skin microbiome, inflammation, and skin toxicities in women with breast cancer receiving moderately hypofractionated radiation therapy. *International Journal of Radiation Oncology, Biology, Physics*, *122*(5), 1175–1185. https://doi.org/10.1016/j.ijrobp.2025.02.044 ⏎

Baker, P., Huang, C., Radi, R., Moll, S. B., Jules, E., & Arbiser, J. L. (2023). Skin barrier function: The interplay of physical, chemical, and immunologic properties. *Cells*, *12*(23), 2745. https://doi.org/10.3390/cells12232745 ⏎

Bangayan, N. J., Shi, B., Trinh, J., Barnard, E., Kasimatis, G., Curd, E., & Li, H. (2020). MG-MLST: Characterizing the microbiome at the strain level in metagenomic data. *Microorganisms*, *8*(5), 684. https://doi.org/10.3390/microorganisms8050684 ⏎

Belizário, J. E. (2025). Role of skin antimicrobial peptides in the pathogenesis of psoriasis, atopic dermatitis and hidradenitis suppurative: Highlights on

dermcidin. *Clinical Immunology Communications*, *7*, 18–26. https://doi.org/10.1016/j.clicom.2024.12.001 ⏎

Bianchi, P., Jacques, C., Theunis, J., Jamin, E. L., Orlandi, C., Cauhape, L., Alvarez-Georges, S., Alves, A., Simcic-Mori, A., Lauze, C., Gravier, E., Carballido, F., Ribet, V., Bessou-Touya, S., & Duplan, H. (2025). Clinical profiling of skin microbiome and metabolome during re-epithelialization. *Scientific Reports*, *15*(1), 22282. https://doi.org/10.1038/s41598-025-07547-9 ⏎

Bier, K., & Schittek, B. (2021). Beneficial effects of coagulase-negative Staphylococci on *Staphylococcus aureus* skin colonization. *Experimental Dermatology*, *30*(10), 1442–1452. https://doi.org/10.1111/exd.14381 ⏎

Blicharz, L., Samborowska, E., Zagożdżon, R., Bukowska-Ośko, I., Czuwara, J., Zych, M., Roszczyk, A., Perlejewski, K., Makowska, K., Nowaczyk, J., Zaremba, M., Kazimierska, K., Waśkiel-Burnat, A., Dadlez, M., Samochocki, Z., Olszewska, M., Radkowski, M., & Rudnicka, L. (2025). Severity of atopic dermatitis is associated with gut-derived metabolites and leaky gut-related biomarkers. *Scientific Reports*, *15*(1), 26146. https://doi.org/10.1038/s41598-025-09520-y ⏎

Bousbaine, D. (2025). The vaccine we all wear: Skin microbiota can be engineered into topical vaccines. *Science*, *389*(6755), 37–38. https://doi.org/10.1126/science.adz0485 ⏎

Bouwstra, J. A., Nădăban, A., Bras, W., MCabe, C., Bunge, A., & Gooris, G. S. (2023). The skin barrier: An extraordinary interface with an exceptional lipid organization. *Progress in Lipid Research*, *92*, 101252. https://doi.org/10.1016/j.plipres.2023.101252 ⏎

Brandwein, M., Fuks, G., Israel, A., Hodak, E., Sabbah, F., Steinberg, D., Bentwich, Z., Shental, N., & Meshner, S. (2021). Biogeographical landscape of the human face skin microbiome viewed in high definition. *Acta Dermato-Venereologica*, *101*(11), 881. https://doi.org/10.2340/00015555-3929 ⏎

Bratborska, A. W., Głuszak, P., Joks, M., Kaźmierska, J., Pazdrowski, J., Polańska, A., Jain, S., Yadav, H., Masternak, M. M., & Dańczak-Pazdrowska, A. (2025). Skin microbiome and radiation-induced skin injury:

Unraveling the relationship, mechanisms, and therapeutic implications. *International Journal of Molecular Sciences*, *26*(11), 5022. https://doi.org/10.3390/ijms26115022 ⏎

Brown, L., Wolf, J. M., Prados-Rosales, R., & Casadevall, A. (2015). Through the wall: Extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nature Reviews Microbiology*, *13*(10), 620–630. https://doi.org/10.1038/nrmicro3480 ⏎

Burns, E. M., Ahmed, H., Isedeh, P. N., Kohli, I., Van Der Pol, W., Shaheen, A., Muzaffar, A. F., Al-Sadek, C., Foy, T. M., Abdelgawwad, M. S., Huda, S., Lim, H. W., Hamzavi, I., Morrow, C. D., Elmets, C. A., & Yusuf, N. (2019). Ultraviolet radiation, both UVA and UVB, influences the composition of the skin microbiome. *Experimental Dermatology*, *28*(2), 136–141. https://doi.org/10.1111/exd.13854 ⏎

Casterline, B. W., & Paller, A. S. (2021). Early development of the skin microbiome: Therapeutic opportunities. *Pediatric Research*, *90*(4), 731–737. https://doi.org/10.1038/s41390-020-01146-2 ⏎

Celoria, V., Rosset, F., Pala, V., Dapavo, P., Ribero, S., Quaglino, P., & Mastorino, L. (2023). The skin microbiome and its role in psoriasis: A review. *Psoriasis: Targets and Therapy*, *13*, 71–78. https://doi.org/10.2147/PTT.S328439 ⏎

Che, Y., Han, J., Harkins, C. P., Hou, P., Conlan, S., Deming, C., Amirkhani, A., Bingham, M. A., Holmes, C. J., Englander, H., Shen, Z., Castelo-Soccio, L., Dimitrova, D., Kanakry, J. A., Bergerson, J. R. E., Notarangelo, L. D., Pittaluga, S., Zhao, C., Dell'Orso, S., … Kong, H. H. (2025). Restoration of the human skin microbiome following immune recovery after hematopoietic stem cell transplantation. *Cell Host & Microbe*, *33*(8), 1412–1427.e5. https://doi.org/10.1016/j.chom.2025.07.002 ⏎

Chen, M., Wang, R., & Wang, T. (2024). Gut microbiota and skin pathologies: Mechanism of the gut–skin axis in atopic dermatitis and psoriasis. *International Immunopharmacology*, *141*, 112658. https://doi.org/10.1016/j.intimp.2024.112658 ⏎

Cheung, C. T., Lancien, U., Corvec, S., Mengeaud, V., Mias, C., Véziers, J., Khammari, A., & Dréno, B. (2024). Pro-inflammatory activity of

Cutibacterium acnes phylotype IA1 and extracellular vesicles: An in vitro study. *Experimental Dermatology*, *33*(8), e15150. https://doi.org/10.1111/exd.15150 ⏎

Chia, M., Ng, A. H. Q., Ravikrishnan, A., Mohamed Naim, A. N., Wearne, S., Common, J., & Nagarajan, N. (2025). Skin metatranscriptomics reveals a landscape of variation in microbial activity and gene expression across the human body. *Nature Biotechnology*, 1–12. https://doi.org/10.1038/s41587-025-02797-4 ⏎

Chudzik, A., Bromke, M. A., Gamian, A., & Paściak, M. (2024). Comprehensive lipidomic analysis of the genus *Cutibacterium*. *mSphere*, *9*(5), e0005424. https://doi.org/10.1128/msphere.00054-24 ⏎

Clanner-Engelshofen, B. M., French, L. E., & Reinholz, M. (2020). *Corynebacterium kroppenstedtii* subsp. Demodicis is the endobacterium of *Demodex folliculorum*. *Journal of the European Academy of Dermatology and Venereology: JEADV*, *34*(5), 1043–1049. https://doi.org/10.1111/jdv.16069 ⏎

Coates, M., Lee, M. J., Norton, D., & MacLeod, A. S. (2019). The skin and intestinal microbiota and their specific innate immune systems. *Frontiers in Immunology*, *10*, 2950. https://doi.org/10.3389/fimmu.2019.02950 ⏎

Constantinides, M. G., Link, V. M., Tamoutounour, S., Wong, A. C., Perez-Chaparro, P. J., Han, S.-J., Chen, Y. E., Li, K., Farhat, S., Weckel, A., Krishnamurthy, S. R., Vujkovic-Cvijin, I., Linehan, J. L., Bouladoux, N., Merrill, E. D., Roy, S., Cua, D. J., Adams, E. J., Bhandoola, A., … Belkaid, Y. (2019). MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science (New York, N.Y.)*, *366*(6464), eaax6624. https://doi.org/10.1126/science.aax6624 ⏎

Cosseau, C., Romano-Bertrand, S., Duplan, H., Lucas, O., Ingrassia, I., Pigasse, C., Roques, C., & Jumas-Bilak, E. (2016). *Proteobacteria* from the human skin microbiota: Species-level diversity and hypotheses. *One Health*, *2*, 33–41. https://doi.org/10.1016/j.onehlt.2016.02.002 ⏎

Cundell, A. M. (2018). Microbial ecology of the human skin. *Microbial Ecology*, *76*(1), 113–120. https://doi.org/10.1007/s00248-016-0789-6 ⏎

Dagnelie, M. A., Corvec, S., Saint-Jean, M., Bourdès, V., Nguyen, J. M., Khammari, A., & Dréno, B. (2018). Decrease in diversity of *Propionibacterium acnes* phylotypes in patients with severe acne on the back. *Acta Dermato-Venereologica*, *98*(2), 262–267. https://doi.org/10.2340/00015555-2847 ⏎

Dagnelie, M. A., Corvec, S., Saint-Jean, M., Nguyen, J.-M., Khammari, A., & Dréno, B. (2019). Cutibacterium acnes phylotypes diversity loss: A trigger for skin inflammatory process. *Journal of the European Academy of Dermatology and Venereology*, *33*(12), 2340–2348. https://doi.org/10.1111/jdv.15795 ⏎

Dagnelie, M. A., Corvec, S., Timon-David, E., Khammari, A., & Dréno, B. (2022). *Cutibacterium acnes* and *Staphylococcus epidermidis*: The unmissable modulators of skin inflammatory response. *Experimental Dermatology*, *31*(3), 406–412. https://doi.org/10.1111/EXD.14467 ⏎

Dagnelie, M. A., Montassier, E., Khammari, A., Mounier, C., Corvec, S., & Dréno, B. (2019). Inflammatory skin is associated with changes in the skin microbiota composition on the back of severe acne patients. *Experimental Dermatology*, *28*(8), 961–967. https://doi.org/10.1111/EXD.13988 ⏎

Dagnelie, M.-A., Corvec, S., Khammari, A., & Dréno, B. (2020). Bacterial extracellular vesicles: A new way to decipher host–microbiota communications in inflammatory dermatoses. *Experimental Dermatology*, *29*(1), 22–28. https://doi.org/10.1111/exd.14050 ⏎

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107 ⏎

Duarte, M., Pedrosa, S. S., Khusial, P. R., & Madureira, A. R. (2024). Exploring the interplay between stress mediators and skin microbiota in shaping age-related hallmarks: A review. *Mechanisms of Ageing and Development*, *220*, 111956. https://doi.org/10.1016/j.mad.2024.111956 ⏎

Ellis, S. R., Nguyen, M., Vaughn, A. R., Notay, M., Burney, W. A., Sandhu, S., Sivamani, R. K., Ellis, S. R., Nguyen, M., Vaughn, A. R., Notay, M., Burney, W. A., Sandhu, S., & Sivamani, R. K. (2019). The skin and gut microbiome and its role in common dermatologic conditions. *Microorganisms*, *7*(11). https://doi.org/10.3390/microorganisms7110550 ⏎

El-Moamly, A., & El-Swify, O. (2025). Raising awareness of Demodex mites: A neglected cause of skin disease. *Infection*, *53*(4), 1273–1298. https://doi.org/10.1007/s15010-025-02521-z ⏎

Fernández-Carro, E., Letsiou, S., Tsironi, S., Chaniotis, D., Ciriza, J., & Beloukas, A. (2025). Alternatives integrating omics approaches for the advancement of human skin models: A focus on metagenomics, metatranscriptomics, and metaproteomics. *Microorganisms*, *13*(8), 1771. https://doi.org/10.3390/microorganisms13081771 ⏎

Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J. A., Schoenfeld, D., Nomicos, E., Park, M., NIH Intramural Sequencing Center Comparative Sequencing Program, Kong, H. H., & Segre, J. A. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, *498*(7454), 367–370. https://doi.org/10.1038/nature12171 ⏎

Foessleitner, P., Pjevac, P., Granser, S., Wisgrill, L., Pummer, L., Eckel, F., Seki, D., Berry, D., Hausmann, B., & Farr, A. (2024). The maternal microbiome in pregnancy, delivery, and early-stage development of neonatal microbiome after cesarean section: A prospective longitudinal study. *Acta Obstetricia et Gynecologica Scandinavica*, *103*(5), 832–841. https://doi.org/10.1111/aogs.14773 ⏎

França, K. (2021). Topical probiotics in dermatological therapy and skincare: A concise review. *Dermatology and Therapy*, *11*(1), 71–77. https://doi.org/10.1007/s13555-020-00476-7 ⏎

Galizia, G., Fortina, A. B., & Semenzato, A. (2024). Seborrheic dermatitis: From microbiome and skin barrier involvement to emerging approaches in dermocosmetic treatment. *Cosmetics*, *11*(6), 208. https://doi.org/10.3390/cosmetics11060208 ⏎

Gao, Z., Perez-Perez, G. I., Chen, Y., & Blaser, M. J. (2010). Quantitation of major human cutaneous bacterial and fungal populations. *Journal of*

Clinical Microbiology, *48*(10), 3575–3581. https://doi.org/10.1128/JCM.00597-10 ⏎

Garcia-Garcerà, M., Garcia-Etxebarria, K., Coscollà, M., Latorre, A., & Calafell, F. (2013). A new method for extracting skin microbes allows metagenomic analysis of whole-deep skin. *PLoS One*, *8*(9), e74914. https://doi.org/10.1371/journal.pone.0074914 ⏎

Garlet, A., Andre-Frei, V., Del Bene, N., Cameron, H. J., Samuga, A., Rawat, V., Ternes, P., & Leoty-Okombi, S. (2024). Facial skin microbiome composition and functional shift with aging. *Microorganisms*, *12*(5), 1021. https://doi.org/10.3390/microorganisms12051021 ⏎

George, C. D., Lee, T., Hollestein, L. M., Asgari, M. M., & Nijsten, T. (2024). Global epidemiology of actinic keratosis in the general population: A systematic review and meta-analysis. *British Journal of Dermatology*, *190*(4), 465–476. https://doi.org/10.1093/bjd/ljad371 ⏎

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., NISC Comparative Sequencing Program, Bouffard, G. G., Blakesley, R. W., Murray, P. R., Green, E. D., Turner, M. L., & Segre, J. A. (2009). Topographical and temporal diversity of the human skin microbiome. *Science*, *324*(5931), 1190–1192. https://doi.org/10.1126/science.1171700 ⏎

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews. Microbiology*, *9*(4), 244–253. https://doi.org/10.1038/nrmicro2537 ⏎

Gupta, A. K., & Kohli, Y. (2004). Prevalence of Malassezia species on various body sites in clinically healthy subjects representing different age groups. *Medical Mycology*, *42*(1), 35–42. https://doi.org/10.1080/13693780310001610056 ⏎

Haddock, N. L., Barkal, L. J., & Bollyky, P. L. (2023). Bacteriophage populations mirror those of bacterial pathogens at sites of infection. *mSystems*, *8*(4), e0049723. https://doi.org/10.1128/msystems.00497-23 ⏎

Hannigan, G. D., Meisel, J. S., Tyldsley, A. S., Zheng, Q., Hodkinson, B. P., SanMiguel, A. J., Minot, S., Bushman, F. D., & Grice, E. A. (2015). The human skin double-stranded DNA virome: Topographical and temporal diversity, genetic enrichment, and dynamic associations with the host

microbiome. *mBio*, *6*(5), e01578-15. 10.1128/mbio.01578-15. https://doi.org/10.1128/mbio.01578-15 ⏎

Harel, N., Ogen-Shtern, N., Reshef, L., Biran, D., Ron, E. Z., & Gophna, U. (2023). Skin microbiome bacteria enriched following long sun exposure can reduce oxidative damage. *Research in Microbiology*, *174*(8), 104138. https://doi.org/10.1016/j.resmic.2023.104138 ⏎

Harker, M., Carvell, A.-M., Marti, V. P. J., Riazanskaia, S., Kelso, H., Taylor, D., Grimshaw, S., Arnold, D. S., Zillmer, R., Shaw, J., Kirk, J. M., Alcasid, Z. M., Gonzales-Tanon, S., Chan, G. P., Rosing, E. A. E., & Smith, A. M. (2014). Functional characterisation of a SNP in the *ABCC11* allele—Effects on axillary skin metabolism, odour generation and associated behaviours. *Journal of Dermatological Science*, *73*(1), 23–30. https://doi.org/10.1016/j.jdermsci.2013.08.016 ⏎

Howard, B., Bascom, C. C., Hu, P., Binder, R. L., Fadayel, G., Huggins, T. G., Jarrold, B. B., Osborne, R., Rocchetta, H. L., Swift, D., Tiesman, J. P., Song, Y., Wang, Y., Wehmeyer, K., Kimball, A. B., & Isfort, R. J. (2022). Aging-associated changes in the adult human skin microbiome and the host factors that affect skin microbiome composition. *Journal of Investigative Dermatology*, *142*(7), 1934–1946.e21. https://doi.org/10.1016/j.jid.2021.11.029 ⏎

Huang, C., Zhuo, F., Guo, Y., Wang, S., Zhang, K., Li, X., Dai, W., Dou, X., & Yu, B. (2025). Skin microbiota: Pathogenic roles and implications in atopic dermatitis. *Frontiers in Cellular and Infection Microbiology*, *14*, 1518811. https://doi.org/10.3389/fcimb.2024.1518811 ⏎

Isler, M. F., Coates, S. J., & Boos, M. D. (2023). Climate change, the cutaneous microbiome and skin disease: Implications for a warming world. *International Journal of Dermatology*, *62*(3), 337–345. https://doi.org/10.1111/ijd.16297 ⏎

Jabeen, M. F., & Hinks, T. S. C. (2023). MAIT cells and the microbiome. *Frontiers in Immunology,* *14*, 1127588. https://doi.org/10.3389/fimmu.2023.1127588 ⏎

Jacobson, M. E., Myles, I. A., Paller, A. S., Eichenfield, L. F., & Simpson, E. L. (2024). A randomized, double-blind, placebo-controlled, multicenter, 16-

week trial to evaluate the efficacy and safety of fb-401 in children, adolescents, and adult subjects (ages 2 years and older) with mild-to-moderate atopic dermatitis. *Dermatology*, *240*(1), 85–94. https://doi.org/10.1159/000532054 ⏎

Jang, H., Matsuda, A., Jung, K., Karasawa, K., Matsuda, K., Oida, K., Ishizaka, S., Ahn, G., Amagai, Y., Moon, C., Kim, S.-H., Arkwright, P. D., Takamori, K., Matsuda, H., & Tanaka, A. (2016). Skin pH is the master switch of kallikrein 5-mediated skin barrier destruction in a murine atopic dermatitis model. *Journal of Investigative Dermatology*, *136*(1), 127–135. https://doi.org/10.1038/JID.2015.363 ⏎

Jo, J.-H., Deming, C., Kennedy, E. A., Conlan, S., Polley, E. C., Ng, W., Segre, J. A., & Kong, H. H. (2016). Diverse human skin fungal communities in children converge in adulthood. *Journal of Investigative Dermatology*, *136*(12), 2356–2363. https://doi.org/10.1016/j.jid.2016.05.130 ⏎

Jo, J.-H., Harkins, C. P., Schwardt, N. H., Portillo, J. A., Zimmerman, M. D., Carter, C. L., Hossen, M. A., Peer, C. J., Polley, E. C., Dartois, V., Figg, W. D., Moutsopoulos, N. M., Segre, J. A., & Kong, H. H. (2021). Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. *Science Translational Medicine*, *13*(625), eabd8077. https://doi.org/10.1126/scitranslmed.abd8077 ⏎

Jo, J.-H., Kennedy, E. A., & Kong, H. H. (2016). Research techniques made simple: Bacterial 16S ribosomal RNA gene sequencing in cutaneous research. *Journal of Investigative Dermatology*, *136*(3), e23–e27. https://doi.org/10.1016/j.jid.2016.01.005 ⏎

Johansson, H. J., Vallhov, H., Holm, T., Gehrmann, U., Andersson, A., Johansson, C., Blom, H., Carroni, M., Lehtiö, J., & Scheynius, A. (2018). Extracellular nanovesicles released from the commensal yeast *Malassezia sympodialis* are enriched in allergens and interact with cells in human skin. *Scientific Reports*, *8*(1), 9182. https://doi.org/10.1038/s41598-018-27451-9 ⏎

Johnson, T., Kang, D., Barnard, E., & Li, H. (2016). Strain-level differences in porphyrin production and regulation in *Propionibacterium acnes* elucidate

disease associations. *mSphere*, *1*(1), e00023-15. https://doi.org/10.1128/mSphere.00023-15 ⏎

Kahraman, M., Akın Polat, Z., Esen, M., & Çoban Gül, F. (2025). The association between the presence of human facial mites Demodex (Acari, Demodicidae) and dermatological symptoms in Rosacea patients in Central Anatolia, Türkiye. *Turkiye Parazitolojii Dergisi*, *48*(4), 240–244. https://doi.org/10.4274/tpd.galenos.2025.14632 ⏎

Kawagishi, Y., Murase, K., Grebenshchikova, A., Iibushi, J., Ma, C., Kimeu, T. M., Minowa-Nozawa, A., Nozawa, T., & Nakagawa, I. (2025). Bacterial extracellular vesicles target different bacterial species, impairing cell division and diminishing their pathogenicity. *Proceedings of the National Academy of Sciences U S A*, *122*(18), e2416652122. https://doi.org/10.1073/pnas.2416652122 ⏎

Kehrmann, J., Koch, F., Zumdick, S., Höwner, A., Best, L., Masshöfer, L., Scharfenberg, S., Zeschnigk, M., Becker, J. C., Schadendorf, D., Buer, J., & Roesch, A. (2023). Reduced staphylococcus abundance characterizes the lesional microbiome of actinic keratosis patients after field-directed therapies. *Microbiology Spectrum*, *11*(3), e04401-22. https://doi.org/10.1128/spectrum.04401-22 ⏎

Keshari, S., Balasubramaniam, A., Myagmardoloonjin, B., Herr, D. R., Negari, I. P., & Huang, C.-M. (2019). Butyric acid from probiotic *Staphylococcus epidermidis* in the skin microbiome down-regulates the ultraviolet-induced pro-inflammatory IL-6 cytokine via short-chain fatty acid receptor. *International Journal of Molecular Sciences*, *20*(18), 4477. https://doi.org/10.3390/ijms20184477 ⏎

Khmaladze, I., Leonardi, M., Fabre, S., Messaraa, C., & Mavon, A. (2020). The skin interactome: A holistic "genome-microbiome-exposome" approach to understand and modulate skin health and aging. *Clinical, Cosmetic and Investigational Dermatology*, *13*, 1021–1040. https://doi.org/10.2147/CCID.S239367 ⏎

Kim, G. K. (2009). Seborrheic dermatitis and *Malassezia* species. *Journal of Clinical and Aesthetic Dermatology*, *2*(11), 14–17. ⏎

Kim, H. J., & Kim, Y. H. (2024). Exploring acne treatments: From pathophysiological mechanisms to emerging therapies. *International Journal of Molecular Sciences*, *25*(10), 5302. https://doi.org/10.3390/ijms25105302 ⏎

Kim, H.-J., Oh, H. N., Park, T., Kim, H., Lee, H. G., An, S., & Sul, W. J. (2022). Aged related human skin microbiome and mycobiome in Korean women. *Scientific Reports*, *12*(1), 2351. https://doi.org/10.1038/s41598-022-06189-5 ⏎

Kim, J.-H., Son, S.-M., Park, H., Kim, B. K., Choi, I. S., Kim, H., & Huh, C. S. (2021). Taxonomic profiling of skin microbiome and correlation with clinical skin parameters in healthy Koreans. *Scientific Reports*, *11*(1), 16269. https://doi.org/10.1038/s41598-021-95734-9 ⏎

Kim, N., Ma, J., Kim, W., Kim, J., Belenky, P., & Lee, I. (2024). Genome-resolved metagenomics: A game changer for microbiome medicine. *Experimental & Molecular Medicine*, *56*(7), 1501–1512. https://doi.org/10.1038/s12276-024-01262-7 ⏎

Kim, S. H., Kim, J. H., Lee, S. J., Jung, M. S., Jeong, D. H., & Lee, K. H. (2022). Minimally invasive skin sampling and transcriptome analysis using microneedles for skin type biomarker research. *Skin Research and Technology*, *28*(2), 322–335. https://doi.org/10.1111/srt.13135 ⏎

Koh, L. F., Ong, R. Y., & Common, J. E. (2022). Skin microbiome of atopic dermatitis. *Allergology International*, *71*(1), 31–39. https://doi.org/10.1016/j.alit.2021.11.001 ⏎

Kortekaas Krohn, I., Callewaert, C., Belasri, H., De Pessemier, B., Diez Lopez, C., Mortz, C. G., O'Mahony, L., Pérez-Gordo, M., Sokolowska, M., Unger, Z., Untersmayr, E., Homey, B., & Gomez-Casado, C. (2024). The influence of lifestyle and environmental factors on host resilience through a homeostatic skin microbiota: An EAACI Task Force Report. *Allergy*, *79*(12), 3269–3284. https://doi.org/10.1111/all.16378 ⏎

Koskinen, K., Pausan, M. R., Perras, A. K., Beck, M., Bang, C., Mora, M., Schilhabel, A., Schmitz, R., & Moissl-Eichinger, C. (2017). First insights into the diverse human archaeome: Specific detection of archaea in the

gastrointestinal tract, lung, and nose and on skin. *mBio*, *8*(6), e00824–17. https://doi.org/10.1128/mBio.00824-17 ⏎

Kubiak, K., Sielawa, H., Chen, W., & Dzika, E. (2018). Endosymbiosis and its significance in dermatology. *Journal of the European Academy of Dermatology and Venereology: JEADV*, *32*(3), 347–354. https://doi.org/10.1111/jdv.14721 ⏎

Kurokawa, I., & Nakase, K. (2020). Recent advances in understanding and managing acne. *F1000Research*, *9*, F1000 Faculty Rev-792. https://doi.org/10.12688/f1000research.25588.1 ⏎

Lacey, N., Delaney, S., Kavanagh, K., & Powell, F. C. (2007). Mite-related bacterial antigens stimulate inflammatory cells in rosacea. *British Journal of Dermatology*, *157*(3), 474–481. https://doi.org/10.1111/j.1365-2133.2007.08028.x ⏎

Lee, H., Jeong, J., Oh, Y., Lee, C.-J., Mun, S., Lee, D.-G., Jo, H., Heo, Y. M., Baek, C., Heo, C. Y., Kang, S. M., & Han, K. (2021). Comparative analysis of human facial skin microbiome between topical sites compared to entire face. *Genes & Genomics*, *43*(12), 1483–1495. https://doi.org/10.1007/s13258-021-01180-2 ⏎

Leung, M. H. Y., Chan, K. C. K., & Lee, P. K. H. (2016). Skin fungal community and its correlation with bacterial community of urban Chinese individuals. *Microbiome*, *4*(1), 46. https://doi.org/10.1186/s40168-016-0192-z ⏎

Li, C., Ravikrishnan, A., Wijaya, I., Naim, A. N. M., Gounot, J.-S., Wearne, S., On, Y. Y., Ho, E. X. P., Ang, Q. Y., Chia, M., Amanda, N. H. Q., Ramaswami, S., Yew, Y. W., Loh, M., Ong, R. Y., Ng, F., Lee, S. H., Chan, J., Team, T. H. S., … Nagarajan, N. (2025). Large-scale skin metagenomics reveals extensive prevalence, coordination, and functional adaptation of skin microbiome dermotypes across body sites. *bioRxiv*, 2025.04.24.650393. https://doi.org/10.1101/2025.04.24.650393 ⏎

Li, M., Kopylova, E., Mao, J., Namkoong, J., Sanders, J., & Wu, J. (2024). Microbiome and lipidomic analysis reveal the interplay between skin bacteria and lipids in a cohort study. *Frontiers in Microbiology*, *15*, 1383656. https://doi.org/10.3389/fmicb.2024.1383656 ⏎

Liang, K., Leong, C., Loh, J. M., Chan, N., Lim, L., Lam, Y. I., Dawson, T. L., & Tey, H. L. (2022). A 3D-printed transepidermal microprojection array for human skin microbiome sampling. *Proceedings of the National Academy of Sciences U S A*, *119*(30), e2203556119. https://doi.org/10.1073/pnas.2203556119 ⏎

Liu, T., Li, S., Ying, S., Tang, S., Ding, Y., Li, Y., Qiao, J., & Fang, H. (2020). The IL-23/IL-17 pathway in inflammatory skin diseases: From bench to bedside. *Frontiers in Immunology*, *11*, 594735. https://doi.org/10.3389/fimmu.2020.594735 ⏎

Ma, L., Zhang, H., Jia, Q., Bai, T., Yang, S., Wang, M., Li, Y., & Shao, L. (2024). Facial physiological characteristics and skin microbiomes changes are associated with body mass index (BMI). *Clinical, Cosmetic and Investigational Dermatology*, *17*, 513–528. https://doi.org/10.2147/CCID.S447412 ⏎

Meisel, J. S., Hannigan, G. D., Tyldsley, A. S., SanMiguel, A. J., Hodkinson, B. P., Zheng, Q., & Grice, E. A. (2016). Skin microbiome surveys are strongly influenced by experimental design. *Journal of Investigative Dermatology*, *136*(5), 947–956. https://doi.org/10.1016/j.jid.2016.01.016 ⏎

Micu, A. E., Popescu, I. A., Halip, I. A., Mocanu, M., Vâță, D., Hulubencu, A. L., Gheucă-Solovăstru, D. F., & Gheucă-Solovăstru, L. (2026). From gut dysbiosis to skin inflammation in atopic dermatitis: Probiotics and the gut–skin axis—clinical outcomes and microbiome implications. *International Journal of Molecular Sciences*, *27*(1), 365. https://doi.org/10.3390/ijms27010365 ⏎

Mim, M. F., Sikder, M. H., Chowdhury, Md. Z. H., Bhuiyan, A.-U.-A., Zinan, N., & Islam, S. M. N. (2024). The dynamic relationship between skin microbiomes and personal care products: A comprehensive review. *Heliyon*, *10*(14), e34549. https://doi.org/10.1016/j.heliyon.2024.e34549 ⏎

Moissl-Eichinger, C., Probst, A. J., Birarda, G., Auerbach, A., Koskinen, K., Wolf, P., & Holman, H.-Y. N. (2017). Human age and skin physiology shape diversity and abundance of Archaea on skin. *Scientific Reports*, *7*(1), 4039. https://doi.org/10.1038/s41598-017-04197-4 ⏎

Moitinho-Silva, L., Degenhardt, F., Rodriguez, E., Emmert, H., Juzenas, S., Möbus, L., Uellendahl-Werth, F., Sander, N., Baurecht, H., Tittmann, L., Lieb, W., Gieger, C., Peters, A., Ellinghaus, D., Bang, C., Franke, A., Weidinger, S., & Rühlemann, M. C. (2022). Host genetic factors related to innate immunity, environmental sensing and cellular functions are associated with human skin microbiota. *Nature Communications*, *13*(1), 6204. https://doi.org/10.1038/s41467-022-33906-5 ⏎

Moskovicz, V., Gross, A., Mizrahi, B., Moskovicz, V., Gross, A., & Mizrahi, B. (2020). Extrinsic factors shaping the skin microbiome. *Microorganisms*, *8*(7), 1023. https://doi.org/10.3390/microorganisms8071023 ⏎

Mukherjee, S., Mitra, R., Maitra, A., Gupta, S., Kumaran, S., Chakrabortty, A., & Majumder, P. P. (2016). Sebum and hydration levels in specific regions of human face significantly predict the nature and diversity of facial skin microbiome. *Scientific Reports*, *6*(1), 36062. https://doi.org/10.1038/srep36062 ⏎

Munteanu, C., Turti, S., & Marza, S. M. (2025). Unraveling the gut–skin axis: The role of microbiota in skin health and disease. *Cosmetics*, *12*(4): 167. https://doi.org/10.3390/cosmetics12040167 ⏎

Myles, I. A., Castillo, C. R., Barbian, K. D., Kanakabandi, K., Virtaneva, K., Fitzmeyer, E., Paneru, M., Otaizo-Carrasquero, F., Myers, T. G., Markowitz, T. E., Moore, I. N., Liu, X., Ferrer, M., Sakamachi, Y., Garantziotis, S., Swamydas, M., Lionakis, M. S., Anderson, E. D., Earland, N. J., … Datta, S. K. (2020). Therapeutic responses to *Roseomonas mucosa* in atopic dermatitis may involve lipid-mediated TNF-related epithelial repair. *Science Translational Medicine*, *12*(560), eaaz8631. https://doi.org/10.1126/scitranslmed.aaz8631 ⏎

Myles, I. A., Earland, N. J., Anderson, E. D., Moore, I. N., Kieh, M. D., Williams, K. W., Saleem, A., Fontecilla, N. M., Welch, P. A., Darnell, D. A., Barnhart, L. A., Sun, A. A., Uzel, G., & Datta, S. K. (2018). First-in-human topical microbiome transplantation with *Roseomonas mucosa* for atopic dermatitis. *JCI Insight*, *3*(9), e120608. https://doi.org/10.1172/jci.insight.120608 ⏎

Nakatsuji, T., Gallo, R. L., Shafiq, F., Tong, Y., Chun, K., Butcher, A. M., Cheng, J. Y., & Hata, T. R. (2021). Use of autologous bacteriotherapy to treat *Staphylococcus aureus* in patients with atopic dermatitis. *JAMA Dermatology*, *157*(8), 978–982. https://doi.org/10.1001/jamadermatol.2021.1311 ⏎

Natarelli, N., Gahoonia, N., & Sivamani, R. K. (2023). Bacteriophages and the microbiome in dermatology: The role of the phageome and a potential therapeutic strategy. *International Journal of Molecular Sciences*, *24*(3), 2695. https://doi.org/10.3390/ijms24032695 ⏎

Ndiaye, C., Bassene, H., Fonkou, M. D. M., Fenollar, F., Lagier, J. C., Raoult, D., & Sokhna, C. (2024). The application of culturomics to explore African skin microbiota. *American Journal of Tropical Medicine and Hygiene*, *111*(6), 1331–1337. https://doi.org/10.4269/ajtmh.23-0165 ⏎

Nguyen, U. T., & Kalan, L. R. (2022). Forgotten fungi: The importance of the skin mycobiome. *Current Opinion in Microbiology*, *70*, 102235. https://doi.org/10.1016/j.mib.2022.102235 ⏎

Notay, M., Saric-Bosanac, S., Vaughn, A. R., Dhaliwal, S., Trivedi, M., Reiter, P. N., Rybak, I., Li, C. C., Weiss, L. B., Ambrogio, L., Burney, W., & Sivamani, R. K. (2020). The use of topical *Nitrosomonas eutropha* for cosmetic improvement of facial wrinkles. *Journal of Cosmetic Dermatology*, *19*(3), 689–693. https://doi.org/10.1111/jocd.13060 ⏎

Ogai, K., Nagase, S., Mukai, K., Iuchi, T., Mori, Y., Matsue, M., Sugitani, K., Sugama, J., & Okamoto, S. (2018). A comparison of techniques for collecting skin microbiome samples: Swabbing versus tape-stripping. *Frontiers in Microbiology*, *9*, 2362. https://doi.org/10.3389/fmicb.2018.02362 ⏎

Oliveira, F. S., Brestelli, J., Cade, S., Zheng, J., Iodice, J., Fischer, S., Aurrecoechea, C., Kissinger, J. C., Brunk, B. P., Stoeckert, C. J., Fernandes, G. R., Roos, D. S., & Beiting, D. P. (2018). MicrobiomeDB: A systems biology platform for integrating, mining and analyzing microbiome experiments. *Nucleic Acids Research*, *46*(D1), D684–D691. https://doi.org/10.1093/nar/gkx1027 ⏎

Pagac, M. P., Davient, B., Plado, L. A., Lam, H. Y. I., Lee, S. M., Ravikrishnan, A., Chua, W. L. E., Muralidharan, S., Sridharan, A., Irudayaswamy, A. S., Srinivas, R., Wearne, S., Mohamed Naim, A. N., Ho, E. X. P., Ng, H. Q. A., Kwah, J. S., Png, E., Bendt, A. K., Wenk, M. R., … Dawson, T. L. (2025). Life stage impact on the human skin ecosystem: Lipids and the microbial community. *npj Biofilms and Microbiomes*, *11*(1), 13. https://doi.org/10.1038/s41522-025-00652-7 ⏎

Park, A., & Heo, T.-H. (2024). Celastrol regulates psoriatic inflammation and autophagy by targeting IL-17A. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *172*, 116256. https://doi.org/10.1016/j.biopha.2024.116256 ⏎

Patra, V., Wagner, K., Arulampalam, V., & Wolf, P. (2019). Skin microbiome modulates the effect of ultraviolet radiation on cellular response and immune function. *iScience*, *15*, 211–222. https://doi.org/10.1016/j.isci.2019.04.026 ⏎

Paul, J. K., Akter, A., Jewel, N. A., Rolin, M. H., Karim, D., Niloy, R. K., & Mondal, S. I. (2025). Exploration of human skin phageome to reveal endolysins and novel antimicrobial peptides for therapeutic applications. *MicrobiologyOpen*, *14*(6), e70115. https://doi.org/10.1002/mbo3.70115 ⏎

Peng, M., & Biswas, D. (2020). Environmental influences of high-density agricultural animal operation on human forearm skin microflora. *Microorganisms*, *8*(10), 1481. https://doi.org/10.3390/microorganisms8101481 ⏎

Pereira, M. S., Redanz, S., & Kriegel, M. A. (2022). Skin deep: The role of the microbiota in cutaneous autoimmunity. *Journal of Investigative Dermatology, Special Issue on Autoimmunity*, *142*(3, Part B), 834–840. https://doi.org/10.1016/j.jid.2021.12.005 ⏎

Perez Perez, G. I., Gao, Z., Jourdain, R., Ramirez, J., Gany, F., Clavaud, C., Demaude, J., Breton, L., & Blaser, M. J. (2016). Body site is a more determinant factor than human population diversity in the healthy skin microbiome. *PloS One*, *11*(4), e0151990. https://doi.org/10.1371/journal.pone.0151990 ⏎

Prajapati, S. K., Lekkala, L., Yadav, D., Jain, S., & Yadav, H. (2025). Microbiome and postbiotics in skin health. *Biomedicines*, *13*(4), Article 4. https://doi.org/10.3390/biomedicines13040791 ⏎

Prast-Nielsen, S., Tobin, A.-M., Adamzik, K., Powles, A., Hugerth, L. W., Sweeney, C., Kirby, B., Engstrand, L., & Fry, L. (2019). Investigation of the skin microbiome: Swabs vs. biopsies. *British Journal of Dermatology*, *181*(3), 572–579. https://doi.org/10.1111/bjd.17691 ⏎

Prieto, J. (2023). Combining targeted metagenomics and culturomics to develop an in vitro skin microbiota model. ⏎

Quaresma, J. A. S. (2019). Organization of the skin immune system and compartmentalized immune responses in infectious diseases. *Clinical Microbiology Reviews*, *32*(4), e00034–18. https://doi.org/10.1128/CMR.00034-18 ⏎

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research,* *41*(Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219 ⏎

Radaschin, D. S., Tatu, A., Iancu, A. V., Beiu, C., & Popa, L. G. (2024). The contribution of the skin microbiome to psoriasis pathogenesis and its implications for therapeutic strategies. *Medicina*, *60*(10), 1619. https://doi.org/10.3390/medicina60101619 ⏎

Robert, C., Cascella, F., Mellai, M., Barizzone, N., Mignone, F., Massa, N., Nobile, V., & Bona, E. (2022). Influence of sex on the microbiota of the human face. *Microorganisms*, *10*(12), 2470. https://doi.org/10.3390/microorganisms10122470 ⏎

Rosenthal, M., Aiello, A., Larson, E., Chenoweth, C., & Foxman, B. (2013). Healthcare workers' hand microbiome may mediate carriage of hospital pathogens. *Pathogens*, *3*(1), 1–13. https://doi.org/10.3390/pathogens3010001 ⏎

Ross, A. A., Doxey, A. C., & Neufeld, J. D. (2017). The skin microbiome of cohabiting couples. *mSystems*, *2*(4), 10.1128/msystems.00043-17. https://doi.org/10.1128/msystems.00043-17 ⏎

Roux, P.-F., Oddos, T., & Stamatas, G. (2022). Deciphering the role of skin surface microbiome in skin health: An integrative multiomics approach reveals three distinct metabolite–microbe clusters. *Journal of Investigative Dermatology*, *142*(2), 469–479.e5. https://doi.org/10.1016/j.jid.2021.07.159 ⏎

Ruffier d'Epenoux, L., Fayoux, E., Veziers, J., Dagnelie, M.-A., Khammari, A., Dréno, B., & Corvec, S. (2024). Biofilm of *Cutibacterium acnes*: A target of different active substances. *International Journal of Dermatology, 63(11)*, 1541–1550. https://doi.org/10.1111/ijd.17194 ⏎

Sachen, K. L., Arnold Greving, C. N., & Towne, J. E. (2022). Role of IL-36 cytokines in psoriasis and other inflammatory skin conditions. *Cytokine*, *156*, 155897. https://doi.org/10.1016/j.cyto.2022.155897 ⏎

Saheb Kashaf, S., Proctor, D. M., Deming, C., Saary, P., Hölzer, M., Taylor, M. E., Kong, H. H., Segre, J. A., Almeida, A., & Finn, R. D. (2022). Integrating cultivation and metagenomics for a multi-kingdom view of skin microbiome diversity and functions. *Nature Microbiology*, *7*(1), 169–179. https://doi.org/10.1038/s41564-021-01011-w ⏎

Schmid, B., Künstner, A., Fähnrich, A., Busch, H., Glatz, M., & Bosshard, P. P. (2022). Longitudinal characterization of the fungal skin microbiota in healthy subjects over a period of 1 year. *Journal of Investigative Dermatology*, *142*(10), 2766–2772.e8. https://doi.org/10.1016/j.jid.2022.03.014 ⏎

Selway, C. A., Mills, J. G., Weinstein, P., Skelly, C., Yadav, S., Lowe, A., Breed, M. F., & Weyrich, L. S. (2020). Transfer of environmental microbes to the skin and respiratory tract of humans after urban green space exposure. *Environment International*, *145*, 106084. https://doi.org/10.1016/j.envint.2020.106084 ⏎

Seong, H. J., & Quince, C. (2025). Geographic variation and host genetics shape the human skin microbiome. *bioRxiv*, 2025.05.01.651599. https://doi.org/10.1101/2025.05.01.651599 ⏎

Shin, H., Pei, Z., Martinez, K. A., Rivera-Vinas, J. I., Mendez, K., Cavallin, H., & Dominguez-Bello, M. G. (2015). The first microbial environment of

infants born by C-section: The operating room microbes. *Microbiome*, *3*, 59. https://doi.org/10.1186/s40168-015-0126-1 ⏎

Shu, M., Wang, Y., Yu, J., Kuo, S., Coda, A., Jiang, Y., Gallo, R. L., & Huang, C.-M. (2013). Fermentation of *Propionibacterium acnes*, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant *Staphylococcus aureus*. *PLoS One*, *8*(2), e55380. https://doi.org/10.1371/journal.pone.0055380 ⏎

Silverberg, J. I., Lio, P. A., Simpson, E. L., Li, C., Brownell, D. R., Gryllos, I., Ng-Cashin, J., Krueger, T., Swaidan, V. R., Bliss, R. L., & Kim, H. D. (2023). Efficacy and safety of topically applied therapeutic ammonia oxidising bacteria in adults with mild-to-moderate atopic dermatitis and moderate-to-severe pruritus: A randomised, double-blind, placebo-controlled, dose-ranging, phase 2b trial. *eClinicalMedicine*, *60*, 102002. https://doi.org/10.1016/j.eclinm.2023.102002 ⏎

Slizovskiy, I. B., Gaire, T. N., Ferm, P. M., Odland, C. A., Dee, S. A., Nerem, J., Bravo, J. E., Kimball, A. D., Boucher, C., & Noyes, N. R. (2025). Reducing skin microbiome exposure impacts through swine farm biosecurity. *GigaScience*, *14*, giaf062. https://doi.org/10.1093/gigascience/giaf062 ⏎

Smith, A., Dumbrava, R., Ghori, N.-U.-H., Foster, R., Campbell, J., Duthie, A., Hoyne, G., Rademaker, M., & Bowen, A. C. (2025). An overview of the skin microbiome, the potential for pathogen shift, and dysbiosis in common skin pathologies. *Microorganisms*, *13*(1), 54. https://doi.org/10.3390/microorganisms13010054 ⏎

Smith, A., Ghori, N.-U.-H., Foster, R., Nicol, M. P., Barnett, T., Pickering, J., Whelan, A., Strunk, T., Wood, F., Raby, E., Fear, M., Weston, S., Campbell, A. J., Hoyne, G. F., & Bowen, A. C. (2024). Optimisation of the sampling method for skin microbiome studies in healthy children: A pilot cohort study. *Frontiers in Microbiomes*, *3*, 1446394. https://doi.org/10.3389/frmbi.2024.1446394 ⏎

Somboonna, N., Wilantho, A., Srisuttiyakorn, C., Assawamakin, A., & Tongsima, S. (2017). Bacterial communities on facial skin of teenage and elderly Thai females. *Archives of Microbiology*, *199*(7), 1035–1042. https://doi.org/10.1007/s00203-017-1375-0 ⏎

Stevens, B. R., & Roesch, L. F. W. (2024). Interplay of human ABCC11 transporter gene variants with axillary skin microbiome functional genomics. *Scientific Reports*, *14*(1), 28037. https://doi.org/10.1038/s41598-024-78711-w ⏎

Suri, Hitakshika, Suri, Harshika, Nagda, N., Misra, T., & Vuppu, S. (2025). Current perspectives on the human skin microbiome: Functional insights and strategies for therapeutic modulation. *Biomedicine & Pharmacotherapy*, *193*, 118655. https://doi.org/10.1016/j.biopha.2025.118655 ⏎

Swaney, M. H., Nelsen, A., Sandstrom, S., & Kalan, L. R. (2023). Sweat and sebum preferences of the human skin microbiota. *Microbiology Spectrum*, *11*(1), e04180–22. https://doi.org/10.1128/spectrum.04180-22 ⏎

Szántó, M., Dózsa, A., Antal, D., Szabó, K., Kemény, L., & Bai, P. (2019). Targeting the gut–skin axis—Probiotics as new tools for skin disorder management? *Experimental Dermatology*, *28*(11), 1210–1218. https://doi.org/10.1111/exd.14016 ⏎

Tirosh, O., Conlan, S., Deming, C., Lee-Lin, S.-Q., Huang, X., Su, H. C., Freeman, A. F., Segre, J. A., & Kong, H. H. (2018). Expanded skin virome in DOCK8-deficient patients. *Nature Medicine*, *24*(12), 1815–1821. https://doi.org/10.1038/s41591-018-0211-7 ⏎

Townsend, E. C., & Kalan, L. R. (2023). The dynamic balance of the skin microbiome across the lifespan. *Biochemical Society Transactions*, *51*(1), 71–86. https://doi.org/10.1042/BST20220216 ⏎

Toyofuku, M., Nomura, N., & Eberl, L. (2019). Types and origins of bacterial membrane vesicles. *Nature Reviews Microbiology*, *17*(1), 13–24. https://doi.org/10.1038/S41579-018-0112-2 ⏎

Toyofuku, M., Schild, S., Kaparakis-Liaskos, M., & Eberl, L. (2023). Composition and functions of bacterial membrane vesicles. *Nature Reviews Microbiology*, *21*(7), 415–430. https://doi.org/10.1038/S41579-023-00875-5 ⏎

Tsurumachi, M., Kamata, Y., Tominaga, M., Ishikawa, J., Hideshima, T., Shimizu, E., Kaneko, T., Suga, Y., & Takamori, K. (2023). Increased production of natural moisturizing factors and bleomycin hydrolase activity

in elderly human skin. *Journal of Dermatological Science*, *110*(1), 2–9. https://doi.org/10.1016/j.jdermsci.2023.03.001 ⏎

Umbach, A. K., Stegelmeier, A. A., & Neufeld, J. D. (2021). Archaea are rare and uncommon members of the mammalian skin microbiome. *mSystems*, *6*(4), e0064221. https://doi.org/10.1128/mSystems.00642-21 ⏎

Van Horn, K. G., Audette, C. D., Tucker, K. A., & Sebeck, D. (2008). Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria. *Diagnostic Microbiology and Infectious Disease*, *62*(4), 471–473. https://doi.org/10.1016/j.diagmicrobio.2008.08.004 ⏎

Vijaya Chandra, S. H., Srinivas, R., Dawson, T. L., & Common, J. E. (2021). Cutaneous *Malassezia*: Commensal, pathogen, or protector? *Frontiers in Cellular and Infection Microbiology*, *10*, 614446. https://doi.org/10.3389/fcimb.2020.614446 ⏎

Wang, Y., Yu, Q., Zhou, R., Feng, T., Hilal, M. G., & Li, H. (2021). Nationality and body location alter human skin microbiome. *Applied Microbiology and Biotechnology*, *105*(12), 5241–5256. https://doi.org/10.1007/s00253-021-11387-8 ⏎

Wang, Z., Wang, X., Shi, Y., Wu, S., Ding, Y., Yao, G., & Chen, J. (2024). Advancements in elucidating the pathogenesis of actinic keratosis: Present state and future prospects. *Frontiers in Medicine*, *11*, 1330491. https://doi.org/10.3389/fmed.2024.1330491 ⏎

Wise, N. M., Wagner, S. J., Worst, T. J., Sprague, J. E., & Oechsle, C. M. (2021). Comparison of swab types for collection and analysis of microorganisms. *MicrobiologyOpen*, *10*(6), e1244. https://doi.org/10.1002/mbo3.1244 ⏎

Wishart, D. S., Oler, E., Peters, H., Guo, A., Girod, S., Han, S., Saha, S., Lui, V. W., LeVatte, M., Gautam, V., Kaddurah-Daouk, R., & Karu, N. (2023). MiMeDB: The Human Microbial Metabolome Database. *Nucleic Acids Research*, *51*(D1), D611–D620. https://doi.org/10.1093/nar/gkac868 ⏎

Wojciechowska, K., & Dos Santos Szewczyk, K. (2025). The skin microbiome and bioactive compounds: Mechanisms of modulation, dysbiosis, and

dermatological implications. *Molecules*, *30*(22), 4363. https://doi.org/10.3390/molecules30224363 ⏎

Wood, D. L. A., Lachner, N., Tan, J.-M., Tang, S., Angel, N., Laino, A., Linedale, R., Lê Cao, K.-A., Morrison, M., Frazer, I. H., Soyer, H. P., & Hugenholtz, P. (2018). A natural history of actinic keratosis and cutaneous squamous cell carcinoma microbiomes. *mBio*, *9*(5), e01432-18. https://doi.org/10.1128/mBio.01432-18 ⏎

Wu, L., Zeng, T., Deligios, M., Milanesi, L., Langille, M. G. I., Zinellu, A., Rubino, S., Carru, C., & Kelvin, D. J. (2020). Age-related variation of bacterial and fungal communities in different body habitats across the young, elderly, and centenarians in Sardinia. *mSphere*, *5*(1), e00558-19. https://doi.org/10.1128/mSphere.00558-19 ⏎

Yao, Z., Moy, R., Allen, T., & Jansen, B. (2017). An adhesive patch-based skin biopsy device for molecular diagnostics and skin microbiome studies. *Journal of Drugs in Dermatology: JDD*, *16*(10), 979–986. ⏎

Ying, S., Zeng, D.-N., Chi, L., Tan, Y., Galzote, C., Cardona, C., Lax, S., Gilbert, J., & Quan, Z.-X. (2015). The influence of age and gender on skin-associated microbial communities in urban and rural human populations. *PLoS One*, *10*(10), e0141842. https://doi.org/10.1371/journal.pone.0141842 ⏎

Yu, T., Xu, X., Liu, Y., Wang, X., Wu, S., Qiu, Z., Liu, X., Pan, X., Gu, C., Wang, S., Dong, L., Li, W., & Yao, X. (2024). Multi-omics signatures reveal genomic and functional heterogeneity of *Cutibacterium acnes* in normal and diseased skin. *Cell Host & Microbe*, *32*(7), 1129–1146.e8. https://doi.org/10.1016/j.chom.2024.06.002 ⏎

Yu, Y., Wen, H., Li, S., Cao, H., Li, X., Ma, Z., She, X., Zhou, L., & Huang, S. (2022). Emerging microfluidic technologies for microbiome research. *Frontiers in Microbiology*, *13*, 906979. https://doi.org/10.3389/fmicb.2022.906979 ⏎

Zhang, D., Hu, J., Gu, B., Cao, B., Lu, J., Chen, Q., Wang, L., Pei, G., Liu, Z.-X., Cheng, M.-G., Gao, S., & Li, X. (2025). BodyMeta: A comprehensive database for microbes under various pathological and physiological

conditions. *Computational and Structural Biotechnology Journal*, *27*, 3685–3692. https://doi.org/10.1016/j.csbj.2025.08.002 ⏎

Zhang, H., Wang, X., Chen, A., Li, S., Tao, R., Chen, K., Huang, P., Li, L., Huang, J., Li, C., & Zhang, S. (2024). Comparison of the full-length sequence and sub-regions of 16S rRNA gene for skin microbiome profiling. *mSystems*, *9*(7), e00399-24. https://doi.org/10.1128/msystems.00399-24 ⏎

12

The Crosstalk between Microbiome and Transcriptome: A Focus on Respiratory Diseases

Pavithra Madamarandawala and Dhammika Magana-Arachchi

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12.1 Introduction

As of the latest definition, the microbiome is a specific population of microorganisms inhabiting a reasonably precise territory that has diverse physicochemical properties (Berg et al., 2020). At present, methods for studying the microbiomes revolve around multi-omics, ranging from high-throughput isolation (culturomics) and visualization (microscopy), to targeting the taxonomic composition (metabarcoding) or addressing the metabolic potential (metabarcoding of functional genes and metagenomics), to analyzing microbial activity (metatranscriptomics, metaproteomics, and metabolomics) (Berg et al., 2020). The human microbiome can be defined as the total collection of microorganisms (including bacteria, viruses, fungi, and single-cell eukaryotes) that live in and on the human body. The

National Institutes of Health (NIH) initiated the Human Microbiome Project (HMP) in 2007 to characterize the diverse population of microbes living on and in the human body, specifically their involvement in human health and disease. Concluded in 2016, the findings from the HMP work instituted a foundational reference point for understanding the complex association between the human microbiome and health, which had remained understudied until then. According to the latest news "The global human microbiome market" (worldwide business environment involving products and services targeting the human microbiome) is estimated to grow from $791 million in 2025 to $6.09 billion by 2035, at a compound annual growth rate (CAGR) of 20.4% during the forecast period, till 2035 (*Human Microbiome Market Outlook 2025–2035—Sciinov Conference*, https://microbiomecongress.org/report/human-microbiome-market-outlook-20252035/).

The transcriptome can be defined as the complete set of transcripts, or all ribonucleic acid (RNA) molecules—such as messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and other noncoding RNAs—present in a specific cell, tissue, or organ at a given time. In comparison to the genome, the transcriptome is dynamic and fluctuates in its gene responses across different situations, tissues, and developmental stages. Hence, the crosstalk between the microbiome and transcriptome can be defined as how a specific population of microorganisms inhabiting a reasonably precise territory (such as a cell, tissue, microorganism, human, animal, or plant) with diverse physicochemical properties influences or exerts its effects on the complete set of all ribonucleic acids (RNAs) changing their gene responses accordingly.

The human microbiome has an extensive diversity and each location of the human body—whether it is skin, gut, lung, or any other site—has its own microbiota or metagenome. Experimental design is crucial in human microbiome research; among the many factors to be considered, it must always be remembered that the microbiome is not static. Hence, depending on the site of the human body, sampling methods need to be decided (Badger et al., 2010). Recent advances in omics in combination with

microbiome data have advanced our knowledge on how a microbiome consisting of both harmless and pathogenic microbes is effective in maintaining disease-free living, whereas an imbalanced or dysbiotic microbiome plays a role in disease dynamics, progression, and the development of targeted therapeutics. According to the literature, the microbiome could also be the key that provides insights into why some individuals are asymptomatic and others have severe symptoms in response to certain diseases, such as severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infections (Michael et al., 2024). However, the main drawback is that most research has been concentrated on the bacterial communities of the microbiome and comparatively only a few studies focus on the other microorganisms, including viruses, fungi, and archaea.

This chapter explores the interplay between the microbiome and the transcriptome in the context of respiratory diseases. It begins with an overview of key concepts, including microorganisms that cause respiratory disease, the human lung transcriptome, and the lung microbiome. The chapter then examines transcriptomic responses in various respiratory illnesses and discusses how alterations in the lung microbiome influence different lung diseases. Subsequently, the role of the human microbiome in individual respiratory diseases is addressed, followed by an in-depth discussion of microbiome–transcriptome crosstalk in respiratory pathology. The chapter concludes with a summary of key findings and outlines future research directions.

12.2 Respiratory Diseases

Diseases affecting the lungs or airways are classified as respiratory diseases, which could be categorized as infectious or non-contagious.

12.2.1 Infectious Respiratory Diseases and the Causative Organisms

Infectious lung diseases are caused by the inhalation of pathogenic viral, bacterial, or other microorganism that damage the pulmonary system, which includes the nose, mouth, pharynx, larynx, trachea, bronchi, and alveoli in lungs.

Pulmonary diseases, their causative organisms, and associated risk factors are summarized in Table 12.1. Common respiratory viral diseases include the common cold or viral rhinitis and influenza, which are caused by the rhinoviruses (RVs), respiratory syncytial viruses (RSVs), influenza A viruses, adenoviruses, and parainfluenza type 3 viruses. Bacterial communicable respiratory diseases include tuberculosis (TB), pneumonia, and whooping cough. Among the known 195 species (Armstrong et al., 2023) belonging to the genus *Mycobacterium*, some are pathogenic or potentially pathogenic to humans. The species belonging to the *Mycobacterium tuberculosis* complex are considered the leading infectious respiratory pathogen globally and serve as the etiological agent for TB. Certain other mycobacterial species, commonly named as non-tuberculous mycobacteria (NTM), cause pulmonary diseases that are non-contagious (Keerthirathne et al., 2016).

TABLE 12.1

Pulmonary Diseases and the Causative or Associated Microorganisms ⏎

Disease	Etiological Agent/s
Viral Diseases	
Common cold	RVs
Common cold	Coronaviruses
Common cold	Enteroviruses (Enterovirus D68 (EV-D68))
Pneumonia	Adenoviruses
Coronavirus disease 2019 (COVID-19)	Severe acute respiratory syndrome coronavirus 2

Disease	Etiological Agent/s
Influenza	RSV
Bronchiolitis	RSV
Measles	Rubeola virus
Viral respiratory illness	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
Rubella (German Measles)	Rubivirus rubellae
SARS	SARS-associated coronavirus (SARS-CoV)
Severe Acute Respiratory Illness (SARI)	Human metapneumovirus (HMPV), influenza A (H1N1), human rhinovirus (HRV) and human adenovirus (HAdV)
Varicella (Chickenpox)	varicella-zoster virus (VZV)
Bacterial Diseases	
Pneumonia (community-acquired)	*Streptococcus pneumoniae*
Pneumonia	*Haemophilus influenzae*
Epiglottitis	*Haemophilus influenzae*
Pneumonia (Hospital-acquired)	*Staphylococcus aureus*
***Streptococcus pyogenes* infection**	*Streptococcus pyogenes*
Atypical pneumonia (Chlamydia Pneumonia = community-acquired)	*Chlamydia* spp. *(C. pneumoniae, C. psittaci, C. trachomatis)*
Diphtheria	*Corynebacterium diphtheriae*

Disease	Etiological Agent/s
Haemophilus influenzae type B (HiB) Disease	*Haemophilus influenzae*, Type b (Hib)
Legionnaires' disease	*Legionella*
Pontiac fever	*Legionella*
Pneumonia (due to Legionnaires' disease)	*Legionella pneumophila*
Pertussis (whooping cough)	*Bordetella pertussis*
Tuberculosis (Human TB)	*M. tuberculosis* complex (*M. tuberculosis, M. africanum, M. bovis, M. canettii, M. caprae)*
Mycoplasma pneumonia, tracheobronchitis, bronchitis	*Mycoplasma pneumoniae, Mycoplasma* spp.
Fungal Diseases	
Pneumocystis Pneumonia	*Pneumocystis jirovecii*
Aspergillosis	*Aspergillus* spp. (*A. fumigatus*)
Candidiasis	*Candida* species (*C. albicans*)
Cryptococcosis	*Cryptococcus neoformans* and Cryptococcus gattii
Histoplasmosis	*Histoplasma capsulatum*
Coccidioidomycosis	*Coccidioides immitis, Coccidioides posadasii*
Blastomycosis	*Blastomyces dermatitidis*
Mucormycosis	*Mucor* spp.
Disease	**Associated Microorganism/s**
Non-Communicable or Chronic Respiratory Diseases	

Disease	Etiological Agent/s
COPD	Bacteria: *Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae* Viruses: RV, RSV, human metapneumovirus (hMPV) Fungi: *Pneumocystis jirovecii*
Asthma	Viruses: RV, RSV, hMPV Bacteria: *Mycoplasma pneumonia, Chlamydia pneumoniae, Streptococcus pneumoniae, and Haemophilus influenzae* Fungi: *Aspergillus fumigatus*
Lung cancer	Bacteria: *M. tuberculosis, Achromobacter xylosoxidans, Streptococcus sinensis* and other *Streptococcus* species and *Staphylococcus sciuri* Viruses: HPV 16 and 18, Epstein-barr virus, Influenza virus Fungi: *Candida* and *Aspergillus* species
Bronchiectasis	Bacteria: NTM, *Pseudomonas aeruginosa, Burkholderia lata and S. dentisani* Fungi: *A. fumigatus*
Cystic fibrosis (CF)	Bacteria: *Pseudomonas aeruginosa, Mycobacteria* Fungi: *A. fumigatus*

Respiratory tract infections caused by *Mycoplasma pneumoniae* are well known, but diverse mycoplasma species, such as *M. orale*, *M. salivarium, M. buccale, M. faucium, M. lipophilum*, and *M. primatum*, can be considered potential pathogens in respiratory diseases including pneumonia, tracheobronchitis, bronchitis, and pharyngitis (Miko et al., 2019; Waites and Atkinson, 2009). *Chlamydophila pneumoniae* is another pathogen that causes pneumonia and bronchitis, and the disease could be acute or chronic. The disease outcome from the obligate intracellular pathogen *Chlamydophila* is determined by a series of pathogen- and host-associated factors, as well as the interaction between organism replication and the host response (Barron, 1988). Whooping cough, which is caused by the human-

specific pathogen *Bordetella pertussis*, is a highly contagious respiratory disease (Nieves & Heininger, 2016).

12.2.2 Chronic Respiratory Diseases and Associated Microorganisms

Chronic diseases, or non-communicable diseases (NCDs), are those that occur due to one or more risk factors of genetic (such as mutations), environmental (such as air pollution), behavioral (such as smoking and alcohol consumption), or metabolic (such as obesity) origin. Major chronic respiratory diseases include chronic obstructive pulmonary disease (COPD), asthma, and lung cancer. As of 2021 statistics, respiratory NCDs account for 4 million deaths worldwide (*Global Burden of Disease 2021_Booklet_FINAL_2024.05.16.Pdf*). In addition to the causative microorganisms of infectious diseases discussed in Section 12.2.1, Table 12.2 also presents the microorganisms reported to be associated with major respiratory NCDs. It is noteworthy that while infection is a risk factor in some NCDs, and it can also opportunistically exacerbate the disease severity in some NCDs. For instance, in COPD infectious disease is a risk factor while bronchiectasis is often caused by severe or repeated infectious lung diseases mainly TB or pneumonia (Chalmers et al. 2018). A detailed version of associated microorganisms in pulmonary diseases is presented in Section 12.3.1.

12.3 Human Lung Microbiome

Situated within the thoracic cavity of the chest, human lungs are the core part of the respiratory system. The respiratory tract is the main gateway for the microorganisms to enter the human body through breathing, which is essential to supply oxygen to the body. According to pulmonary studies, as the upper and lower respiratory tracts are continuous, they may share microbiota, and the nasal microbiome contributes partially to the composition of the lung microbiome (C.-X. Li et al., 2024). Previously, it

was assumed that the human lower respiratory tract was sterile in healthy individuals. However, the use of cutting-edge techniques has now proven that a diverse community of microorganisms resides in the human lung. This microbiota plays an important role not only in disease causation, but also in immune mediation and disease progression (Ekanayake et al., 2020). Maintaining a balanced respiratory microbiome is essential for lung health, as dysbiosis of the respiratory tract has been associated with a wide range of infectious and non-infectious respiratory diseases.

Various sampling procedures are being utilized to evaluate the respiratory tract microbiome, including oral swabs, pharyngeal secretions, sputum, endotracheal aspirate (ETA), bronchoalveolar lavage (BAL) fluid, microlavage (mBAL), bronchoscopy aspiration, bronchial biopsies, and exhaled breath condensates (Belizário et al., 2023). The lung microbiome has been assessed using both culture-dependent and culture-independent advanced molecular techniques, such as metagenomics, for the organism identification. Metagenomic sequencing can be done either by (1) targeting a specific region of the organism's genome, such as the 16S rRNA for bacteria and archaea, the internal transcribed spacer (ITS) for fungi, and 18S for eukaryotes like protists, or (2) using an unbiased approach to sequence the entire genomic content in the sample via shotgun sequencing. Although there are no active, lung microbiome-specific databases, most of the lung metagenomics data are publicly available through common databases, such as the National Center for Biotechnology Information (NCBI), and microbiome-specific databases, as stated in Table 12.2. Molecular methods for identifying the respiratory microbiome are noteworthy, but concurrently basic microbiological culture is still vital to confirm the viability of pathogens (Ekanayake et al., 2020).

Knowledge about the composition of the lung microbiome is vital in treating many lung diseases. The mucosal surface of 50–75 cm^3 of the respiratory tract is colonized by bacteria, archaea, viruses, bacteriophages, and fungal species (Belizário et al., 2023). It has been shown that respiratory bacterial diversity is higher in diseased populations compared to healthy ones. The predominant bacterial phyla in the lung microbiome are

similar to those in the gut, mainly Firmicutes and Bacteroidetes followed by Proteobacteria and Actinobacteriota, while the main identified fungi are typically environmental, such as Ascomycota (*Aspergillus, Cladosporium, Eremothecium*, and *Vanderwaltozyma)* and Microsporidia (*Systenostrema*) (Enaud et al., 2020). Frequently the lung microbiome overlaps with the oral microbiome, but it has been observed that *Prevotella* occurs in high numbers in the oral cavity and lesser in healthy lungs (Belizário et al., 2023). The study of the healthy lung microbiome is problematic due to the difficulty in performing invasive sampling procedures on non-diseased individuals. A study characterizing human non-malignant lung tissue identified *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteriota* at the phylum level. At the genus level, five *Proteobacteria* genera, *Acinetobacter*, *Pseudomonas*, and *Ralstonia*, plus one unknown genus each from the families Comamonadaceae and Oxalobacteraceae were identified. Furthermore, findings confirm that the lung microbiota is distinct in comparison to microbes in the mouth, skin, or even the nose (Yu et al., 2016). According to Belizario et al. (2023), in internal lung tissues, the estimated bacterial count is around 10^2 bacteria per gram of tissue, with a high abundance of *Prevotella* spp., *Veillonella* spp., *Streptococcus* spp., and *Tropheryma whipplei* (Belizário et al., 2023). In another study using metagenomics, researchers identified *Streptococcus dentisani* (31.3%), *Rothia mucilaginosa* (12%) and *S. salivarius* (8.1%) in highest abundance in species level for healthy samples (Ekanayake et al., 2020).

Though this chapter focuses on the respiratory diseases, researchers have discovered that bacteria inside malignant cells have the potential to inactivate cancer treatments, highlighting the importance of microbiome studies in patients diagnosed with cancer. A study on lung cancer patients' microbiomes revealed that the phyla Proteobacteria and Firmicutes dominated the bacterial composition, and the three most abundant bacterial species were *Achromobacter xylosoxidans*, *Streptococcus sinensis* and *Staphylococcus sciuri* with abundances of 35.30%, 20.50%, and 11.00%, respectively (Ekanayake et al., 2020).

TABLE 12.2
Common Databases on Human Lung Transcriptome and Microbiome ⏎

Database/Link	Content in the Current Version (As of September 2025)
Microbiome Databases	
Disbiome https://disbiome.ugent.be/home	*Contains data from* 375 diseases including respiratory diseases. Covers microbial composition changes across diseases. (Janssens et al., 2018)
mBodyMap https://mbodymap.microbiome.cloud	*Contains data from* within 22 body sites including *human lungs*. Information on microbes across the lung and their association with lung diseases can be found. (*mBodyMap: A Curated Database for Microbes across Human Body and Their Associations with Health and Diseases | Nucleic Acids Research | Oxford Academic*, n.d.)
MicrobiomeDB https://microbiomedb.org/mbio/app	*Contains data from* various microbiome studies *including lung*. (*MicrobiomeDB: A Systems Biology Platform for Integrating, Mining and Analyzing Microbiome Experiments | Nucleic Acids Research | Oxford Academic*, n.d.)
Lung transcriptomics databases	
Human lung cell atlas https://hlca.sf.czbiohub.org	*Contains data from* 58 cell populations of the lung (~75,000 human lung and blood cells) (Travaglini et al., 2020) *derived using* droplet- and plate-based scRNA-seq

Database/Link	Content in the Current Version (As of September 2025)
The integrated Human Lung Cell Atlas (HLCA) Link Note: This is a part of the major database the human cell atlas. It also contains a COVID-19 cell atlas www.covid19cellatlas.org	HCLA *contains data from* 49 datasets (486 subjects) (Sikkema et al., 2023) *derived using* single-cell transcriptomics.
Lung Gene Expression Analysis (LGEA) database and web portal Link to LGEA	*Contains data from* human and mouse lungs (Healthy and diseased) *derived using* both bulk- and scRNA-seq (Du et al., 2021)
Lung cell atlas www.lungcellatlas.org	*Contains data from* healthy human lungs (Upper and lower parenchyma), trachea and bronchi *derived using* sc/snRNA-seq and ST (Madissoon et al., 2023)
Lung map www.lungmap.net	*Contains data from* healthy and diseased human, Rhesus and mouse lungs *derived using* bulk and single-cell multiomics (Gaddis et al., 2024)
uniLUNG https://lung.unifiedcellatlas.org	*Contains data from* 62 datasets, 17 diseases and 58 cell types *derived using* single-cell transcriptomics (F. Wu et al., 2024)
Lung endothelial cell atlas www.lungendothelialcellatlas.com	*Contains data* from 6 datasets from 73 subjects *derived using* scRNA-seq. comparison data from mouse lungs available (Schupp et al., 2021)
Pulmonary Disease-Specific Transcriptomics Databases	

Database/Link	Content in the Current Version (As of September 2025)
Idiopathic Pulmonary Fibrosis (IPF) Cell Atlas www.ipfcellatlas.com	*Contains data from* 4 datasets of 134 subjects; healthy, and diseased (IPF, chronic hypersensitivity pneumonitis (CHP), nonspecific interstitial pneumonitis (NSIP), systemic sclerosis (SSC), polymyositis (PM), sarcoidosis, and COPD) *derived using* scRNA-seq (Neumark et al., 2020)
COPD cell atlas www.copdcellatlas.com	*Contains data from* COPD alveolar niche of 17 patients and 15 healthy controls *derived using* scRNA-seq (Sauler et al., 2022)
Columbia University/NYP COVID-19 Lung Atlas Link	*Contains data from* 19 COVID-19 autopsy lungs and seven pre-pandemic controls *derived using* snRNA-seq (*A Molecular Single-Cell Lung Atlas of Lethal COVID-19 | Nature*, n.d.)
Lung cancer explorer https://lce.biohpc.swmed.edu/lungcancer/	*Contains data from* over 6700 patients in 56 studies (Wang et al., 2021)
scMoresDB (A comprehensive database of single-cell multi-omics data for human respiratory diseases) http://liwzlab.ifr.fidt.top:61010/scmoresdb/#/Home	*Contains data from* 69 datasets of healthy and diseased individuals *derived using* scRNA-seq, ST and RNA-seq (Chen et al., 2024)

Notes: It should be noted that most of these databases are continuously developing with added features and new data as they become available. In addition, even though not interactive or sorted as the databases in Table 12.2, one could also access human/other species' lung transcriptomics data through UCSC cell browser (www.nupulmonary.org/resources/), the lung transcriptome section of the human protein atlas

(www.proteinatlas.org/humanproteome/tissue/lung#the_lung_transcriptome) and lung datasets of 10• genomics (www.10xgenomics.com/datasets?configure%5BhitsPerPage%5D=50&configure%5BmaxValuesPerFacet%5D=1000&query=Lung). An interactive database derived using scRNA-seq data of only mouse lungs is also available at https://theislab.github.io/LungAgingAtlas/, the lung aging atlas (Angelidis et al., 2019).

12.3.1 Lung Microbiome and Different Respiratory Diseases

12.3.1.1 Structure and Function of the Respiratory Tract

The structure and function of the lower respiratory tract composed of trachea and lungs (bronchi, bronchioles, and alveoli) are different from the upper respiratory tract supporting the airflow and gaseous exchanges, both providing physical and chemical barriers maintaining sterility in the lungs (Losada et al., 2010). Conductive airways are covered by a ciliated epithelium scattered with diverse types of secretory cells, that release mucins, surfactants and variety of proteins and also immune cells when sensing the microbial presence responding with innate immunity (Belizário et al., 2023). In contrast to the conducting airways, the lung epithelium is protected neither by cilia nor mucus, but is covered by surfactant proteins, maintaining the surface tension, thereby facilitating efficient gas exchange (Losada et al., 2010). Alveolar macrophages, lymphocytes (T and B cells), dendritic cells, adhesion molecules on the epithelium, ciliated and non-ciliated (clara) secretory cells, and basal cells of the lungs, and natural killer (NK) cells collectively protect the lungs from potential pathogens (Belizário et al., 2023).

12.3.1.2 Effect of Different Respiratory Diseases on Lung Microbiome

With the advent of new technologies, researchers are now able to explore the interactions between the respiratory diseases (chronic and infectious) and the flora of the lung. How the healthy active lung microbiota gets

affected with the chronic respiratory diseases, such as asthma, COPD, lung cancer, cystic fibrosis (CF), or bronchiectasis, and the effect of communicable respiratory pathogens on the lung microbiome are the main areas of interest for the development of personalized medicine.

12.3.1.2.1 Asthma and Wheezing

Asthma is categorized as inflammation or swelling of the lungs and the contraction of the airways which is reversible, with causative factors being either environmental or genetic in nature. Respiratory diseases caused by viruses, bacteria, and fungi have been associated with the induction and development of asthma. Asthma exacerbations have been attributed mainly to viral infections, with the primary viruses being RV, and RSV, which cause pneumonia and bronchiolitis leading to wheezing in infants and children that can progress to asthma in later years. The human metapneumovirus (hMPV) has been identified in asthmatic exacerbations in children and adults. Bacterial infections such as *Mycoplasma pneumonia* and *Chlamydia pneumoniae* are also associated with asthmatic exacerbations. In addition, fungal exposure has also been identified as a causative agent for asthma exacerbations (Losada et al., 2010). Many bacterial species have been associated with asthma, but the role of bacteria in the initiation and development of asthma is found to be complicated (Losada et al., 2010). A study recorded colonization of *Streptococcus pneumoniae*, and *Haemophilus influenzae* from the hypopharyngeal regions of neonates, whose mothers suffered from asthma. They were found to be at risk of recurrent wheezing and developing asthma by the age of five (Bisgaard et al., 2007). Considering fungi, it has been recorded that for one species, *Aspergillus fumigatus*, 20 allergens participate in human airway allergies, and chronic colonization leads to severe asthma (Losada et al., 2010).

12.3.1.2.2 Cystic Fibrosis

Cystic fibrosis (CF) is an inherited severe autosomal recessive disease where microbes chronically colonize the lower airways. Researchers have identified organisms both cultivable and fastidious from respiratory samples collected from CF patients demonstrating the presence of a diverse composition of the microorganisms in relation to the healthy lungs. These organisms include viruses, bacteria such as *Pseudomonas aeruginosa*, *Mycobacterium* species, and *A. fumigatus* (Losada et al., 2010). In CF patients, the interplay between these organisms, the lung microbiome, and the structure of the upper and lower respiratory system—comprising tissues and cells as a whole—is a complex ecological niche. The presence of thick mucus in CF patients prevents the removal of microorganisms and debris from the lungs, as these get trapped in the mucus. It is also recorded that the number of neutrophils that secrete defensins is high in the colonized site in addition to the secondary metabolites produced by these microorganisms, which cause damage to the epithelial membranes of the airways and the lungs (Rada, 2017).

12.3.1.2.3 Bronchiectasis

Bronchiectasis is categorized as the chronic widening and swelling of the airways with recurrent infections (Chalmers et al. 2018). Literature suggests an association of NTM and fungi, mainly *A. fumigatus*, with the disease bronchiectasis. However, the pathological significance of the fungi in relation to bronchiectasis is minimal, except for a relatively small number of idiopathic bronchiectasis patients with allergic bronchopulmonary aspergillosis (ABPA) (Losada et al., 2010). A study by Ekanayake et al. (2020) recorded Proteobacteria as the most abundant phylum in bronchiectasis patients, while *Pseudomonas aeruginosa* was recorded as the highest abundant species followed by *Burkholderia lata* and *S. dentisani*.

12.3.1.2.4 Chronic Obstructive Pulmonary Disease

COPD is defined as "*a disease state characterized by airflow limitation that is not fully reversible*" by the Global Initiative for Chronic Obstructive

Lung Disease (GOLD). In comparison to the healthy lung microbiome colonization by the bacteria such as *Haemophilus influenzae, Pseudomonas aeruginosa*, and *Streptococcus pneumoniae;* viruses including RV, RSV, and hMPV; and fungal species including *Pneumocystis jirovecii* have been associated—either as a single organism or in combination—with stable COPD or during exacerbations, increasing disease severity with a high organism load (Ren et al., 2018).

12.3.1.2.5 Pneumonia

The etiological agents for community-acquired pneumonia (CAP) and hospital acquired pneumonia (HAP) are *Streptococcus pneumoniae* and *Staphylococcus aureus* respectively in addition to many other bacterial species (Belizário et al., 2023). Viruses including adenoviruses and hMPV, as well as fungal species from the genus *Pneumocystis*, are also identified as a causative agent in pneumonia (Table 12.1). In patients with SARS-CoV-2-associated pneumonia, the bacterial species that reside in the upper respiratory tract—such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*—were detected causing bacterial co-infections. Additionally, the fungal pathogens reported included *candidiasis*, *aspergillosis*, and *Pneumocystis jirovecii* (Belizário et al., 2023).

Clinical symptoms of bacterial and viral pneumonia often overlap, making accurate identification of the causative organism essential for effective treatment (Belizário et al., 2023). In addition, treatment outcomes depend on distinguishing whether the detected microorganism is a resident member of the lung microbiome or an exogenous pathogen. Immunocompromised individuals, including post-transplant patients and those with TB or HIV infection, are particularly susceptible to pneumonia due to impaired immune function.

Lung transplantation is the only therapeutic possibility for patients with end-stage lung disease; however, it has been shown that the success rate is much lower in comparison to other solid organ transplants (R. Li et al.,

2024). A comparative analysis of paired nasal swabs and BAL fluid from 17 early postoperative lung transplant patients identified differences in Shannon diversity and beta diversity in the microbiomes of the two sites. According to the findings, the upper respiratory tract harbors more *Corynebacterium*, *Acinetobacter* and *Pseudomonas* and the lower respiratory tract harbors more *Ralstonia, Enterococcus, Stenotrophomonas*, and *Pedobacter* (R. Li et al., 2024). As pathogenic organisms were recorded from both sites and researchers postulate that both sites could reflect the disease characteristics of the transplanted lung. They also postulate that the differences were due to the sampling sites rather than the sampling of the individuals (R. Li et al., 2024).

12.3.2 Role Played by Human Microbiome on Respiratory Diseases

Although the association between disease states and the human microbiome has been well recognized, definitive evidence establishing microbial composition as a direct cause of disease remains limited. It is understood that a stable equilibrium must be maintained within the microbiome among commensal organisms and potential pathogens. Scientific studies have shown that microbial taxonomic groups present in healthy individuals often undergo proportional and abnormal shifts during disease conditions (Badger et al., 2010). Studies have shown how diet influences the gut microbiome (Espírito Santo et al., 2021). As is evident in the gut, skin and urinary tract microbiomes, the lung microbiome also has links to diet, gender, genetic polymorphisms, and environmental conditions (Belizário et al., 2023).

It is observed that along with pulmonary diseases, there is dysbiosis of the intestinal flora and an immune-inflammatory response. The abdominal flora and its metabolites are directly or indirectly involved in the immune regulation of the host in lung diseases (Ma et al., 2022). The diarrhea symptoms that occurred due to the changes in the gut microbiota in COVID-19 patients were globally evident during the pandemic. Researchers hypothesize that the transfer of bacteria from the

gastrointestinal (GI) tract microbiome to the lungs via reflux, lymphatic, or systemic circulation may contribute to inflammatory airway diseases (Enaud et al., 2020; Ma et al., 2022). Same as the gut–brain axis, scientists advocate that though anatomically different the gut–lung axis connecting the intestine and lung alveoli also exercise similar biological functions (Ma et al., 2022).

The lung microbiome of patients with ventilator-associated pneumonia (VAP) differs significantly from that of healthy individuals, characterized by reduced alpha and beta diversity. This reduction is attributed to the loss of potentially protective commensal microorganisms and increased colonization by Proteobacteria, which are presumed to originate from the gut, thereby disrupting the normal equilibrium of the lung microbiota (Belizário et al., 2023). However, several hypotheses have been proposed regarding the source of this dysbiosis. Some suggest that alterations in the upper digestive tract, particularly within oropharyngeal microbial communities, facilitate pathogen invasion and lead to respiratory disease, while others propose that the intestinal microbiome contributes broadly to the development of bacterial pneumonia (Belizário et al., 2023). Specifically, in Pseudomonas pneumonia, it has been postulated that the organism translocates from the gut rather than from the oropharynx (Belizário et al., 2023).

In acute respiratory distress syndrome (ARDS) patients, dysbiosis in the lung microbiome has been detected due to the enrichment of gut-associated bacteria of the Enterobacteriaceae family, such as the Gram-negative bacteria *Escherichia coli*, and *Klebsiella pneumoniae* (Dickson et al., 2015) while gut and lung microbial changes cause gradual exacerbation and severity of COPD (Chiu et al., 2022).

Antibiotic resistance is a global issue and many bacterial strains that are causative agents for respiratory diseases are categorized as resistant to primary antibiotics. Thus, the therapeutic use of probiotics, such as *Lactobacillus rhamnosus, Bacillus subtilis, Enterococcus faecalis*, *Faecalibacterium prausnitzii, and Akkermansia muciniphila*, has attracted attention as an alternative treatment for respiratory diseases (Belizário et al.,

2023). The review by Espírito Santo et al. in detail discusses how dietary components, nutritional patterns, and the gut microbiota that influence lung immunity and lung diseases.

12.4 Human Lung Transcriptome

Microanatomically speaking, human lung itself is complex bearing more than 40 different reported cell types (Montoro et al. 2018; Franks et al. 2008). Each of these cell types varies in its cellular makeup, localization, and their contribution to respiratory and non-respiratory functions of the lungs. Hence, understandably, the transcriptome, the real-time profile of RNA, may differ from one cell type to another. They may also express different transcriptomic signatures during lung development. The Gene Ontology database lists different biological pathways and processes associated with lung development (such as lung alveolus development, and the regulation of lung morphogenesis) with lists of genes vital for each particular process or pathway (Gene Ontology Consortium, 2004). A further advanced database based on multi-omics studies is the Lung Cell Atlas (see Table 12.2 for the link and details), which lists studies on healthy lung transcriptome (Madissoon et al., 2023). Spatial transcriptomics (ST), single-cell/single-nucleus-RNA sequencing (sc/sn-RNA-seq), and VDJ sequencing (VDJ-seq) have characterized micro- and macroanatomical tissue compartments in the lungs (Madissoon et al., 2023), cell-cell communication patterns, developmental trajectories and defects from the early pseudoglandular to the alveolar stage (Marconett et al. 2013.; Chen et al. 2025). Developmental lung transcriptomics is specifically important for human lung organoid generation (Kim et al., 2024) and lung regeneration therapeutics for patients suffering from pulmonary damage (Martins et al., 2024).

12.4.1 Current Techniques Used to Study Lung Transcriptome

With the advent of next-generation sequencing, an enormous amount of information on human transcriptomics, including that of the human lungs, became available. RNA-sequencing is one such widely used technique which can produce single-organism transcriptomes (either total RNA, just mRNA, or other noncoding RNA). It can detect transcript levels at the single-cell level (single-cell RNA-seq or scRNA-seq) or single-nucleus level (single-nucleus RNA sequencing or snRNA-seq or sNuc-seq), or in bulk. It should be noted that bulk RNA-seq generates data as an average of the cell population analyzed, and in comparison to single-cell transcriptomics, the number of genes detected per sample by bulk RNA-seq is high. On the other hand, single-cell transcriptomics can detect cellular heterogeneity and rare cell populations using a small sample input. Combining this with the metagenomics described in Section 3 above, one might also identify the specific microbial communities in association with imbalances in host transcript levels. However, it is essential to note that metagenomics detects the DNA of both transcriptionally active (actively transcribing organisms contributing to metabolic output) and inactive microorganisms (dormant or dead cells, or extracellular DNA that does not contribute to metabolic output), thereby masking the true contributors (Singer et al., 2017). An advanced form of RNA-sequencing called metatranscriptomics can overcome this limitation as it is capable of analyzing all expressed RNA at any given time. As this technique can also detect host transcripts, it can be used to explore host-microbiome interactions (Westermann et al., 2017). Another powerful technique used in lung and other transcriptome research is ST, which can quantify and map the transcriptome within the physical location of the tissue itself. This can provide information on intercellular interactions and genes that are activated or suppressed in the context of the tissue microenvironment (Williams et al., 2022). Currently, one can also analyze T- and B-cell receptor clonotypes through a technique called V(D)J sequencing, a modified version of scRNA-seq. This DNA level technique assesses the diversity and frequency of immunoglobulins using primers targeting V and J genes (Chovanec et al., 2018). It is essential to note that every technique

referred to above may have limitations, such as the inability to detect less-active microbes, small sample size, limited barcoding capacity, and difficulty to obtain pulmonary disease-associated specimens or controls.

It is important to note that, while bulk sequencing of RNA extracted from a population of cells in lung tissue generates important data as an average of that cell population, it is critical to understand what happens at the single-cell level and link these interactions to understand the bigger picture of a healthy lung function and its status during a pulmonary disease. Single-cell transcriptomics has also filled knowledge gaps in the field regarding cellular diversity and the detection of rare cell populations. For these reasons, the majority of the studies on the healthy or diseased lung transcriptome prioritize either scRNA-seq or snRNA-seq and ST as shown in the list of common databases on the human lung transcriptome in Table 12.2.

12.4.2 Behavior of Lung Transcriptome on Different Respiratory Diseases

Transcriptome studies on diseased lung samples have generated enormous amounts of information on differentially expressed genes and noncoding RNAs, enriched pathways biological processes, and regulatory networks in study groups of diseased individuals and healthy controls. A current trend is also to use sc/sn-RNA-seq and/or ST, or to perform meta-analyses to integrate data from different transcriptomic techniques and/or studies to increase sample size and thus improve statistical power. Figure 12.1 shows findings from different transcriptomic studies in two major respiratory diseases: COPD and TB.

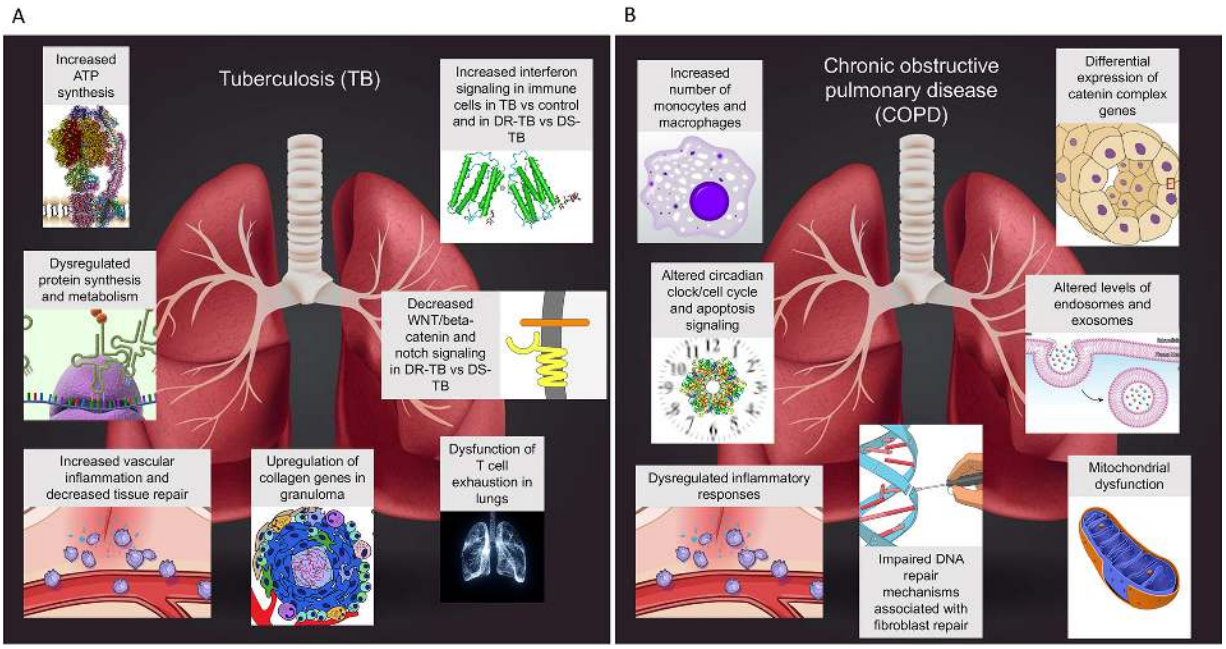

FIGURE 12.1 Findings from different transcriptomic studies conducted on two key respiratory diseases: TB and COPD. (A) Transcriptomic changes identified in the studies conducted on TB (increased host immune response, inflammation, downregulated tissue repair, dysregulated translation, metabolism, and dysregulated cell-signaling pathways). (B) Transcriptomic changes identified in COPD studies—differential expression of catenin complex genes, genes associated with plasma membrane components including endosomes and exosomes, an increased number of monocyte/macrophages, dysregulated inflammatory responses, impaired fibroblast repair, and mitochondrial dysfunction. ⏎

12.4.2.1 Tuberculosis

TB is one of the top ten respiratory infections in the world and hence, is widely studied, including its resulting transcriptomic changes. Major transcriptomic changes identified in the majority of studies include an increased host immune response (influx of regulatory T cells, neutrophils, and exhausted $CD8^+$ T cells) (Wang et al., 2023), inflammation, downregulated tissue repair (Fonseca et al., 2025), dysregulated translation and metabolism (Coppola et al., 2021), and dysregulated cell-signaling pathways such as interferon signaling (Wang et al., 2023) (Figure 12.1A).

The TB-related transcriptomics may be immune cell specific and present intercellular crosstalk (Wang et al., 2023). A recent ST study revealed the ST of TB granulomas, demonstrating shifts of the immune environment from B-cell predominance to myeloid cell predominance. They further showed that in all granulomas, collagen genes were upregulated (Qiu et al., 2024). A study using scRNA-seq demonstrated that the reason why people with a history of TB infection experience progressive damage to lung tissues is increased post-TB vascular inflammation. Specifically, FOXO3 signaling and NF-kB dependent thromboinflammation were decreased in post-TB patients (Sun et al., 2025). When the infecting *M. tuberculosis* strain resists the anti-TB drugs, it impacts the host immunity by downregulating defense-related pathways such as WNT/beta-catenin signaling, Notch signaling and microtubule nucleation creating a favorable environment for its survival (Madamarandawala et al., 2023). Further, transcriptomics can track the treatment efficacy of anti-TB drugs and suggest synergistic drugs in personalized medicine (Ma et al., 2019).

12.4.2.2 Chronic Obstructive Pulmonary Disease

The transcriptomic profile of patients with COPD is characterized by the differential expression of genes involved in the catenin complex, plasma membrane–associated components (including endosomes and exosomes), increased monocyte and macrophage populations, dysregulated inflammatory signaling, impaired fibroblast-mediated tissue repair, and mitochondrial dysfunction (Ditz et al., 2021; Heo et al., 2025; Sauler et al., 2022) (Figure 12.1B). As with other respiratory diseases, transcriptomic approaches are increasingly used to evaluate treatment efficacy in COPD. Although aerobic training has been shown to improve pulmonary function in COPD patients, transcriptomic analyses indicate limited effectiveness in promoting the repair of damaged alveolar structures (J. Li et al., 2024). Furthermore, Wu et al. (2022) reported that targeting prostaglandin E2 (EP) and prostacyclin (IP) receptors holds therapeutic potential for COPD management and lung regeneration (Wu et al., 2022).

Transcriptomic studies have also identified or proposed therapeutic targets for several other respiratory diseases: the CXCR1, CXCR2, and MMP9 genes in asthma as targets of the drug reparixin (Yan et al., 2024); TXN, PIGR and the novel genes CYP24A1, and PLAT as target genes in cystic fibrosis (Sun & Zhou, 2025); the combined action of IL-15, IL-4, and IL-7 for T-cell expansion during COVID-19 (Durkee-Shock et al., 2022); and the PSAT1, PSRC1, COLEC12, PLLP, and HP genes in lung cancer (Liao et al., 2024).

Reasons for drug resistance observed in several respiratory diseases have also been uncovered lately, specifically in relation to lung cancer. In small cell lung cancer, the WNT pathway is altered, as was seen in drug-resistant TB patients (J. Zhang et al., 2022). Another study showed that the tumor microenvironments of non-small-cell lung cancer patients differentially express the two genes: CD24 and CYP1B1 (W. Zhang et al., 2022). Differential expression of long noncoding RNAs is also known to play a role in drug susceptibility in lung adenocarcinoma (Perez-Medina et al., 2024).

12.5 The Crosstalk between Microbiome and Transcriptome in Respiratory Diseases

The discussion about the crosstalk between the microbiome and the immune system started in the past decade. However, the interest shown by researchers in the interrelation among the microbiome, transcriptomics, and immunity is still on the rise. While the lung microbiome can shape the lung transcriptome, any changes to the lung transcriptome due to risk factors other than microorganisms may reshape the lung microbiome as well. This never-ending interplay between the microbiome and transcriptome decides the host immunity and, thus, the overall health of an individual.

12.5.1 Lung Microbiome Shapes the Lung Transcriptome and Host Immunity

Microbial dysbiosis during a pulmonary disease can reshape the lung transcriptome and thereby decide the output host immune defense. Microbiome studies have demonstrated how it affects the immune pathways such as innate immunity, inflammasomes, and the development of regulatory T cells (Zeevi et al., 2016). Research has generated knowledge on how the microbiome as well as microbiome metabolites influence the host immunity, the influence of the maternal microbiome on offspring health, and how therapies can be modulated due to easy manipulation of the microbiome in comparison to the genome (Zeevi et al., 2016).

The type and the load of a particular bacterial species can determine the outcome of a pulmonary disease depending on its effect on host transcript levels (Table 12.3). For instance, in COPD, the percentages of certain transcriptionally active bacterial species are proportionate to the COPD exacerbation rate (Ren et al., 2018). The authors further highlight the importance of understanding the relationship between the COPD exacerbations and the load of bacterial species when designing future therapeutics. In several other studies, it was found that infection of the lung by one microorganism may result in transcriptomic changes that favor co-infections by other microorganisms, as was seen with pneumococcal superinfection during RV-induced asthma (Bedke et al., 2012).

TABLE 12.3

Some Examples of the Crosstalk between Lung Microbiome and Lung Transcriptome

Pulmonary Disease	Differentially Expressed Genes and/or Pathway/Biological process (If Noted)	Microorganism Associated (If Reported Any)	Notes on the Crosstalk

Pulmonary Disease	Differentially Expressed Genes and/or Pathway/Biological process (If Noted)	Microorganism Associated (If Reported Any)	Notes on the Crosstalk
Early-stage lung adenocarcinoma (ES-LUAD)	Transcription factors: DCN, MME, ADH1B, ADRA1A, **FHL1**, **CDO1**, SERPING1, HS6ST2, SGCG, GLB1L2, PRSS22, **GRIA1**, DTX2, RHPN1	*Saccharomyces paradoxus* (An opportunistic pathogen)	Interaction between the fungi and the transcription factors specifically the prognostic genes (in bold), is associated with host survival
COPD	Increased lymphocyte production	*Pseudomonas, Veillonella*	High bacterial load is associated with elevated lymphocyte production increasing the rate of COPD exacerbation
	Activation of the Th17 immune response	*Streptococcus, Rothia*.	Low rate of COPD exacerbation
	Downregulation of miR-122 and miR-130a leads to upregulation of their target IL-17a	NA	microbiome changes during COPD changes the levels of specific miRNA leading to COPD exacerbation
Asthma	Transforming growth factor (TGF)-β	RV, *Streptococcus pneumoniae*	Impair antiviral immunity favoring bacterial co-transfection such as pneumococcal superinfection

Pulmonary Disease	Differentially Expressed Genes and/or Pathway/Biological process (If Noted)	Microorganism Associated (If Reported Any)	Notes on the Crosstalk
Airborne bacterial or viral infection	Host immunity, protein folding and endoplasmic reticulum stress, macrophage function, protein/cytokine response and iron homeostasis	Airborne virus/bacteria	Gene expression pattern shown in response to air pollution in patients who acquired a post-bacterial infection varied with those who acquired a post-viral infection
	Expression of miR-146b and miR-29c, reduced antiviral response	Influenza virus	Some pulmonary miRNA levels shift as a consequence of gut microbiome changes during the use of drugs against influenza virus
$PM_{2.5}$ induced pulmonary fibrosis	Shift of specific miRNA levels results IL-17 and HIF-1 signaling	Airborne virus/bacteria	IL-17 and HIF-1 signaling favors the survival of opportunistic pathogens
COVID-19	Expression of SARS-CoV-2 receptor, ACE2, antiviral response	SARS-CoV-2	Cigarette smoke upregulates the SARS-CoV-2 receptor, ACE2 in a dose-dependent manner, alcohol use reduces host antiviral response

12.5.2 Host Transcriptome Reshapes the Host Microbiome and Host Immunity

In addition to an existing infectious disease, other risk factors for respiratory diseases include those of genetic (such as mutations), environmental (such as air pollution and allergens), behavioral (such as smoking and alcohol consumption), occupational (such as exposure to asbestos and silica), or metabolic (such as obesity) origin. While the link between the above risk factors and the onset of respiratory disease was unknown for some time, transcriptomics has unraveled risk-factor-specific host transcriptional changes that may dysregulate the host immune system, thereby increasing susceptibility to infections and co-infections.

12.5.2.1 Exposure to Air Pollution

Exposure to particulate matter, which are small solid or liquid droplets of anthropogenic origin—increases the risk of acquiring infectious respiratory diseases. This occurs due to the increased load of airborne microorganisms in polluted air, favorable atmospheric conditions presented by polluted air for opportunistic airborne pathogens (Thilakarathne et al. 2021), and the negative impact on the host's innate immune system (Croft et al. 2021). Exposure to air pollutants alters the expression of host genes important in immunity, protein folding, endoplasmic reticulum stress, macrophage function, protein/cytokine response, and iron homeostasis (Bauer et al., 2012; Harrod et al., 2003; Yang et al., 2018). The expression patterns of these pathways also vary with the type of pollutant. Interestingly, the gene expression pattern shown in response to air pollution in patients who acquired a post-bacterial infection varied from those who acquired a post-viral infection (Croft et al. 2021).

12.5.2.2 Smoking and Consumption of Alcohol

Impact of the cigarette smoke on lung transcriptome have been demonstrated to be specific to cell compartments, strains (in murine studies) and the duration of exposure (Siamwala et al., 2023; Faherty et al. 2024). In

macrophages, cigarette smoking downregulates genes involved in immune cell trafficking, phagosome formation, response to pathogens, and histone or lysine methylation, while it upregulates genes involved in oxidative phosphorylation and sirtuin signaling. Furthermore, significant differences in the transcriptomic profiles of airspace macrophages were observed between smokers and non-smokers, highlighting the profound impact of cigarette smoke exposure on macrophage biology (Faherty et al. 2024). Cigarette smoke also upregulates the SARS-CoV-2 receptor, angiotensin converting enzyme 2 (ACE2), in a dose-dependent manner, thus creating a favorable environment within the individual's body for SARS-CoV-2 infection (Smith et al., 2020).

Long-term use of alcohol can dysregulate the immune pathways not just in the liver but also in the lungs, thereby increasing susceptibility to or the exacerbation of infections such as SARS-CoV-2, TB, and pneumonia. This can lead to prolonged healing and higher mortality rates from respiratory diseases (Oord et al., 2022; Trevejo-Nunez et al., 2015). A recent study demonstrated that the lungs are more sensitive to transcriptomic changes upon chronic alcohol usage than the liver, specifically showcasing a low abundance of immune cell populations, a reduced antiviral response (specifically to SARS-CoV-2) and reduced mTOR signaling (Lewis et al., 2023; Pommerolle et al., n.d.). Transcriptomically, male lungs showed increased sensitivity to alcohol usage compared to female lungs (Pommerolle et al., n.d.).

12.5.3 Pulmonary Noncoding RNA-Lung Microbiome Interaction

In addition to mRNAs, noncoding RNAs have also been reported to be involved in regulating the relationship between the host and its microbiome, as has been observed in the gut (Behrouzi et al., 2020) and the intestine (Nakata et al., 2017). Following these footsteps, researchers in the field of pulmonary research have also identified crosstalk between microRNA (miRNA) and the lung microbiome. miRNAs are small noncoding RNAs

which are known for their predominant roles in mRNA repression and translation inhibition. An mRNA may be targeted by more than one miRNA and one miRNA may target more than one mRNA. There are nearly 2800 miRNAs annotated so far, but not all are expressed at all times (Gao et al., 2025). A pathogen or a group of pathogens may find it advantageous to trigger the expression of a specific miRNA, which could lead to the downregulation of mRNAs associated with host defense. Alternatively, the pathogens, may suppress the expression of a specific miRNA, leading to the upregulation of its target mRNA, which—if expressed abnormally—may exacerbate a pathological condition. According to Zhu et al. (2020), as a consequence of microbiome changes during COPD, the two miRNAs miR-122 and miR-130a are downregulated leading to the upregulation of one of their common targets, interleukin 17A (IL-17A). IL-17A is a proinflammatory cytokine generally associated with autoimmune diseases; however, it triggers the expression of other interleukins that can impair lung tissue, worsening the existing pathological condition (Bozinovski and Vlahos, 2015). miRNA levels were also found to be altered in association with microbiota shifts in pollutant-induced respiratory diseases, such as with $PM_{2.5}$ (Jia et al., 2022). Specifically, differentially expressed miRNAs between $PM_{2.5}$-induced pulmonary fibrosis cases and control were found to be enriched in IL-17 and hypoxia-inducible factor-1 (HIF-1) signaling, two pathways which can be modulated in favor of microbial survival (Jia et al., 2022; Kuwabara et al., 2017; Thompson et al., 2017).

12.5.4 Gut–Lung Axis Microbiome and Its Crosstalk with Transcriptome

The human body itself is very complex, and every system communicates to function together as a whole organism. Thus, while it is important to understand what happens in one part of the body (which is commonly done in most of basic research labs), it is also essential to consider the fact that these organs are connected and may influence the outcomes of one another. This chapter mostly covers the extent of the lung microbiome, the lung

transcriptome and their crosstalk. However, one must note that the lung microbiome and/or transcriptome may undergo changes as a result of microbiome shifts in other parts of the body, as is seen in association with the gut-lung axis. The GI tract harbors the highest noted microbial content in the human body, and thus, its effect on connected body organs such as the lungs is unarguably higher.

Transcriptionally regulated networks identified in the oral–gut–lung axis in association with lung and gut cancer are reported to be enriched in the "microbial interaction" biological process (Otálora-Otálora et al., 2024). In 2018, Pang et al. found that the intestinal microbiome shifts in response to antibiotics taken against the influenza virus and that this is linked to a shift in the pulmonary miRNA expression profile which may worsen under antibiotic misuse. They further reported that pulmonary miRNAs—specifically miR-146b and miR-29c—expressed in such a scenario may interrupt host immunity against the virus.

Alternatively, it is also worth noting that pathogens that primarily infect the lung can result in transcriptomic changes in other parts of the body and, thus, be diagnosed using these identified biomarkers. For example, pulmonary TB may result in the differential expression of mRNAs in blood (Berry et al., 2010; Madamarandawala et al., 2023) and sputum (Lai et al., 2021).

12.6 Conclusion

Regardless of whether the causative respiratory pathogen is viral, bacterial, or fungal, infection or colonization of the respiratory tract profoundly disrupts the equilibrium of the resident healthy microbiome. This disturbance leads to an imbalance in the relative proportions of commensal and pathogenic microorganisms within the respiratory microbiota, thereby contributing to the development and progression of pulmonary diseases. The severity of this dysbiosis is further exacerbated by an increased microbial load in the lungs. At the same time, emerging evidence highlights

the protective roles of certain microorganisms against respiratory and systemic diseases. Therefore, transcriptomic studies are essential for gaining a comprehensive understanding of the complex interactions between the respiratory tract microbiome and invading respiratory pathogens.

12.7 Recommendations

Site-specific microbiome and transcriptomic analyses are vital for understanding of the diverse respiratory diseases, accurate diagnosis, and predicting therapeutics.

References

Angelidis, I., Simon, L. M., Fernandez, I. E., Strunz, M., Mayr, C. H., Greiffo, F. R., Tsitsiridis, G., Ansari, M., Graf, E., Strom, T.-M., Nagendran, M., Desai, T., Eickelberg, O., Mann, M., Theis, F. J., & Schiller, H. B. (2019). An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. *Nature Communications*, *10*(1), 963. https://doi.org/10.1038/s41467-019-08831-9 ⏎

Armstrong, D. T., Eisemann, E., & Parrish, N. (2023). A brief update on mycobacterial taxonomy, 2020 to 2022. *Journal of Clinical Microbiology*, *61*(4), e0033122. https://doi.org/10.1128/jcm.00331-22 ⏎

Badger, J., Ng, P., & Venter, J. (2010). The human genome, microbiomes, and disease. In *Metagenomics of the Human Body* Springer, New York, NY (pp. 1–14). https://doi.org/10.1007/978-1-4419-7089-3_1 ⏎

Barron, A. L. (1988). *Microbiology of Chlamydia*. CRC Press. USA. 258. ⏎

Bauer, R. N., Diaz-Sanchez, D., & Jaspers, I. (2012). Effects of air pollutants on innate immunity: The role of Toll-like receptors and nucleotide-binding oligomerization domain–like receptors. *Journal of*

Allergy and Clinical Immunology, *129*(1), 14–24. https://doi.org/10.1016/j.jaci.2011.11.004 ⏎

Bedke, N., Sammut, D., Green, B., Kehagia, V., Dennison, P., Jenkins, G., Tatler, A., Howarth, P. H., Holgate, S. T., & Davies, D. E. (2012). Transforming growth factor-beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response. *PLoS One*, *7*(9), e44580. https://doi.org/10.1371/journal.pone.0044580 ⏎

Behrouzi, A., Ashrafian, F., Mazaheri, H., Lari, A., Nouri, M., Riazi Rad, F., Hoseini Tavassol, Z., & Siadat, S. D. (2020). The importance of interaction between microRNAs and gut microbiota in several pathways. *Microbial Pathogenesis*, *144*, 104200. https://doi.org/10.1016/j.micpath.2020.104200 ⏎

Belizário, J., Garay-Malpartida, M., & Faintuch, J. (2023). Lung microbiome and origins of the respiratory diseases. *Current Research in Immunology*, *4*, 100065. https://doi.org/10.1016/j.crimmu.2023.100065 ⏎

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: Old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0 ⏎

Berry, M. P. R., Graham, C. M., McNab, F. W., Xu, Z., Bloch, S. A. A., Oni, T., Wilkinson, K. A., Banchereau, R., Skinner, J., Wilkinson, R. J., Quinn, C., Blankenship, D., Dhawan, R., Cush, J. J., Mejias, A., Ramilo, O., Kon, O. M., Pascual, V., Banchereau, J., … O'Garra, A. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, *466*(7309), 973–977. https://doi.org/10.1038/nature09247 ⏎

Bisgaard, H., Hermansen, M. N., Buchvald, F., Loland, L., Halkjaer, L. B., Bønnelykke, K., Brasholt, M., Heltberg, A., Vissing, N. H., Thorsen, S. V., Stage, M., & Pipper, C. B. (2007). Childhood asthma after bacterial

colonization of the airway in neonates. *New England Journal of Medicine*, *357*(15), 1487–1495. https://doi.org/10.1056/NEJMoa052632 ⏎

Bozinovski, S., & Vlahos, R. (2015). Multifaceted role for IL-17A in the pathogenesis of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *191*(11), 1213–1214. ⏎

Chalmers, J. D., Chang, A. B., Chotirmall, S. H., Dhar, R., & McShane, P. J. (2018). Bronchiectasis. *Nature Reviews Disease Primers*, *4*(1), 45. ⏎

Chen, K., Han, Y., Wang, Y., Zhou, D., Wu, F., Cai, W., Zheng, S., Xiao, Q., Zhang, H., & Li, W. (2024). scMoresDB: A comprehensive database of single-cell multi-omics data for human respiratory system. *iScience*, *27*(4), 109567. https://doi.org/10.1016/j.isci.2024.109567 ⏎

Chen, M., Lv, J., Zhang, Q., Gong, Q., Zhao, T., Chen, Z., *et al*. (2025). Spatial transcriptomics unveils the blueprint of mammalian lung development. *Genomics Proteomics Bioinformatics*, qzaf053. ⏎

Chiu, Y.-C., Lee, S.-W., Liu, C.-W., Lan, T.-Y., & Wu, L. S.-H. (2022). Relationship between gut microbiota and lung function decline in patients with chronic obstructive pulmonary disease: A 1-year follow-up study. *Respiratory Research*, *23*(1), 10. https://doi.org/10.1186/s12931-022-01928-8 ⏎

Chovanec, P., Bolland, D. J., Matheson, L. S., Wood, A. L., Krueger, F., Andrews, S., & Corcoran, A. E. (2018). Unbiased quantification of immunoglobulin diversity at the DNA level with VDJ-seq. *Nature Protocols*, *13*(6), 1232–1252. https://doi.org/10.1038/nprot.2018.021 ⏎

Coppola, M., Lai, R. P.-J., Wilkinson, R. J., & Ottenhoff, T. H. M. (2021). The in vivo transcriptomic blueprint of *Mycobacterium tuberculosis* in the lung. *Frontiers in Immunology, 12*, 763364. https://doi.org/10.3389/fimmu.2021.763364 ⏎

Croft, D. P., Burton, D. S., Nagel, D. J., Bhattacharya S, Falsey AR, Georas SN, Hopke PK, Johnston CJ, Kottmann RM, Litonjua AA, Mariani TJ, Rich DQ, Thevenet-Morrison K, Thurston SW, Utell MJ, McCall MN.

(2021). The effect of air pollution on the transcriptomics of the immune response to respiratory infection. *Scientific Reports*, *11*, 19436. doi: 10.1038/s41598-021-98729-8 ⏎

Dickson, R. P., Erb-Downward, J. R., & Huffnagle, G. B. (2015). Homeostasis and its disruption in the lung microbiome. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *309*(10), L1047–1055. https://doi.org/10.1152/ajplung.00279.2015 ⏎

Ditz, B., Boekhoudt, J. G., Aliee, H., Theis, F. J., Nawijn, M., Brandsma, C.-A., Hiemstra, P. S., Timens, W., Tew, G. W., Grimbaldeston, M. A., Neighbors, M., Guryev, V., Berge, M. van den, & Faiz, A. (2021). Comparison of genome-wide gene expression profiling by RNA Sequencing versus microarray in bronchial biopsies of COPD patients before and after inhaled corticosteroid treatment: Does it provide new insights? *ERJ Open Research*, *7*(2), 00104-2021. https://doi.org/10.1183/23120541.00104-2021 ⏎

Du, Y., Ouyang, W., Kitzmiller, J. A., Guo, M., Zhao, S., Whitsett, J. A., & Xu, Y. (2021). Lung gene expression analysis web portal version 3: Lung-at-a-glance. *American Journal of Respiratory Cell and Molecular Biology*, *64*(1), 146–149. https://doi.org/10.1165/rcmb.2020-0308LE ⏎

Durkee-Shock, J., Lazarski, C. A., Jensen-Wachspress, M. A., Zhang, A., Son, A., Kankate, V. V., Field, N. E., Webber, K., Lang, H., Conway, S. R., Hanley, P. J., Bollard, C. M., Keller, M. D., & Schwartz, D. M. (2022). Transcriptomic analysis reveals optimal cytokine combinations for SARS-CoV-2-specific T cell therapy products. *Molecular Therapy—Methods & Clinical Development*, *25*, 439–447. https://doi.org/10.1016/j.omtm.2022.04.013 ⏎

Ekanayake, A., Madegedara, D., Chandrasekharan, V., & Magana-Arachchi, D. (2020). Respiratory bacterial microbiota and individual bacterial variability in lung cancer and bronchiectasis patients. *Indian Journal of Microbiology*, *60*(2), 196–205. https://doi.org/10.1007/s12088-019-00850-w ⏎

Enaud, R., Prevel, R., Ciarlo, E., Beaufils, F., Wieërs, G., Guery, B., & Delhaes, L. (2020). The gut–lung axis in health and respiratory diseases: A place for inter-organ and inter-kingdom crosstalks. *Frontiers in Cellular and Infection Microbiology*, *10*, 9. https://doi.org/10.3389/fcimb.2020.00009 ⏎

Espírito Santo, C., Caseiro, C., Martins, M. J., Monteiro, R., & Brandão, I. (2021). Gut microbiota, in the halfway between nutrition and lung function. *Nutrients*, *13*(5), 1716. https://doi.org/10.3390/nu13051716 ⏎

Faherty, L., Zhang, W. Z., Salih, M. M., Robinson, E. K., Perez, E., Kim, K., Carpenter, S., & Cloonan, S. M. (2024). Transcriptomic analysis reveals distinct effects of cigarette smoke on murine airspace and bone-marrow derived macrophages. *Respiratory Research*, *25*(1), 322. ⏎

Fonseca, K. L., Lozano, J. J., Despuig, A., Habgood-Coote, D., Sidorova, J., Aznar, D., Arias, L., Del Río-Álvarez, Á., Carrillo-Reixach, J., Goff, A., Wildner, L. M., Gogishvili, S., Nikolaishvili, K., Shubladze, N., Avaliani, Z., Tapia, G., Rodríguez-Martínez, P., Cardona, P.-J., Martinón-Torres, F., … Vilaplana, C. (2025). Unravelling the transcriptome of the human tuberculosis lesion and its clinical implications. *Nature Communications*, *16*(1), 5028. https://doi.org/10.1038/s41467-025-60255-w ⏎

Franks, T. J., Colby, T. V., Travis, W. D., Tuder, R. M., Reynolds, H. Y., Brody, A. R., Cardoso, W. V., Crystal, R. G., Drake, C. J., Engelhardt, J., Frid, M., Herzog, E., Mason, R., Phan, S. H., Randell, S. H., Rose, M. C., Stevens, T., Serge, J., Sunday, M. E., Voynow, J. A., Weinstein, B. M., Whitsett, J., & Williams, M. C. (2008). Resident cellular components of the human lung: Current knowledge and goals for research on cell phenotyping and function. *Proceedings of the American Thoracic Society*, *5*(7), 763–766. ⏎

Gaddis, N., Fortriede, J., Guo, M., Bardes, E. E., Kouril, M., Tabar, S., Burns, K., Ardini-Poleske, M. E., Loos, S., Schnell, D., Jin, K., Iyer, B., Du, Y., Huo, B.-X., Bhattacharjee, A., Korte, J., Munshi, R., Smith, V., Herbst, A., … Salomonis, N. (2024). LungMAP portal ecosystem:

Systems-level exploration of the lung. *American Journal of Respiratory Cell and Molecular Biology, 70(2), 129*–139. https://doi.org/10.1165/rcmb.2022-0165OC ⏎

Gao, F., Wang, F., Chen, Y., Deng, B., Yang, F., Cao, H., Chen, J., Chen, H., Qi, F., & Kapranov, P. (2025). The human genome encodes a multitude of novel miRNAs. *Nucleic Acids Research*, *53*(4), gkaf070. ⏎

GBD_2021_Booklet_FINAL_2024.05.16.pdf. (n.d.). Retrieved October 28, 2025, from www.healthdata.org/sites/default/files/2024-05/GBD_2021_Booklet_FINAL_2024.05.16.pdf

Gene Ontology Consortium. (2004). The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Research*, *32*(suppl_1), D258–D261. https://doi.org/10.1093/nar/gkh036 ⏎

Harrod, K. S., Jaramillo, R. J., Rosenberger, C. L., Wang, S.-Z., Berger, J. A., McDonald, J. D., & Reed, M. D. (2003). Increased susceptibility to RSV infection by exposure to inhaled diesel engine emissions. *American Journal of Respiratory Cell and Molecular Biology*, *28*(4), 451–463. https://doi.org/10.1165/rcmb.2002-0100OC ⏎

Heo, Y., Kim, J., Hong, S.-H., & Kim, W. J. (2025). Single cell transcriptomics in blood of patients with chronic obstructive pulmonary disease. *BMC Pulmonary Medicine*, *25*, 19. https://doi.org/10.1186/s12890-024-03475-y ⏎

Human Microbiome Market Outlook 2025–2035—Sciinov Conference. (n.d.). Retrieved October 28, 2025, from https://microbiomecongress.org/report/human-microbiome-market-outlook-20252035/ ⏎

Janssens, Y., Nielandt, J., Bronselaer, A., Debunne, N., Verbeke, F., Wynendaele, E., Van Immerseel, F., Vandewynckel, Y.-P., De Tré, G., & De Spiegeleer, B. (2018). Disbiome database: Linking the microbiome to disease. *BMC Microbiology*, *18*(1), 50. https://doi.org/10.1186/s12866-018-1197-5 ⏎

Jia, Q., Li, Q., Wang, Y., Zhao, J., Jiang, Q., Wang, H., Xue, W., Zhu, Z., & Tian, L. (2022). Lung microbiome and transcriptome reveal mechanisms

underlying PM2.5 induced pulmonary fibrosis. *Science of the Total Environment*, *831*, 154974. https://doi.org/10.1016/j.scitotenv.2022.154974 ⏎

Keerthirathne, T. P., Magana-Arachchi, D. N., Madegedara, D., & Sooriyapathirana, S. S. (2016). Real time PCR for the rapid identification and drug susceptibility of Mycobacteria present in Bronchial washings. *BMC Infectious Diseases*, *16*(1), 607. https://doi.org/10.1186/s12879-016-1943-y ⏎

Kim, J., Eo, E.-Y., Kim, B., Lee, H., Kim, J., Koo, B.-K., Kim, H.-J., Cho, S., Kim, J., & Cho, Y.-J. (2024). Transcriptomic analysis of air–liquid interface culture in human lung organoids reveals regulators of epithelial differentiation. *Cells*, *13*(23), 1991. https://doi.org/10.3390/cells13231991 ⏎

Kuwabara, T., Ishikawa, F., Kondo, M., & Kakiuchi, T. (2017). The role of IL-17 and related cytokines in inflammatory autoimmune diseases. *Mediators of Inflammation*, *2017*, 3908061. https://doi.org/10.1155/2017/3908061 ⏎

Lai, R. P. J., Cortes, T., Marais, S., Rockwood, N., Burke, M. L., Garza-Garcia, A., Horswell, S., Sesay, A. K., O'Garra, A., Young, D. B., & Wilkinson, R. J. (2021). Transcriptomic characterization of tuberculous sputum reveals a host Warburg effect and microbial cholesterol catabolism. *mBio*, *12*(6), e0176621. ⏎

Lewis, S. A., Cinco, I. R., Doratt, B. M., Blanton, M. B., Hoagland, C., Newman, N., Davies, M., Grant, K. A., & Messaoudi, I. (2023). Chronic alcohol consumption dysregulates innate immune response to SARS-CoV-2 in the lung. *eBioMedicine*, *97*, 104812. https://doi.org/10.1016/j.ebiom.2023.104812 ⏎

Li, C.-X., Lv, M., Liu, H.-Y., Lin, Y.-X., Pan, J.-B., You, C.-X., & Su, J. (2024). Comparison of the upper and lower airway microbiome in early postoperative lung transplant recipients. *Microbiology Spectrum*, *12*(6), e0379123. https://doi.org/10.1128/spectrum.03791-23 ⏎

Li, J., Chen, C., Li, P., Zhang, X., Liu, X., Wu, W., & Gu, W. (2024). Lung transcriptomics reveals the underlying mechanism by which aerobic training enhances pulmonary function in chronic obstructive pulmonary disease. *BMC Pulmonary Medicine*, *24*(1), 154. https://doi.org/10.1186/s12890-024-02967-1 ⏎

Li, R., Li, J., & Zhou, X. (2024). Lung microbiome: New insights into the pathogenesis of respiratory diseases. *Signal Transduction and Targeted Therapy*, *9*(1), 19. https://doi.org/10.1038/s41392-023-01722-y ⏎

Liao, Z., Jia, P., Li, Y., Zheng, Z., & Zhang, J. (2024). Exploring potential therapeutic targets for small cell lung cancer based on transcriptomics combined with Mendelian randomization analysis. *Frontiers in Immunology*, *15*, 1464259. https://doi.org/10.3389/fimmu.2024.1464259 ⏎

Losada, L., Ghedin, E., Morris, A., Chu, H. W., & Nierman, W. C. (2010). The human lung microbiome. *Metagenomics of the Human Body*, *11*, 117–143. https://doi.org/10.1007/978-1-4419-7089-3_7 ⏎

Ma, P.-J., Wang, M.-M., & Wang, Y. (2022). Gut microbiota: A new insight into lung diseases. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *155*, 113810. https://doi.org/10.1016/j.biopha.2022.113810 ⏎

Ma, S., Jaipalli, S., Larkins-Ford, J., Lohmiller, J., Aldridge, B. B., Sherman, D. R., & Chandrasekaran, S. (2019). Transcriptomic signatures predict regulators of drug synergy and clinical regimen efficacy against tuberculosis. *mBio*, *10*(6), e02627-19. 10.1128/mbio.02627-19. https://doi.org/10.1128/mbio.02627-19 ⏎

Madamarandawala, P., Rajapakse, S., Gunasena, B., Madegedara, D., & Magana-Arachchi, D. (2023). A host blood transcriptional signature differentiates multi-drug/rifampin-resistant tuberculosis (MDR/RR-TB) from drug susceptible tuberculosis: A pilot study. *Molecular Biology Reports*, *50*(4), 3935–3943. https://doi.org/10.1007/s11033-023-08307-6 ⏎

Madissoon, E., Oliver, A. J., Kleshchevnikov, V., Wilbrey-Clark, A., Polanski, K., Richoz, N., Ribeiro Orsi, A., Mamanova, L., Bolt, L., Elmentaite, R., Pett, J. P., Huang, N., Xu, C., He, P., Dabrowska, M., Pritchard, S., Tuck, L., Prigmore, E., Perera, S., … Meyer, K. B. (2023). A spatially resolved atlas of the human lung characterizes a gland-associated immune niche. *Nature Genetics*, *55*(1), 66–77. https://doi.org/10.1038/s41588-022-01243-4 ⏎

Marconett, C. N., Zhou, B., Rieger, M. E., Selamat, S. A., Dubourd, M., Fang, X., Lynch, S. K., Stueve, T. R., Siegmund, K. D., Berman, B. P., Borok, Z., & Laird-Offringa, I. A. (2013). Integrated transcriptomic and epigenomic analysis of primary human lung epithelial cell differentiation. *PLoS Genetics*, *9*(6), e1003513. ⏎

Martins, L. R., Sieverling, L., Michelhans, M., Schiller, C., Erkut, C., Grünewald, T. G. P., Triana, S., Fröhling, S., Velten, L., Glimm, H., & Scholl, C. (2024). Single-cell division tracing and transcriptomics reveal cell types and differentiation paths in the regenerating lung. *Nature Communications*, *15*(1), 2246. https://doi.org/10.1038/s41467-024-46469-4 ⏎

mBodyMap: A curated database for microbes across human body and their associations with health and diseases | Nucleic Acids Research | Oxford Academic. (n.d.). Retrieved October 28, 2025, from https://academic.oup.com/nar/article/50/D1/D808/6413603

Michael, O. S., Adetunji, J. B., Badmus, O. O., Areola, E. D., Oshinjo, A. M., Adetunji, C. O., & Oyewole, O. A. (2024). Chapter 5—COVID-19 and microbiome. In C. O. Adetunji, O. S. Michael, N. Esiobu, & R. E. Aluko (Eds.), *An Introduction to the Microbiome in Health and Diseases* (pp. 71–95). Academic Press, UK. https://doi.org/10.1016/B978-0-323-91190-0.00005-9 ⏎

MicrobiomeDB: A systems biology platform for integrating, mining and analyzing microbiome experiments | Nucleic Acids Research | Oxford Academic. (n.d.). Retrieved October 28, 2025, from https://academic.oup.com/nar/article/46/D1/D684/4584629 ⏎

Miko, B. A., Pereira, M. R., & Safdar, A. (2019). Respiratory tract infections: Sinusitis, bronchitis, and pneumonia. In A. Safdar (Ed.), *Principles and Practice of Transplant Infectious Diseases* (pp. 339–349). Springer. USA. https://doi.org/10.1007/978-1-4939-9034-4_20 ⏎

Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birket, S. E., Yuan, F., Chen, S., Leung, H. M., Villoria, J., Rogel, N., Burgin, G., Tsankov, A. M., Waghray, A., Slyper, M., Waldman, J., Nguyen, L., Dionne, D., Rozenblatt-Rosen, O., Tata, P. R., Mou, H., Shivaraju, M., Bihler, H., Mense, M., Tearney, G. J., Rowe, S. M., Engelhardt, J. F., Regev, A., & Rajagopal, J. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature*, *560*(7718), 319–324. ⏎

Nakata, K., Sugi, Y., Narabayashi, H., Kobayakawa, T., Nakanishi, Y., Tsuda, M., Hosono, A., Kaminogawa, S., Hanazawa, S., & Takahashi, K. (2017). Commensal microbiota-induced microRNA modulates intestinal epithelial permeability through the small GTPase ARF4. *Journal of Biological Chemistry*, *292*(37), 15426–15433. https://doi.org/10.1074/jbc.M117.788596 ⏎

Neumark, N., Cosme, C., Rose, K.-A., & Kaminski, N. (2020). The idiopathic pulmonary fibrosis cell atlas. *American Journal of Physiology—Lung Cellular and Molecular Physiology*, *319*(6), L887–L892. https://doi.org/10.1152/ajplung.00451.2020 ⏎

Nieves, D.J. and Heininger, U. (2016). Bordetella pertussis. In *Emerging Infections 10* (eds W.M. Scheld, J.M. Hughes and R.J. Whitley). (pp. 311–339). John Wiley & Sons, Ltd., UK. https://doi.org/10.1128/9781555819453.ch17 ⏎

Oord, E. J. C. G. van den, Xie, L. Y., Zhao, M., Aberg, K. A., & Clark, S. L. (2022). A single-nucleus transcriptomics study of alcohol use disorder in the nucleus accumbens. *medRxiv*, 2022.06.16.22272431. https://doi.org/10.1101/2022.06.16.22272431 ⏎

Otálora-Otálora, B. A., Payán-Gómez, C., López-Rivera, J. J., Pedroza-Aconcha, N. B., Aristizábal-Guzmán, C., Isaza-Ruget, M. A., & Álvarez-Moreno, C. A. (2024). Global transcriptomic network analysis of the

crosstalk between microbiota and cancer-related cells in the oral–gut–lung axis. *Frontiers in Cellular and Infection Microbiology*, *14*, 1425388. https://doi.org/10.3389/fcimb.2024.1425388 ⏎

Pang, P., Yu, B., Shi, Y., Deng, L., Xu, H., Wu, S., & Chen, X. (2018). Alteration of intestinal flora stimulates pulmonary microRNAs to interfere with host antiviral immunity in influenza. *Molecules*, *23*(12), 3151. https://doi.org/10.3390/molecules23123151 ⏎

Perez-Medina, M., Lopez-Gonzalez, J. S., Benito-Lopez, J. J., Ávila-Ríos, S., Soto-Nava, M., Matias-Florentino, M., Méndez-Tenorio, A., Galicia-Velasco, M., Chavez-Dominguez, R., Meza-Toledo, S. E., & Aguilar-Cazares, D. (2024). Transcriptomic analysis reveals early alterations associated with intrinsic resistance to targeted therapy in lung adenocarcinoma cell lines. *Cancers*, *16*(13), 2490. https://doi.org/10.3390/cancers16132490 ⏎

Pommerolle, L., Arif, M., Behee, M., Appolonia, C. N., Basu, A., Wolf, K. M., Zawatsky, C. N., Johnson, N., Rivellini, O., Park, J. K., & Cinar, R. (n.d.). Chronic alcohol intake compromises lung immunity by altering immunometabolism in humans and mouse models. *American Journal of Respiratory Cell and Molecular Biology*, *71*(5), 559–576. https://doi.org/10.1165/rcmb.2024-0086OC ⏎

Qiu, X., Zhong, P., Yue, L., Li, C., Yun, Z., Si, G., Li, M., Chen, Z., Tan, Y., & Bao, P. (2024). Spatial transcriptomic sequencing reveals immune microenvironment features of *Mycobacterium tuberculosis* granulomas in lung and omentum. *Theranostics*, *14*(16), 6185–6201. https://doi.org/10.7150/thno.99038 ⏎

Rada, B. (2017). Interactions between neutrophils and *Pseudomonas aeruginosa* in cystic fibrosis. *Pathogens*, *6*(1), 10. https://doi.org/10.3390/pathogens6010010 ⏎

Ren, L., Zhang, R., Rao, J., Xiao, Y., Zhang, Z., Yang, B., Cao, D., Zhong, H., Ning, P., Shang, Y., Li, M., Gao, Z., & Wang, J. (2018). Transcriptionally active lung microbiome and its association with

bacterial biomass and host inflammatory status. *mSystems*, *3*(5), e00199-18. https://doi.org/10.1128/mSystems.00199-18 ⏎

Sauler, M., McDonough, J. E., Adams, T. S., Kothapalli, N., Barnthaler, T., Werder, R. B., Schupp, J. C., Nouws, J., Robertson, M. J., Coarfa, C., Yang, T., Chioccioli, M., Omote, N., Cosme, C., Poli, S., Ayaub, E. A., Chu, S. G., Jensen, K. H., Gomez, J. L., … Rosas, I. O. (2022). Characterization of the COPD alveolar niche using single-cell RNA sequencing. *Nature Communications*, *13*(1), 494. https://doi.org/10.1038/s41467-022-28062-9 ⏎

Schupp, J. C., Adams, T. S., Cosme, C., Raredon, M. S. B., Yuan, Y., Omote, N., Poli, S., Chioccioli, M., Rose, K.-A., Manning, E. P., Sauler, M., DeIuliis, G., Ahangari, F., Neumark, N., Habermann, A. C., Gutierrez, A. J., Bui, L. T., Lafyatis, R., Pierce, R. W., … Kaminski, N. (2021). Integrated single-cell atlas of endothelial cells of the human lung. *Circulation*, *144*(4), 286–302. https://doi.org/10.1161/CIRCULATIONAHA.120.052318 ⏎

Siamwala, J. H., Mossman, J. A., Schorl, C., Borgas, D., Sakhatskyy, P., Rand, D. M., Lu, Q., & Rounds, S. (2023). Strain-dependent lung transcriptomic differences in cigarette smoke and LPS models of lung injury in mice. *Physiological Genomics*, *55*(6), 259–274. https://doi.org/10.1152/physiolgenomics.00152.2022 ⏎

Sikkema, L., Ramírez-Suástegui, C., Strobl, D. C., Gillett, T. E., Zappia, L., Madissoon, E., Markov, N. S., Zaragosi, L.-E., Ji, Y., Ansari, M., Arguel, M.-J., Apperloo, L., Banchero, M., Bécavin, C., Berg, M., Chichelnitskiy, E., Chung, M., Collin, A., Gay, A. C. A., … Theis, F. J. (2023). An integrated cell atlas of the lung in health and disease. *Nature Medicine*, *29*(6), 1563–1577. https://doi.org/10.1038/s41591-023-02327-2 ⏎

Singer, E., Wagner, M., & Woyke, T. (2017). Capturing the genetic makeup of the active microbiome in situ. *ISME Journal*, *11*(9), 1949–1963. https://doi.org/10.1038/ismej.2017.59 ⏎

Smith, J. C., Sausville, E. L., Girish, V., Yuan, M. L., Vasudevan, A., John, K. M., & Sheltzer, J. M. (2020). Cigarette smoke exposure and inflammatory signaling increase the expression of the SARS-CoV-2 receptor ACE2 in the respiratory tract. *Developmental Cell*, *53*(5), 514–529.e3. https://doi.org/10.1016/j.devcel.2020.05.012 ⏎

Sun, G., Li, K., Ping, J., Zhao, L., Cui, C., Wu, J., Xie, L., Yao, X., Xu, G., Ma, S., Fan, Y., Wang, Q., Yang, D., Luo, B., Liu, H., Yang, J., Zhang, W., Song, W., Zhao, G., … Liu, G.-H. (2025). A single-cell transcriptomic atlas reveals senescence and inflammation in the post-tuberculosis human lung. *Nature Microbiology*, *10*(8), 2073–2091. https://doi.org/10.1038/s41564-025-02050-3 ⏎

Sun, G., & Zhou, Y.-H. (2025). Identifying novel therapeutic targets in cystic fibrosis through advanced single-cell transcriptomics analysis. *Computers in Biology and Medicine*, *187*, 109748. https://doi.org/10.1016/j.compbiomed.2025.109748 ⏎

Thilakarathne, S. M. N. K., Ekanayake, A., Madamarandawala, P. S., *et al*. (2021). Impact of haze events on airborne bacterial consortia—A case study. *SN Applied Sciences*, *3*, 14. ⏎

Thompson, A. A. R., Dickinson, R. S., Murphy, F., Thomson, J. P., Marriott, H. M., Tavares, A., Willson, J., Williams, L., Lewis, A., Mirchandani, A., Dos Santos Coelho, P., Doherty, C., Ryan, E., Watts, E., Morton, N. M., Forbes, S., Stimson, R. H., Hameed, A. G., Arnold, N., … Walmsley, S. R. (2017). Hypoxia determines survival outcomes of bacterial infection through HIF-1alpha dependent re-programming of leukocyte metabolism. *Science Immunology*, *2*(8), eaal2861. https://doi.org/10.1126/sciimmunol.aal2861 ⏎

Travaglini, K. J., Nabhan, A. N., Penland, L., Sinha, R., Gillich, A., Sit, R. V., Chang, S., Conley, S. D., Mori, Y., Seita, J., Berry, G. J., Shrager, J. B., Metzger, R. J., Kuo, C. S., Neff, N., Weissman, I. L., Quake, S. R., & Krasnow, M. A. (2020). A molecular cell atlas of the human lung from single cell RNA sequencing. *bioRxiv*, 742320. https://doi.org/10.1101/742320 ⏎

Trevejo-Nunez, G., Kolls, J. K., & de Wit, M. (2015). Alcohol use as a risk factor in infections and healing. *Alcohol Research: Current Reviews*, *37*(2), 177–184. ⏎

Waites, K. B., & Atkinson, T. P. (2009). The role of Mycoplasma in upper respiratory infections. *Current Infectious Disease Reports*, *11*(3), 198–206. ⏎

Wang, L., Ma, H., Wen, Z., Niu, L., Chen, X., Liu, H., Zhang, S., Xu, J., Zhu, Y., Li, H., Chen, H., Shi, L., Wan, L., Li, L., Li, M., Wong, K.-W., & Song, Y. (2023). Single-cell RNA-sequencing reveals heterogeneity and intercellular crosstalk in human tuberculosis lung. *Journal of Infection*, *87*(5), 373–384. https://doi.org/10.1016/j.jinf.2023.09.004 ⏎

Wang, Y., Shen, J., & Huang, J. (2021). Identification of prognostically relevant gene signatures in lung squamous cell carcinoma by Lung Cancer Explorer. *Translational Cancer Research*, *10*(5), 2009–2022. https://doi.org/10.21037/tcr-21-222 ⏎

Westermann, A. J., Barquist, L., & Vogel, J. (2017). Resolving host–pathogen interactions by dual RNA-seq. *PLoS Pathogens*, *13*(2), e1006033. https://doi.org/10.1371/journal.ppat.1006033 ⏎

Williams, C. G., Lee, H. J., Asatsuma, T., Vento-Tormo, R., & Haque, A. (2022). An introduction to spatial transcriptomics for biomedical research. *Genome Medicine*, *14*(1), 68. ⏎

Wu, F., Cai, W., Tang, H., Zheng, S., Zhang, H., Chen, Y., Han, Y., Zhou, D., Wang, R., Ye, M., You, R., Chen, A., Li, J., Zhang, X., & Li, W. (2024). A universal single-cell transcriptomics atlas of human lung decodes multiple pulmonary diseases. *bioRxiv*, 2024.12.17.628654. https://doi.org/10.1101/2024.12.17.628654 ⏎

Wu, X., Bos, I. S. T., Conlon, T. M., Ansari, M., Verschut, V., van der Koog, L., Verkleij, L. A., D'Ambrosi, A., Matveyenko, A., Schiller, H. B., Königshoff, M., Schmidt, M., Kistemaker, L. E. M., Yildirim, A. Ö., & Gosens, R. (2022). A transcriptomics-guided drug target discovery strategy identifies receptor ligands for lung regeneration. *Science Advances*, *8*(12), eabj9949. https://doi.org/10.1126/sciadv.abj9949 ⏎

Yan, Q., Zhang, X., Xie, Y., Yang, J., Liu, C., Zhang, M., Zheng, W., Lin, X., Huang, H., Liu, X., Jiang, Y., Zhan, S., & Huang, X. (2024). Bronchial epithelial transcriptomics and experimental validation reveal asthma severity-related neutrophilc signatures and potential treatments. *Communications Biology*, *7*(1), 181. https://doi.org/10.1038/s42003-024-05837-y ⏎

Yang, J., Chen, Y., Yu, Z., Ding, H., & Ma, Z. (2018). Changes in gene expression in lungs of mice exposed to traffic-related air pollution. *Molecular and Cellular Probes*, *39*, 33–40. https://doi.org/10.1016/j.mcp.2018.03.005 ⏎

Yu, G., Gail, M. H., Consonni, D., Carugno, M., Humphrys, M., Pesatori, A. C., Caporaso, N. E., Goedert, J. J., Ravel, J., & Landi, M. T. (2016). Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. *Genome Biology*, *17*(1), 163. https://doi.org/10.1186/s13059-016-1021-1 ⏎

Zeevi, D., Korem, T., & Segal, E. (2016). Talking about cross-talk: The immune system and the microbiome. *Genome Biology*, *17*(1), 50. https://doi.org/10.1186/s13059-016-0921-4 ⏎

Zhang, J., Zhang, H., Zhang, L., Li, D., Qi, M., Zhang, L., Yu, H., Wang, D., Jiang, G., Wang, X., Zhu, X., & Zhang, P. (2022). Single-cell transcriptome identifies drug-resistance signature and immunosuppressive microenvironment in metastatic small cell lung cancer. *Advanced Genetics*, *3*(2), 2100060. https://doi.org/10.1002/ggn2.202100060 ⏎

Zhang, W., Ke, Y., Liu, X., Jin, M., & Huang, G. (2022). Drug resistance in NSCLC is associated with tumor micro-environment. *Reproductive Biology*, *22*(3), 100680. https://doi.org/10.1016/j.repbio.2022.100680 ⏎

Zhu, K., Zhou, S., Xu, A., Sun, L., Li, M., Jiang, H., Zhang, B., Zeng, D., Fei, G., & Wang, R. (2020). Microbiota imbalance contributes to COPD deterioration by enhancing IL-17a production via miR-122 and miR-30a. *Molecular Therapy. Nucleic Acids*, *22*, 520–529. https://doi.org/10.1016/j.omtn.2020.09.017 ⏎

13

The Role of the Microbiome in Psychiatric Disorders

Lucas C. Pedro, Maria Fernanda Ebs, Gabriel S. Mondo, Jessica L. Fernandes, and Gislaine Z. Réus

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13.1 Introduction

Topics such as non-communicable chronic diseases (NCDs) are widely discussed within scientific health research settings. A quick search for terms like "diabetes mellitus" or "chronic obstructive pulmonary disease" on PubMed can easily yield over one million results in scientific publications. However, data released by the World Health Organization (WHO, 2022a) have increasingly drawn scientific attention to a particular field of inquiry: psychiatric disorders. According to this data, an estimated one billion people are currently living with some form of mental disorder. Among them, approximately 301 million suffer from anxiety disorders, while 280 million live with depressive disorders.

In this context, psychiatric disorders have taken a prominent place in discussions about integrative healthcare and translational research.

Scientists continue to strive for a better understanding of the pathophysiology, treatment strategies, diagnostic criteria, and preventive approaches for these conditions. That said, defining what constitutes a mental disorder can be challenging due to its complex etiologies, poorly delineated disease trajectories, and highly individualized patient histories.

In general terms, the American Psychiatric Association (APA) defines mental disorders as health conditions characterized by changes in emotions, behavior, cognition, and thought processes, with or without impairment in social, occupational, or familial functioning (American Psychiatric Association, 2022). The most common psychiatric disorders often share similar symptoms, such as drastic mood changes and difficulties in daily functioning, yet they also exhibit distinctive features. In Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), the most popular manual for psychiatric disorders diagnosis, organized by the APA, major depressive disorder (MDD), commonly referred to as depression, is marked by anhedonia, hopelessness, sadness, irritability, and excessive guilt. Additional symptoms, such as sleep disturbances, are also frequently observed. In anxiety disorders, including generalized anxiety disorder (GAD) and post-traumatic stress disorder, symptoms tend to revolve around excessive worry about the future, racing and obsessive thoughts, and cognitive disruption, often leading to functional impairment. Two other primary psychiatric conditions are bipolar disorder and schizophrenia. In bipolar disorder, individuals experience extreme mood swings, alternating between depressive episodes and manic phases characterized by heightened irritability, excessive energy, impulsivity, and hypersexuality. Schizophrenia, on the other hand, involves a set of complex and severe symptoms such as auditory and visual hallucinations, persecutory delusions, disorganized thinking, and intense agitation.

It is also important to note that the diagnosis of these disorders is almost exclusively clinical, due to the lack of reliable biochemical tests capable of identifying or aiding in psychiatric assessments. Therefore, patient care is typically based on a combination of psychotherapy and pharmacotherapy, with additional interventions such as electroconvulsive therapy (ECT) or

deep brain stimulation (DBS) being recommended in cases where treatment resistance is evident (APA, 2022; Asir et al., 2024).

Understanding the neurobiology of psychiatric disorders may contribute to the development of better treatments and more accurate diagnostic tools, ultimately enhancing therapeutic outcomes and improving patient quality of life. However, scientific research continues to face significant obstacles in this area, ranging from ethical and methodological limitations in human studies to the inherent complexity of the brain itself. When faced with such challenges, science often benefits from taking a step back and reassessing its frameworks. Recently, attention has shifted to an unexpected yet promising area of research in psychiatry: the human microbiota.

Defined by Marchesi and Ravel (2015) as a dense community of microscopic organisms, primarily bacteria, living in symbiosis within restricted anatomical niches, the human microbiota is increasingly recognized for its systemic impact on health. With this perspective, researchers, such as Verma et al. (2024), in their study of the gut–brain axis in mental health, have begun exploring the potential links between microbiota composition and psychiatric disorders. Among the various microbiota sites in the human body, three stand out for their microbial density and correlation with health and homeostasis: the pulmonary, oral, and, perhaps most significantly, the gut microbiota.

The pulmonary microbiota consists not only of bacteria but also viruses and fungi. Although research in this field is relatively recent, studies by Li et al. (2024) have revealed strong associations between microbial imbalances and the progression of diseases such as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis, indicating that dysbiosis in the lung microbiota may influence symptoms, disease course, and prognosis.

Moving anatomically upward, the oropharyngeal microbiota, which is home to over 700 species (according to the Human Oral Microbiome Database), affects host physiology in various ways, including the modulation of salivary enzymes (such as amylase) and even influencing taste perception. Furthermore, evidence from studies like that of Baker et al.

(2024) highlights correlations between systemic diseases and dysbiosis in the oral microbiota. For instance, periodontitis-causing pathogens can enter the bloodstream, triggering heightened inflammatory and immune responses.

Finally, the star of microbiota research, the gut microbiota, plays a key role in regulating intestinal function and has garnered particular interest among neuroscientists studying psychiatry and human behavior. This growing fascination stems from the close anatomical and functional connection between the brain and the gut, a relationship now conceptualized as the gut–brain axis, wherein a complex microbial ecosystem interacts bidirectionally with neural and immune pathways. Studies such as those by Yang et al. (2023) demonstrate that rats exposed to experimental models of gut dysbiosis exhibit psychiatric symptoms and systemic inflammation. Clinical and theoretical evidence highlights that modulating the gut microbiota can regulate depressive and anxious behaviors, as comprehensively reviewed by Nikolova et al. (2021). Furthermore, Macrea et al. (2023), highlighted the therapeutic potential of prebiotic, probiotic, and postbiotic interventions in depressive disorders, observing that traditional antidepressant medications do not appear to interfere negatively with the mechanisms of these biotic substances.Despite mounting evidence and growing scientific interest, there remains a need for deeper exploration into the underlying mechanisms of microbiota–systemic interactions, especially in relation to psychiatric disorders. Could these microscopic organisms living inside us hold answers to longstanding questions in mental health? Might they represent a promising pathway for intervention?

13.2 Psychiatric Disorders

According to the WHO, genuine mental health extends beyond the mere absence of psychiatric disorders. In a broader sense, mental well-being enables individuals to cope with challenges, build healthy relationships, and

perform more effectively in both work and life overall (WHO, 2022a). In 2022, the most comprehensive global survey on mental health conducted to date underscored the urgency of addressing this topic. The findings revealed that, by 2019, approximately 970 million people worldwide were living with some form of mental disorder. The highest prevalence rates were associated with anxiety and depressive disorders, affecting 301 million and 280 million individuals, respectively. The same report identified depression as the second leading cause of disability worldwide, while anxiety ranked sixth (World Health Organization, 2022b). Additionally, 40 million people live with bipolar disorder and 24 million with schizophrenia (World Health Organization, 2022a).

It is essential to highlight that mental illness often causes significant impairments in patients' lives. Surprisingly, the damage can be equal to or even greater than that caused by physical health conditions. Among individuals with psychiatric disorders under outpatient care, 71% experienced severely compromised quality of life, and 60% reported severe impairment in functioning (IsHak et al., 2024). Furthermore, psychiatric conditions also increase the risk of physical illnesses, prolonged hospitalization, and mortality (Richmond-Rakerd et al., 2021).

Multiple factors may contribute to the development of mental disorders, including traumatic life experiences, poverty, limited access to education, and poor living and working conditions. The environment in which an individual is situated can make them susceptible to mental illness, depending on the context (Alegría et al., 2023; Morrison & Frank, 2023). Social connections are also widely recognized as essential to mental health. In addition to being an intrinsic human need for survival and overall health, they promote increased resilience, personal satisfaction, well-being, and even life expectancy (Holt-Lunstad, 2024). Conversely, experiences of discrimination or social rejection can negatively impact self-esteem and perceived self-worth, thereby increasing the risk of developing mental disorders (Gnanapragasam et al., 2023). Genetic factors may also play a role. Emerging research suggests that genetic variants, even those with seemingly minimal effects, can collectively increase genetic vulnerability.

Moreover, a single gene may be associated with multiple mental disorders (Andreassen et al., 2023; Mallard et al., 2023; Morneau-Vaillancourt et al., 2025).

In addition, it is essential not to overlook the stigma that surrounds psychiatric disorders. Frequently, fear of criticism pushes the search for treatment into the background. Patients themselves may internalize narratives and adopt imposed labels, leading to a distorted self-perception (Fox & Earnshaw, 2023). At this point, the role of healthcare professionals and research in the field becomes essential, not only to demystify the topic but also to facilitate access to information and clarify the pathways toward mental health care (Subramaniam, 2024).

13.2.1 Anxiety Disorders

Physiologically, anxiety can be considered hyperexcitability of fear circuits (Rosen & Schulkin, 2022). Indeed, the recruitment of the central nervous system, the upregulation of adrenaline/noradrenaline, and the subsequent tachycardia, sweating, and alterations in focus and memory facilitate an instinctive self-protective reaction in real danger situations (Hamm, 2020). Likewise, the amygdala plays a role in detecting threats and modulating the physiological response to them (Šimić et al., 2021). However, prolonged or permanent hypersensitivity to fear promotes heightened vigilance and exaggerated reactivity to seemingly threatening stimuli. As a result, the amygdala remains chronically activated, and the inhibitory capacity of the hippocampus and prefrontal cortex progressively decreases (Rosen & Schulkin, 2022). Consequently, an overload of the hypothalamic–pituitary–adrenal axis (HPA) and pro-inflammatory cytokines will disrupt the body's homeostatic balance (Santamaría-García et al., 2025). This process is deleterious to the organism and may increase the risk of anxiety disorders (Abend, 2023).

Regarding clinical diagnosis, the main symptoms of pathological anxiety include excessive fear and anxiety, muscle tension, reduced concentration and memory, fatigue, irritability, shortness of breath, dizziness, and sleep

disturbances. The intensity, duration, and resulting impairments are decisive in determining the severity of the condition (APA, 2022). Anxiety disorders, however, are classified into subtypes. The main ones are GAD, panic disorder, social anxiety disorder, specific phobia, and agoraphobia. It is important to note that obsessive-compulsive disorder and post-traumatic stress disorder are now classified in separate categories (O'Leary & Khan, 2024). Anxiety subtypes are differentiated primarily by their core characteristics. GAD involves excessive and recurrent worry about various topics. Social anxiety disorder is marked by excessive fear of exposure, judgment, and criticism in social contexts (Szuhany & Simon, 2022). Specific phobia refers to an intense, persistent, and irrational fear directed toward a particular situation, such as flying or driving. Panic disorder is characterized by sudden, unexpected episodes of intense anxiety, without apparent cause, accompanied by severe physiological fear responses or a sense of impending death. Agoraphobia is defined as intense fear or avoidance of situations where escape may be intricate or help unavailable during a crisis (APA, 2022).

Generally, treatment involves combining psychotherapy and pharmacotherapy, particularly in cases of moderate to severe severity (Stephenson et al., 2023). First-line medications include serotonin–norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs), due to their efficacy and low incidence of side effects (Szuhany & Simon, 2022). Effective options include escitalopram, duloxetine, venlafaxine, and pregabalin (Fagan & Baldwin, 2023), the latter being a potential alternative when traditional antidepressants result in intolerance or lack of efficacy (Cardoner et al., 2025). In therapeutic terms, cognitive behavioral therapy (CBT) is the most widely used approach (Papola et al., 2024). This method aims to develop anxiety management skills while reshaping behaviors. Restructuring anxious thoughts and beliefs (Stephenson et al., 2023). The effects of psychotherapy often persist for up to one year after treatment completion (Flückiger et al., 2022; van Dis et al., 2020). It is worth mentioning that mindfulness-based practices produce positive outcomes. One study found responses comparable to those

achieved with escitalopram use (Hoge et al., 2023). Furthermore, standardized protocols employing neurofeedback have also significantly reduced anxiety symptoms, underscoring the importance of alternative treatments (Lotfinia et al., 2025).

13.2.2 Major Depressive Disorder

MDD is a complex disorder characterized by a wide range of symptoms. The primary features include depressed mood and loss of interest in daily activities. Common symptoms also include sleep disturbances, fatigue, difficulty concentrating, significant weight loss or gain, psychomotor and appetite changes (APA, 2022), functional impairment, low energy, irritability (Martin-Key et al., 2021), reduced libido, and menstrual irregularities (Wu et al., 2024). In more severe cases, there is a risk of suicidal thoughts, planning, or attempts (Olgiati et al., 2023). The core diagnostic criteria include the persistence of symptoms for at least two weeks, the mandatory presence of depressed mood or loss of interest, a minimum of five concurrent symptoms, and significant impairment in the individual's life (APA, 2022).

Currently, it is considered that dysregulation of the brain's monoaminergic system leads to depressive symptoms (Akil & Nestler, 2023). In this context, an imbalance in dopamine (responsible for pleasure and reward), serotonin (associated with relaxation and sleep), and norepinephrine (involved in the fight-or-flight response) is regarded as the basis of this disorder (Jiang et al., 2022). However, elevated levels of pro-inflammatory cytokines, chemokines, acute-phase proteins (Pastis et al., 2024), TNF-α, and IL-17 are also associated with depressive symptoms (Tsuboi et al., 2024). In addition, the relationships between genetic factors, stress exposure, inflammation, and depression have been increasingly investigated in depth (Gabbay et al., 2024; Gal et al., 2023).

The multifactorial nature of depression underscores the complexity and challenges inherent in its treatment. Therapeutic approaches comprise a range of modalities, including psychotherapy, pharmacotherapy, ECT

(Karrouri et al., 2021), DBS, and transcranial magnetic stimulation (Akil & Nestler, 2023). Despite these diverse options, first-line treatments globally continue to be SSRIs, SNRIs, tricyclic antidepressants, and monoamine oxidase inhibitors (Gao et al., 2025).

However, standard treatments prove insufficient in some instances, necessitating alternative therapeutic strategies. Ketamine has emerged as a promising option, demonstrating a rapid onset of action and sustained efficacy in alleviating depressive symptoms (Kim et al., 2024). DBS has also shown favorable outcomes; a five-year cohort study reported significant and enduring improvements in depressive symptoms for a group of patients (Aibar-Durán et al., 2022). Another investigation documented a 47% long-term reduction in depression scale scores following DBS treatment (Reddy et al., 2024). ECT remains a fundamental intervention, frequently associated with symptom remission and rapid response in cases of treatment-resistant or severe depression (Hsieh, 2023). The same applies to transcranial magnetic stimulation, which has been demonstrated to be effective when administered according to validated protocols (Chen et al., 2025a).

It is essential to highlight that depression stands as the leading cause of suicide worldwide (Kim et al., 2024). Preventive measures include training in problem-solving skills, promoting resilience, enhancing social connectedness, and identifying, supporting, and protecting individuals at risk. Additionally, facilitating access to healthcare services and reducing the stigma associated with seeking help are critical components of prevention efforts (Henry, 2021). Among these strategies, safety planning has demonstrated efficacy in reducing suicidal ideation or behaviors, alleviating depression severity and hopelessness, and lowering hospitalization rates. Moreover, this approach can be adapted to diverse clinical and cultural contexts (Ferguson et al., 2022).

13.2.3 Bipolar Disorder

Bipolar disorder is characterized by distinct episodes of significant mood disturbance, ranging from mania to hypomania, and often including depressive episodes (APA, 2022). Manic episodes are considered psychiatric emergencies, frequently necessitating hospitalization to manage severe agitation and prevent potential harm to the patient (Ankit Jain & Paroma Mitra, 2025). The manic phase is marked by inflated self-esteem, reduced need for sleep, accelerated thought and speech, psychomotor agitation, and an urgent need to remain active, often manifesting in reckless or risky behaviors such as excessive spending or sexual risk-taking. In contrast, hypomania represents a milder manifestation of manic symptoms, typically causing less functional impairment and not requiring hospitalization (APA, 2022).

Multiple factors are thought to contribute to the development of bipolar disorder, including genetic, environmental, inflammatory, and endocrinological influences (Oliva et al., 2025). A large-scale study demonstrated its polygenic nature, with each genetic variant exerting a small effect that, in combination, substantially increases the likelihood of developing the disorder. The same study reported stronger genetic correlations between bipolar disorder type I and schizophrenia, whereas type II showed greater genetic proximity to depression (Mullins et al., 2021).

Bipolar disorder is associated with limbic hyperactivity, suggesting emotional dysregulation. In stressful situations, exaggerated connectivity between the prefrontal cortex and amygdala is observed, unlike in healthy individuals (Kirsch et al., 2022). Neurotransmitter function, including that of dopamine, serotonin, and noradrenaline, is impaired (Nikolaus et al., 2017). Manic patients show reduced dopamine reuptake, with severity of symptoms correlating with increased dopaminergic hyperactivity (Yatham et al., 2022). During depressive episodes, lower plasma dopamine β-hydroxylase activity, which converts dopamine to noradrenaline, is linked to more severe symptoms, indicating dopaminergic and noradrenergic dysfunction (Sun et al., 2021). Additionally, lower serotonin levels predict

better lithium response, suggesting that medication-induced serotonin stabilization mitigates symptom severity (Ananth et al., 2020).

This condition is diagnostically classified into distinct categories. Type I is defined by the presence of at least one manic episode lasting a minimum of seven days or exhibiting sufficient severity to justify hospitalization. Although depressive episodes are common within this subtype, they are not essential for diagnosis. Conversely, bipolar disorder type II is characterized by recurrent major depressive episodes alternating with periods of hypomania lasting at least four days (Greenwood, 2020). Differential diagnosis is essential, particularly regarding depression. Bipolar disorder often begins with depressive episodes, and failure to distinguish between these conditions can result in delayed or incorrect diagnosis (Scott et al., 2022), thereby increasing the risk of suicide, treatment resistance, and negative impacts on the patient's life.

Unfortunately, bipolar disorder leads to diverse functional impairments that vary with mood state. Depressive episodes impair relationships and occupational performance, whereas manic or hypomanic symptoms often disrupt financial management and impulse control. Still, autonomy deficits persist across all phases (Grunze & Born, 2020; Strejilevich et al., 2019). Even in remission, patients may experience difficulties in autonomy, cognition, and work performance (Pereira et al., 2025), with attentional, executive, and processing functions frequently compromised, possibly due to reduced cortical thickness (Little et al., 2024). The quality of life and overall health remain lower in both symptomatic and remitted patients compared to healthy individuals (Rhee et al., 2023).

In this regard, the faster and more efficient the diagnosis and treatment, the better the prognosis. From a pharmacological perspective, antidepressants alone are not effective. Mood stabilizers, such as lithium, valproate, and lamotrigine, are commonly used (Nierenberg et al., 2023). Among these, lamotrigine has demonstrated efficacy in maintaining stability, reducing relapse rates, and preventing symptom recurrence (Haenen et al., 2024). Atypical antipsychotics also play an essential role in bipolar disorder management (Escudero et al., 2020), including quetiapine,

aripiprazole, asenapine, lurasidone, and cariprazine (Nierenberg et al., 2023). Unfortunately, relapses and treatment discontinuation remain common in the context of bipolar disorder. Concerning long-term treatment, dropout rates become even more prevalent, with half of patients discontinuing within three to four years (Jung et al., 2024; Moon et al., 2012). Thus, psychotherapy and psychoeducation are valuable tools for reducing symptoms, mitigating the disorder's impact, preventing relapse, and improving treatment adherence (Hautzinger, 2024; Rabelo et al., 2021; Valdivieso-Jiménez, 2023).

13.2.4 Schizophrenia

Schizophrenia is a psychiatric disorder characterized by a wide range of symptoms that substantially impair overall functioning. Positive symptoms, exaggerated experiences absent in healthy individuals, include delusions and hallucinations (APA, 2022; Torres, 2024), while negative symptoms involve functional blunting, such as reduced emotional and verbal expressiveness, social withdrawal, avolition, and anhedonia (Torres, 2024). Cognitive deficits, including impairments in attention, memory, concentration, and social skills, as well as catatonia and stereotypies, are also common (Harris, 2023).

The disorder typically emerges during adolescence, often preceded by perceptual and cognitive changes that signal its onset (Zhang et al., 2025). Particular attention is given to the decline in sociability and the presence of negative symptoms, which, remarkably, may persist for up to a year before the full emergence of schizophrenia (Buciuc et al., 2022). Although the so-called prodromal symptoms are challenging to recognize, timely intervention is crucial and may prevent psychotic episodes (Powers et al., 2020). Regarding diagnostic classification, at least two of the positive, negative, or cognitive symptoms are required with a minimum duration of one month. Moreover, the presence of hallucinations or delusions, and prominent functional decline is essential for confirming the diagnosis (APA, 2022).

Several factors may contribute to the development of schizophrenia; it even suggests that it results from the interaction of biological and environmental factors (Nieto et al., 2023). A large-scale study analyzing 320,000 individuals identified 287 genetic regions associated with the disorder, particularly those involved in synaptic and neurological regulation, with emphasis on glutamatergic signaling and neural plasticity. These findings reinforce both the polygenic nature of schizophrenia and the role of dysfunctional synaptic communication in predisposition to the disorder (Trubetskoy et al., 2022). Clinically, patients experiencing a first psychotic episode often present dopaminergic hyperactivity, neuronal excitability linked to glutamatergic dysregulation, and impaired GABAergic inhibition. In contrast, individuals at high risk of psychosis do not exhibit this combined profile, suggesting that the psychotic episode itself arises only when glutamate, GABA, and dopamine are simultaneously dysregulated (Sigvard et al., 2023). Furthermore, patients with schizophrenia show dopaminergic alterations in the striatum, a core region of the disorder. These changes were not observed in healthy individuals (Schiulaz et al., 2025).

Additionally, environmental, behavioral, and psychological factors can increase the likelihood of developing schizophrenia. A study conducted with young individuals who visited emergency services following substance use found a higher risk of schizophrenia compared to the general population. Patients who experienced a psychotic episode after substance use had a 163-fold higher risk of schizophrenia compared to healthy individuals. At the same time, those without a psychotic episode had an almost tenfold higher risk (Myran et al., 2023). A history of mental disorders in first-degree relatives, higher levels of stress, and childhood traumas, such as neglect or physical and emotional abuse, can also contribute. One study found that the prevalence of these experiences was higher among individuals with schizophrenia than in healthy controls (Vila-Badia et al., 2022). This illustrates the inherent biological and environmental interaction in schizophrenia, where repeated exposure to stress progressively increases stress sensitivity, anxiety levels, and

excitability, ultimately leading to a higher risk of developing the disorder (Nieto et al., 2023).

It is also essential to highlight the relevance of differential diagnosis, since hallucinations and delusions are not exclusive to schizophrenia. Rognli et al. (2023) emphasize that psychosis can be induced using alcohol, drugs, or medications, for example. Young individuals with a history of cannabis use and recurrent emergency visits are more susceptible to developing schizophrenia following a substance-induced psychotic episode (Rognli et al., 2023). Psychosis can also occur in some cases of bipolar disorder, although the symptom patterns differ across conditions. In schizophrenia, negative symptoms may increase the risk of psychosis. In contrast, in bipolar disorder type I, positive symptoms tend to be more prominent, an observation that underscores the importance of distinguishing between these disorders (Kim et al., 2025).

Regarding treatment, a combination of medication, psychotherapy, and social rehabilitation is recommended (Wright et al., 2020). Antipsychotics are classified into two groups: typical, or first-generation, which primarily act by blocking dopamine receptors, and atypical, or second-generation, which act as antagonists at both serotonin and dopamine receptors (Lieberman & Kane, 2009). First-generation antipsychotics include trifluoperazine, prochlorperazine, triflupromazine, and haloperidol, while second-generation agents include risperidone, olanzapine, quetiapine, aripiprazole, lurasidone, and clozapine (Krutika Chokhawala & Stevens, 2023).

The efficacy of psychotherapy has also been well established. A study focusing on CBT demonstrated that sessions could reduce overall symptom severity, even in treatment-resistant patients (Salahuddin et al., 2024). Group psychotherapy has also been shown to be an effective intervention (Guaiana et al., 2022). Additionally, CBT-based psychoeducation has been found to improve treatment adherence and mitigate aggression in patients with schizophrenia (Can & Budak, 2025).

Ultimately, psychosocial rehabilitation interventions are crucial in facilitating the reintegration of patients into their communities and society.

Social skills training programs, which incorporate therapeutic and communication techniques, help maintain interpersonal relationships and reduce the stress caused by social interactions (Almerie et al., 2015). Similarly, cognitive remediation practices aimed at enhancing memory, concentration, and other aspects of daily functioning are effective, yielding long-lasting results that provide tangible benefits for patients' everyday lives (Bowie et al., 2020). In more severe cases, supported employment strategies are also valuable, as they prepare individuals for the labor market and help them find competitive and suitable jobs, promoting autonomy (Frederick & VanderWeele, 2019). Psychoeducation activities for family members and caregivers are likewise crucial, as they address questions, alleviate distress, and enhance the effectiveness of care and treatment. Thus, the integration of pharmacological treatment, psychotherapy, and social reintegration can address not only the disorder itself but also the unique needs of everyone.

13.3 Microbiome

Homo sapiens are intrinsically symbiotic organisms. Traditionally, it was believed that humans were born practically sterile and, upon contact with the microbial environment, they begin to acquire and diversify their microbiota, thereby developing their immune system (Malard et al., 2021). However, this dogma of a sterile uterus has been challenged by recent evidence suggesting the presence of microorganisms in the placenta, amniotic fluid, and umbilical cord in term pregnancies, indicating the possibility of an initial intrauterine microbial exposure. Nevertheless, subsequent studies have raised methodological criticisms and argued against the existence of fetal intestinal colonization in utero, leaving the issue unresolved. Thus, although birth and the postnatal period are recognized as critical events for the assembly of the neonatal microbial community, the extent of prenatal colonization remains the subject of intense scientific debate (Milani et al., 2017).

The initial composition of this microbial community is related to genetic factors and varies among individuals. The first significant exposure to microorganisms occurs during passage through the birth canal, followed by colonization through breast milk after birth. The infant's microbiome is highly correlated with that of the mother. Still, it undergoes modifications over time due to the exposome, including diet and environmental factors that can promote health or increase disease susceptibility (Agrawal et al., 2025). The study of the human microbiome has consolidated current knowledge regarding the importance of the microbiota in lifelong health (Requena & Velasco, 2021). Consequently, this field advanced significantly with the launch of the Human Microbiome Project in 2007, which mapped microbial diversity across different body niches (Turnbaugh et al., 2007).

The human microbiota refers to the community of microorganisms, including bacteria, archaea, viruses, and eukaryotes, which occupy distinct body niches. In contrast, the microbiome is the complete set of microbial genomes residing in the host (Ogunrinola et al., 2020). The microbial communities that comprise the microbiota form complex ecological networks both among themselves and with the host, and can be classified as mutualistic, commensal, or, in some cases, parasitic. These relationships are not static and may shift in response to changes in the environment, diet, immune status, or other host-related factors (Drew et al., 2021). For example, the gut exhibits cooperative mechanisms such as metabolic cross-feeding, in which metabolites produced by one species serve as substrates for another process that is crucial for maintaining the functional stability of the microbial community (Wang et al., 2023).

Trillions of bacteria are present in the human microbiota, interacting daily at various sites, including the skin, urogenital tract, pharynx, oral cavity, respiratory system, and, most notably, the gastrointestinal tract, which harbors the most remarkable diversity and density of microorganisms (Malard et al., 2021; Shah et al., 2025). Across these environments, the microbiota establishes a symbiotic relationship with the host, contributing significantly to physiological development early on, as well as influencing nutritional and immune functions and protection against pathogens

throughout life (Requena & Velasco, 2021). The microbiota participates in trophic, barrier, metabolic, immunological, and signaling functions in virtually all organs (Malard et al., 2021). Its transition from a healthy state to one associated with disease, characterized by imbalances in microbial populations and/or functions, is termed dysbiosis (Govender & Ghai, 2025; Requena & Velasco, 2021; Talapko et al., 2024). As a result of these biological activities, microorganisms are considered integral components of the body, driving changes from birth to death. The human microbiome is constantly evolving in response to intrinsic and extrinsic host factors, such as age, nutrition, lifestyle, hormonal changes, genetic background, and underlying diseases, which are the primary determinants of its composition at any given time (Ogunrinola et al., 2020). Understanding the human microbiome, therefore, requires an integrated approach that considers the diversity of body niches and the dynamic interactions established between the host and its microbiome. Among these niches, the oral, pulmonary, and gut microbiota are particularly relevant for bidirectional communication with the central nervous system and for modulating both physiological and pathological processes (Figure 13.1).

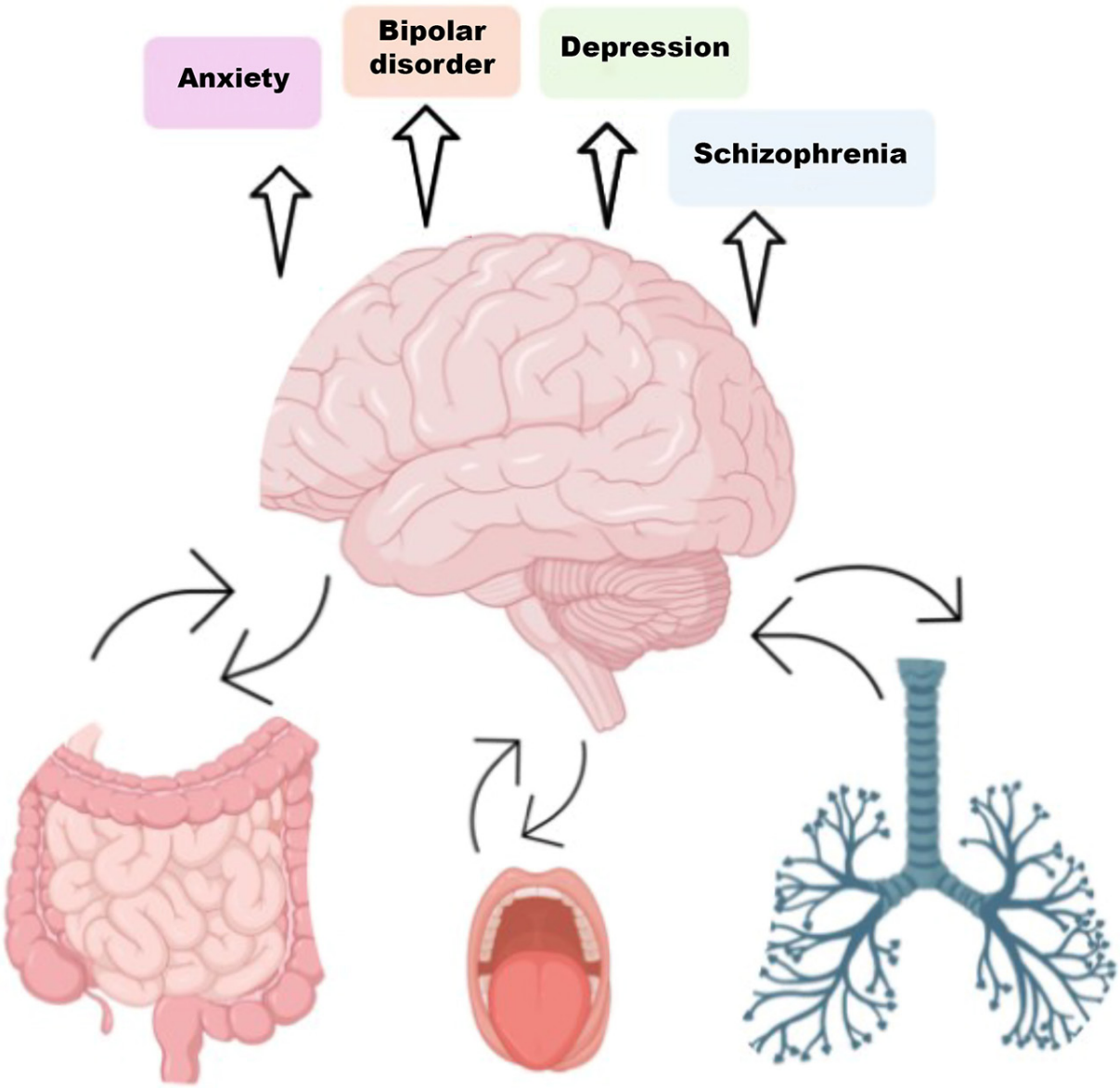

FIGURE 13.1 There is a bidirectional communication between the brain and gut-microbiota, brain and mouth-microbiota, and brain and lung-microbiota. In individuals with schizophrenia, anxiety, depression, and bipolar disorder, changes in the microbiota composition and in its metabolism are reported. In addition, microbiota dysfunction is associated with neurotransmitter dysregulation, increased inflammation, HPA axis dysfunctions, impaired blood–brain barrier integrity, and deficits in neuroplasticity and neuronal connectivity in psychiatric conditions. ⏎

13.3.1 Oral Microbiome and Brain

The human oral cavity comprises a variety of distinct microhabitats, including teeth, gingival sulcus, attached gingiva, tongue, buccal mucosa, lips, hard palate, and soft palate. Contiguous anatomical structures such as the tonsils, pharynx, proximal esophagus, Eustachian tube, middle ear, nasal passages, and paranasal sinuses share continuity with the oral cavity and may harbor related microbial communities. The oral microbiome is defined as the collection of microorganisms present in the oral cavity and these anatomical extensions, with most investigations and samples derived from intraoral sites (Dewhirst et al., 2010).

The Cultivated Oral Bacteria Genome Reference includes 1,089 bacterial species isolated under 34 culture conditions from dental biofilms, saliva, and tongue samples (Li et al., 2023). Technological advances, such as next-generation sequencing, have identified approximately 770 microbial species in the human oral cavity, including bacteria, viruses, fungi, protozoa, and archaea (Sureda et al., 2020). Among the most prevalent bacterial genera are *Actinomyces, Prevotella, Streptococcus, Fusobacterium, Leptotrichia, Corynebacterium, Veillonella, Rothia, Capnocytophaga, Selenomonas*, and *Treponema*. However, the composition of the oral microbiome shifts according to the health status of the environment (Liu et al., 2012). In terms of diversity, the oral microbiome is surpassed only by the gut microbiome, playing a central role in maintaining both oral and systemic homeostasis (Deo & Deshmukh, 2019). Beyond its local role in oral health, the oral microbial community can influence neurological processes through systemic and immunological mechanisms. Alterations in this community, frequently observed in conditions such as periodontitis and oral cancers, result in complex host–pathogen interactions that may trigger inflammatory responses with systemic repercussions.

Chronic periodontitis, primarily caused by bacteria of the red complex, is characterized by oral microbial dysbiosis and local inflammation, which contributes to a systemic inflammatory burden (Chen et al., 2025b). Pathogenic changes in the oral microbiome can be modulated by diet in the context of obesity, thereby exacerbating periodontal inflammation. Obesity itself is linked to a low-grade systemic inflammatory state, characterized by

elevated levels of pro-inflammatory cytokines, dysfunctional immune responses, and dysregulated adipokine profiles (Chen et al., 2025b). Moreover, oral microbial dysbiosis is associated with increased production of polyamines and acid phosphatase (ACP) activity, alongside reduced antioxidant activity of superoxide dismutase (SOD) and catalase (Rashid et al., 2025).

The oral microbiome may induce neuroinflammation through multiple pathways: oral pathogens and their products can translocate to the brain via circulation or peripheral nerves; pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) can cross or modulate the blood–brain barrier, increasing its permeability; and complement activation (C3a, C5a) can amplify local inflammatory responses. These processes promote the activation of microglia and astrocytes, as well as the excessive secretion of inflammatory mediators and metalloproteinases, ultimately leading to neuronal dysfunction and contributing to neurodegeneration (Wang et al., 2019b).

In mice, chronic periodontitis induced oral and gut dysbiosis, disruption of intestinal and blood–brain barriers, and systemic and cerebral increases in LPS and pro-inflammatory cytokines, leading to glial activation, neuroinflammation, and cognitive decline mediated by the LPS/TLR4/NF-κB pathway (Xue et al., 2020). This redox imbalance, by exacerbating periodontal inflammation, may also amplify systemic and neuroinflammatory responses, supporting the role of the oral microbiome as a modulator of the oral–brain axis. Furthermore, clinical and experimental evidence suggests that alterations in the oral microbiome are associated with neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Other brain-related disorders have occasionally been implicated in oral microbial dysbiosis, including schizophrenia, MDD, and bipolar disorder (Skallevold et al., 2023).

13.3.2 Lung Microbiome and Brain

The lungs, in direct contact with the external environment, harbor a diverse range of microorganisms, including bacteria, archaea, fungi, viruses, and

protozoa. The relative abundance of these species varies considerably, resulting in distinct microbial compositions between the upper and lower respiratory tracts (Budden et al., 2019). Generally, these organisms can be classified into two groups: pathogenic and commensal. Pathogens can cause or exacerbate disease by invading host tissues, producing toxins, triggering inflammatory responses, and evading immune defenses. Conversely, commensals coexist with the host in a dynamic equilibrium maintained by factors such as nutrient competition and reciprocal regulation through the exchange of metabolites. The lung microbiome encompasses the entire collection of microbes found in the airways, together with their genomes and metabolites. It is increasingly recognized as a key element in maintaining respiratory health as well as in the development and progression of disease (Wang et al., 2025).

Growing evidence indicates that the lung microbiome does not function in isolation but is strongly modulated by the gut–lung axis. A balanced gut microbiome regulates the respiratory mucosa, whereas intestinal dysbiosis can directly impact the lungs through the microaspiration of microorganisms or metabolites, thereby favoring the development of respiratory diseases (Budden et al., 2017; Marsland et al., 2015).

In contrast, the lung–brain axis comprises a complex, bidirectional communication network that critically integrates respiratory and neurological functions. This axis has gained importance in understanding pathophysiological conditions that affect both the lungs and the brain (Huang et al., 2025). Bidirectional communication may occur via five potential mechanisms: direct translocation of microorganisms, effects of lung microbes on systemic immunity, vagal signaling, modulation through the HPA axis, and metabolic alterations (Bajinka et al., 2022). Dysbiosis is a factor associated with several pulmonary and neurological disorders (Bajinka et al., 2022). Understanding this axis is crucial for enhancing the prevention and treatment of neurorespiratory disorders, a niche that is often overlooked in current interventions (Huang et al., 2025).

13.3.3 Gut Microbiome and Brain

The gut microbiota is highly diverse, comprising up to 1,000 species and, depending on the species, a variable number of strains (Eisenstein, 2020; Trueba et al., 2024). Dominant genera in the infant gut microbiota include *Bifidobacterium, Veillonella, Streptococcus, Citrobacter, Escherichia, Bacteroides*, and *Clostridium* (Milani et al., 2017). Curiously, in the adult gut microbiome, the phyla Firmicutes and Bacteroidetes predominate, representing approximately 90% of the species, whereas Actinobacteria, Fusobacteria, Proteobacteria, and Verrucomicrobia are present in smaller proportions), accounting for about 10% of the species (Arumugam et al., 2011). In contrast, Actinobacteria, Fusobacteria, Proteobacteria, and Verrucomicrobia are present in smaller proportions (Arumugam et al., 2011; Rinninella et al., 2019). Among them, *Clostridium* species (Firmicutes) are particularly abundant, along with *Bacteroides* and *Prevotella* (Bacteroidetes) (Rinninella et al., 2019).

The intestinal immune system, comprising innate and adaptive mechanisms, establishes dynamic interactions with symbiotic microorganisms, promoting homeostasis through a mutually beneficial relationship. It tolerates commensal microbiota while preserving the ability to mount pro-inflammatory responses against invading pathogens. Maintaining this symbiotic balance is fundamental for host health, whereas its disruption increases susceptibility to infections and sepsis (Malard et al., 2021). Furthermore, the gut microbiota plays a pivotal role not only in gastrointestinal function but also in regulating mood, pain, and anxiety through communication with the brain, as represented by the bidirectional gut–brain axis (Arneth, 2018; Di Napoli et al., 2025). Specifically, the gut microbiota has been shown to modulate behavior and brain processes, including pain perception, prefrontal myelination, stress response, and brain biochemistry (Di Napoli et al., 2025).

Brain connectivity refers to the patterns and strength of interactions between brain regions, which sustain neural communication, coordinated activity, and neuroplasticity, often altered in neurological disorders. The gut

microbiota is thought to modulate this connectivity through multiple pathways. Among them, microbial metabolites such as short-chain fatty acids (SCFAs) can cross the intestinal barrier, exert neuroprotective effects, and influence synaptic plasticity. The microbiota also regulates the synthesis of neurotransmitters, including serotonin and GABA, which are essential for cognitive and emotional functions. Another mechanism involves immune modulation: dysbiosis promotes the release of pro-inflammatory mediators that induce systemic inflammation and impair brain function. Additionally, by altering intestinal barrier integrity and permeability, the microbiota may facilitate the translocation of bacterial products into the circulation, thereby indirectly affecting neural connectivity (Di Napoli et al., 2025).

The gut–brain axis also represents a bidirectional pathway linking gastrointestinal functions to cognitive and emotional processes through neural, immune, endocrine, and humoral mechanisms. The gut microbiota modulates this communication by regulating intestinal barrier integrity, vagal afferent signaling, and neurotransmitter synthesis (including serotonin, GABA, and catecholamines), as well as producing metabolites with neuroendocrine and immunological effects. Probiotics have been shown to restore tight junction integrity, reduce inflammation, and alter brain neurochemistry. Conversely, dysbiosis disrupts these pathways and is associated with psychiatric and neurodegenerative disorders, including MDD, schizophrenia, and Parkinson's disease (Arneth, 2018).

13.4 Major Depressive Disorder and Microbiome

Zheng et al. (2016) have demonstrated that gut microbiome dysfunctions can induce depressive-like behavior in mice, suggesting that the microbiota —the ensemble of microorganisms residing within the body—appears to exert a direct influence on brain function, behavior, and emotional regulation.

The so-called microbiota–gut–brain axis has become one of the most promising paradigms for understanding mood disorders such as depression and bipolar disorder. While most research has focused on gut microbiota, recent studies have also highlighted the roles of oral and pulmonary microbiomes in this intricate communication system.

Although the current body of evidence does not definitively clarify whether intestinal dysbiosis is a cause or merely a consequence of depression, studies such as that by Liu et al. (2023) demonstrate that individuals with depression often exhibit a distinctly different microbial composition compared to healthy individuals. In particular, beneficial anti-inflammatory bacteria, such as *Faecalibacterium prausnitzii* and *Bifidobacterium*, are typically reduced, while pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β, are elevated. This low-grade chronic inflammation, commonly observed in depressive states, may therefore be directly linked to gut dysbiosis. These findings open avenues for therapeutic strategies aimed at restoring microbial balance as part of a comprehensive treatment plan for depression.

For decades, mental health researchers have focused their efforts almost exclusively on the brain, investigating neurotransmitters, neural circuits, and psychological trauma as potential roots of depression. In this model, the body often appeared as a passive vessel for mental phenomena. However, a recent shift in mental health science is disrupting this paradigm, suggesting that one of the most compelling keys to understanding depression may lie in an unexpected place: the gut.

There, among trillions of bacteria, unfolds one of the most fascinating relationships in modern biology: the gut–brain axis. This intricate communication system, mediated by chemical signals, immune pathways, hormones, and neural impulses, positions the gastrointestinal tract as a central actor in mood regulation (Liu et al., 2023).

Researchers such as Radjabzadeh et al. (2022) and Valles-Colomer et al. (2019) have been closely examining the microbiota of individuals diagnosed with depression. What they are finding, surprisingly consistently, is an alteration in microbial composition. Beneficial bacteria, such as

Faecalibacterium, Coprococcus, and *Butyricicoccus*, which are known for their anti-inflammatory properties and for strengthening the intestinal barrier, are frequently reduced. In their place, there is an increased presence of microorganisms, such as *Eggerthella, Streptococcus, Enterococcus*, and *Flavonifractor*, many of which are associated with inflammatory processes and metabolic disturbances.

In studies such as the one conducted by Amin et al. (2023), a connection was found between mitochondrial activity during the Krebs cycle, where cellular energy molecules are formed, and microbiota composition. Depressed individuals exhibited increased pyruvate and decreased citrate levels, correlating with the presence of bacteria from the phyla *Pseudomonadota* and *Bacteroidota*.

These microbes are not passive residents; they actively participate in the production of molecules that influence brain function, including neurotransmitters and SCFAs. They also modulate stress response and immune system activity. In other words, these bacteria are not just passengers; they are living components of our emotional regulation system (Reyes-Martínez et al., 2023).

Clinical studies and meta-analyses, comprising hundreds of patients and dozens of research papers, such as the ones published by Kelly et al. (2016) and Pan et al. (2025), have shown that these altered bacterial profiles are not merely a consequence of depression but may also serve as predisposing factors. In animal models, for example, healthy mice that received microbiota transplants from depressed individuals began to exhibit depression-like behaviors, as demonstrated by Liu et al. (2020).

One of the most important is the production of neuroactive metabolites. Gut bacteria are capable of synthesizing or modulating the production of neurotransmitters such as serotonin, dopamine, and GABA. It is well known, for instance, that approximately 90% of the body's serotonin is produced in the gut, rather than in the brain. However, its functions differ both locally and systemically, as demonstrated by Terry and Margolis (2016).

Another pathway involves systemic inflammation. When dysbiosis occurs, the intestinal barrier may become compromised, allowing toxic substances, such as lipopolysaccharides (LPS), to enter the bloodstream. This results in low-grade inflammation that can affect the brain, impairing neuroplasticity, disrupting mood regulation, and promoting anhedonia (Carlessi et al., 2021; Réus et al., 2023; Vakili et al., 2022).

Also noteworthy is the HPA axis, our central stress response system. Healthy microbiota helps maintain the balance of this axis. When disrupted, the HPA axis may lead to excessive release of cortisol, the stress hormone, which has been linked to various depressive states (Figure 13.1). The absence or imbalance of microbiota, as demonstrated in germ-free animal models, exacerbates this dysfunction, leading to exaggerated stress responses (Sudo et al., 2004; Wu et al., 2020, 2021).

Finally, the microbiota also impacts tryptophan metabolism, a precursor of serotonin. Tryptophan can follow neuroprotective or neurotoxic metabolic pathways, and according to research by Tian et al. (2022), the microbiota plays a key role in determining which pathway is prioritized. In depressed individuals, tryptophan often shifts toward the production of kynurenine, a compound that can induce neurodegeneration and inflammation. Interestingly, this process was shown to be mitigated by the supplementation of two beneficial bacteria, *Bifidobacterium* and *Lactobacillus* (Tian et al., 2022; Zhang et al., 2021).

The good news is that, unlike genetics or past trauma, the microbiota is highly modifiable. Diet, stress, sleep, physical activity, antibiotics, and the consumption of fermented foods can significantly alter bacterial composition within days or weeks. Studies testing probiotics, including beneficial strains such as *Lactobacillus helveticus* and *Bifidobacterium longum* (Schaub et al., 2022), have demonstrated reductions in symptoms of mild to moderate depression. Remarkably, some of these benefits persisted even weeks after discontinuing supplementation, as shown by Tian et al. (2022). While more robust clinical trials are still needed, initial results are promising.

The Mediterranean diet, rich in fiber, olive oil, vegetables, whole grains, and fruits, was the aim point of a study conducted by Oddo et al. (2022), and the results showed that this dietary behavior had protective effects on mood. This is not just a "healthy" diet in the conventional sense; it is microbiota-friendly, nourishing the microorganisms that, in turn, nourish the brain.

On the horizon are more radical approaches, such as fecal microbiota transplantation (FMT) (Wang et al., 2019). Although still primarily used in cases like *Clostridium difficile* infections, its application in neuropsychiatric disorders is beginning to be explored, with intriguing experimental results.

It is fascinating to consider that a significant part of our emotional well-being may depend on microorganisms we cannot even see. This is not to say, of course, that depression is simply a gut disease. The human mind is profoundly complex. Psychological trauma, social relationships, life history, genetics, and environmental factors remain essential to understanding and treating mental illness. However, ignoring the role of the body, specifically the microbiota, would be a mistake. Mental health care must increasingly include listening to the gut. After all, it is saying something important, and we are finally starting to hear it.

13.5 Bipolar Disorder and Microbiome

Traditionally, bipolar disorder etiology has been attributed to dysregulation of neurotransmitters such as dopamine and serotonin, disruptions to circadian rhythms, inflammatory processes, and genetic factors, like those found in other neuropsychiatric conditions. However, as noted by Sublette et al. (2021), there is growing evidence that bipolar disorder is not merely a brain-based disorder. Alterations in the gut microbiota may play a significant role in its pathophysiology. This emerging field centers around the microbiota–gut–brain axis, which integrates immune, neuroendocrine, and metabolic pathways that connect the gut to the central nervous system.

Painold et al. (2019) found that patients hospitalized during depressive episodes of bipolar disorder had significantly reduced gut microbial diversity, along with a decrease in *Faecalibacterium* and *Ruminococcaceae* butyrate-producing bacteria, which have anti-inflammatory properties. These findings suggest that microbial imbalances may contribute to the disorder's pro-inflammatory profile.

Similarly, a systematic review has reinforced that a reduction in microbial diversity is one of the most consistent findings in patients with bipolar disorder (Sublette et al., 2021). This reduction is often accompanied by an increase in *Actinobacteria* and *Bacteroides*, which may be associated with dysfunction in energy metabolism. Additionally, Coello et al. (2019) reported an increased abundance of *Flavonifractor* in bipolar patients compared to healthy controls and unaffected relatives, suggesting the presence of specific microbial signatures in individuals with the disorder. Nevertheless, current evidence remains limited.

Imagine a classic assembly-line factory during the Industrial Revolution, where each part of the final product relies on the success of the previous step. Biochemical processes in the body work in a similar chain. Gut bacteria perform vital functions. For example, the previously mentioned *F. prausnitzii* ferments indigestible dietary fibers from foods like apples and oats, producing butyrate in the colon that strengthens the intestinal barrier by tightening cellular junctions. This restricts the passage of toxins into systemic circulation, reducing oxidative stress and inflammatory byproducts and thereby improving the body's overall inflammatory profile.

In this context, McGuiness et al. (2022) reported that individuals with bipolar disorder not yet stabilized through treatment exhibited reduced levels of *Roseburia*, *Coprococcus*, *Ruminococcus*, and *Faecalibacterium*, bacteria closely linked to butyrate production. This reduction correlated negatively with depressive symptom severity, suggesting that microbiota dysfunction may increase systemic inflammation and oxidative stress, potentially contributing to depressive features, a biochemical "assembly line."

More recently, Huang et al. (2023) observed that bipolar disorder patients in a depressive phase had elevated levels of *Bacilli*, *Lactobacillales*, and *Veillonella*, as well as reduced *Dorea*. These changes were associated with elevated inflammatory markers such as IL-6 and TNF-α, indicating that a broad range of microbial phyla and species may be involved in BD pathophysiology, each playing individual or synergistic roles.

Going beyond correlation, Xu et al. (2023) used Mendelian randomization to investigate potential causal relationships between gut microbiota and bipolar disorder. They found that genera such as *Prevotella 7* and *Roseburia* were associated with an increased risk of bipolar disorder, while *Ruminiclostridium 5* appeared to exert a protective effect.

According to Painold et al. (2019), dysbiosis in bipolar disorder may increase intestinal permeability, also known as a "leaky gut," allowing LPS to enter the systemic circulation. This activates the immune system, leading to increased levels of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α. Systemic inflammation, in turn, impacts the central nervous system, triggering microglial activation, compromising the blood–brain barrier, and impairing synaptic plasticity.

The group led by Misiak (2020) demonstrated that the gut microbiota regulates the HPA axis. In chronic stress contexts, excessive cortisol production can disrupt the microbiota. Conversely, when dysbiosis occurs due to non-stress-related causes, such as increased intestinal permeability, there is a tendency toward HPA hyperactivation, resulting in hypercortisolemia and heightened stress reactivity, a common finding in bipolar disorder patients.

Lucidi et al. (2021) suggested that gut microbiota affects tryptophan metabolism, a key precursor of serotonin. In bipolar disorder, tryptophan is often diverted away from serotonin synthesis toward the kynurenine pathway, resulting in the accumulation of neurotoxic metabolites. This shift is driven by the enzyme indoleamine-2,3-dioxygenase (IDO), which becomes hyperactive in inflammatory environments, further evidence of the microbiota's influence on mental health.

As emphasized by McIntyre et al. (2021), bacterial species can synthesize neuroactive compounds: *Lactobacillus* and *Bifidobacterium* produce GABA; *Streptococcus* and *Enterococcus* produce serotonin; *Bacillus* synthesizes dopamine. Therefore, microbial composition directly affects the availability of neurotransmitters relevant to bipolar disorder (Figure 13.1).

In a pilot study conducted by Reininghaus et al. (2020a, 2020b), the results supported the hypothesis that probiotic supplementation may help stabilize cognitive function in individuals with bipolar disorder, which could, in turn, enhance their psychosocial, occupational, and financial functioning. However, the implementation of this potential new treatment remains challenging due to the considerable variability in the composition of the human gut microbiota. Additionally, a double-blind, placebo-controlled trial led by Jenny Borkent and her team evaluated the effect of probiotic administration in 131 participants, observing positive effects on gut permeability and inflammatory markers (Borkent et al., 2024). Another trial, conducted by Reininghaus et al. (2020a, 2020b), used a different probiotic, OMNi-BiOTiC Stress Repair, to evaluate gastrointestinal symptoms and psychiatric outcomes. Results showed a reduction in depressive mood reactivity, ruminative thinking, and manic symptoms.

Still in its experimental phase, a case study reported by Parker et al. (2022) described significant clinical improvement in a bipolar patient following FMT. While promising, such findings must be interpreted with caution due to the anecdotal nature of case reports. The same study notes that the improvement was sustained and non-recurring, contrasting with typical placebo responses, which are often brief and transient.

13.6 Anxiety Disorders and Microbiome

Research has explored this relationship, identifying several potential causes, with stress emerging as a key factor influencing both anxiety and the microbiota–brain axis. Early stress can trigger anxious behaviors in

adulthood, favoring the development of anxiety and contributing to gut dysbiosis, reducing microbial diversity and compromising the production of neuroactive metabolites, such as SCFA and tryptophan, which influence synaptic plasticity and emotional regulation (Bertollo et al., 2025; Borrego-Ruiz & Borrego, 2025; De Palma et al., 2015; Socała et al., 2021). Thus, early and chronic stress emerges as a critical factor for both the development of anxiety and the bidirectional dysregulation between microbiota and brain.

The relationship between the microbiome and behavior is bidirectional, as stress and emotional states can influence gut microbial composition through the release of stress hormones and sympathetic neurotransmitters, which in turn modulate gut physiology and alter the microbial environment (Malan-Müller et al., 2023; Montiel-Castro et al., 2013). Therefore, studies have shown that specific microbial populations are associated with anxiety and exhibit changes in their composition in individuals with this condition (Figure 13.1).

A study of 198 Spanish individuals revealed that patients with anxiety had lower microbial diversity, as measured by the Simpson's index (Malan-Müller et al., 2023). Specifically, individuals with GAD demonstrated lower microbiota richness and diversity, characterized by a reduction in *Firmicutes* spp. and SCFA-producing microorganisms, alongside an increase in *Fusobacteria* and *Bacteroidetes* (Jiang et al., 2018). Similar results were observed in animal models, where mice exhibiting anxious behaviors showed significantly lower levels of *Firmicutes*, corroborating the association between anxiety and alterations in gut microbial composition (Huang et al., 2018). Hence, the literature indicates that anxious individuals and mice exhibit lower microbial diversity, characterized by a reduction in *Firmicutes* and an increase in *Bacteroidetes* and Fusobacteria. Some genera, such as *Prevotella* and *Lactobacillus*, are positively associated with anxiety. In contrast, others exhibit an inverse correlation, suggesting that modulating the gut microbiota may be a strategic approach for preventing anxiety (Xiong et al., 2023). Future

studies should explore the species level, as different species within the same genus may have distinct effects.

13.7 Schizophrenia and Microbiome

Individuals with schizophrenia have distinct gut microbiota profiles compared to healthy controls. Studies indicate a reduction in beneficial butyrate-producing bacteria, such as *Blautia*, and an increase in pro-inflammatory or potentially pathogenic taxa, including *Proteus, Clostridium, Megasphaera*, and *Succinivibrio*. These microbial alterations have been linked to brain changes, including decreased gray matter volume and impaired neural connectivity, as well as cognitive deficits and a greater severity of clinical symptoms (Wang et al., 2024; Wu et al., 2025; Xu et al., 2020).

Neuroimaging studies have shown correlations between these microbial alterations and brain changes. For example, greater Proteus abundance was associated with smaller gray matter volume and lower cognitive scores (Wu et al., 2025). Changes in brain connectivity have also been linked to modifications in the microbiome (Lin et al., 2023; Wang et al., 2024). The proposed mechanisms involve inflammatory and metabolic pathways. Pro-inflammatory bacteria have been correlated with increased cytokines (Wu et al., 2024), while changes were observed in relevant metabolic pathways, including lipid metabolism, SCFA synthesis, and neurotransmitter pathways such as GABA and tryptophan (Wang et al., 2024; Zhu et al., 2020).

Some specific intestinal bacterial species, such as *Lactobacillus fermentum*, *Enterococcus faecium*, and *Alkaliphilus oremlandii*, appear to be unique to individuals with schizophrenia. Certain microbial groups, including *Lachnospiraceae, Veillonella, Collinsella, Lactobacillus, Succinivibrio*, and *Corynebacterium*, have been found to exhibit a positive correlation with the severity of schizophrenia symptoms. In contrast, others, such as *Coprococcus, Ruminococcus, Roseburia, Adlercreutzia, Anaerostipes*, and *Faecalibacterium*, have shown a negative correlation

(Xiong et al., 2023). These findings suggest that alterations in the composition of the intestinal microbiota and its metabolic activities are strongly linked to the clinical manifestations and neural alterations observed in schizophrenia, indicating potential targets for future therapeutic strategies.

13.8 Conclusion and Future Directions

Research into the role of the microbiome in psychiatric disorders has made promising progress, but still faces significant limitations. One of the biggest challenges is the difficulty in establishing clear cause-and-effect relationships: we do not know for sure whether dysbiosis is a trigger or a consequence of psychiatric conditions. Furthermore, factors such as diet, medication use, and stress can alter the gut microbiota, creating a complex feedback loop that is difficult to understand fully.

Another relevant point is the significant variability of the human microbiome. Its composition is shaped by factors such as genetics, age, diet, lifestyle, and environment, making it challenging to define a universal "healthy microbiome" and generalize results across different populations. Studies on anxiety, for example, suggest a reduction in microbial diversity; however, the specific species involved vary between individuals. Therefore, future research should move beyond correlations and focus on understanding the specific functions of different species and strains.

To overcome these challenges, some research directions stand out. Integrated multiomics approaches: (1) Combining metagenomics, metabolomics, proteomics, and transcriptomics can provide a more comprehensive understanding of how the microbiome impacts host health at the molecular level. This type of integration can help identify relevant metabolic pathways, such as the kynurenine pathway in depression, which appears to be modulated by the microbiota. (2) Longitudinal studies and clinical trials: it is essential to conduct long-term studies and randomized, double-blind, placebo-controlled clinical trials to consistently assess the

impact of interventions such as probiotics, prebiotics, and FMT on psychiatric symptoms. Initial results, such as the case of FMT in bipolar disorder, are intriguing but still anecdotal. (3) Exploring other microbial niches: although the gut–brain axis is the most extensively studied, we should not neglect microbiomes from other sites, such as the oral and pulmonary microbiomes. The oral microbiome, for example, has been linked to neuroinflammation and neurodegenerative diseases through systemic inflammatory responses. Mapping these connections can broaden our understanding of the microbiome's global influence on mental health. (4) Personalized and precision medicine: given the uniqueness of each person's microbiome, future therapies will likely need to be tailored to individual needs. Identifying specific microbial signatures for different psychiatric conditions could pave the way for the development of "psychobiotics" or other tailored interventions that act synergistically with each person's microbial and metabolic profile. In short, understanding the complex network of interactions between the microbiome and the brain is a challenging but fundamental path. Progress in this field can transform not only our understanding of psychiatric disorders but also the way we think about and implement more effective and individualized therapeutic strategies.

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References

Abend, R. (2023). Understanding anxiety symptoms as aberrant defensive responding along the threat imminence continuum. *Neuroscience & Biobehavioral Reviews*, *152*, 105305. https://doi.org/10.1016/j.neubiorev.2023.105305 ⏎

Agrawal, P., Mendhey, P., Kumar, R., Patel, S., Kaushik, P. K., Dadsena, A., & Kumar, S. (2025). Introduction to microbiomes in health and diseases. *International Review of Cell and Molecular Biology*, *394*, 1–42. https://doi.org/10.1016/BS.IRCMB.2024.12.010 ⏎

Aibar-Durán, J. Á., Rodríguez Rodríguez, R., de Diego Adeliño, F. J., Portella, M. J., Álvarez-Holzapfel, M. J., Martín Blanco, A., Puigdemont Campos, D., & Molet Teixidó, J. (2022). Long-term results of deep brain stimulation for treatment-resistant depression: outcome analysis and correlation with lead position and electrical parameters. *Neurosurgery*, *90*(1), 72–80. https://doi.org/10.1227/NEU.0000000000001739 ⏎

Akil, H., & Nestler, E. J. (2023). The neurobiology of stress: vulnerability, resilience, and major depression. *Proceedings of the National Academy of Sciences U S A*, *120*(49), e2312662120. https://doi.org/10.1073/pnas.2312662120 ⏎

Alegría, M., Alvarez, K., Cheng, M., & Falgas-Bague, I. (2023). Recent advances on social determinants of mental health: looking fast forward. *American Journal of Psychiatry*, *180*(7), 473–482. https://doi.org/10.1176/appi.ajp.20230371 ⏎

Almerie, M. Q., Okba Al Marhi, M., Jawoosh, M., Alsabbagh, M., Matar, H. E., Maayan, N., & Bergman, H. (2015). Social skills programmes for schizophrenia. *Cochrane Database of Systematic Reviews*, *2015*(6), CD009006. https://doi.org/10.1002/14651858.CD009006.pub2 ⏎

American Psychiatric Association. (2022). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5-TR (5th ed.)*. American Psychiatric Association Publishing. ⏎

Amin, N., Liu, J., Bonnechere, B., MahmoudianDehkordi, S., Arnold, M., Batra, R., Chiou, Y.-J., Fernandes, M., Ikram, M. A., Kraaij, R., Krumsiek, J., Newby, D., Nho, K., Radjabzadeh, D., Saykin, A. J., Shi, L., Sproviero, W., Winchester, L., Yang, Y., & van Duijn, C. M. (2023). Interplay of metabolome and gut microbiome in individuals with major depressive disorder vs control individuals. *JAMA Psychiatry*, *80*(6), 597. https://doi.org/10.1001/jamapsychiatry.2023.0685 ⏎

Ananth, M., Bartlett, E. A., DeLorenzo, C., Lin, X., Kunkel, L., Vadhan, N. P., Perlman, G., Godstrey, M., Holzmacher, D., Ogden, R. T., Parsey, R. V., & Huang, C. (2020). Prediction of lithium treatment response in bipolar depression using 5-HTT and 5-HT1A PET. *European Journal of Nuclear Medicine and Molecular Imaging*, *47*(10), 2417–2428. https://doi.org/10.1007/s00259-020-04681-6 ⏎

Andreassen, O. A., Hindley, G. F. L., Frei, O., & Smeland, O. B. (2023). New insights from the last decade of research in psychiatric genetics: discoveries, challenges and clinical implications. *World Psychiatry*, *22*(1), 4–24. https://doi.org/10.1002/wps.21034 ⏎

Arneth, B. M. (2018). Gut–brain axis biochemical signalling from the gastrointestinal tract to the central nervous system: gut dysbiosis and altered brain function. *Postgraduate Medical Journal*, *94*(1114), 446–452. https://doi.org/10.1136/POSTGRADMEDJ-2017-135424 ⏎

Arumugam, M., Raes, J., Pelletier, E., Paslier, D. Le, Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., … Zeller, G. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180. https://doi.org/10.1038/nature09944 ⏎

Asir, B., Boscutti, A., Fenoy, A. J., & Quevedo, J. (2024). *Deep Brain Stimulation (DBS) in Treatment-Resistant Depression (TRD): Hope and Concern* (pp. 161–186). https://doi.org/10.1007/978-981-97-4402-2_9 ⏎

Bajinka, O., Simbilyabo, L., Tan, Y., Jabang, J., & Saleem, S. A. (2022). Lung–brain axis. *Critical Reviews in Microbiology*, *48*(3), 257–269. https://doi.org/10.1080/1040841X.2021.1960483 ⏎

Baker, J. L., Mark Welch, J. L., Kauffman, K. M., McLean, J. S., & He, X. (2024). The oral microbiome: diversity, biogeography and human health. *Nature Reviews Microbiology*, *22*(2), 89–104. https://doi.org/10.1038/s41579-023-00963-6 ⏎

Bertollo, A. G., Santos, C. F., Bagatini, M. D., & Ignácio, Z. M. (2025). Hypothalamus–pituitary–adrenal and gut-brain axes in biological

interaction pathway of the depression. *Frontiers in Neuroscience*, *19*, 1541075. https://doi.org/10.3389/fnins.2025.1541075 ⏎

Borkent, J., Ioannou, M., Neijzen, D., Haarman, B. C. M., & Sommer, I. E. C. (2024). Probiotic formulation for patients with bipolar or schizophrenia spectrum disorder: a double-blind, randomized placebo-controlled trial. *Schizophrenia Bulletin, 51(6)*, 1568–1580. https://doi.org/10.1093/schbul/sbae188 ⏎

Borrego-Ruiz, A., & Borrego, J. J. (2025). Early life stress and gut microbiome dysbiosis: a narrative review. *Stresses*, *5*(2), 38. https://doi.org/10.3390/stresses5020038 ⏎

Bowie, C. R., Bell, M. D., Fiszdon, J. M., Johannesen, J. K., Lindenmayer, J.-P., McGurk, S. R., Medalia, A. A., Penadés, R., Saperstein, A. M., Twamley, E. W., Ueland, T., & Wykes, T. (2020). Cognitive remediation for schizophrenia: an expert working group white paper on core techniques. *Schizophrenia Research*, *215*, 49–53. https://doi.org/10.1016/j.schres.2019.10.047 ⏎

Buciuc, A.-G., Tapoi, C., Sindrilaru, M.-I.-T., Grecu, A., & Petcu, C. (2022). Prodromal phase and first episode psychosis in schizophrenia: early signs and diagnosis. *European Psychiatry*, *65*(S1), S777–S778. https://doi.org/10.1192/j.eurpsy.2022.2008 ⏎

Budden, K. F., Gellatly, S. L., Wood, D. L. A., Cooper, M. A., Morrison, M., Hugenholtz, P., & Hansbro, P. M. (2017). Emerging pathogenic links between microbiota and the gut–lung axis. *Nature Reviews Microbiology*, 15(1), 55–63.

Budden, K. F., Shukla, S. D., Rehman, S. F., Bowerman, K. L., Keely, S., Hugenholtz, P., Armstrong-James, D. P. H., Adcock, I. M., Chotirmall, S. H., Chung, K. F., & Hansbro, P. M. (2019). Functional effects of the microbiota in chronic respiratory disease. *Lancet Respiratory Medicine*, *7*(10), 907–920. https://doi.org/10.1016/S2213-2600(18)30510-1 ⏎

Can, S. Y., & Budak, F. K. (2025). The effect of cognitive behavioural therapy–based psychoeducation on medication adherence and aggression in individuals diagnosed with schizophrenia: an experimental study.

Journal of Psychiatric and Mental Health Nursing, *32*(2), 445–456. https://doi.org/10.1111/jpm.13127 ⏎

Cardoner, N., Gutiérrez-Rojas, L., Saiz, P., Lahera, G., Álvarez-Mon, M. Á., Alonso Ortega, P., & Pérez-Páramo, M. (2025). Does pregabalin offer potential as a first-line therapy for generalized anxiety disorder? A meta-analysis of efficacy, safety, and cost-effectiveness. *Frontiers in Pharmacology*, *16*, 1483770. https://doi.org/10.3389/fphar.2025.1483770 ⏎

Carlessi, A. S., Borba, L. A., Zugno, A. I., Quevedo, J., & Réus, G. Z. (2021). Gut microbiota–brain axis in depression: the role of neuroinflammation. *European Journal of Neuroscience*, *53*(1), 222–235. https://doi.org/10.1111/ejn.14631 ⏎

Chen, L., Fukuda, A. M., Jiang, S., Leuchter, M. K., van Rooij, S. J. H., Widge, A. S., McDonald, W. M., & Carpenter, L. L. (2025a). Treating depression with repetitive transcranial magnetic stimulation: a clinician's guide. *American Journal of Psychiatry*, *182*(6), 525–541. https://doi.org/10.1176/appi.ajp.20240859 ⏎

Chen, T. P., Yu, H. C., Lin, W. Y., & Chang, Y. C. (2025b). Bidirectional association between obesity and chronic periodontitis: inflammatory pathways and clinical implications. *Journal of Dental Sciences*, *20*(4), 2021–2025. https://doi.org/10.1016/J.JDS.2025.07.009

Chokhawala, K., & Stevens, L. (2023). *Antipsychotic Medications*. StatPearls Publishing.

Coello, K., Hansen, T. H., Sørensen, N., Munkholm, K., Kessing, L. V., Pedersen, O., & Vinberg, M. (2019). Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives. *Brain, Behavior, and Immunity*, *75*, 112–118. https://doi.org/10.1016/j.bbi.2018.09.026 ⏎

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., Denou, E., Silva, M. A., Santacruz, A., Sanz, Y., Surette, M. G., Verdu, E. F., Collins, S. M., & Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice.

Nature Communications, *6*(1), 7735. https://doi.org/10.1038/ncomms8735 ⏎

Deo, P. N., & Deshmukh, R. (2019). Oral microbiome: unveiling the fundamentals. *Journal of Oral and Maxillofacial Pathology*, *23*(1), 122–128. https://doi.org/10.4103/JOMFP.JOMFP_304_18 ⏎

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*(19), 5002–5017. https://doi.org/10.1128/jb.00542-10 ⏎

Di Napoli, A., Pasquini, L., Visconti, E., Vaccaro, M., Rossi-Espagnet, M. C., & Napolitano, A. (2025). Gut-brain axis and neuroplasticity in health and disease: a systematic review. *Radiologia Medica*, *130*(3), 327–358. https://doi.org/10.1007/s11547-024-01938-0 ⏎

Drew, G. C., Stevens, E. J., & King, K. C. (2021). Microbial evolution and transitions along the parasite–mutualist continuum. *Nature Reviews. Microbiology*, *19*(10), 623. https://doi.org/10.1038/S41579-021-00550-7 ⏎

Eisenstein, M. (2020). The hunt for a healthy microbiome. *Nature*, *577*(7792), S6–S8. https://doi.org/10.1038/D41586-020-00193-3 ⏎

Escudero, M. A. G., Gutiérrez-Rojas, L., & Lahera, G. (2020). Second generation antipsychotics monotherapy as maintenance treatment for bipolar disorder: a systematic review of long-term studies. *Psychiatric Quarterly*, *91*(4), 1047–1060. https://doi.org/10.1007/s11126-020-09753-2 ⏎

Fagan, H. A., & Baldwin, D. S. (2023). Pharmacological treatment of generalised anxiety disorder: current practice and future directions. *Expert Review of Neurotherapeutics*, *23*(6), 535–548. https://doi.org/10.1080/14737175.2023.2211767 ⏎

Ferguson, M., Rhodes, K., Loughhead, M., McIntyre, H., & Procter, N. (2022). The effectiveness of the safety planning intervention for adults experiencing suicide-related distress: a systematic review. *Archives of*

Suicide Research, *26*(3), 1022–1045. https://doi.org/10.1080/13811118.2021.1915217 ⏎

Flückiger, C., Carratta, K., Del Re, A. C., Probst, G., Vîslă, A., Gómez Penedo, J. M., & Wampold, B. E. (2022). The relative efficacy of bona fide cognitive behavioral therapy and applied relaxation for generalized anxiety disorder at follow-up: a longitudinal multilevel meta-analysis. *Journal of Consulting and Clinical Psychology*, *90*(4), 339–352. https://doi.org/10.1037/ccp0000717 ⏎

Fox, A. B., & Earnshaw, V. A. (2023). The relationship between mental illness stigma and self-labeling. *Psychiatric Rehabilitation Journal*, *46*(2), 127–136. https://doi.org/10.1037/prj0000552 ⏎

Frederick, D. E., & VanderWeele, T. J. (2019). Supported employment: meta-analysis and review of randomized controlled trials of individual placement and support. *PLoS One*, *14*(2), e0212208. https://doi.org/10.1371/journal.pone.0212208 ⏎

Gabbay, V., Ely, B. A., Vileisis, J. N., Petrovic, Z., Cicvaric, A., Asnis, G. M., Kim-Schulze, S., & Radulovic, J. (2024). Immune and neural response to acute social stress in adolescent humans and rodents. *Translational Psychiatry*, *14*(1), 306. https://doi.org/10.1038/s41398-024-03008-5 ⏎

Gal, Z., Torok, D., Gonda, X., Eszlari, N., Anderson, I. M., Deakin, B., Juhasz, G., Bagdy, G., & Petschner, P. (2023). Inflammation and blood–brain barrier in depression: interaction of *CLDN5* and *IL6* gene variants in stress-induced depression. *International Journal of Neuropsychopharmacology*, *26*(3), 189–197. https://doi.org/10.1093/ijnp/pyac079 ⏎

Gao, K., Oruc, E. B., & Koparal, B. (2025). Pharmacological monotherapy for depressive disorders: current and future—a narrative review. *Medicina*, *61*(4), 558. https://doi.org/10.3390/medicina61040558 ⏎

Gnanapragasam, S. N., Astill Wright, L., Pemberton, M., & Bhugra, D. (2023). Outside/inside: social determinants of mental health. *Irish*

Journal of Psychological Medicine, *40*(1), 63–73. https://doi.org/10.1017/ipm.2021.49 ⏎

Govender, P., & Ghai, M. (2025). Population-specific differences in the human microbiome: factors defining the diversity. *Gene*, *933*, 148923. https://doi.org/10.1016/J.GENE.2024.148923 ⏎

Greenwood, T. A. (2020). Creativity and bipolar disorder: a shared genetic vulnerability. *Annual Review of Clinical Psychology*, *16*(1), 239–264. https://doi.org/10.1146/annurev-clinpsy-050718-095449 ⏎

Grunze, H., & Born, C. (2020). The impact of subsyndromal bipolar symptoms on patient's functionality and quality of life. *Frontiers in Psychiatry*, *11*, 510. https://doi.org/10.3389/fpsyt.2020.00510 ⏎

Guaiana, G., Abbatecola, M., Aali, G., Tarantino, F., Ebuenyi, I. D., Lucarini, V., Li, W., Zhang, C., & Pinto, A. (2022). Cognitive behavioural therapy (group) for schizophrenia. *Cochrane Database of Systematic Reviews*, *2022*(7), CD009608. https://doi.org/10.1002/14651858.CD009608.pub2 ⏎

Haenen, N., Kamperman, A. M., Prodan, A., Nolen, W. A., Boks, M. P., & Wesseloo, R. (2024). The efficacy of lamotrigine in bipolar disorder: a systematic review and meta-analysis. *Bipolar Disorders*, *26*(5), 431–441. https://doi.org/10.1111/bdi.13452 ⏎

Hamm, A. O. (2020). Fear, anxiety, and their disorders from the perspective of psychophysiology. *Psychophysiology*, *57*(2), e13474. https://doi.org/10.1111/psyp.13474 ⏎

Harris, A. (2023). Approach to schizophrenia. *Internal Medicine Journal*, *53*(4), 473–480. https://doi.org/10.1111/imj.16068 ⏎

Hautzinger, M. (2024). Adjuvant psychotherapies to prevent relapse in bipolar disorder. *JAMA Psychiatry*, *81*(9), 855. https://doi.org/10.1001/jamapsychiatry.2024.1310 ⏎

Henry, M. (2021). Suicide prevention: a multisectorial public health concern. *Preventive Medicine*, *152*, 106772. https://doi.org/10.1016/j.ypmed.2021.106772 ⏎

Hoge, E. A., Bui, E., Mete, M., Dutton, M. A., Baker, A. W., & Simon, N. M. (2023). Mindfulness-based stress reduction vs escitalopram for the treatment of adults with anxiety disorders. *JAMA Psychiatry*, *80*(1), 13. https://doi.org/10.1001/jamapsychiatry.2022.3679 ⏎

Holt-Lunstad, J. (2024). Social connection as a critical factor for mental and physical health: evidence, trends, challenges, and future implications. *World Psychiatry*, *23*(3), 312–332. https://doi.org/10.1002/wps.21224 ⏎

Hsieh, M. H. (2023). *Electroconvulsive therapy for treatment-resistant depression* (pp. 69–90). https://doi.org/10.1016/bs.pbr.2023.01.004 ⏎

Huang, E., Kang, S., Park, H., Park, S., Ji, Y., & Holzapfel, W. H. (2018). Differences in anxiety levels of various murine models in relation to the gut microbiota composition. *Biomedicines*, *6*(4), 113. https://doi.org/10.3390/biomedicines6040113 ⏎

Huang, S., Zhou, Y., Ji, H., Zhang, T., Liu, S., Ma, L., Deng, D., Ding, Y., Han, L., Shu, S., Wang, Y., & Chen, X. (2025). Decoding mechanisms and protein markers in lung–brain axis. *Respiratory Research*, *26*(1), 190. https://doi.org/10.1186/S12931-025-03272-Z ⏎

Huang, T., Shang, Y., Dai, C., Zhang, Q., Hu, S., & Xie, J. (2023). Gut microbiota and its relation to inflammation in patients with bipolar depression: a cross-sectional study. *Annals of General Psychiatry*, *22*(1), 21. https://doi.org/10.1186/s12991-023-00453-2 ⏎

IsHak, W. W., Mirocha, J., Dang, J., Vanle, B., Metrikin, B., Tessema, K., & Danovitch, I. (2024). Quality of life and functioning impairments across psychiatric disorders in adults presenting for outpatient psychiatric evaluation and treatment. *Psychiatric Research and Clinical Practice*, *6*(3), 68–77. https://doi.org/10.1176/appi.prcp.20230064 ⏎

Jain, A., Mitra, P. (2025). Bipolar Disorder. [Updated 2023 Feb 20]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558998/.

Jiang, H., Zhang, X., Yu, Z., Zhang, Z., Deng, M., Zhao, J., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized

anxiety disorder. *Journal of Psychiatric Research*, *104*, 130–136. https://doi.org/10.1016/j.jpsychires.2018.07.007 ⏎

Jiang, Y., Zou, D., Li, Y., Gu, S., Dong, J., Ma, X., Xu, S., Wang, F., & Huang, J. H. (2022). Monoamine neurotransmitters control basic emotions and affect major depressive disorders. *Pharmaceuticals*, *15*(10), 1203. https://doi.org/10.3390/ph15101203 ⏎

Jung, W., Moon, E., Lim, H. J., Park, J. M., Lee, B. D., Lee, Y. M., Jeong, H., Suh, H., & Kim, K. (2024). The dropout rates and associated factors in patients with mood disorders in long-term naturalistic treatment. *Clinical Psychopharmacology and Neuroscience*, *22*(2), 263–275. https://doi.org/10.9758/cpn.23.1089 ⏎

Karrouri, R., Hammani, Z., Benjelloun, R., & Otheman, Y. (2021). Major depressive disorder: Validated treatments and future challenges. *World Journal of Clinical Cases*, *9*(31), 9350–9367. https://doi.org/10.12998/wjcc.v9.i31.9350 ⏎

Kelly, J. R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P. J., Beers, S., Scott, K., Moloney, G., Hoban, A. E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J. F., & Dinan, T. G. (2016). Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research*, *82*, 109–118. https://doi.org/10.1016/j.jpsychires.2016.07.019 ⏎

Kim, J.-W., Suzuki, K., Kavalali, E. T., & Monteggia, L. M. (2024). Ketamine: mechanisms and relevance to treatment of depression. *Annual Review of Medicine*, *75*(1), 129–143. https://doi.org/10.1146/annurev-med-051322-120608 ⏎

Kim, Y. H., Jang, J., Kang, N., Jeong, J. H., Kim, J., Ahn, Y. M., Kim, Y. S., & Kim, S. H. (2025). Revealing differential psychotic symptoms in schizophrenia and bipolar I disorder by manifold learning and network analyses. *Translational Psychiatry*, *15*(1), 194. https://doi.org/10.1038/s41398-025-03403-6 ⏎

Kirsch, D. E., Preston, A., Tretyak, V., Le, V., Weber, W., Strakowski, S. M., & Lippard, E. T. C. (2022). Neural functional connectivity changes to psychosocial stress in young adults with bipolar disorder and preliminary associations with clinical trajectories. *Bipolar Disorders*, *24*(3), 298–309. https://doi.org/10.1111/bdi.13127 ⏎

Li, R., Li, J., & Zhou, X. (2024). Lung microbiome: new insights into the pathogenesis of respiratory diseases. *Signal Transduction and Targeted Therapy*, *9*(1), 19. https://doi.org/10.1038/s41392-023-01722-y ⏎

Li, W., Liang, H., Lin, X., Hu, T., Wu, Z., He, W., Wang, M., Zhang, J., Jie, Z., Jin, X., Xu, X., Wang, J., Yang, H., Zhang, W., Kristiansen, K., Xiao, L., & Zou, Y. (2023). A catalog of bacterial reference genomes from cultivated human oral bacteria. *npj Biofilms and Microbiomes*, *9*(1), 1–13. https://doi.org/10.1038/s41522-023-00414-3 ⏎

Lieberman, J. A., & Kane, J. M. (2009). *Kaplan & Sadock's Comprehensive Textbook of Psychiatry* (9th ed., Vol. 2). Lippincott Williams & Wilkins. ⏎

Lin, D., Fu, Z., Liu, J., Perrone-Bizzozero, N., Hutchison, K. E., Bustillo, J., Du, Y., Pearlson, G., & Calhoun, V. D. (2023). *Association between the oral microbiome and brain resting state connectivity in schizophrenia*. https://doi.org/10.1101/2023.12.22.573165 ⏎

Little, B., Flowers, C., Blamire, A., Thelwall, P., Taylor, J., Gallagher, P., Cousins, D. A., & Wang, Y. (2024). Multivariate brain-cognition associations in euthymic bipolar disorder. *Bipolar Disorders*, *26*(6), 604–616. https://doi.org/10.1111/bdi.13484 ⏎

Liu, B., Faller, L. L., Klitgord, N., Mazumdar, V., Ghodsi, M., Sommer, D. D., Gibbons, T. R., Treangen, T. J., Chang, Y. C., Li, S., Stine, O. C., Hasturk, H., Kasif, S., Segrè, D., Pop, M., & Amar, S. (2012). Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PLoS One*, *7*(6), e37919. https://doi.org/10.1371/JOURNAL.PONE.0037919 ⏎

Liu, L., Wang, H., Chen, X., Zhang, Y., Zhang, H., & Xie, P. (2023). Gut microbiota and its metabolites in depression: from pathogenesis to

treatment. *EBioMedicine*, *90*, 104527. https://doi.org/10.1016/j.ebiom.2023.104527 ⏎

Liu, S., Guo, R., Liu, F., Yuan, Q., Yu, Y., & Ren, F. (2020). Gut microbiota regulates depression-like behavior in rats through the neuroendocrine-immune-mitochondrial pathway. *Neuropsychiatric Disease and Treatment*, *16*, 859–869. https://doi.org/10.2147/NDT.S243551 ⏎

Lotfinia, S., Shahkaram, H., Yaseri, A., Kianimoghadam, A. S., Sarani Yaztappeh, J., & Masjedi Arani, A. (2025). A clinical trial comparing the efficacy of two EEG-based neurofeedback protocols for generalized anxiety disorder: sensory motor rhythm and alpha-theta. *Iranian Journal of Psychiatry and Behavioral Sciences*, *19*(2), e158451. https://doi.org/10.5812/ijpbs-158451 ⏎

Lucidi, L., Pettorruso, M., Vellante, F., Di Carlo, F., Ceci, F., Santovito, M. C., Di Muzio, I., Fornaro, M., Ventriglio, A., Tomasetti, C., Valchera, A., Gentile, A., Kim, Y.-K., Martinotti, G., Fraticelli, S., Di Giannantonio, M., & De Berardis, D. (2021). Gut microbiota and bipolar disorder: an overview on a novel biomarker for diagnosis and treatment. *International Journal of Molecular Sciences*, *22*(7), 3723. https://doi.org/10.3390/ijms22073723 ⏎

Macrea, C., Ilias, T., Fratila, O., Roxana, B., & Hocopan, C. (2023). Probiotics and the microbiota–gut–brain axis: focus on patients with depression. A review of current research. *Biomedical Papers*, *167*(3), 219–224. https://doi.org/10.5507/bp.2023.024

Malan-Müller, S., Valles-Colomer, M., Palomo, T., & Leza, J. C. (2023). The gut–microbiota–brain axis in a Spanish population in the aftermath of the COVID-19 pandemic: microbiota composition linked to anxiety, trauma, and depression profiles. *Gut Microbes*, *15*(1), 2162306. https://doi.org/10.1080/19490976.2022.2162306 ⏎

Malard, F., Dore, J., Gaugler, B., & Mohty, M. (2021). Introduction to host microbiome symbiosis in health and disease. *Mucosal Immunology*, *14*(3), 547–554. https://doi.org/10.1038/S41385-020-00365-4 ⏎

Mallard, T. T., Grotzinger, A. D., & Smoller, J. W. (2023). Examining the shared etiology of psychopathology with genome-wide association studies. *Physiological Reviews*, *103*(2), 1645–1665. https://doi.org/10.1152/physrev.00016.2022 ⏎

Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: a proposal. *Microbiome*, *3*(1), 31. https://doi.org/10.1186/s40168-015-0094-5 ⏎

Marsland, B. J., Trompette, A., & Gollwitzer, E. S. (2015). The Gut–Lung Axis in Respiratory Disease. *Annals of the American Thoracic Society*, *12*(Supplement_2), S150–S156. https://doi.org/10.1513/AnnalsATS.201503-133AW

Martin-Key, N. A., Mirea, D.-M., Olmert, T., Cooper, J., Han, S. Y. S., Barton-Owen, G., Farrag, L., Bell, E., Eljasz, P., Cowell, D., Tomasik, J., & Bahn, S. (2021). Toward an extended definition of major depressive disorder symptomatology: digital assessment and cross-validation study. *JMIR Formative Research*, *5*(10), e27908. https://doi.org/10.2196/27908 ⏎

McGuinness, A. J., Davis, J. A., Dawson, S. L., Loughman, A., Collier, F., O'Hely, M., Simpson, C. A., Green, J., Marx, W., Hair, C., Guest, G., Mohebbi, M., Berk, M., Stupart, D., Watters, D., & Jacka, F. N. (2022). A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Molecular Psychiatry*, *27*(4), 1920–1935. https://doi.org/10.1038/s41380-022-01456-3 ⏎

McIntyre, R. S., Subramaniapillai, M., Shekotikhina, M., Carmona, N. E., Lee, Y., Mansur, R. B., Brietzke, E., Fus, D., Coles, A. S., Iacobucci, M., Park, C., Potts, R., Amer, M., Gillard, J., James, C., Anglin, R., & Surette, M. G. (2021). Characterizing the gut microbiota in adults with bipolar disorder: a pilot study. *Nutritional Neuroscience*, *24*(3), 173–180. https://doi.org/10.1080/1028415X.2019.1612555 ⏎

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Palacio, S. D., Montes, S. A., Mancabelli, L., Lugli, G. A.,

Rodriguez, J. M., Bode, L., de Vos, W., Gueimonde, M., Margolles, A., van Sinderen, D., & Ventura, M. (2017). The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews: MMBR*, *81*(4), e00036-17. https://doi.org/10.1128/MMBR.00036-17 ⏎

Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L., & Samochowiec, J. (2020). The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *102*, 109951. https://doi.org/10.1016/j.pnpbp.2020.109951 ⏎

Montiel-Castro, A. J., González-Cervantes, R. M., Bravo-Ruiseco, G., & Pacheco-López, G. (2013). The microbiota–gut–brain axis: neurobehavioral correlates, health and sociality. *Frontiers in Integrative Neuroscience*, *7*, 70. https://doi.org/10.3389/fnint.2013.00070 ⏎

Moon, E., Chang, J. S., Kim, M. Y., Seo, M. H., Cha, B., Ha, T. H., Choi, S., Cho, H. S., Park, T., & Ha, K. (2012). Dropout rate and associated factors in patients with bipolar disorders. *Journal of Affective Disorders*, *141*(1), 47–54. https://doi.org/10.1016/j.jad.2012.02.025 ⏎

Morneau-Vaillancourt, G., Palaiologou, E., Polderman, T. J. C., & Eley, T. C. (2025). Research review: a review of the past decade of family and genomic studies on adolescent mental health. *Journal of Child Psychology and Psychiatry*, *66*(6), 910–927. https://doi.org/10.1111/jcpp.14099 ⏎

Morrison, L., & Frank, C. J. (2023). Social determinants of mental and behavioral health. *Primary Care: Clinics in Office Practice*, *50*(4), 679–688. https://doi.org/10.1016/j.pop.2023.04.003 ⏎

Mullins, N., Forstner, A. J., O'Connell, K. S., Coombes, B., Coleman, J. R. I., Qiao, Z., Als, T. D., Bigdeli, T. B., Børte, S., Bryois, J., Charney, A. W., Drange, O. K., Gandal, M. J., Hagenaars, S. P., Ikeda, M., Kamitaki, N., Kim, M., Krebs, K., Panagiotaropoulou, G., … Andreassen, O. A. (2021). Genome-wide association study of more than 40,000 bipolar disorder cases provides new insights into the underlying biology. *Nature*

Genetics, *53*(6), 817–829. https://doi.org/10.1038/s41588-021-00857-4 ⏎

Myran, D. T., Harrison, L. D., Pugliese, M., Solmi, M., Anderson, K. K., Fiedorowicz, J. G., Perlman, C. M., Webber, C., Finkelstein, Y., & Tanuseputro, P. (2023). Transition to schizophrenia spectrum disorder following emergency department visits due to substance use with and without psychosis. *JAMA Psychiatry*, *80*(11), 1169. https://doi.org/10.1001/jamapsychiatry.2023.3582 ⏎

Nierenberg, A. A., Agustini, B., Köhler-Forsberg, O., Cusin, C., Katz, D., Sylvia, L. G., Peters, A., & Berk, M. (2023). Diagnosis and treatment of bipolar disorder. *JAMA*, *330*(14), 1370. https://doi.org/10.1001/jama.2023.18588 ⏎

Nieto, L., Domínguez-Martínez, T., Navarrete, L., Rosel-Vales, M., Saracco-Álvarez, R., Celada-Borja, C., Rascón-Gasca, M. L., & Samperio, L. G. M. (2023). The effect of stressful life events on the risk for psychosis: differences between Mexican at clinical and familial high risk. *Frontiers in Psychiatry*, *14*, 1254993. https://doi.org/10.3389/fpsyt.2023.1254993 ⏎

Nikolaus, S., Müller, H.-W., & Hautzel, H. (2017). Different patterns of dopaminergic and serotonergic dysfunction in manic, depressive and euthymic phases of bipolar disorder. *Nuklearmedizin*, *56*(05), 191–200. https://doi.org/10.3413/Nukmed-0893-17-04 ⏎

Oddo, V. M., Welke, L., McLeod, A., Pezley, L., Xia, Y., Maki, P., Koenig, M. D., Kominiarek, M. A., Langenecker, S., & Tussing-Humphreys, L. (2022). Adherence to a Mediterranean diet is associated with lower depressive symptoms among U.S. adults. *Nutrients*, *14*(2), 278. https://doi.org/10.3390/nu14020278 ⏎

Ogunrinola, G. A., Oyewale, J. O., Oshamika, O. O., & Olasehinde, G. I. (2020). The human microbiome and its impacts on health. *International Journal of Microbiology*, *2020*(1), 8045646. https://doi.org/10.1155/2020/8045646 ⏎

O'Leary, K. B., & Khan, J. S. (2024). Pharmacotherapy for anxiety disorders. *Psychiatric Clinics of North America*, *47*(4), 689–709. https://doi.org/10.1016/j.psc.2024.04.012 ⏎

Olgiati, P., Fanelli, G., & Serretti, A. (2023). Clinical correlates and prognostic implications of severe suicidal ideation in major depressive disorder. *International Clinical Psychopharmacology*, *38*(4), 201–208. https://doi.org/10.1097/YIC.0000000000000461 ⏎

Oliva, V., Fico, G., De Prisco, M., Gonda, X., Rosa, A. R., & Vieta, E. (2025). Bipolar disorders: an update on critical aspects. *Lancet Regional Health—Europe*, *48*, 101135. https://doi.org/10.1016/j.lanepe.2024.101135 ⏎

Painold, A., Mörkl, S., Kashofer, K., Halwachs, B., Dalkner, N., Bengesser, S., Birner, A., Fellendorf, F., Platzer, M., Queissner, R., Schütze, G., Schwarz, M. J., Moll, N., Holzer, P., Holl, A. K., Kapfhammer, H., Gorkiewicz, G., & Reininghaus, E. Z. (2019). A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disorders*, *21*(1), 40–49. https://doi.org/10.1111/bdi.12682 ⏎

Pan, B., Pan, Y., Huang, Y.-S., Yi, M., Hu, Y., Lian, X., Shi, H.-Z., Wang, M., Xiang, G., Yang, W.-Y., Liu, Z., & Xia, F. (2025). Efficacy and safety of gut microbiome-targeted treatment in patients with depression: a systematic review and meta-analysis. *BMC Psychiatry*, *25*(1), 64. https://doi.org/10.1186/s12888-024-06438-z ⏎

Papola, D., Miguel, C., Mazzaglia, M., Franco, P., Tedeschi, F., Romero, S. A., Patel, A. R., Ostuzzi, G., Gastaldon, C., Karyotaki, E., Harrer, M., Purgato, M., Sijbrandij, M., Patel, V., Furukawa, T. A., Cuijpers, P., & Barbui, C. (2024). Psychotherapies for generalized anxiety disorder in adults. *JAMA Psychiatry*, *81*(3), 250. https://doi.org/10.1001/jamapsychiatry.2023.3971 ⏎

Parker, G., Spoelma, M. J., & Rhodes, N. (2022). Faecal microbiota transplantation for bipolar disorder: a detailed case study. *Bipolar Disorders*, *24*(5), 559–563. https://doi.org/10.1111/bdi.13187 ⏎

Pastis, I., Santos, M. G., & Paruchuri, A. (2024). Exploring the role of inflammation in major depressive disorder: beyond the monoamine hypothesis. *Frontiers in Behavioral Neuroscience*, *17*, 1282242. https://doi.org/10.3389/fnbeh.2023.1282242 ⏎

Pereira, I. M. B., Mantovani, L. M., Frota, G. A., Wenceslau, R. R., Matos, J. C., Cruz, B. F., Teixeira, A. L., & Barbosa, I. G. (2025). Social functioning in bipolar disorder: investigating the role played by comorbid physical illnesses and cognition. *Dementia & Neuropsychologia*, *19*, e20240188. https://doi.org/10.1590/1980-5764-dn-2024-0188 ⏎

Powers AR, Addington J, Perkins DO, Bearden CE, Cadenhead KS, Cannon TD, Cornblatt BA, Mathalon DH, Seidman LJ, Tsuang MT, Walker EF, McGlashan TH, Woods SW. (2020). Duration of the psychosis prodrome. *Schizophr Res*. *216*:443–449. doi: 10.1016/j.schres.2019.10.051

Rabelo, J. L., Cruz, B. F., Ferreira, J. D. R., Viana, B. de M., & Barbosa, I. G. (2021). Psychoeducation in bipolar disorder: a systematic review. *World Journal of Psychiatry*, *11*(12), 1407–1424. https://doi.org/10.5498/wjp.v11.i12.1407 ⏎

Rashid, M. H., Kumar, S. P., Rajan, R., & Mamillapalli, A. (2025). Salivary microbiota dysbiosis and elevated polyamine levels contribute to the severity of periodontal disease. *BMC Oral Health*, *25*(1), 1–11. https://doi.org/10.1186/S12903-024-05381-5/FIGURES/4 ⏎

Reddy, S., Kabotyanski, K. E., Hirani, S., Liu, T., Naqvi, Z., Giridharan, N., Hasen, M., Provenza, N. R., Banks, G. P., Mathew, S. J., Goodman, W. K., & Sheth, S. A. (2024). Efficacy of deep brain stimulation for treatment-resistant depression: systematic review and meta-analysis. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *9*(12), 1239–1248. https://doi.org/10.1186/s12903-024-05381-5 ⏎

Reininghaus, E. Z., Wetzlmair, L.-C., Fellendorf, F. T., Platzer, M., Queissner, R., Birner, A., Pilz, R., Hamm, C., Maget, A., Koidl, C., Riedrich, K., Klampfer, K., Ferk, K., & Dalkner, N. (2020a). The impact

of probiotic supplements on cognitive parameters in euthymic individuals with bipolar disorder: a pilot study. *Neuropsychobiology*, *79*(1), 63–70. https://doi.org/10.1159/000492537 ⏎

Reininghaus, E. Z., Wetzlmair, L.-C., Fellendorf, F. T., Platzer, M., Queissner, R., Birner, A., Pilz, R., Hamm, C., Maget, A., Rieger, A., Prettenhofer, A., Wurm, W., Mörkl, S., & Dalkner, N. (2020b). Probiotic treatment in individuals with euthymic bipolar disorder: a pilot-study on clinical changes and compliance. *Neuropsychobiology*, *79*(1), 71–79. https://doi.org/10.1159/000493867 ⏎

Requena, T., & Velasco, M. (2021). The human microbiome in sickness and in health. *Revista Clínica Española (English Edition)*, *221*(4), 233–240. https://doi.org/10.1016/J.RCENG.2019.07.018 ⏎

Réus, G. Z., Manosso, Luana. M., Quevedo, J., & Carvalho, A. F. (2023). Major depressive disorder as a neuro-immune disorder: origin, mechanisms, and therapeutic opportunities. *Neuroscience & Biobehavioral Reviews*, *155*, 105425. https://doi.org/10.1016/j.neubiorev.2023.105425 ⏎

Reyes-Martínez, S., Segura-Real, L., Gómez-García, A. P., Tesoro-Cruz, E., Constantino-Jonapa, L. A., Amedei, A., & Aguirre-García, M. M. (2023). Neuroinflammation, microbiota–gut–brain axis, and depression: the vicious circle. *Journal of Integrative Neuroscience*, *22*(3), 65. https://doi.org/10.31083/j.jin2203065 ⏎

Rhee, T. G., Gillissie, E. S., Nierenberg, A. A., & McIntyre, R. S. (2023). Association of current and remitted bipolar disorders with health-related quality of life: findings from a nationally representative sample in the US. *Journal of Affective Disorders*, *321*, 33–40. https://doi.org/10.1016/j.jad.2022.10.025 ⏎

Richmond-Rakerd, L. S., D'Souza, S., Milne, B. J., Caspi, A., & Moffitt, T. E. (2021). Longitudinal associations of mental disorders with physical diseases and mortality among 2.3 million New Zealand citizens. *JAMA Network Open*, *4*(1), e2033448. https://doi.org/10.1001/jamanetworkopen.2020.33448 ⏎

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, *7*(1), 14. https://doi.org/10.3390/MICROORGANISMS7010014 ⏎

Rognli, E. B., Heiberg, I. H., Jacobsen, B. K., Høye, A., & Bramness, J. G. (2023). Transition from substance-induced psychosis to schizophrenia spectrum disorder or bipolar disorder. *American Journal of Psychiatry*, *180*(6), 437–444. https://doi.org/10.1176/appi.ajp.22010076 ⏎

Rosen, J. B., & Schulkin, J. (2022). Hyperexcitability: from normal fear to pathological anxiety and trauma. *Frontiers in Systems Neuroscience*, *16*, 727054. https://doi.org/10.3389/fnsys.2022.727054 ⏎

Salahuddin, N. H., Schütz, A., Pitschel-Walz, G., Mayer, S. F., Chaimani, A., Siafis, S., Priller, J., Leucht, S., & Bighelli, I. (2024). Psychological and psychosocial interventions for treatment-resistant schizophrenia: a systematic review and network meta-analysis. *Lancet Psychiatry*, *11*(7), 545–553. https://doi.org/10.1016/S2215-0366(24)00136-6 ⏎

Santamaría-García, H., Migeot, J., Medel, V., Hazelton, J. L., Teckentrup, V., Romero-Ortuno, R., Piguet, O., Lawor, B., Northoff, G., & Ibanez, A. (2025). Allostatic interoceptive overload across psychiatric and neurological conditions. *Biological Psychiatry*, *97*(1), 28–40. https://doi.org/10.1016/j.biopsych.2024.06.024 ⏎

Schaub, A.-C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., Doll, J. P. K., Yamanbaeva, G., Mählmann, L., Brand, S., Beglinger, C., Borgwardt, S., Raes, J., Schmidt, A., & Lang, U. E. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. *Translational Psychiatry*, *12*(1), 227. https://doi.org/10.1038/s41398-022-01977-z ⏎

Schiulaz, A., Nordio, G., Giacomel, A., Easmin, R., Bettinelli, A., Selvaggi, P., Williams, S., Turkheimer, F., Jauhar, S., Howes, O., Veronese, M., Angelescu, I., Bloomfield, M., Bonoldi, I., Borgan, F.,

Dahoun, T., D'Ambrosio, E., Demjaha, A., Donocik, J., … Vano, L. (2025). Radiomic analysis of striatal [18F]FDOPA PET imaging in patients with psychosis for the identification of antipsychotic response. *Molecular Imaging and Biology*, *27*(3), 365–378. https://doi.org/10.1007/s11307-025-02014-3 ⏎

Scott, J., Graham, A., Yung, A., Morgan, C., Bellivier, F., & Etain, B. (2022). A systematic review and meta-analysis of delayed help-seeking, delayed diagnosis and duration of untreated illness in bipolar disorders. *Acta Psychiatrica Scandinavica*, *146*(5), 389–405. https://doi.org/10.1111/acps.13490 ⏎

Shah, A. B., Cho, H., & Shim, S. H. (2025). Exploring the bioactive landscape: peptides and non-peptides from the human microbiota. *npj Biofilms and Microbiomes 2025 11:1*, *11*(1), 1–22. https://doi.org/10.1038/s41522-025-00713-x ⏎

Sigvard, A. K., Bojesen, K. B., Ambrosen, K. S., Nielsen, M. Ø., Gjedde, A., Tangmose, K., Kumakura, Y., Edden, R., Fuglø, D., Jensen, L. T., Rostrup, E., Ebdrup, B. H., & Glenthøj, B. Y. (2023). Dopamine synthesis capacity and GABA and glutamate levels separate antipsychotic-naïve patients with first-episode psychosis from healthy control subjects in a multimodal prediction model. *Biological Psychiatry Global Open Science*, *3*(3), 500–509. https://doi.org/10.1016/j.bpsgos.2022.05.004 ⏎

Šimić, G., Tkalčić, M., Vukić, V., Mulc, D., Španić, E., Šagud, M., Olucha-Bordonau, F. E., Vukšić, M., & R. Hof, P. (2021). Understanding emotions: origins and roles of the amygdala. *Biomolecules*, *11*(6), 823. https://doi.org/10.3390/biom11060823 ⏎

Skallevold, H. E., Rokaya, N., Wongsirichat, N., & Rokaya, D. (2023). Importance of oral health in mental health disorders: an updated review. *Journal of Oral Biology and Craniofacial Research*, *13*(5), 544–552. https://doi.org/10.1016/J.JOBCR.2023.06.003 ⏎

Socała, K., Doboszewska, U., Szopa, A., Serefko, A., Włodarczyk, M., Zielińska, A., Poleszak, E., Fichna, J., & Wlaź, P. (2021). The role of

microbiota–gut–brain axis in neuropsychiatric and neurological disorders. *Pharmacological Research*, *172*, 105840. https://doi.org/10.1016/j.phrs.2021.105840 ⏎

Stephenson, C., Moghimi, E., Shao, Y., Kumar, A., Yee, C. S., Miller, S., Stefatos, A., Gholamzadehmir, M., Abbaspour, Z., Jagayat, J., Shirazi, A., Gizzarelli, T., Gutierrez, G., Khan, F., Patel, C., Patel, A., Yang, M., Omrani, M., & Alavi, N. (2023). Comparing the efficacy of electronic cognitive behavioral therapy to medication and combination therapy for generalized anxiety disorder: a quasi-experimental clinical trial. *Frontiers in Psychiatry*, *14*, 1194955. https://doi.org/10.3389/fpsyt.2023.1194955 ⏎

Strejilevich, S., Szmulewicz, A., Igoa, A., Marengo, E., Caravotta, P., & Martino, D. (2019). Episodic density, subsyndromic symptoms, and mood instability in late-life bipolar disorders: a 5-year follow-up study. *International Journal of Geriatric Psychiatry*, *34*(7), 950–956. https://doi.org/10.1002/gps.5094 ⏎

Sublette, M. E., Cheung, S., Lieberman, E., Hu, S., Mann, J. J., Uhlemann, A., & Miller, J. M. (2021). Bipolar disorder and the gut microbiome: a systematic review. *Bipolar Disorders*, *23*(6), 544–564. https://doi.org/10.1111/bdi.13049 ⏎

Subramaniam, M. (2024). Stigma and mental health: overcoming barriers. *Singapore Medical Journal*, *65*(10), 533–535. https://doi.org/10.4103/singaporemedj.SMJ-2024-166 ⏎

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *Journal of Physiology*, *558*(1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388 ⏎

Sun, Z., Bo, Q., Mao, Z., Li, F., He, F., Pao, C., Li, W., He, Y., Ma, X., & Wang, C. (2021). Reduced plasma dopamine-β-hydroxylase activity is associated with the severity of bipolar disorder: a pilot study. *Frontiers in Psychiatry*, *12*, 566091. https://doi.org/10.3389/fpsyt.2021.566091 ⏎

Sureda, A., Daglia, M., Argüelles Castilla, S., Sanadgol, N., Fazel Nabavi, S., Khan, H., Belwal, T., Jeandet, P., Marchese, A., Pistollato, F., Forbes-Hernandez, T., Battino, M., Berindan-Neagoe, I., D'Onofrio, G., & Nabavi, S. M. (2020). Oral microbiota and Alzheimer's disease: do all roads lead to Rome? *Pharmacological Research*, *151*, 104582. https://doi.org/10.1016/J.PHRS.2019.104582 ⏎

Szuhany, K. L., & Simon, N. M. (2022). Anxiety disorders. *JAMA*, *328*(24), 2431. https://doi.org/10.1001/jama.2022.22744 ⏎

Talapko, J., Erić, S., Meštrović, T., Stipetić, M. M., Juzbašić, M., Katalinić, D., Bekić, S., Muršić, D., Flam, J., Belić, D., Lešić, D., Fureš, R., Markanović, M., & Škrlec, I. (2024). The impact of oral microbiome dysbiosis on the aetiology, pathogenesis, and development of oral cancer. *Cancers*, *16*(17), 2997. https://doi.org/10.3390/CANCERS16172997 ⏎

Terry, N., & Margolis, K. G. (2016). *Serotonergic Mechanisms Regulating the GI Tract: Experimental Evidence and Therapeutic Relevance* (pp. 319–342). https://doi.org/10.1007/164_2016_103 ⏎

Tian, P., Chen, Y., Zhu, H., Wang, L., Qian, X., Zou, R., Zhao, J., Zhang, H., Qian, L., Wang, Q., Wang, G., & Chen, W. (2022). *Bifidobacterium breve* CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: a randomized clinical trial. *Brain, Behavior, and Immunity*, *100*, 233–241. https://doi.org/10.1016/j.bbi.2021.11.023 ⏎

Torres, F. (2024, March). What is Schizophrenia? American Psychiatric Association. https://www.psychiatry.org/patients-families/schizophrenia/what-is-schizophrenia ⏎

Trubetskoy, V., Pardiñas, A. F., Qi, T., Panagiotaropoulou, G., Awasthi, S., Bigdeli, T. B., Bryois, J., Chen, C.-Y., Dennison, C. A., Hall, L. S., Lam, M., Watanabe, K., Frei, O., Ge, T., Harwood, J. C., Koopmans, F., Magnusson, S., Richards, A. L., Sidorenko, J., … van Os, J. (2022). Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature*, *604*(7906), 502–508. https://doi.org/10.1038/s41586-022-04434-5 ⏎

Trueba, G., Cardenas, P., Romo, G., & Gutierrez, B. (2024). Reevaluating human–microbiota symbiosis: strain-level insights and evolutionary perspectives across animal species. *BioSystems*, *244*, 105283. https://doi.org/10.1016/J.BIOSYSTEMS.2024.105283 ⏎

Tsuboi, H., Sakakibara, H., Minamida-Urata, Y., Tsujiguchi, H., Hara, A., Suzuki, K., Miyagi, S., Nakamura, M., Takazawa, C., Kannon, T., Zhao, J., Shimizu, Y., Shibata, A., Ogawa, A., Suzuki, F., Kambayashi, Y., Konoshita, T., Tajima, A., & Nakamura, H. (2024). Serum TNFα and IL-17A levels may predict increased depressive symptoms: findings from the Shika Study cohort project in Japan. *BioPsychoSocial Medicine*, *18*(1), 20. https://doi.org/10.1186/s13030-024-00317-5 ⏎

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature*, *449*(7164), 804. https://doi.org/10.1038/NATURE06244 ⏎

Vakili, K., Fathi, M., Yaghoobpoor, S., Sayehmiri, F., Nazerian, Y., Nazerian, A., Mohamadkhani, A., Khodabakhsh, P., Réus, G. Z., Hajibeygi, R., & Rezaei-Tavirani, M. (2022). The contribution of gut–brain axis to development of neurological symptoms in COVID-19 recovered patients: a hypothesis and review of literature. *Frontiers in Cellular and Infection Microbiology*, *12*, 983089. https://doi.org/10.3389/fcimb.2022.983089 ⏎

Valdivieso-Jiménez, G. (2023). Eficacia de la Terapia Cognitiva Conductual Para el Trastorno Bipolar: una Revisión Sistemática. *Revista Colombiana de Psiquiatría*, *52*(3), 213–224. https://doi.org/10.1016/j.rcp.2021.05.006 ⏎

van Dis, E. A. M., van Veen, S. C., Hagenaars, M. A., Batelaan, N. M., Bockting, C. L. H., van den Heuvel, R. M., Cuijpers, P., & Engelhard, I. M. (2020). Long-term outcomes of cognitive behavioral therapy for anxiety-related disorders. *JAMA Psychiatry*, *77*(3), 265. https://doi.org/10.1001/jamapsychiatry.2019.3986 ⏎

Verma, A., Inslicht, S. S., & Bhargava, A. (2024). Gut-brain axis: role of microbiome, metabolomics, hormones, and stress in mental health disorders. *Cells*, *13*(17), 1436. https://doi.org/10.3390/cells13171436 ⏎

Vila-Badia, R., Del Cacho, N., Butjosa, A., Serra Arumí, C., Esteban Santjusto, M., Abella, M., Cuevas-Esteban, J., Morelló, G., Pardo, M., Muñoz-Samons, D., Usall, J., Abella, M., Ahicart, A., Alvarez, M., Arranz, B., Barneda, V., Bañuelos, M., Bogas, J. L., Bonilla, R., … Vives, L. (2022). Prevalence and types of childhood trauma in first episode psychosis patients. Relation with clinical onset variables. *Journal of Psychiatric Research*, *146*, 102–108. https://doi.org/10.1016/j.jpsychires.2021.12.033 ⏎

Wang, J.-W., Kuo, C.-H., Kuo, F.-C., Wang, Y.-K., Hsu, W.-H., Yu, F.-J., Hu, H.-M., Hsu, P.-I., Wang, J.-Y., & Wu, D.-C. (2019a). Fecal microbiota transplantation: review and update. *Journal of the Formosan Medical Association*, *118*, S23–S31. https://doi.org/10.1016/j.jfma.2018.08.011 ⏎

Wang, R. P. H., Ho, Y. S., Leung, W. K., Goto, T., & Chang, R. C. C. (2019b). Systemic inflammation linking chronic periodontitis to cognitive decline. *Brain, Behavior, and Immunity*, *81*, 63–73. https://doi.org/10.1016/J.BBI.2019.07.002

Wang, S., Mu, L., Yu, C., He, Y., Hu, X., Jiao, Y., Xu, Z., You, S., Liu, S. L., & Bao, H. (2023). Microbial collaborations and conflicts: unraveling interactions in the gut ecosystem. *Gut Microbes*, *16*(1), 2296603. https://doi.org/10.1080/19490976.2023.2296603 ⏎

Wang, X., Bi, L., Li, J., Liu, D., Li, W., Wang, Z., & Pan, X. (2025). Dynamic balance of the lung microbiome in health and respiratory diseases. *Chinese Medical Journal*. https://doi.org/10.1097/CM9.0000000000003712 ⏎

Wang, Z., Yuan, X., Zhu, Z., Pang, L., Ding, S., Li, X., Kang, Y., Hei, G., Zhang, L., Zhang, X., Wang, S., Jian, X., Li, Z., Zheng, C., Fan, X., Hu, S., Shi, Y., & Song, X. (2024). Multiomics analyses reveal microbiome–gut–brain crosstalk centered on aberrant gamma-aminobutyric acid and

tryptophan metabolism in drug-naïve patients with first-episode schizophrenia. *Schizophrenia Bulletin*, *50*(1), 187–198. https://doi.org/10.1093/schbul/sbad026 ⏎

World Health Organization. (2022a). *Mental Health…* www.Who.Int/News-Room/Fact-Sheets/Detail/Mental-Health-Strengthening-Our-Response ⏎

World Health Organization. (2022b). *World mental health report: transforming mental health for all*. www.Who.Int/Publications/i/Item/9789240049338 ⏎

Wright, A., Browne, J., Mueser, K. T., & Cather, C. (2020). Evidence-based psychosocial treatment for individuals with early psychosis. *Child and Adolescent Psychiatric Clinics of North America*, *29*(1), 211–223. https://doi.org/10.1016/j.chc.2019.08.013 ⏎

Wu, H., Jiawei, X., Wen, Z., Han, Y., Liu, Y., Chen, S., Ye, Z., Li, J., Xie, L., & Wu, X. (2025). Microbiome–gut–brain profiles in schizophrenia and their potential link to cognitive performance: findings from a case–control study. *Schizophrenia Bulletin*, *51*(6), 1679–1692. https://doi.org/10.1093/schbul/sbaf028 ⏎

Wu, H., Liu, Y., Han, Y., Liu, B., Chen, S., Ye, Z., Li, J., Xie, L., & Wu, X. (2024). Integrated analysis of gut microbiome, inflammation, and neuroimaging features supports the role of microbiome–gut–brain crosstalk in schizophrenia. *Schizophrenia Bulletin Open*, *5*(1), sgae026. https://doi.org/10.1093/schizbullopen/sgae026 ⏎

Wu, Q., Xu, Z., Song, S., Zhang, H., Zhang, W., Liu, L., Chen, Y., & Sun, J. (2020). Gut microbiota modulates stress-induced hypertension through the HPA axis. *Brain Research Bulletin*, *162*, 49–58. https://doi.org/10.1016/j.brainresbull.2020.05.014 ⏎

Wu, W.-L., Adame, M. D., Liou, C.-W., Barlow, J. T., Lai, T.-T., Sharon, G., Schretter, C. E., Needham, B. D., Wang, M. I., Tang, W., Ousey, J., Lin, Y.-Y., Yao, T.-H., Abdel-Haq, R., Beadle, K., Gradinaru, V., Ismagilov, R. F., & Mazmanian, S. K. (2021). Microbiota regulate social

behaviour via stress response neurons in the brain. *Nature*, *595*(7867), 409–414. https://doi.org/10.1038/s41586-021-03669-y ⏎

Xiong, R.-G., Li, J., Cheng, J., Zhou, D.-D., Wu, S.-X., Huang, S.-Y., Saimaiti, A., Yang, Z.-J., Gan, R.-Y., & Li, H.-B. (2023). The role of gut microbiota in anxiety, depression, and other mental disorders as well as the protective effects of dietary components. *Nutrients*, *15*(14), 3258. https://doi.org/10.3390/nu15143258 ⏎

Xu, R., Liu, S., Li, L., Zhang, Y., Luo, G., Fang, B., & Wang, X. (2023). Causal effects of gut microbiota on the risk of bipolar disorder: a Mendelian randomization study. *Frontiers in Microbiomes*, *2*, 1249518. https://doi.org/10.3389/frmbi.2023.1249518 ⏎

Xu, R., Wu, B., Liang, J., He, F., Gu, W., Li, K., Luo, Y., Chen, J., Gao, Y., Wu, Z., Wang, Y., Zhou, W., & Wang, M. (2020). Altered gut microbiota and mucosal immunity in patients with schizophrenia. *Brain, Behavior, and Immunity*, *85*, 120–127. https://doi.org/10.1016/j.bbi.2019.06.039 ⏎

Xue, L., Zou, X., Yang, X. Q., Peng, F., Yu, D. K., & Du, J. R. (2020). Chronic periodontitis induces microbiota–gut–brain axis disorders and cognitive impairment in mice. *Experimental Neurology*, *326*, 113176. https://doi.org/10.1016/J.EXPNEUROL.2020.113176 ⏎

Yang, D.-F., Huang, W.-C., Wu, C. W., Huang, C.-Y., Yang, Y.-C. S. H., & Tung, Y.-T. (2023). Acute sleep deprivation exacerbates systemic inflammation and psychiatry disorders through gut microbiota dysbiosis and disruption of circadian rhythms. *Microbiological Research*, *268*, 127292. https://doi.org/10.1016/j.micres.2022.127292 ⏎

Yatham, L. N., Liddle, P. F., Gonzalez, M., Saraf, G., Vafai, N., Lam, R. W., & Sossi, V. (2022). A positron emission tomography study of dopamine transporter density in patients with bipolar disorder with current mania and those with recently remitted mania. *JAMA Psychiatry*, *79*(12), 1217. https://doi.org/10.1001/jamapsychiatry.2022.3541 ⏎

Zhang, X., Chen, S., Zhang, M., Ren, F., Ren, Y., Li, Y., Liu, N., Zhang, Y., Zhang, Q., & Wang, R. (2021). Effects of fermented milk containing *Lacticaseibacillus paracasei* strain shirota on constipation in patients

with depression: a randomized, double-blind, placebo-controlled trial. *Nutrients*, *13*(7), 2238. https://doi.org/10.3390/nu13072238 ⏎

Zhang, Z., Ma, X., Ouyang, L., Li, Z., Liu, W., He, Y., Lv, J., Chen, X., & Yuan, L. (2025). A frame network study of first-episode schizophrenia, ultra-high risk, and healthy populations. *Schizophrenia*, *11*(1), 110. https://doi.org/10.1038/s41537-025-00658-2 ⏎

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N. D., Licinio, J., Wei, H., & Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*, *21*(6), 786–796. https://doi.org/10.1038/mp.2016.44 ⏎

Zhu, F., Ju, Y., Wang, W., Wang, Q., Guo, R., Ma, Q., Sun, Q., Fan, Y., Xie, Y., Yang, Z., Jie, Z., Zhao, B., Xiao, L., Yang, L., Zhang, T., Feng, J., Guo, L., He, X., Chen, Y., … Ma, X. (2020). Metagenome-wide association of gut microbiome features for schizophrenia. *Nature Communications*, *11*(1), 1612. https://doi.org/10.1038/s41467-020-15457-9 ⏎

14

Microbial Dynamics and Functions in Tumor Microenvironments

Afsana Parveen Jahir Hussain, Madhi Oli Ramamurthy, Poorvi Subramanian, Sreenidhi Mohanvelu, Loganayaki Periyasamy, Sivasubramani Narayanan, Sheeja Aravindan, and Natarajan Aravindan

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14.1 Introduction and Historical Perspective

For much of the twentieth century, the prevailing dogma in oncology maintained that solid tumors were sterile environments. This assumption stemmed from early histopathological observations and the inability to culture microbes from tumor tissues using conventional microbiological techniques. The conceptual framework of cancer biology focused exclusively on genetic mutations, epigenetic alterations, and dysregulated signaling pathways within host cells, leaving little room to consider microbial contributions beyond well-established infectious etiologies such as *Helicobacter pylori* in gastric cancer or human papillomavirus (HPV) in cervical cancer. The historical perspective on microbes in cancer began to shift as accumulating evidence linked chronic infections with oncogenesis. Seminal discoveries in the late twentieth century demonstrated that certain pathogens could initiate or promote malignant transformation through mechanisms involving chronic inflammation, genomic instability, and immune modulation. These findings, however, were restricted to high-biomass infections and did not challenge the prevailing sterility paradigm for most solid tumors. The advent of next-generation sequencing (NGS) technologies in the early 2000s marked a turning point. Metagenomic and metatranscriptomic approaches enabled the detection of microbial nucleic acids at extremely low abundance, revealing

unexpected microbial signatures in tumor specimens previously considered sterile. Initial reports were met with skepticism, as concerns about contamination and technical artifacts loomed large. Rigorous methodological refinements, including the use of randomized blanks, dual-indexing, and orthogonal validation through imaging techniques such as fluorescence in situ hybridization (FISH) and RNAscope, gradually strengthened confidence in these observations. By the 2010s, large-scale pan-cancer analyses began to uncover consistent patterns of microbial presence across diverse tumor types, including breast, pancreatic, lung, and colorectal cancers (CRC). These studies demonstrated that intratumoral microbes are not random contaminants but exhibit tissue-specific tropisms and functional associations with tumor biology. For example, *Fusobacterium nucleatum* emerged as a prominent player in CRC, implicated in modulating Wnt/β-catenin signaling, promoting epithelial barrier dysfunction, and fostering an immunosuppressive microenvironment. Similarly, intracellular bacteria have been observed within breast cancer (BCa) cells and associated with enhanced metastatic potential, suggesting a direct role in tumor progression. Technological advances have further refined our understanding of microbial localization within the tumor microenvironment (TME). Spatial transcriptomics and single-cell sequencing have revealed that microbes can reside intracellularly within cancer cells, immune cells, and stromal compartments, as well as extracellularly in necrotic regions and fibrotic stroma. These microniches are shaped by gradients of oxygen, pH, and nutrients, imposing selective pressures that favor anaerobic and facultative anaerobic taxa. Such insights underscore the complexity of tumor ecosystems and challenge simplistic models of host–microbe interactions. The recognition of fungal and viral communities within tumors has expanded the scope of intratumoral microbiome research. Pan-cancer mycobiome studies have identified *Candida* and other genera at extremely low abundance, often correlating with inflammatory signatures and adverse clinical outcomes. Similarly, while emerging data suggest that nonclassical viral sequences may also inhabit tumor tissues, established oncogenic viruses like HPV, Epstein–Barr virus (EBV), and Hepatitis B virus (HBV) remain central to cancer etiology with potential implications for immune modulation and therapeutic response. Today, the field stands at an inflection point. The sterility dogma has been decisively overturned, and intratumoral microbes are increasingly recognized as integral components of the TME. Yet, critical questions persist regarding causality, functional significance, and translational potential. Are these microbes passive passengers, opportunistic colonizers, or active drivers of tumor biology? Addressing these questions will require interdisciplinary approaches combining microbiology, oncology, immunology, and computational biology, supported by standardized protocols and robust contamination controls. Overall, the historical trajectory from presumed sterility to acknowledged microbial complexity reflects a

broader evolution in cancer research, away from a gene-centric paradigm to a systems-level perspective that embraces the multifaceted interplay between the host and microbiome. This expanded view not only enriches our understanding of tumor biology but also opens new avenues for diagnostics, prognostics, and therapeutics in precision oncology.

14.2 Methodological Pillars and Validation for Tumor Microbiome

The study of tumor-associated microbiomes presents unique methodological challenges due to the extremely low biomass of microbial communities within solid tumors. Unlike gut or oral microbiomes, where microbial load is high, intratumoral microbes exist at levels that are orders of magnitude lower than host deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). This disparity necessitates rigorous experimental design, contamination control, and multimodal validation to ensure that a detected microbial signal reflects true biological presence rather than noise. Early evidence for intratumoral microbes emerged from the reanalysis of bulk RNA sequencing (RNA-seq) and whole-genome sequencing (WGS) datasets, where microbial reads were identified alongside host sequences. Contemporary studies employ metagenomic and metatranscriptomic sequencing, often leveraging assembly-based strategies to improve specificity and reduce false positives (Figure 14.1). Computational pipelines incorporate stringent filtering steps, including the removal of low-complexity reads, alignment to comprehensive microbial reference databases, and the application of decontamination algorithms such as “decontam” to distinguish true signals from reagent or environmental contaminants. Dual-indexing and randomized sample processing further mitigate index hopping and batch effects. Low-biomass microbiome research is highly susceptible to contamination from reagents, laboratory environments, and sequencing platforms. Best practices include the use of multiple negative controls (kit blanks, extraction blanks), spike-in standards for normalization, and randomized sample allocation across batches. Reporting frameworks now recommend explicit documentation of control performance, sequencing depth, and read budgets allocated to microbial detection. Studies should also disclose contamination filtering criteria and provide raw data for independent verification. To confirm microbial presence beyond sequencing, orthogonal validation using imaging techniques is essential. FISH targeting bacterial 16S ribosomal RNA (rRNA), RNAscope for microbial transcripts, and immunohistochemistry (IHC) for microbial antigens provide visual evidence of microbes within tumor architecture. Spatial transcriptomics and proteogenomics add further resolution, enabling the co-localization of microbial signals with host cell phenotypes and microenvironmental features such as hypoxia or immune infiltration (Figure 14.1). Together, these

approaches reduce reliance on sequencing alone and strengthen causal inference by linking microbes to specific tumor niches. Recent commentaries advocate for integrated frameworks combining sequencing, imaging, and culture-based methods where feasible. While culturing intratumoral microbes remains challenging, its success in select cases underscores the importance of multimodal strategies. Spatial multiomics, including single-cell RNA-seq coupled with microbial read recovery, promises unprecedented insight into host-microbe interactions at cellular resolution (Figure 14.1). Standardization efforts, such as proposed checklists for low-biomass microbiome studies, aim to harmonize protocols and improve reproducibility across cohorts and platforms. In summary, methodological rigor is the cornerstone of tumor microbiome research. By combining contamination-aware sequencing pipelines with orthogonal imaging and spatial validation, studies can confidently distinguish true intratumoral residents from artifacts. These methodological pillars not only safeguard scientific integrity but also lay the foundation for translational advances, including microbial biomarkers and therapeutic interventions in oncology.

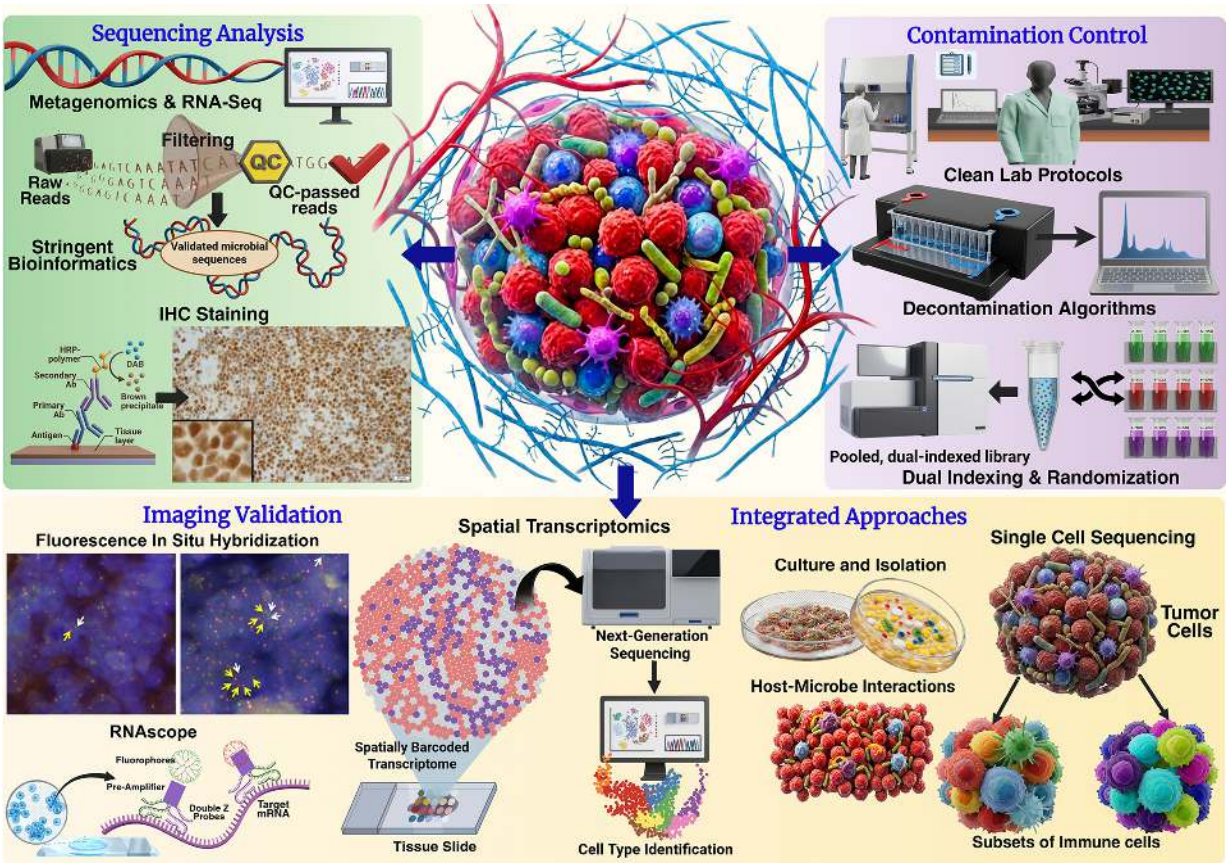

FIGURE 14.1 Schematic overview of contamination-controlled multi-modal approach for tumor microbiome detection and validation. ⏎

14.3 Contamination Control Methods in Tumor Microbiome Research

Contamination control is the cornerstone of low-biomass microbiome research, particularly in the context of tumor samples where the microbial load is extremely low compared with host nucleic acids. Without rigorous controls, false-positive microbial signals can arise from reagents, laboratory environments, sequencing platforms, or computational artifacts. Common contamination sources include DNA extraction kits, polymerase chain reaction (PCR) reagents, and sequencing adapters, which often harbor trace microbial DNA. Environmental contaminants from laboratory surfaces, airborne particles, and operator handling can also introduce exogenous sequences, while index hopping during multiplexed sequencing and barcode misassignment further complicate interpretation. These factors are particularly problematic in low-biomass samples, where contaminant reads can outnumber true microbial signals. Preventive measures begin with the physical separation of pre- and post-PCR workflows, the use of dedicated clean rooms, and the implementation of ultraviolet (UV) sterilization and deoxyribonuclease (DNase) treatment for work surfaces and equipment. Reagent selection is critical, as low-background extraction kits and certified clean consumables reduce background noise. Further, randomization of sample processing across batches minimizes systematic biases, while dual-indexing strategies mitigate index hopping. Bioinformatic pipelines incorporate algorithms to identify and remove contaminant taxa based on prevalence patterns across controls and samples. Tools such as “decontam” use statistical models to distinguish true biological signals from contaminants by leveraging frequency and prevalence data. Additional strategies include filtering taxa that correlate with the sequencing batch or reagent lot, and excluding organisms known to be common kit contaminants (e.g., *Ralstonia* and *Bradyrhizobium*). Assembly-based approaches further reduce false positives by requiring contiguous microbial genome fragments rather than isolated reads. To ensure reproducibility, studies must report the number and type of controls used, contamination filtering criteria, and read budgets allocated to microbial detection. Emerging guidelines recommend documenting raw and processed data, along with metadata on reagent lots and sequencing runs. Visual summaries of contamination profiles across controls and samples enhance transparency and allow independent verification. The most robust studies combine preventive laboratory practices with computational decontamination and orthogonal validation (e.g., imaging and spatial transcriptomics). Future directions include the development of standardized contamination reference databases, machine learning models for contaminant prediction, and harmonized reporting checklists for low-biomass microbiome studies. Together, these advances will strengthen confidence in inter- and intratumoral microbial discoveries and accelerate their translation into clinical applications (Figure 14.1).

14.4 Localization: Intracellular versus Extracellular Niches within the TME

Across multiple tumor types, microbes are now documented inside cancer and immune cells (intracellular) as well as outside cells within the stromal/extracellular matrix (ECM), necrotic cores, and hypoxic microniches of the TME. Multiple routes that deliver microbes to tumors include hematogenous translocation from the gut or oral cavities, local invasion across disrupted mucosa, and cell-associated trafficking (e.g., microbes traveling with tumor cells or within phagocytes). Once present, intracellular niches shelter microbes from humoral defenses and antibiotics, while stromal/ECM niches (especially hypoxic zones) confer metabolic compatibility for anaerobes and protection via biofilm matrices, both of which are repeatedly observed in tumor contexts (Figure 14.2). The pronounced desmoplasia of pancreatic ductal adenocarcinoma (PDAC) offers abundant ECMs and cancer-associated fibroblasts (CAFs) networks that may scaffold microbial residency and interactions with immune cells, consistent with observed microbiome-immunity correlations (Yang et al., 2023, Bullman, 2023, Battaglia et al., 2024, Montanari et al., 2025, Edwards et al., 2021, Riquelme et al., 2019, Meng et al., 2025, Bautista et al., 2025c, Gigi et al., 2025). This conclusion rests on convergent evidence from (1) contamination-aware sequencing and assembly, (2) culture-independent in situ hybridization and imaging (16S rRNA FISH and RNAscope), (3) spatial transcriptomics to co-localize microbial and host transcripts, (4) targeted imaging mass cytometry or multiplex immunofluorescence for phenotyping adjacent immune/stromal cells, and (5) functional studies in animal models and human cohorts (Yang et al., 2023, Bullman, 2023, Battaglia et al., 2024, Cisar et al., 2023, Rademacher et al., 2025, Strack, 2022). Originally, the large-scale re-analyses of human tumor datasets and dedicated prospective cohorts produced compelling evidence for intracellular bacteria. Subsequently, synthesized microscopy, spatial data, and imaging-based spatial transcriptomics (multiplexed error-robust fluorescence in situ hybridization (MERFISH), sequential FISH (seqFISH), and in situ sequencing (ISS)) at the single-cell and subcellular resolution indicated localization within cancer and immune cells, while also describing extracellular colonization of stromal territories. Collectively, the evidence supports intrinsic microbial communities, albeit at an extremely low abundance and with substantial heterogeneity by cancer type (Yang et al., 2023, Bullman, 2023, Schorr et al., 2023). In addition, spatially resolved pan-cancer work in metastatic tumors further maps organ-specific tropisms and hypoxia-associated enrichment of anaerobes, aligning with the concept that tumor biophysics (oxygen tension, pH, and ECM density) shapes where microbes persist inside cells or protected stromal niches. Reviews focused on immuno-oncology similarly conclude that

intratumoral microbes modulate the tumor immune microenvironment (TIME), emphasizing both intracellular residency and stromal distribution as key determinants of function (Yang et al., 2023, Battaglia et al., 2024, Liu et al., 2024) (Figure 14.2).

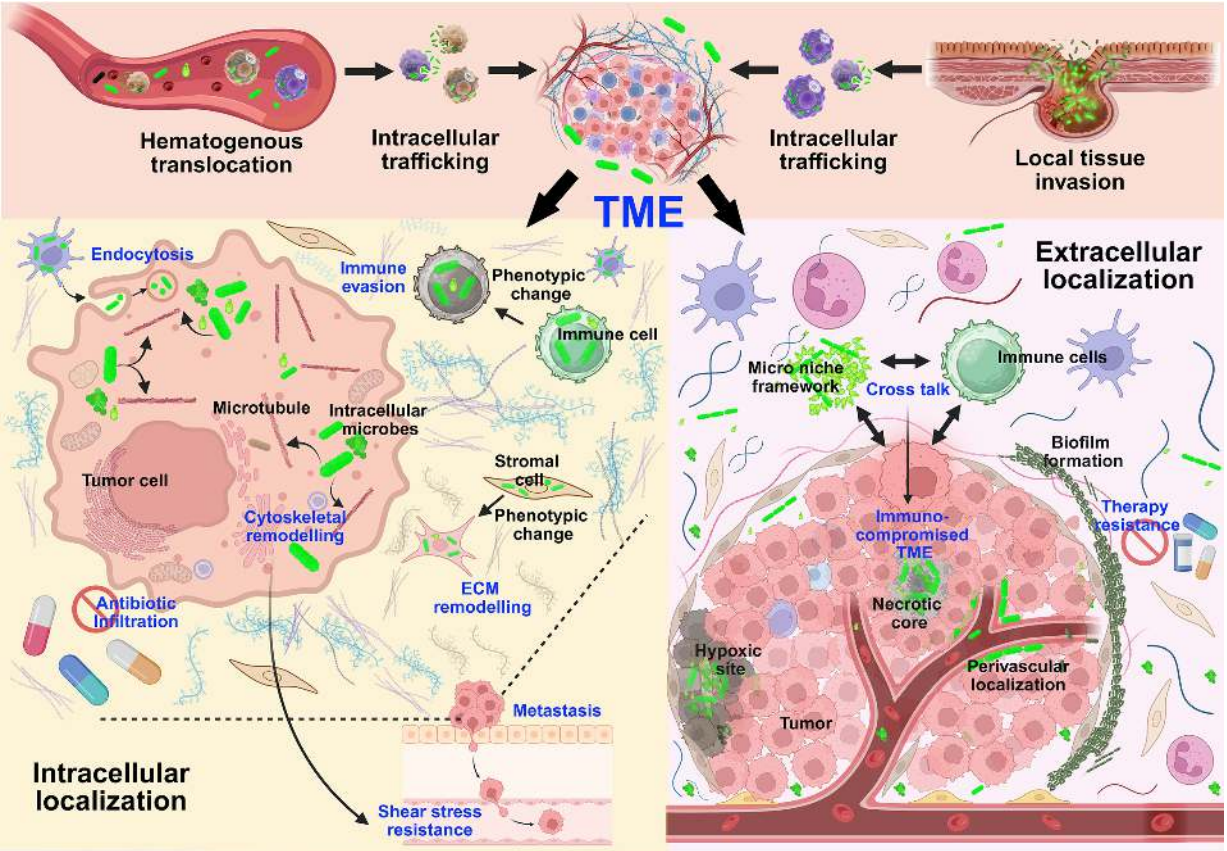

FIGURE 14.2 Intracellular and extracellular localization of microbial niches in the TME. ⏎

14.4.1 Intracellular Presence

A landmark study demonstrated that BCa tumors harbor conserved intracellular bacterial profiles in both mice and humans. Selectively depleting intratumoral bacteria reduced lung metastasis without affecting primary tumor growth, implying a specific role of bacteria in dissemination rather than initiation. Mechanistically, intracellular bacteria remodeled the actin cytoskeleton of circulating tumor cells, enhancing their resistance to mechanical shear stress in circulation and thereby sustaining metastatic seeding, which is direct evidence of functional intracellular symbiosis aiding cancer cell survival (Figure 14.2). Contemporary studies independently contextualized these experiments, noting that bacteria were predominantly cytosolic within tumor cells and that their presence promoted metastasis; they also cautioned against simplistic antimicrobial interventions without mechanistic specificity (Fu et al., 2022b, Longmore, 2022). In

parallel, cross-tumor evidence studies profiled that bacteria could reside within both cancer cells and tumor-infiltrating immune cells, laying out hypothesized entry routes (endocytosis and cell-to-cell transfer) and persistence strategies (immune evasion and metabolic coupling). Further studies emphasized that such intracellular colonization is quantifiable and aligns with taxa observed in human tumors, reinforcing its translational relevance (Schorr et al., 2023, Zhu et al., 2022, Fu et al., 2022a) (Figure 14.2). A pathobiont, *F. nucleatum*, linked to CRC progression, has been detected not only in the malignant epithelium but also associated with immune and stromal compartments, with mechanistic work showing how *F. nucleatum* shapes immune cell phenotypes and therapy responses. Emerging spatial studies report *F. nucleatum*'s ability to invade or adhere to CAFs, driving an inflammatory CAF program that enhances invasion, illustrating intracellular/juxtacellular interactions beyond the tumor epithelium (Li et al., 2025, Jiang et al., 2025b, Karta et al., 2025). Pan-cancer mycobiome studies documented fungal DNA and intracellular fungal staining within cancer and immune cells across many cancers, supporting a role for intracellular or close apposed fungi in immune modulation. Further, imaging across tissue microarrays showed fungi to be ubiquitous yet at low abundance, frequently intracellular, and often co-localized with bacterial communities (Leake, 2022, Dohlman et al., 2022, Li and Saxena, 2022).

14.4.2 Extracellular/Stromal Presence

Intratumoral microbes are also observed outside cells, within stromal ECM, perivascular spaces, necrotic cores, and hypoxic gradients, where anaerobes preferentially persist (Figure 14.2). The metastasis-wide pan-cancer resource demonstrated anaerobe enrichment in hypoxic tumors and neutrophil-associated diversity, implying that extracellular microenvironments (oxygen tension, immune infiltration) shape microbiome composition and function. Based on this, a microniche framework concept has been synthesized, indicating that tumors host embedded microbial consortia whose locations (stromal vs. intracellular) confer distinct survival advantages and interaction potentials (Bullman, 2023, Battaglia et al., 2024) (Figure 14.2). For example, within gastrointestinal malignancies, biofilm-like aggregates and mucosal-adherent communities have been associated with epithelial injury and pro-tumor inflammation. Recent studies connected biofilm biology to immune evasion, chronic inflammation, and ECM remodeling that collectively favor stromal persistence in the TME. To that end, cutting-edge perspectives propose that cancer-associated biofilms provide a protective matrix for bacteria and fungi in stromal/extracellular compartments, complicating eradication and contributing to persistent inflammatory signaling (Montanari et al., 2025, Choi et al., 2023, Kushwaha et al., 2024). In tumors with intense desmoplasia,

tumor tissues harbor distinct bacteria whose diversity correlates with long-term survival; influences immune infiltration; and reshapes the tumor's stromal ecosystem, consistent with stromal/extracellular colonization facilitating immune crosstalk. The dense stroma (CAFs and a collagen-rich ECM) is itself a major determinant of immune exclusion and therapy resistance, making microbial residency in these compartments especially consequential for drug penetration and local immunity (Edwards et al., 2021, Riquelme et al., 2019).

14.5 Evidence for Microbial Presence in Solid Tumors

Accumulating evidence demonstrates that many human solid tumors harbor a distinct and non-random microbiome, with microbial DNA and viable bacteria detected in many adult and pediatric cancer types (Figure 14.3). These intratumoral microbial communities display tumor type- and anatomical site–specific patterns, suggesting selective colonization. Here, we discuss the presence and species composition of tumor-associated microbes, their trafficking routes, and modes of entry into tumors, the mechanisms governing their localization and persistence, their functional roles in tumor biology and host immunity, and their clinical relevance, including diagnostic and therapeutic implications.

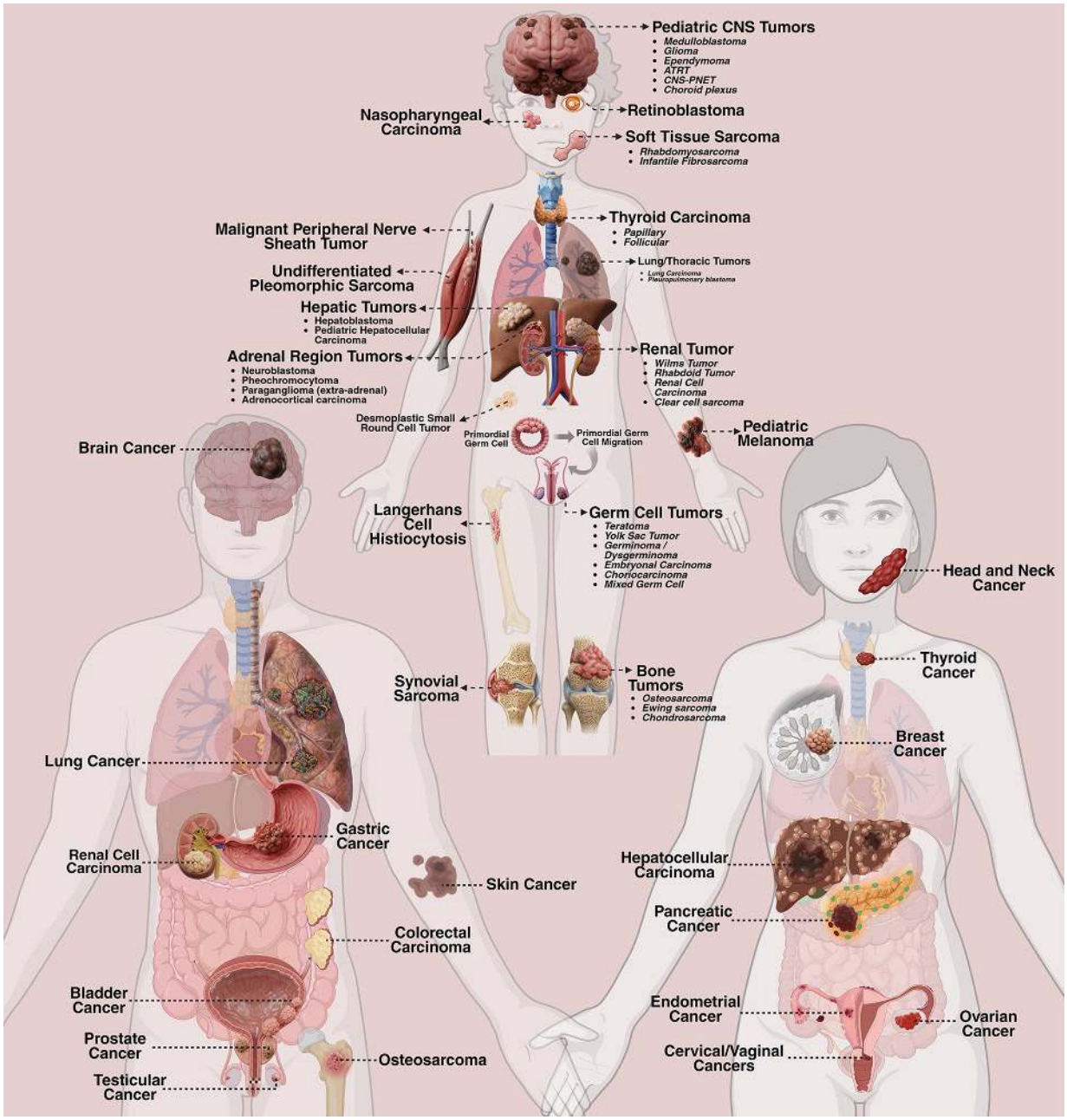

FIGURE 14.3 Tumor-associated microbiomes in human cancer. ⏎

14.5.1 Brain Cancer

For decades, the central nervous system (CNS) and intracranial tumors were treated as microbiologically "sterile" compartments, largely because the blood–brain barrier (BBB) was assumed to exclude microorganisms and conventional culture rarely recovered microbes from brain tissues. However, such a conceptual view was challenged by a large, prospective, multi-institutional study of gliomas and brain metastases that

related bacterial 16S rRNA and lipopolysaccharide (LPS) signals in malignant brain tumors to host immune-metabolic gene expression patterns. In parallel, another study profiling 322 brain tumors reported tumor-type-specific and location-specific microbial signatures, and it documented higher bacterial richness and diversity in brain metastases than in glioblastoma (GBM), suggesting that the intracranial microbial landscape varies by both primary origin and neuroanatomical region (Gigi et al., 2025, Morad et al., 2025, Jawahar et al., 2025). These findings matter, since the brain TME, already defined by hypoxia, necrosis, profound myeloid predominance, and variable lymphocyte exclusion, may include microbial components that intersect with innate immune sensing, antigen processing, metabolic reprogramming, and therapy response. Importantly, because brain tumors represent low-biomass ecosystems, the field has also matured methodologically as recent studies emphasize contamination control and transparent reporting as prerequisites for credible claims of intratumoral microbes (Morad et al., 2025, Lu et al., 2024, Fierer et al., 2025). The brain tumor microbiome typically refers to microbial DNA/RNA, proteins, products, or cellular structures detected within tumor tissue, including tumor cells and infiltrating stromal/immune compartments, rather than microbes colonizing mucosal surfaces. Studies explicitly highlighted the distinction between viable "microbiota" and "microbial components," noting that culture may be negative even when microbial nucleic acids or cell wall products are detectable. This framing is particularly relevant to CNS tumors where microbial loads are expected to be low and viability may be compromised by hypoxia, immune surveillance, prior antibiotics, perioperative prophylaxis, and tissue processing (Morad et al., 2025, Jawahar et al., 2025, Austin et al., 2026). The most compelling studies triangulate these signals with imaging and spatial analyses to show that microbial signals are not uniformly distributed, but instead cluster in specific regions and cellular neighborhoods (Gigi et al., 2025, Morad et al., 2025, Jawahar et al., 2025). The strongest human evidence comes from works demonstrating bacterial signals localized to tumor, immune, and stromal cells in gliomas and brain metastases. In particular, these spatial studies linked bacterial signals to antimicrobial and immune-metabolic transcriptional programs across regions and cell neighborhoods, supporting the possibility that microbial components participate in shaping the brain tumor immune milieu (Morad et al., 2025, Jawahar et al., 2025). Complementing this, GBM has been shown to possess distinct microbial signatures compared with metastases and its microbial pathway enrichment patterns differ by tumor type. In particular, tumors that metastasized to the brain harbor higher bacterial richness and diversity than GBM, metastases in posterior brain regions exhibit greater diversity than anterior lesions, introducing a neuroanatomical dimension to tumor microbial ecology. This is consistent with the concept that intracranial tumors differ in vascularization, BBB disruption, necrotic burden, and immune cell composition

across regions, factors that could plausibly influence microbial persistence and detectability (Gigi et al., 2025, Morad et al., 2025). These studies advance the field beyond "is there a signal?" toward "what is its spatial distribution, cellular localization, and potential function?" (Gigi et al., 2025, Morad et al., 2025).

Compared with the evidence for bacteria, the evidence for fungi within brain tumors is less, yet is emerging. A multicompartment study analyzed tumor, tumor-adjacent tissue, and blood with internal transcribed spacer (ITS) sequencing and reported diverse fungal genera within tumor tissues, showing *Alternaria, Malassezia, Schizophyllum*, and *Tomentella* as common detections (Figure 14.4). Owing to the low-biomass, these readouts should be interpreted cautiously and require replication with strict contamination controls and orthogonal validation (Fierer et al., 2025, Austin et al., 2026, Sipos et al., 2025). However, pan-cancer mycobiome analyses emphasize that fungi, although low in abundance, can be immunologically potent, and that fungal detection is methodologically challenging (due to ITS primer bias, reagent contamination, and database limitations). For brain tumors, these constraints are amplified by limited tissue quantity and a high host nucleic acid background (Li and Saxena, 2022, Fierer et al., 2025, Ding et al., 2025).

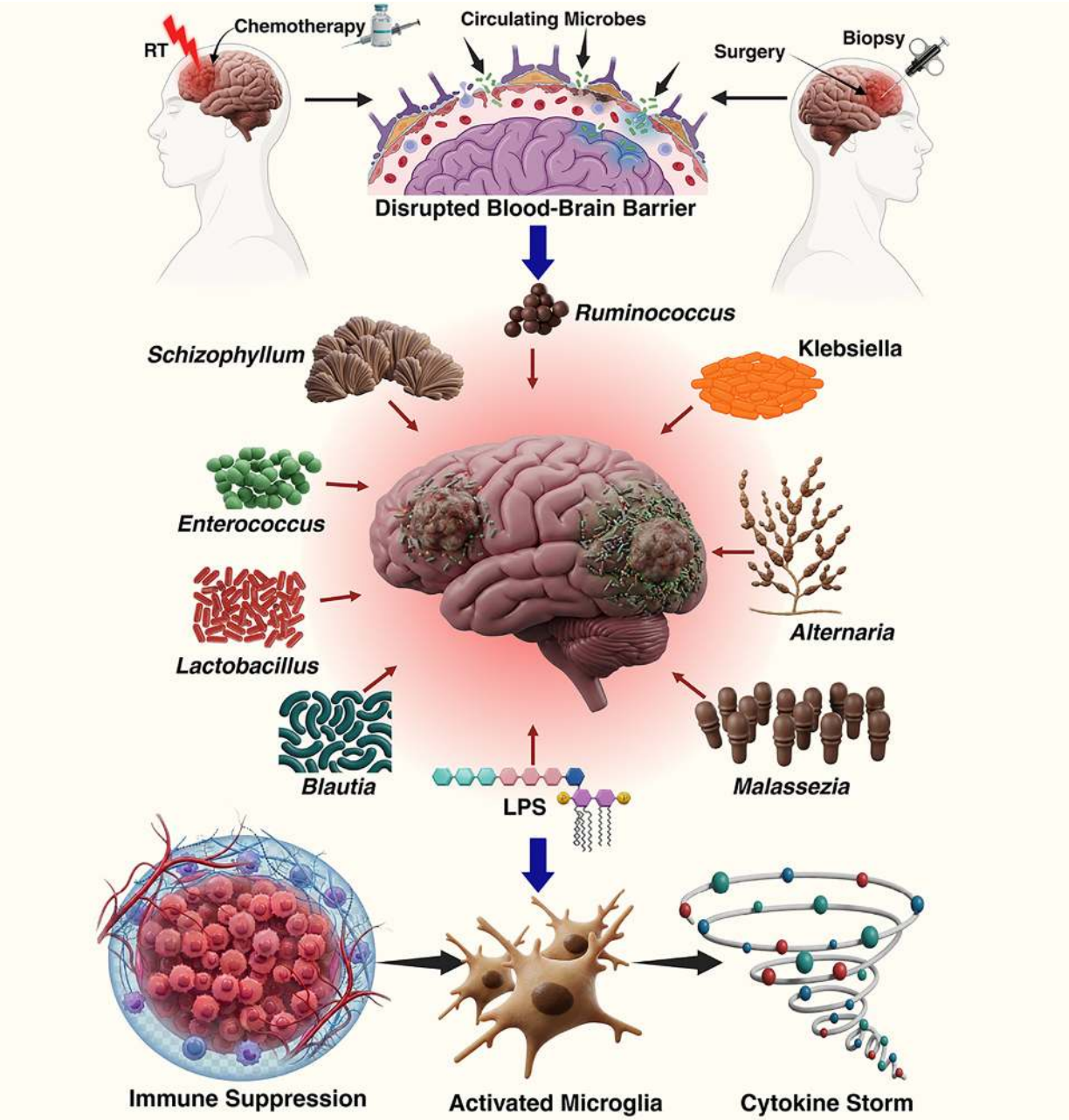

FIGURE 14.4 Microbial composition and biological consequences in brain cancer. ⏎

14.5.1.1 Mechanisms

Brain tumors, especially GBM, are dominated by microglia and macrophages (including monocyte-derived macrophages), with variable T-cell infiltration. Spatial and single-cell studies emphasize regional immune heterogeneity and niche-specific programs (hypoxia, angiogenesis, and necrosis) that influence immune suppression and therapy resistance. Within this context, bacterial products such as LPS could act as ligands for toll-like receptors (TLRs) pathways in tumor and immune cells, potentially shifting

cytokine tone, antigen presentation capacity, or checkpoint expression (Liu et al., 2024, Morad et al., 2025, Ruiz-Moreno et al., 2025, Fu et al., 2025a). Consistent with this, studies reported correlations between bacterial 16S rRNA signals and "immune-metabolic" signatures at regional and cellular scales, suggesting that microbial elements could help pattern immune activation/suppression within the tumor. These observations align with general intratumoral microbiota frameworks in which microbes can either stimulate antitumor responses or promote immune escape depending on context (Morad et al., 2025, Jawahar et al., 2025, Zhang et al., 2025a, Wang et al., 2025b). Second, GBM is metabolically plastic; spatial transcriptomics and atlas studies highlight hypoxia-driven niches associated with poor prognosis and therapy resistance. The brain tumor microbial signal is linked to immune-metabolic programs, raising the hypothesis that microbial components could intersect with metabolic stress responses, for instance, by modulating redox, lipid metabolism, or inflammatory metabolism in myeloid cells. While direct causal mechanisms in brain tumors remain to be proven, analogous intratumoral microbiome literature shows that microbes can influence oncogenic signaling, inflammatory cascades, and drug metabolism in other cancers (Morad et al., 2025, Jawahar et al., 2025, Ruiz-Moreno et al., 2025, Fu et al., 2025a, Vella et al., 2025, Xue et al., 2023). A particularly provocative clinical angle is that microbial variation might correlate with responses to standard GBM therapy (surgery + radiotherapy + temozolomide) or to emerging immunotherapies; studies indicated associations between microbial features, tumor biology, and clinical outcomes and highlighted the need for mechanistic follow-up (Gigi et al., 2025) (Figure 14.4).

14.5.1.2 Biomarker and Therapeutic Implications

Intratumoral microbial signals could be integrated with spatial immune markers to stratify tumors by immune-metabolic state, myeloid activation, or predicted therapy resistance. Microbial signals correlate with regional host transcriptional programs and realized tumor-type and location-specific microbial patterns, both of which are promising for biomarker development (Gigi et al., 2025, Morad et al., 2025). Pan-cancer tumor-resident bacterial studies propose strategies to eradicate pathogenic tumor-resident bacteria or harness bacteria as delivery vehicles; however, brain tumor applications face unique constraints (the BBB, neurosurgical safety, low-biomass, and uncertainty about causality). The most realistic near-term translational direction is mechanism-guided association, mapping microbial components to immune suppression signatures (e.g., checkpoint expression and macrophage polarization), and testing whether these associations predict response to radiation, temozolomide, or immunotherapies in prospective cohorts (Morad et al., 2025, Fu et al., 2025a, Zhang et al., 2025b, Vella et al., 2025). Further studies emphasize that robust microbial detection

pipelines in low-biomass tumors are a prerequisite for translational claims, implying that clinical studies must incorporate contamination controls and orthogonal validation from the start (Jawahar et al., 2025, Fierer et al., 2025). That being said, the field's next step is to transform association into mechanism, defining how microbial components intersect with myeloid biology, hypoxia niches, and therapy resistance in GBM and metastases, while maintaining the highest standards of contamination control that low-biomass CNS tissues demand. Crucially, moving forward, it is critical to address key gaps, including: (1) Are detected bacterial/fungal signals viable microbes, dormant forms, or molecular remnants, and does viability matter for immune modulation? (Morad et al., 2025, Jawahar et al., 2025); (2) How do microbial elements reach the CNS tumor niche (hematogenous trafficking, immune cell carriage, or a disrupted BBB), and do routes differ between GBM and metastases? (Gigi et al., 2025, Morad et al., 2025); (3) Do microbial components causally alter myeloid polarization, antigen presentation, or checkpoint pathways, or are they epiphenomena of necrosis and BBB breakdown? (Jawahar et al., 2025, Austin et al., 2026); (4) Are microbial signatures linked to survival, temozolomide resistance, radioresistance, or immunotherapy response, after controlling for tumor subtype and treatment (Gigi et al., 2025, Morad et al., 2025)? Addressing these knowledge gaps could substantially deepen our understanding of brain tumor evolution.

14.5.2 Head and Neck Cancer

Head and neck cancer (HNC) arises from the mucosal epithelium of the oral cavity, pharynx (including the oropharynx), and larynx—sites that are continuously exposed to diverse microbial communities and environmental carcinogens such as tobacco and alcohol. Within head and neck oncology, microbial investigations have historically emphasized oral and salivary communities; yet there is increasing interest in the intratumoral (tissue-resident) microbiome, which comprises microbial material present within the tumor tissue itself and is often measured using decontaminated RNA-seq/whole-genome approaches—such as The Cancer Microbiome Atlas (TCMA) or targeted assays in clinical cohorts. This distinction matters because tissue-localized microbes can interact more directly with the TME, immune infiltrates, and therapy exposure than luminal communities (Meng et al., 2025, Lu et al., 2024, Wang et al., 2022a, Hamada et al., 2023). Pan-cancer efforts have enabled the extraction of microbial signatures from tumor sequencing data using contamination-aware pipelines. In head and neck squamous cell carcinoma (HNSCC), analyses leveraging TCMA and The Cancer Genome Atlas (TCGA) have reported distinct microbial profiles between tumor tissues and adjacent normal tissues, as well as associations with clinicopathologic

features (Wang et al., 2022b, Hamada et al., 2023). These results reinforce the concept of tumor-associated dysbiosis at the tissue level and suggest tumor-type and site-specific microbial patterns rather than random contamination (Wang et al., 2022a). Beyond computational inference, clinical studies have measured specific microbes directly in tumor specimens. For example, *F. nucleatum* DNA has been detected in formalin-fixed paraffin-embedded head and neck tumor samples using sensitive quantitative methods, and associations with survival endpoints have been explored in contemporary cohorts. Although directionality varies across studies and subsites, such work supports the feasibility of detecting microbial signals in HNSCC tissue and motivates mechanistic exploration (Datorre et al., 2025, Bayrak et al., 2025). HNC is not a single disease entity; it includes oral cavity SCC, oropharyngeal SCC (often HPV-associated), laryngeal SCC, and others. Studies emphasize that microbial profiles can vary by tumor subsite, HPV status, and local tissue ecology (keratinization, oxygenation, and salivary exposure), consistent with anatomic niche selection. Moreover, HNSCC tumor microbiome signatures have been reported to vary with clinical parameters such as stage and histologic grade, supporting potential links to tumor progression (Meng et al., 2025, Wang et al., 2022a, Sahin et al., 2025, Hamada et al., 2024).

14.5.2.1 Trafficking and Modes of Entry

Because head and neck tumors arise in microbe-rich mucosal regions, multiple plausible routes may explain how microbial cells or products appear within tumor tissues. This includes: (1) *direct mucosal invasion and barrier disruption:* HNSCC develops in chronically inflamed mucosa where epithelial barrier integrity can be compromised by carcinogens, dysplasia, ulceration, and tumor-associated necrosis. Such changes can facilitate microbial translocation from the surface biofilm into deeper tissue compartments (Meng et al., 2025, Irfan et al., 2020); (2) *biofilm-associated infiltration:* Oral pathogens and commensals form structured biofilms; biofilm fragments and microbial aggregates may penetrate disrupted epithelium or persist within tumor-associated crypts and necrotic regions (Meng et al., 2025, Irfan et al., 2020); (3) *iatrogenic introduction during diagnostic or therapeutic procedures:* Biopsy, instrumentation, and surgical manipulation can introduce surface microbes into tissue planes. While this does not negate biological relevance, it underscores the importance of rigorous sampling controls and spatial validation (Lu et al., 2024, Fierer et al., 2025); (4) *immune cell-mediated "hitchhiking" or trafficking:* Microbial components can be taken up by dendritic cells or macrophages and transported into inflamed tissues; this is conceptually consistent with broader intratumoral microbiota models in which microbes or microbial products are found in association with host cells within tumors (Yang et al., 2023, Lu et al., 2024); and (5) *hematogenous seeding from oral sites:* Transient

bacteremia can occur after dental manipulation or even routine activities in periodontal disease. In principle, circulating microbes may seed permissive tumor niches, although definitive causal demonstrations in HNSCC remain limited (Meng et al., 2025, Sahin et al., 2025). Importantly, because intratumoral microbial biomass can be low, contamination control and reporting standards are critical when studying tumor tissues, particularly in sequencing-based assays (Lu et al., 2024, Fierer et al., 2025) (Figure 14.5).

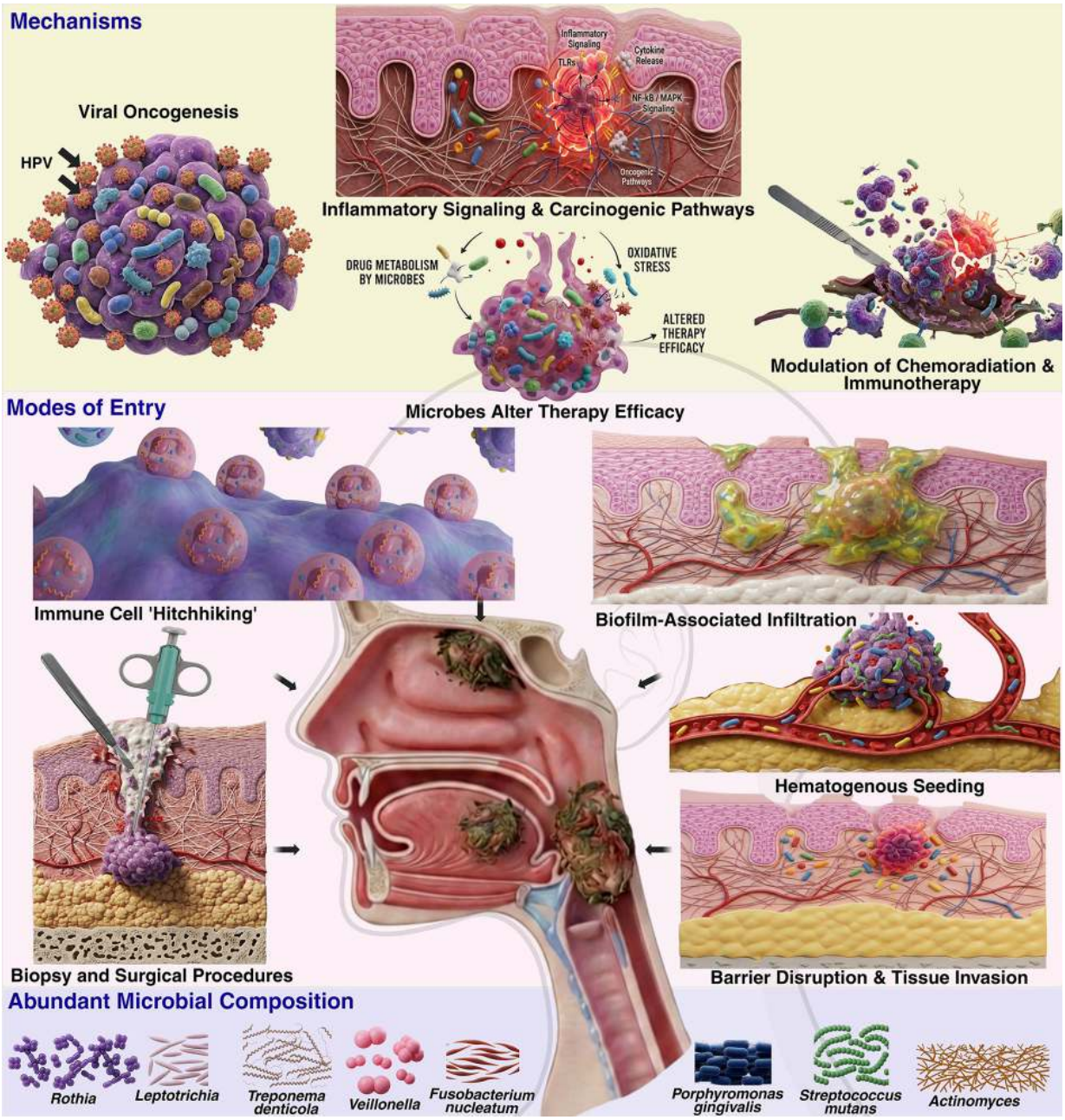

FIGURE 14.5 Microbial contributions to oncogenic pathways and therapy response in head and neck cancer. ⏎

14.5.2.2 Composition and Spatial Localization

Across tissue-based HNSCC datasets, certain repeatedly discussed taxa tend to overlap with oral mucosal communities but display shifted abundances in the tumor compared with adjacent normal tissue. Studies leveraging TCMA and TCGA data showed an enrichment of *Fusobacterium* and *Treponema*, with a parallel reduction in *Streptococcus, Rothia, Actinomyces*, and *Veillonella* in the tumor vs. normal tissue

(Wang et al., 2022b, Datorre et al., 2025). More importantly, studies identified multiple taxa with diagnostic classification potential—with an area under the curve (AUC) > 0.7—for tumor versus normal discrimination. On the other hand, *Actinomyces* and *Rothia* are differentially distributed between tumor and normal tissues in TCMA/TCGA re-analyses and prognostic exploration studies (Wang et al., 2022b, Hamada et al., 2023). Because findings vary by sampling method (tumor core vs. margin vs. surface), decontamination strategy, and subsite composition, these taxa lists should be interpreted as candidate signals requiring orthogonal validation (e.g., via FISH, spatial methods, culture, and targeted PCR). Methodological reviews stress the need for multimodality confirmation to strengthen causal inference (Lu et al., 2024, Fierer et al., 2025).

14.5.2.3 Mechanisms

Intratumoral microbes may exert effects through inflammation, immune modulation, genotoxic stress, and metabolic rewiring, with the net effect varying by microbial load, community structure, and host context. Major mechanistic pathways modified by the intratumoral microbiome in HNSCC include: (1) *inflammatory signaling and carcinogenic pathways:* Oral-associated bacteria can activate host pattern recognition receptors (PRRs), such as TLRs, leading to NF-κB/mitogen-activated protein kinase (MAPK) pathway activation, cytokine production, and a pro-tumor inflammatory milieu. Studies focused on oral microbiome-cancer interactions highlight that organisms such as *Porphyromonas gingivalis* and *F. nucleatum* can promote tumorigenic functions in experimental settings, including enhanced proliferation and invasion in oral cancer models (Meng et al., 2025, Irfan et al., 2020); (2) *immune microenvironment shaping (immunosuppression vs. immune activation):* Intratumoral microbes can shape immune infiltration patterns. HNSCC-focused studies describe dual roles: microbes may either stimulate antitumor immunity or contribute to immunosuppressive conditions depending on composition and bacterial burden. In oropharyngeal HNSCC cohorts receiving checkpoint blockade, early-clinical-trial-linked analyses suggest that total intratumoral bacterial abundance (not necessarily individual taxa) may correlate with an immunosuppressive phenotype and immunotherapy resistance, emphasizing the importance of microbial load as a biological variable (Meng et al., 2025, Sahin et al., 2025, Silver et al., 2025); (3) *chemoradiation and immunotherapy modulation:* Microbes within tumors can alter therapeutic efficacy by metabolizing drugs, influencing oxidative stress responses, or modulating immune activation. Broad intratumoral microbiota studies describe how intratumoral microbes may influence treatment responses, including immunotherapy outcomes, via immune and metabolic mechanisms. In HNSCC, investigators show how microbiome alterations may relate to radiotherapy/chemotherapy effects and treatment toxicities (e.g., mucositis), supporting

clinical interest in microbial markers and interventions, though many studies still assess oral communities rather than tumor tissue directly (Yang et al., 2023, Lu et al., 2024, Sahin et al., 2025, Makarewicz et al., 2024); and (4) *viral oncogenesis and tumor microbiome:* The HNSCC ecosystem includes oncogenic viruses. High-risk HPV (hrHPV), particularly relevant in oropharyngeal cancers, is a major etiologic driver and shapes the tumor immune landscape and prognosis. Studies typically consider HPV as part of the broader microbial milieu influencing HNSCC behavior (Datorre et al., 2025, Sahin et al., 2025) (Figure 14.5).

14.5.2.4 Clinical Relevance

Tissue microbiome signatures can discriminate tumor from adjacent normal samples in computational datasets. Several studies suggest links between intratumoral taxa and outcomes. For instance, TCMA/TCGA analyses reported that certain genera (e.g., *Leptotrichia*), stratified survival in HNSCC and were associated with differential tumor gene expression pathways, illustrating one approach to linking microbial signals with tumor biology. Additionally, organism-specific tissue assays (e.g., for *F. nucleatum*) have been correlated with survival endpoints in HNSCC cohorts (Hamada et al., 2023, Datorre et al., 2025, Bayrak et al., 2025). Further, bioinformatics analyses have indicated that intratumoral microbiome patterns relate to classical HNSCC risk factors and clinical features. TCMA/TCGA frameworks report associations between microbial signatures and variables such as stage, grade, and demographic factors, supporting the idea that microbial features may reflect or influence tumor phenotype (Wang et al., 2022a, Hamada et al., 2024). If validated, tissue microbial features could be incorporated into multiomic stratification models (combining microbial metrics with host transcriptomics or immune scores) to predict risk, progression, or therapy response in HNSCC. More importantly, this could lead to the development of intratumoral-microbe-targeting strategies as emerging therapeutic platforms, including use of selective antimicrobials or localized approaches to reduce harmful taxa or total bacterial burden, while exercising caution to avoid unintended dysbiosis or mucosal injury (Meng et al., 2025, Lu et al., 2024); use of probiotics (conceptual at present for intratumoral targeting) (Hamada et al., 2023); or use of bacteria-based delivery systems (engineered bacteria or bacterial vesicles) (Meng et al., 2025).

Owing to the unparalleled significance of the tumor microbiome in HNSCC, current global efforts are focused on determining whether microbes are localized to tumor cells, immune cells, stroma, necrotic cores, or superficial contamination layers; harmonizing collection (tumor core vs. surface vs. margin), processing, and reporting across subsites (oral cavity vs. oropharynx vs. larynx) to resolve inconsistent signatures (Fierer et al., 2025, Sahin et al., 2025); using controlled preclinical models (including oral

carcinogenesis models) to test whether candidate taxa modulate epithelial transformation, immune phenotypes, or therapy response (Meng et al., 2025, Xie et al., 2025a); and combining microbial features with HPV status, immune infiltration measures, and host omics to test causality, function, and significance.

14.5.3 Thyroid Cancer

Thyroid cancer is the most common endocrine malignancy and includes papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), and anaplastic thyroid cancer (ATC), each with distinct molecular drivers and clinical behavior. Although thyroid tissue is a low-microbial-biomass niche, interest in the thyroid tumor microbiome is rising for several reasons. First, PTC is generally indolent yet clinically heterogeneous, and features such as extrathyroidal extension, invasion status, and lymph node metastasis strongly influence recurrence risk and management. Second, thyroid cancers arise in an endocrine organ with unique local biology (iodine handling, thyroid hormone synthesis, oxidative chemistry), creating a microenvironment where microbial molecules, if present, could modulate inflammation, immune tone, and tumor behavior. Third, intratumoral microbiome science is increasingly viewed as clinically actionable, with potential roles in biomarker development and therapy response in several cancers (Yang et al., 2023, Lu et al., 2024, Wang et al., 2025b, Yuan et al., 2022, Liu et al., 2025c). One of the most prominent tissue-focused studies profiled PTC tumors via 16S rRNA seq, reported detectable microbial abundance across all patients, and further showed that tumor microbial diversity and composition were associated with invasion-related clinical staging. Specifically, the study reported higher alpha diversity in more advanced lesions (T3/T4) compared with T1/T2, and beta diversity differences consistent with stage-associated community shifts. Furthermore, this study identified a set of eight bacterial genera that, in combination, displayed predictive capacity for invasion status (using a receiver operating characteristic (ROC) curve, with the ROC/AUC reported in the study), supporting the hypothesis that tumor-associated microbial features may track with clinically relevant PTC phenotypes (Yuan et al., 2022, Liu et al., 2025b). Beyond descriptive profiling, emerging work integrates microbial abundance with host gene-expression and mechanistic assays. A recent study linked reduced abundance of a specific intratumoral taxon (*Delftia lacustris*) with host gene-expression patterns—including methyltransferase-like 3 (*METTL3*))—and tested functional consequences in PTC cell models, providing an example of a proposed microbe–host regulatory axis. While this requires validation across multicenter cohorts, it illustrates the field's move from association to hypothesis-driven mechanisms (Lu et al., 2024, Qiu et al., 2026).

Screening studies across intratumoral microbiota in thyroid cancer have emphasized that thyroid intratumoral microbiome research is evolving, framing intratumoral microbial functions in immune regulation and TME modulation of thyroid cancer (Liu et al., 2025b, Ataollahi et al., 2024).

14.5.3.1 Trafficking and Modes of Entry

The thyroid is not directly connected to a large luminal microbial compartment, so models of microbial entry emphasize translocation, trafficking, and iatrogenic introduction. Thyroid-specific studies outline plausible routes by which microbes (or microbial components) could appear in tumor tissues, including bloodstream transport and immune-cell-mediated carriage. Predominantly, five routes are commonly considered, including (1) *hematogenous seeding (bloodborne delivery):* Transient bacteremia and circulating microbial components can seed permissive tumor niches, particularly in inflamed tissues with altered vasculature (Yang et al., 2023, Liu et al., 2025b); (2) *immune-cell-mediated trafficking ("hitchhiking"):* Dendritic cells and macrophages can internalize microbial products and transport them to inflamed tissue sites; (3) *lymphatic trafficking:* The thyroid has robust lymphatic drainage, and immune/microbial cargo can move through lymphatic networks, relevant to PTC where lymph node involvement is common (Liu et al., 2025b, Ataollahi et al., 2024); (4) *direct extension from adjacent microbe-exposed sites (rare, context-dependent):* While less direct than aerodigestive sites, the neck region hosts skin and upper airway microbiota; surgical planes and tissue disruption may create opportunities for transfer (Liu et al., 2025b); and (5) *iatrogenic introduction during clinical procedures:* Fine-needle aspiration, biopsy, surgery, and specimen handling can introduce microbes. Because some of these pathways overlap with contamination risk, thyroid tumor microbiome studies must explicitly address "mode of entry" hypotheses while simultaneously documenting procedures and controls to reduce artifactual signals (Fierer et al., 2025) (Figure 14.6).

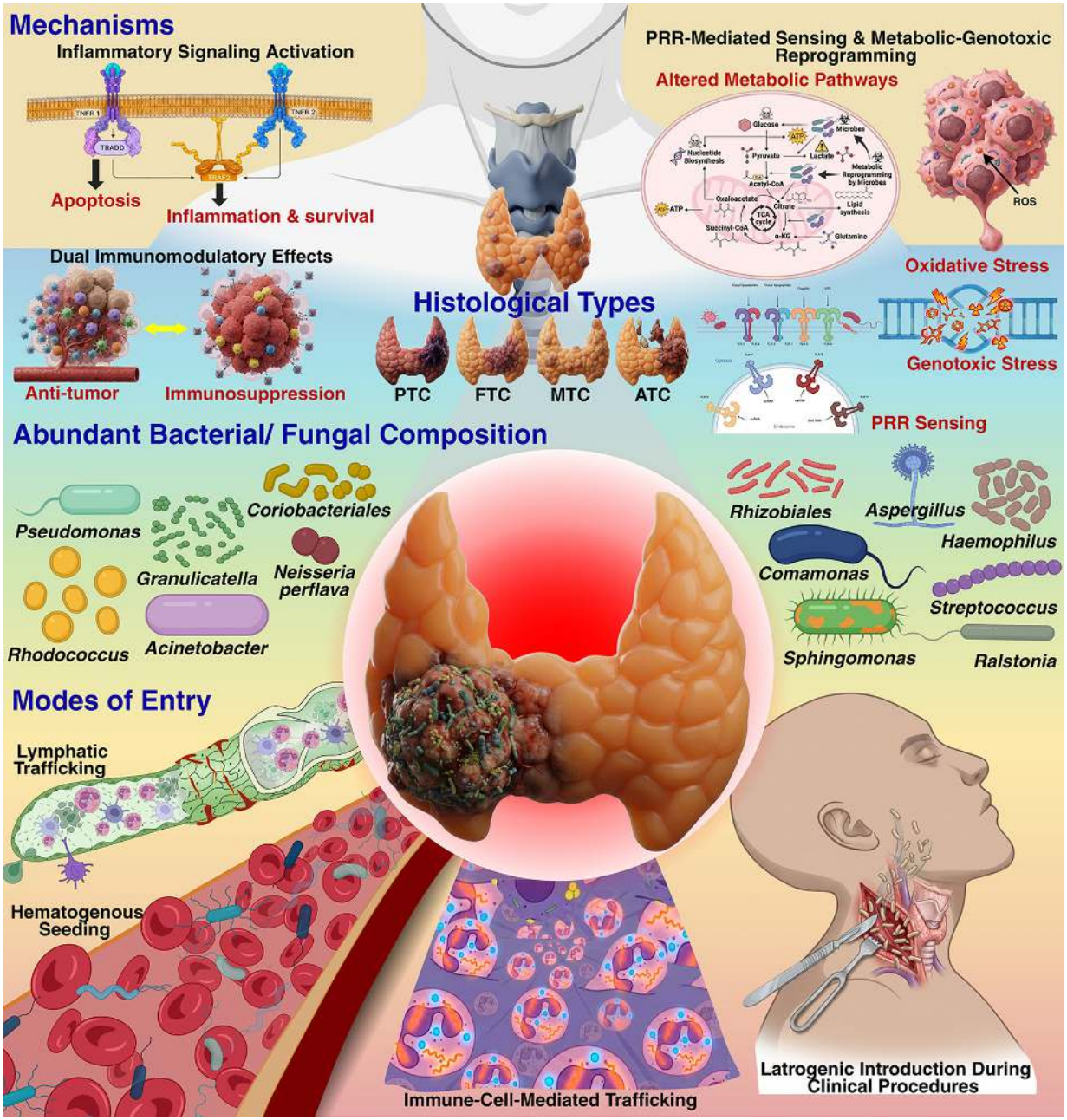

FIGURE 14.6 Biological consequence of intratumoral microbiome in thyroid cancer. ⏎

14.5.3.2 Tumor-Associated Taxa and Tumor-Type Specificity

In the PTC 16S rRNA sequencing study, the tumor microbiome included several prominent genera, and the relative abundance of some taxa differed by clinical stage. For instance, the study reported *Pseudomonas* as a dominant genus overall, with stage-associated differences in abundance, and identified taxa enriched in T1/T2 versus taxa enriched in T3/T4 disease (including taxa that contributed to the eight-genus predictive signature). These results suggest that intratumoral microbial structure may vary with

tumor progression or invasive phenotype (Yuan et al., 2022, Liu et al., 2025b). Most tissue-level thyroid tumor microbiome evidence currently centers on PTC, reflecting its prevalence and the availability of surgical specimens. However, studies have emphasized that different thyroid cancer subtypes likely create distinct TMEs (e.g., aggressiveness and necrosis in ATC, and neuroendocrine biology in MTC) that may influence microbial persistence, immune signaling, and detectability, yet direct comparative tissue microbiome datasets remain limited (Yuan et al., 2022, Liu et al., 2025b, Ataollahi et al., 2024).

14.5.3.3 Mechanisms

The most plausible mechanistic pathways mirror those discussed across the intratumoral microbiome literature, adapted to the thyroid cancer context. Intratumoral microbial molecules can engage PRRs and activate inflammatory programs (e.g., NF-κB, MAPK, and extracellular signal-regulated kinase (ERK)), promoting cytokine production and a pro-tumor inflammatory milieu in susceptible contexts. Thyroid-focused studies discuss the possibility that the intratumoral microbiota could influence thyroid cancer development through inflammatory and immune regulatory routes, though direct causal demonstrations remain limited (Yang et al., 2023, Liu et al., 2025b, Ataollahi et al., 2024, Wu et al., 2023). Intratumoral microbiota can either enhance antitumor immunity (e.g., through innate immune activation) or promote immune suppression (e.g., via anti-inflammatory programs or T-cell dysfunction), depending on microbial composition and burden. A broader study emphasized the intratumoral microbiome's "dual role" in immune modulation and highlighted implications for prognosis and treatment. Thyroid cancer studies proposed that microbial effects may intersect with thyroid tumor immune states and could potentially influence progression and therapeutic response (Yang et al., 2023, Wang et al., 2025b, Liu et al., 2025b, Ataollahi et al., 2024, Wu et al., 2023). Likewise, intratumoral microbiota frameworks described mechanisms such as reactive oxygen species (ROS) modulation, genotoxic metabolites, and metabolic pathway shifts affecting cancer cell physiology and the TME. In PTC tissue profiling, functional inference suggested differences in predicted metabolic pathways between less invasive and more invasive disease groups, supporting the hypothesis that tumor-associated microbes may correlate with metabolic programs relevant to invasion (Yang et al., 2023, Lu et al., 2024, Yuan et al., 2022). A notable thyroid-specific aspect is the intersection with thyroid function and autoimmunity. The PTC tissue study reported correlations between microbial genera and thyroid hormones and thyroid autoantibody markers, raising hypotheses that the endocrine-immune context might relate to tumor-associated microbial patterns. Similarly, studies highlighted thyroid autoimmunity and the hormonal milieu as potentially relevant modifiers of the tumor ecosystem, though

causality remains to be established (Yuan et al., 2022, Liu et al., 2025b, Ataollahi et al., 2024) (Figure 14.6).

14.5.3.4 Clinical Relevance

If robustly validated, intratumoral microbial signatures could augment thyroid nodule risk stratification, complementing imaging and cytology by providing tissue-based ecological features associated with invasion or progression. In PTC, a multitaxa signature demonstrated discriminatory capacity for invasion-related staging in tissue profiling, suggesting the feasibility of biomarker development (Yuan et al., 2022, Liu et al., 2025b). Because invasion status, extrathyroidal extension, and nodal disease influence outcomes, microbial correlates of these features could hold prognostic value. The PTC intratumoral microbiome study linked microbial diversity and composition differences to advanced lesions, motivating longitudinal and recurrence-focused validation. Potential translational directions for thyroid cancer include: (1) microbiome-informed patient stratification (combining microbial metrics with host transcriptomics and immune infiltration) to refine risk or predict therapy response (Lu et al., 2024, Wang et al., 2025b); (2) microbe-targeted interventions (e.g., selective antimicrobials or local modulation), which remain speculative in thyroid cancer and would require careful evaluation to avoid unintended effects (Wang et al., 2025b, Liu et al., 2025b); and (3) mechanism-guided targeting of microbe-linked pathways, exemplified by studies proposing microbe–host axes (e.g., taxon abundance associated with host regulators) that might be therapeutically actionable if causally validated (Lu et al., 2024, Qiu et al., 2026). Across these approaches, the low-biomass nature of thyroid tissue mandates conservative translational claims until signals are confirmed by spatial methods and replicated with stringent controls (Fierer et al., 2025).

Overall, the tumor microbiome in thyroid cancer requires several research priorities, including: (1) spatial localization and viability evidence, to determine whether microbial signals localize to tumor cells, immune cells, stroma, or necrotic areas using imaging/spatial approaches; (2) standardized thyroid tissue workflows, to harmonize sampling (tumor core vs. margin), collection timing, and negative controls, following low-biomass guidelines, to reduce inter-study inconsistency; (3) subtype- and stage-stratified cohorts, to expand beyond PTC to FTC, MTC, and ATC and compare aggressive vs. indolent disease with matched controls; and (4) mechanistic causality, using controlled in vitro and in vivo models to test whether candidate taxa or microbial burden modulate invasion, immune suppression, or endocrine signaling, advancing from association to causation.

14.5.4 Lung Cancer

Lung cancer arises in a uniquely microbe-exposed organ where inhaled air, particulates, and microaspirated oral communities continuously interact with the airway epithelium and immune system. With evolving paradigm that the intratumoral microbiome (bacteria, fungi, viruses, and their products) acts as a functional component of the TME, which is particularly relevant for mucosa-adjacent organs such as the lung (Wong-Rolle et al., 2021, Zhao et al., 2025), there microbes are known to shape tumor evolution, immunity, and therapy response. The major conceptual leap in intratumoral microbiome science from pan-cancer profiling has unveiled that the tumor type, subtype, and host exposures, including smoking, are associated with distinct microbial patterns. In lung cancer, studies have moved beyond detection to mapping where microbes reside (tumor cells vs. immune cells, or airway vs. parenchyma) and which host programs covary with bacterial burden, including oncogenic pathways and immune suppression signatures (Zhao et al., 2020, 2025, Wong-Rolle et al., 2022). Because lung tumors are low-biomass samples compared with airway secretions, rigorous contamination controls are essential. Recent recommendations outline best practices to prevent and report contamination in low-biomass microbiomes, which are directly applicable to lung tumor tissue work (Fierer et al., 2025).

14.5.4.1 Composition and Spatial Ecology

Multiple tissue-based studies indicate that lung tumors harbor bacterial signatures that can differ from adjacent nonmalignant lung. A systematic meta-analysis integrating public 16S rRNA datasets reported consistent decreases in several taxa in tumor tissue compared with tumor-adjacent normal lung (e.g., the *Actinobacteria* phylum and specific families and genera), supporting the idea that tumor development is accompanied by local microbial remodeling within lung tissue. A separate non-small cell lung cancer (NSCLC) tissue study explicitly unveiled that microbial communities vary substantially between individuals and that "enteric/potentially pathogenic/pro-inflammatory" bacteria were more frequently detected in cancer tissue than paired adjacent tissue, highlighting heterogeneity and the need for patient-level resolution rather than one "universal" tumor microbiome (Fierer et al., 2025, Najafi et al., 2021, Liu et al., 2020, Dumont-Leblond et al., 2021). More importantly, spatial transcriptomics in lung cancer has described how spatial approaches uncover microenvironmental niches (the tumor core vs. the invasive front, and tertiary lymphoid structures) that cannot be resolved by bulk sequencing alone, an important prerequisite for mapping microbes to specific tumor neighborhoods (Kang et al., 2025, Cui et al., 2025). To that end, a landmark spatial meta-transcriptomics study of early-stage lung cancer used a method that simultaneously quantified host gene expression and microbial targets across TMEs. It reported that the bacterial burden was significantly higher in

tumor cells than in immune cells or stroma, and that bacterial burden increased along a gradient from tumor-adjacent normal lung and tertiary lymphoid structures to tumor cells and then to airways, consistent with airway-proximal entry and selective enrichment in malignant compartments. Importantly, bacterial burden correlated with oncogenic signaling (e.g., β-catenin) in tumor cells, suggesting that intratumoral bacteria may associate with pro-tumor transcriptional programs in situ (Wong-Rolle et al., 2021, Zhao et al., 2025, Wong-Rolle et al., 2022). More recently, computational single-cell approaches that mine microbial reads from scRNA-seq data (with explicit filtering of contaminants and false positives) have been used to infer which host cell types are "microbe-associated" across primary lung cancer, adjacent tissue, and metastases, proposing that bacteria-associated cells outnumber fungi-associated cells across these lung cancer-related tissues and that the distribution of microbe-associated cells shifts with progression and metastasis. As lung tumors exist at the interface of airways and parenchyma, several studies examine the tracheobronchial compartment as a plausible local source of microbes that may enter tumor tissue. A metagenomic case-control study collected tracheal lavage after intubation along alongside sterilely collected tumor and adjacent lung; it observed differences in lavage beta diversity between lung cancer cases and controls and reported an enrichment of oral commensals (e.g., *Granulicatella adiacens* and *Neisseria subflava*) in lavage among cases, consistent with microaspiration and airway-level ecological shifts in lung cancer. Although this study focuses on tracheobronchial lavage rather than tumor tissue per se, it provides a plausible local reservoir and supports airway-tumor microbial continuity that is distinct from any intestinal explanation (Wong-Rolle et al., 2022, Liu et al., 2020, Bailey et al., 2024).

Fungi are increasingly recognized as low-abundance but immunologically active tumor residents. A pan-cancer mycobiome analysis reported tumor-associated fungal signals across cancers and identified specific fungal associations (e.g., *Blastomyces*) in lung cancer. Similarly, complementary pan-cancer work found that fungi can be detected across many cancers and that fungal communities may co-occur with bacteriomes and immune states, often providing prognostic information (Dohlman et al., 2022, Narunsky-Haziza et al., 2022, Zong et al., 2023). Lung-cancer focused studies also highlighted tumor-resident fungal involvement in immune modulation and clinical outcomes. For example, an integrated single-cell analysis implicated intratumoral fungi in immunosuppressive microenvironments and noted a reported association of *Aspergillus sydowii* enrichment with adverse lung cancer outcomes. Given the challenges of fungal detection (e.g., ITS bias and environmental contamination), contemporary mycobiome studies recommend multimodal validation and careful interpretation, especially in low-biomass lung cancer tissues (Fierer et al., 2025, Ding et al., 2025, Zhao et al., 2025, Zong et al., 2023) (Figure 14.7).

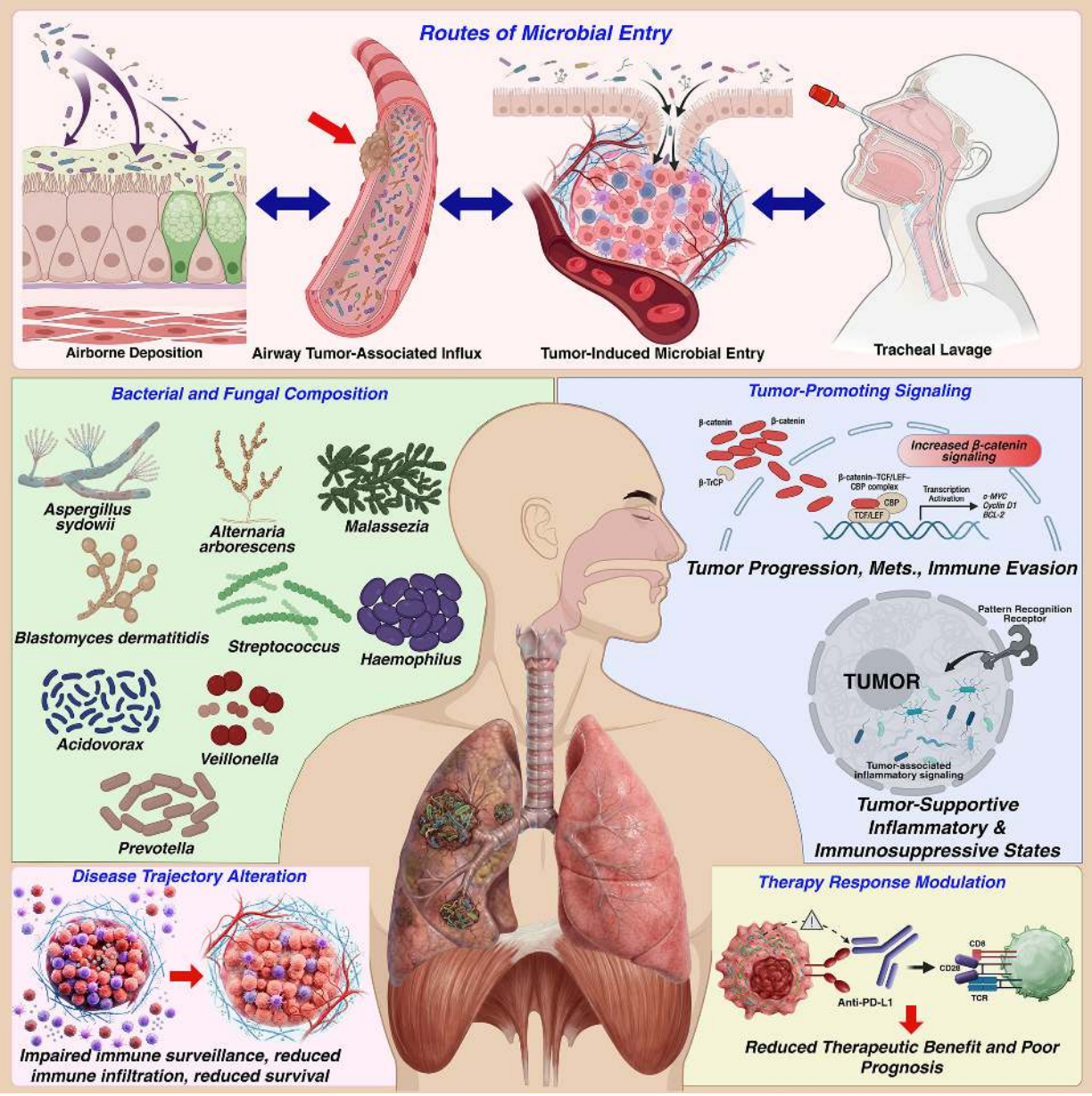

FIGURE 14.7 Tumor–microbial influence on lung cancer progression and therapy response. ⏎

14.5.4.2 Mechanisms

Intratumoral microbes can shape the TIME through PRR signaling, cytokine circuits, and immune cell recruitment. Lung cancer intratumoral microbiome studies emphasize that tumor-resident microbes may promote tumorigenesis and immune evasion by altering immune infiltration and inflammatory signaling, and may influence responses to therapies, especially immunotherapy. This is consistent with broader intratumoral microbiome studies that described how tumor microbiota can modulate innate and

adaptive immunity, affecting prognosis and therapy response across cancers and providing mechanistic templates relevant to lung cancer (Wang et al., 2025b, Zhao et al., 2025, Yang et al., 2025a, Xie et al., 2025a). A key mechanistic pattern is the association between higher intratumoral bacterial burden and altered oncogenic/immune programs. The spatial meta-transcriptomics lung study showed a tumor-cell-enriched bacterial burden and correlations with β-catenin signaling, an axis often linked to immune exclusion and tumor progression, suggesting one route by which bacteria could influence tumor–immune interactions directly within tumor cells (Zhao et al., 2025, Wong-Rolle et al., 2022). Furthermore, emerging evidence suggests that intratumoral microbial features correlate with survival and immune states in lung adenocarcinoma. To that end, matched tumor microbiome and host transcriptome lung adenocarcinoma study (n = 478) found that survival-risk groups defined by immune marker genes differed in intratumoral microbial community characteristics. The high-risk group had higher alpha diversity and an enrichment of lung cancer-associated genera such as *Streptococcus*, while network analyses suggested more active bacteria–immune crosstalk patterns in low-risk tumors. These findings reinforce a model in which intratumoral microbial composition is intertwined with immune contexture and clinical trajectory (Zhao et al., 2025, Shi et al., 2024). A growing conceptual debate in tumor microbiome research is whether total intratumoral microbial abundance is more clinically relevant than the presence of specific taxa. Although affirmative mechanistic evidence in this direction currently comes from other cancers, preliminary observations argue that total intratumoral bacterial abundance can drive neutrophil accumulation and adaptive immune depletion, thereby promoting resistance to PD-L1 blockade, and that abundance rather than taxonomic identity was the key variable in those models. Lung cancer studies discussing immunotherapy resistance increasingly incorporate this "burden" concept as one plausible explanation for inconsistent taxa associations across cohorts (Silver et al., 2025, Zhao et al., 2025, Yang et al., 2025a) (Figure 14.7).

14.5.4.3 Clinical Significance

From a translational standpoint, microbial signatures may serve as diagnostic or prognostic markers. Emerging literature are links lung microbiota, including tumor-associated communities and microbial components, with lung cancer initiation, progression, metastasis, and the potential for microbiome-based biomarkers. The tracheobronchial lavage metagenomics pilot study adds a clinically feasible sampling compartment (airway lavage) that differs between cases and controls and may indicate local ecological changes relevant to risk stratification, though it requires validation in larger cohorts (Liu et al., 2020, Bailey et al., 2024, Kashyap et al., 2024). There is evidence and plausible mechanisms for local or intratumoral microbial modulation of

PD-1/PD-L1 therapy via effects on immune cell activation, antigen presentation, and checkpoint expression. The microbiota-PD-1/PD-L1 interplay emphasizes that microbial communities across body sites including the lung and the TME, can influence checkpoint signaling and immunotherapy outcomes (Figure 14.7). Meanwhile, lung-cancer-specific intratumoral microbiota reviews explicitly frame tumor microbes and their metabolites as modulators of the TIME that may help explain variability in immune checkpoint inhibitor (ICI) response and resistance (Wang et al., 2025b, Zhao et al., 2025, Yang et al., 2025a, Lu et al., 2025c). Given that antibiotic effects are often interpreted through gut dysbiosis, they are also relevant to tumor microbiome work as a major confounder and potential modifier of host immunity and airway ecology. A large analysis of advanced NSCLC treated with chemo-immunotherapy or ICI monotherapy reported that antibiotic exposure around therapy initiation was associated with a worse response rate and shorter progression-free and overall survival, including in chemo-immunotherapy patients, and supported antibiotic stewardship in oncology practice. In contrast, an earlier multicenter analysis suggested that antibiotic-exposed NSCLC patients preserved efficacy outcomes with first-line chemo-immunotherapy, illustrating ongoing controversy and the need to account carefully for timing, indication bias (infection severity), and treatment context. Regardless of the mechanism, these studies underscore why lung tumor microbiome studies must document antibiotic exposure and include it in modeling (Fierer et al., 2025, Austin et al., 2026, Cortellini et al., 2021, Elkrief et al., 2024). Conceptually, microbiome-targeted strategies within the lung tumor ecosystem could aim to (1) reduce harmful intratumoral microbial burden, (2) neutralize microbial products that promote immune suppression, or (3) harness bacteria as engineered delivery vehicles. Studies on tumor-resident bacteria describe how intracellular bacteria can influence metastasis and therapy efficiency and discuss engineered or synthetic biology approaches to target tumor-resident microbes. Lung-cancer-specific intratumoral microbiota studies similarly discuss microbiota-targeted strategies (e.g., rational antibiotic use, engineered bacteria) in the context of overcoming immunotherapy resistance, while noting that clinical translation requires stronger causal evidence (Vella et al., 2025, Zhao et al., 2025, Yang et al., 2025a, Luo et al., 2025) (Figure 14.7).

Overall, the "tumor microbiome" concept in lung cancer has progressed from detection to functional hypothesis generation, aided by pan-cancer evidence of intracellular tumor bacteria and lung-specific spatial and transcriptomic studies that link bacterial burden to tumor-cell oncogenic programs and immune contexture. Tissue and airway studies support the idea that local respiratory ecology, including microaspiration-associated taxa, intersects with lung cancer biology in a manner distinct from intestinal explanations. Clinically, intratumoral microbial signatures (and possibly burden) are

emerging as candidate biomarkers for prognosis and therapy response, but translation will depend on rigorous low-biomass controls, orthogonal validation, and prospective cohorts (Fierer et al., 2025, Zhao et al., 2025, Wong-Rolle et al., 2022, Bailey et al., 2024, Shi et al., 2024, Kashyap et al., 2024). Despite rapid progress, several gaps remain unresolved and require a better understanding. These include: (1) whether intratumoral microbes drive oncogenic signaling and immune escape, or whether tumor conditions (hypoxia, necrosis, and altered vasculature) select for particular microbes as spatial studies linking burden to tumor-cell programs are suggestive but need experimental validation (Zhao et al., 2025, Wong-Rolle et al., 2022); (2) whether tumor-cell intracellular bacteria the primary actors, or whether airway communities and immune-cell-associated microbes dominate functional effects given that evidence indicates tumor-cell enrichment, but airway gradients and lavage signatures suggest local seeding routes (Wong-Rolle et al., 2022); (3) whether total intratumoral microbial abundance a better predictor of the TIME suppression than specific taxa since early evidence suggests abundance may matter, but this requires lung-specific mechanistic confirmation (Silver et al., 2025, Yang et al., 2025a); and (4) which fungi are truly intratumoral in lung cancer (vs. contaminants) and how they sculpt immune niches because pan-cancer work implicates fungal signatures in lung tumors, but tumor-localized functional studies are still limited (Dohlman et al., 2022, Ding et al., 2025).

14.5.5 Breast Cancer

BCa is a heterogeneous disease with distinct molecular subtypes (e.g., hormone receptor-positive, HER2-positive, and triple-negative breast cancer (TNBC)), and variable responses to therapy. This heterogeneity extends beyond tumor genomics and host factors to the TME, which includes immune and stromal components, and increasingly, tumor-resident microbes as an additional ecological layer (Vella et al., 2025, Nejman et al., 2020, Tzeng et al., 2021). Historically, breast tissue was often assumed to be sterile. Over the past decade, multiple studies using culture-independent approaches (primarily 16S rRNA gene sequencing, qPCR, and computational microbial signal extraction from cancer datasets) have reported microbial DNA signatures in normal breast tissue, tumor-adjacent tissue, and breast tumors. The field has also emphasized that tumor tissue is frequently a low-biomass environment, making rigorous contamination controls essential for confident inference (Fierer et al., 2025, Nejman et al., 2020, Tzeng et al., 2021, Rad et al., 2025, Wang et al., 2025b). A landmark pan-cancer study analyzed tumors and matched adjacent normal tissues across seven cancer types and reported that each cancer type harbors a distinct tumor microbiome; notably, BCa displayed a particularly rich and diverse intratumoral bacterial signal. This work

also reported that intratumoral bacteria are mostly intracellular, detectable within both cancer cells and immune cells, supporting the concept that microbes can be spatially embedded within tumor ecosystems rather than merely contaminating surfaces (Nejman et al., 2020, Greathouse et al., 2020). A large study of fresh-frozen surgical specimens profiled breast tissue microbiota across tumor, tumor-adjacent normal, high-risk, and healthy control tissues, and found that multiple genera differed by tissue type and clinical variables (e.g., stage, grade, receptor status, lymphovascular invasion, and node positivity). Importantly, this work integrated microbial data with immune readouts (NanoString immunology gene expression and multiplex cytokine analysis) and constructed microbe-immune correlation networks, providing evidence that breast tissue bacteria may associate with local immune signatures in tumors and benign tissues (Tzeng et al., 2021). A systematic review and meta-analysis synthesizing 16S rRNA sequencing studies (and incorporating TCGA-BCa data) reported that breast tissue microbiota commonly includes the phyla *Proteobacteria, Firmicutes, Actinobacteriota*, and *Bacteroidota*, with *Staphylococcus* and *Corynebacterium* frequently detected across studies. This meta-analytic consolidation is valuable because individual studies often vary by sampling site, processing methods, and decontamination approaches, factors that can shift the apparent microbial profile, especially in low-biomass tissue environments (Fierer et al., 2025, Rad et al., 2025). Furthermore, reanalysis of publicly available breast tissue microbiome datasets using updated pipelines have identified additional differentially abundant taxa compared with original analyses and highlight the importance of reproducible, modern workflows in breast microbiome research. A separate multi-study analysis reported that some taxa (e.g., *Cutibacterium*, including *Cutibacterium acnes*) were reduced in BCa tissues relative to adjacent or normal tissues and may have diagnostic utility; it also explored potential antitumor effects in experimental settings (Sohail et al., 2023, Zhou et al., 2025) (Figure 14.8).

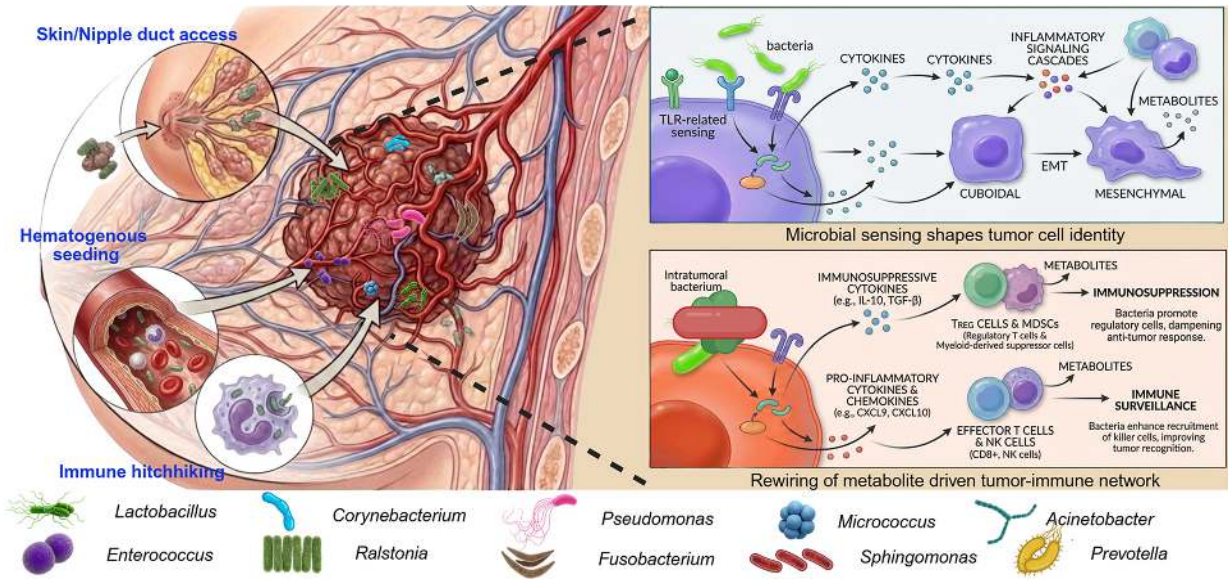

FIGURE 14.8 Microbial seeding and tumor conditioning in BCa. ⏎

14.5.5.1 Trafficking and Modes of Entry

Unlike luminal organs, breast tissue does not host a large internal lumen; thus, tumor-associated microbes arise through multiple nonexclusive entry routes (Vella et al., 2025, Xu et al., 2025, Chang et al., 2024). Key routes include: (1) *skin/nipple-duct access and local translocation*, where the breast is anatomically connected to the external environment via nipple ducts, and microbes may move along ductal structures into tissue microenvironments, especially with inflammation, barrier disruption, or tissue remodeling during tumorigenesis (Tzeng et al., 2021, Chang et al., 2024); (2) *hematogenous seeding*, where circulating microbes or microbial products may seed tumors, particularly given tumor vascular abnormalities, and immune cell trafficking (Wang et al., 2025b, Vella et al., 2025, Nejman et al., 2020); (3) *immune-cell-mediated trafficking ("hitchhiking")*, as intratumoral bacteria are reported to be largely intracellular and detectable in immune cells, consistent with carriage of microbial components via myeloid cells into tumors (Wang et al., 2025b, Vella et al., 2025, Nejman et al., 2020); and (4) *iatrogenic introduction*, where biopsy and surgery can introduce microbes into low-biomass tissues; low-biomass guidelines emphasize documenting and controlling these risks (Fierer et al., 2025, Yeo et al., 2024). These models are not mutually exclusive, and future work using spatial and single-cell approaches is increasingly emphasized to distinguish resident intratumoral microbes from procedural artifacts and to map localization to cancer cells, immune subsets, or stromal compartments (Vella et al., 2025, Nejman et al., 2020, Wang et al., 2025b) (Figure 14.8).

14.5.5.2 BCa Tumor Microbiome Composition and Subtype/Site Specificity

Multiple studies report that breast tissue microbiota differs between tumors and non-malignant tissues (adjacent normal or healthy controls), and that specific genera shift in abundance across tissue states (Table 14.1). For instance, the large breast tissue study reported that *Propionibacterium* (*Cutibacterium*) and *Staphylococcus* were depleted in tumors and correlated with immune signatures (e.g., negative association with oncogenic immune features and positive correlations with T-cell activation-related genes for some taxa). Among these, *C. acnes* stands out because it is frequently observed in breast tissue and serves as both a diagnostic feature and a potential contributor to antitumor activity. A multi-study analysis also reported a reduced abundance of *Cutibacterium* and *C. acnes* in BCa tissue compared with adjacent/normal tissue and presented experimental evidence that *C. acnes* supernatant may exhibit antitumor effects in BCa models. Complementary evidence from fresh tissue cohorts indicates that *Propionibacterium/Cutibacterium* abundance correlates with immune-activation-related genes, including those involved in T-cell activation, suggesting a plausible immune-supportive function within breast tissue ecosystems. From a molecular function standpoint, these findings support a model in which *C. acnes* (or strains thereof) could contribute to a more immune-alert tissue state, and its depletion in tumors could reflect the loss of a protective ecological structure. Cross-study meta-analysis also supports the repeated detection of *Staphylococcus* and *Corynebacterium* across datasets, although relative abundance patterns vary by study design and processing (Tzeng et al., 2021, Rad et al., 2025, Sohail et al., 2023). BCa exhibits profound subtype heterogeneity, highlighting that intratumoral microbial signatures can associate with molecular subtypes and may reflect selective ecological pressures within distinct TMEs (e.g., hypoxia, immune infiltration, and stromal composition). The pan-cancer Science study also reported correlations of intratumoral bacteria with tumor subtypes and clinical variables, motivating subtype-stratified BCa microbiome analyses (Nejman et al., 2020, Greathouse et al., 2020, Xu et al., 2025, Chang et al., 2024, Jiang et al., 2025b).

TABLE 14.1

Composition, Expression, and Spatial Localization of Intratumoral Microbiome in BCa

Genus	Species	Direction in Tumor	Evidence Tier	Remarks
Firmicutes				

Genus	Species	Direction in Tumor	Evidence Tier	Remarks
Staphylococcus	*S. epidermidis*, *S. aureus*	↓ or altered	**High**	Frequently detected across tumor and adjacent tissue; often depleted in tumors; immune associations reported (Kodikara et al., 2025, Ahmad et al., 2025b)
Streptococcus	*S. mitis*, *S. anginosus*	↓	**High**	Present in benign tissue networks; lost in tumors; T-cell correlations reported (Kodikara et al., 2025)
Enterococcus	—	Variable	Moderate	Intracellular reports in tumor cells/immune cells (Yeo et al., 2024)
Lactobacillus	—	Variable	Moderate	Low abundance; context-dependent
Clostridium	—	Rare	Exploratory	Low-frequency signal
Actinobacteriota				
Corynebacterium	—	Variable	**High**	Repeatedly detected across cohorts and meta-analyses (Kodikara et al., 2025, Ahmad et al., 2025b)
Cutibacterium	*C. acnes*	↓	**High**	Reduced in tumors vs. adjacent/normal; potential anti-tumor associations (Yeo et al., 2024, Ahmad et al., 2025b)
Micrococcus	—	Variable	Moderate	Skin/duct associated origin plausible
Brevibacterium	—	Low	Exploratory	Detected in some tumor cohorts
Proteobacteria				
Ralstonia	—	↑ (subset)	**High**	Common TCGA-BCa and fresh tissue signal; contamination-aware confirmed in multiple analyses (Yeo et al., 2024, Ahmad et al., 2025b)
Pseudomonas	—	↑	**High**	Intratumoral and intracellular detection; enriched in pan-cancer breast analyses (Yeo et al., 2024)
Burkholderia	—	↓	Moderate	Reduced in tumors vs. adjacent in meta-analysis (Yeo et al., 2024)
Sphingomonas	—	Variable	Moderate	Detected in multiple tissue studies

Genus	Species	Direction in Tumor	Evidence Tier	Remarks
Acinetobacter	—	Variable	Moderate	Reported in TCGA and fresh tissue
Haemophilus	—	↓	Moderate	Reduced in tumor tissue in sequencing studies (Ahmad et al., 2025b)
Neisseria	—	Low	Exploratory	Low frequency detection
Bacteroidota				
Prevotella	—	Variable	Moderate	Low abundance tissue signal
Bacteroides	—	Rare	Exploratory	Often excluded as gut-dominant unless tissue-validated
Fusobacteriota				
Fusobacterium	*F. nucleatum*	↑ (subset)	Moderate	Intracellular tumor bacteria reported in pan-cancer analyses, including breast (Yeo et al., 2024)
Other Bacteria (Low Abundance but Reported)				
Anaerococcus	Firmicutes		Moderate	Hub genus in benign tissue, absent in tumors (Kodikara et al., 2025)
Caulobacter	Proteobacteria		Moderate	Lost in tumor immune networks (Kodikara et al., 2025)
Granulicatella	Firmicutes		Exploratory	Tumor stage associations reported
Moraxella	Proteobacteria		Exploratory	Low level signal
Rothia	Actinobacteriota		Exploratory	Rare detection
Group	**Taxa**		**Evidence**	**Notes**
Viruses and Fungi (Reported but Limited Evidence)				
Viruses	HPV DNA (rare), bacteriophages		Exploratory	Low-frequency detection in tissue
Fungi	*Malassezia* sp.		Exploratory	Occasional breast tissue detection

14.5.5.3 Mechanisms

Mechanistic models proposed in breast-specific tumor microbiome studies converge on a few key pathways: immune modulation, inflammation, metabolic and epigenetic

influence, and therapy interactions (Wang et al., 2025b, Vella et al., 2025, Jiang et al., 2025b). As with innate sensing, inflammation, and TME remodeling, microbial components can engage host PRRs (e.g., TLR-related sensing), stimulating inflammatory signaling cascades and cytokine networks. Breast-focused studies emphasized that intratumoral microbes may influence inflammation, the epithelial-mesenchymal transition (EMT), and local metabolite production that shapes tumor progression (Wang et al., 2025b; Vella et al., 2025; Xu et al., 2025; Chang et al., 2024; Yeo et al., 2024). A distinctive advance in BCa microbiome work is its integration with immune profiling. The breast tissue study showed that microbial-immune networks differed by tissue type, with more interconnected structures in benign tissues and altered network topology in tumors; it also identified taxa that were hubs in benign tissue networks but absent in cancer-associated networks. These findings support the idea that tumor development may be accompanied by a shift in the local ecological immune interaction map, potentially influencing immune surveillance or immunosuppression (Wang et al., 2025b, Vella et al., 2025, Tzeng et al., 2021). In parallel, BCa microbiome studies describe microbial metabolites and enzymatic activities as potential modulators of host signaling and gene regulation, including epigenetic and metabolic pathways relevant to cancer progression and therapy response. Moreover, emerging studies argue that the tumor-resident microbiota can influence therapeutic response by altering drug metabolism, modulating oxidative stress, or reshaping immune activation. BCa-specific studies emphasize associations between intratumoral dysbiosis and resistance to chemotherapy, immunotherapy, and endocrine therapy, and they discuss early-stage strategies for microbiome targeting to overcome resistance, while acknowledging the need for causality and safety evaluations (Wang et al., 2025b, Xu et al., 2025, Jiang et al., 2025b, Sabeel et al., 2025) (Figure 14.8).

14.5.5.4 Clinical Relevance: Biomarkers, Prognosis, and Therapeutic Opportunities

Breast tissue microbial profiles have shown potential for distinguishing tumor from adjacent normal or healthy tissue states in multiple datasets, including meta-analytic and multi-study reanalysis work, suggesting the possibility of microbial biomarkers that complement existing pathology and molecular profiling (Rad et al., 2025, Sohail et al., 2023, Zhou et al., 2025). Associations between microbial taxa and prognostic clinicopathological features (stage, grade, receptor status, lymphovascular invasion, and node positivity) have been reported in large breast tissue cohorts, and microbial patterns have been correlated with immune features, indicating potential relevance to prognosis and immune phenotyping (Wang et al., 2025b, Tzeng et al., 2021). Furthermore, BCa studies increasingly discuss strategies for local tumor microbiome modulation, including the selective depletion of tumor-associated pathogens, engineered bacteria or bacterial

products, and precision delivery approaches. Nanoplatform studies, for instance, describe microbiome-targeted delivery and antimicrobial strategies aimed at modulating tumor-associated microbes while preserving beneficial communities, an attractive concept given the risks of nonspecific antibiotics. These concepts remain largely preclinical and require careful assessment of safety, ecological consequences, and efficacy in humans (Fierer et al., 2025, Vella et al., 2025, Xu et al., 2025, Jiang et al., 2025b, Sabeel et al., 2025).

In light of this understanding, the next generation of studies must address: (1) spatial validation and cellular localization, to confirm whether key taxa are localized intracellularly in cancer cells or immune cells, and map distribution within tumor core vs. margin vs. stroma using spatial and single-cell approaches; (2) standardized low-biomass workflows, to implement stringent controls and reporting consistent with low-biomass guidelines; (3) subtype-stratified cohorts and longitudinal designs, to evaluate microbial signatures within hormone-receptor^{+}, HER2^{+}, and TNBC subtypes and across treatment timelines to distinguish prognostic from predictive biomarkers; and (4) mechanistic causality low, to move beyond correlations by combining clinical sampling with organoid/co-culture systems and controlled preclinical models.

14.5.6 Colorectal Cancer

CRC has traditionally been examined through the frameworks of genetics, epigenetics, and the TME. Yet a major conceptual shift emerged with the recognition that CRC tumors harbor their own distinct, tissue-resident microbial ecosystems collectively termed the tumor microbiome. This discovery has fundamentally reshaped our understanding of CRC biology. Unlike the gut microbiome, which reflects luminal microbial communities, the tumor microbiome refers specifically to microbes embedded within the tumor tissue itself. These organisms reside intracellularly within cancer cells, including the cytoplasm and perinuclear regions, as well as within immune cells, tumor-associated macrophages, hypoxic niches, fibroblasts, extracellular compartments, and particularly at the invasive fronts where they can potentiate EMT and metastatic dissemination. Advanced spatial imaging reveals that these microbes are not randomly distributed; instead, they exhibit defined spatial patterning that suggests functional specialization within the evolving TME. High-resolution sequencing and imaging studies provide evidence that these microbes are viable, metabolically active, and functionally involved in tumor biology. Recent pan-cancer study established that CRC tumors harbor their own distinct microbial signatures, characterized by anaerobic, invasive, and biofilm-forming bacterial species, mucus-associated aggregates, intratumoral fungi, and prominent tumor-associated viruses (Nejman et al., 2020). These

organisms are not merely surface contaminants but are spatially localized within tumor tissue and immune cell compartments. Spatially resolved sampling across individual CRC lesions demonstrates that the intratumoral microbiome is remarkably homogeneous within a given tumor, indicating that a single biopsy can reliably capture the overall microbial community structure. This intralesional uniformity reduces concerns that micro-spatial variability might obscure associations between tumor-resident microbes and pathological or molecular features. It also supports the practical integration of tumor microbiome profiling into routine diagnostic and pathology workflows.

14.5.6.1 Trafficking and Modes of Entry

In CRC, the tumor microbiome is increasingly understood as an actively trafficked and selectively retained community rather than a passive byproduct of local tissue breakdown. Microorganisms gain access to developing neoplastic lesions through multiple, nonexclusive routes that exploit the altered vascular, immune, and stromal architecture of tumors. Circulating bacteria and bacterial fragments can translocate across compromised endothelial barriers and lodge within the hypoxic, nutrient-rich tumor core, where aberrant angiogenesis and leaky vasculature reduce clearance. Myeloid cells, particularly macrophages and neutrophils, may function as cellular vectors, internalizing microbes at distant sites and subsequently homing to tumors in response to chemokine gradients, thereby delivering viable organisms or immunostimulatory components directly into the malignant niche. Once present, tumor-specific ecological pressures, including an acidic pH, necrosis, and immune tolerance, favor the persistence of select taxa capable of intracellular survival, biofilm formation, or metabolic coupling with cancer cells. These microbes are localized in invasive fronts, glandular structures, or intracellular compartments, where they modulate DNA damage, epigenetic regulation, and inflammatory signaling. Thus, microbial trafficking into CRC reflects a dynamic, tumor-driven process that integrates vascular permeability, immune cell migration, and microbial fitness, shaping cancer progression and therapeutic response.

14.5.6.2 Composition

The tumor microbiome in CRC represents a distinct microbial assemblage that is spatially, functionally, and ecologically different from microorganisms found in adjacent nonmalignant tissue or luminal contents. Across independent studies, the CRC tumor microbiome is dominated by specific bacterial lineages with demonstrated oncogenic or immunomodulatory potential (Table 14.2). Anaerobic and microaerophilic bacteria are particularly prominent, consistent with the hypoxic and necrotic architecture of malignant lesions. Certain taxa display preferential localization to the tumor epithelium,

invasive fronts, or immune infiltrates, suggesting spatial stratification within tumors. Importantly, microbial composition can vary by tumor stage, molecular subtype, and anatomical location, yet converges on a limited set of recurrent organisms across cohorts. The composition of the tumor microbiome is not uniform across all CRCs. Right-sided and left-sided tumors can harbor distinct microbial signatures, and tumors characterized by microsatellite instability or chromosomal instability exhibit differing microbial profiles. Furthermore, intratumoral heterogeneity is common: microbial density and diversity vary between the tumor core, invasive margin, and surrounding stroma. Such spatial heterogeneity suggests that localized microenvironmental factors, including oxygen gradients, immune pressure, and nutrient availability, strongly shape microbial composition (Figure 14.9).

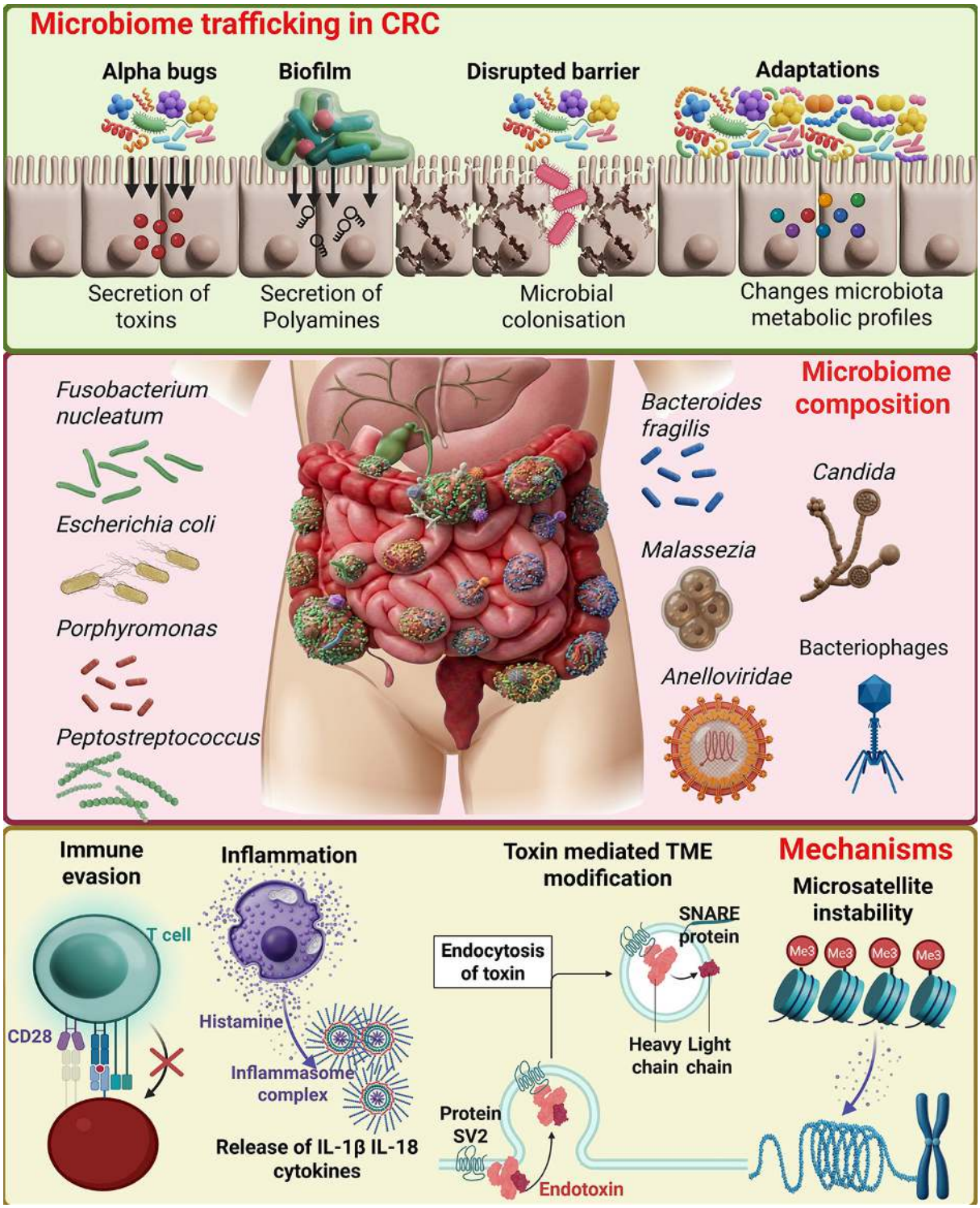

FIGURE 14.9 Biological consequence of tumor microbiome in CRC. ⏎

TABLE 14.2

Commonly Reported Intra-Tumoral Microbial Taxa in CRC ⏎

Microbial Group	Representative Taxa	Tumor-Associated Features	Functional Relevance within Tumors
Bacteria (anaerobic)	*F. nucleatum*	Enriched within tumor glands and invasive fronts; often intracellular	Promotes inflammatory signaling, immune evasion, and tumor cell proliferation
Bacteria (facultative anaerobic)	*Escherichia coli* (pks$^+$ strains)	Localized to tumor epithelium and stromal regions	Produces genotoxins that induce DNA damage and genomic instability
Bacteria (oral associated)	*Porphyromonas*, *Peptostreptococcus*	Selectively retained in malignant tissues	Associated with tumor progression and altered immune infiltration
Bacteria (biofilm forming)	*Bacteroides fragilis* (enterotoxigenic strains)	Patchy intratumoral distribution	Activates oncogenic signaling and inflammatory cascades
Fungi	*Candida* sp. *Malassezia* sp.	Low abundance but recurrent in tumor tissue	Modulates immune activation and inflammatory tone
Viruses	Anelloviridae, bacteriophages	Embedded within tumor-associated microbial networks	Shapes bacterial community structure and gene transfer
Intracellular microbes	Mixed bacterial taxa	Reside within cancer or immune cells	Enhance survival under immune pressure and therapeutic stress

14.5.6.3 Mechanisms

Intratumoral bacteria are frequently found within the intracellular compartments of both cancer cells and infiltrating immune cells, where they actively influence cellular behavior by modulating stress-response pathways, metabolic programs, and signaling networks in a tumor-type-specific manner (Nejman et al., 2020). Increasing evidence across multiple malignancies, including CRC, demonstrates that these intracellular microbial communities exhibit compositions that correlate with tumor subtype, host exposures such as smoking, and therapeutic responsiveness. This pattern underscores that tumor-resident bacteria are not incidental contaminants but are functionally integrated components of the TME, contributing to its biological heterogeneity and clinical behavior (Nejman et al., 2020). CRC tissue profiling reveals a low biomass but structured microbial ecosystem with the identification of key tumor-associated taxa, including *F. nucleatum*. *F. nucleatum* is highly enriched in CRC known to be a highly invasive. This pro-oncogenic bacteria specifically invades tumor cells using its adhesin (*Fusobacterium* adhesin A (FadA)*)*, which binds to the E-cadherin (E-cad) on the colorectal epithelial cells. This binding triggers the E-cad/β-catenin signaling, which in

turn promotes the CRC cell proliferation and pro-tumorigenic transcriptional signatures (Lu et al., 2025c). It reshapes the TME by recruiting myeloid-derived suppressor cells (MDSCs) that activate the TLR4–PAK1–β-catenin inflammatory signaling axis, ultimately driving a pro-invasive and pro-survival cellular phenotype (Lu et al., 2025a). Further, it disrupts the tumor suppressor hippo pathway by inhibiting the kinase cascade (MST1/2 → LATS1/2), which allows unphosphorylated Yes-associated proteins (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) to translocate into the nucleus. This translocation activates genes that reduces apoptosis and promotes cell division, survival, migration, metastasis, and stem-like or regenerative programs (Noorbakhsh et al., 2021). This hippo pathway disruption enhances autophagy and protect CRC cells from 5-fluorouracil (5-fu) and oxaliplatin, directly modulating drug efficacy and driving chemoresistance (Lu et al., 2025c). A landmark study demonstrated that *F. nucleatum* can accompany CRC cells to distant metastatic sites, maintaining its presence within liver metastases, thereby supporting the concept that certain microbes may prime metastatic niches, travel intracellularly with disseminating tumor cells, and actively promote metastasis (Bullman et al., 2017). *F. nucleatum* also suppresses natural killer (NK) cell cytotoxicity via Fap2–TIGIT interactions, promoting immune evasion and tumor progression (Gur et al., 2015). *Peptostreptococcus anaerobius* activates NFκB signaling by recruiting MDSCs and interacting with integrin $\alpha_2\beta_1$ on CRC cells, which in turn enhances CRC progression and creates an immunosuppressive TME (Gu et al., 2023). This bacterial intertumoral presence promotes EMT by this MDSC accumulation and IL-23 secretion, fostering chemoresistance to both oxaliplatin and anti-PD-1 therapy by activating the STAT3-EMT pathways in tumor cells (Yu et al., 2026). Recognition of these intratumoral microbes as potent immunomodulatory agents has spurred growing interest in their influence on immunotherapy outcomes and combination treatment strategies. Emerging evidence suggests that tumor-associated microbial signatures may function as biomarkers for immunotherapy stratification, helping identify patients most likely to benefit from specific immune-based therapies (Figure 14.9).

Bacteroides fragilis (BF, specifically enterotoxigenic strains) found within tumor tissue is associated with DNA damage; it produces BF toxin, which cleaves E-cad, increases epithelial permeability, and activates inflammatory signaling in the colonic mucosa, promoting a tumor-prone microenvironment (Purcell et al., 2017). Experimental models demonstrate that enterotoxigenic BF (ETBF) can directly induce CRC, while human studies associate mucosal positivity for BFT with early neoplastic changes and colorectal neoplasia. Together, these findings implicate ETBF as both an initiator and promoter of carcinogenesis within the tumor-associated microbiota (Boleij et al., 2015). *Campylobacter* sp. (especially *C. jejuni)* were identified in tumor tissue

and associated with invasive phenotypes. *C. jejuni* produces genotoxicity via cytolethal distending toxin (CDT), a DNase-like toxin that induces DNA double-strand breaks (DSBs), gene mutations, chromosomal aberrations, and cell-cycle arrest, thereby driving genomic instability and DNA damage in CRC cells (Yu et al., 2026). *Campylobacter* is enriched in primary CRC from patients with metastasis, and CDT activates the JAK2-STAT3-MMP9 inflammatory signaling pathway that enhances metastasis and can stimulate IL-8–driven inflammatory responses, ultimately promoting tumor progression (He et al., 2024). Tumor microbes play a vital role in metabolic reprogramming by modulating hypoxia adaptation, polyamine metabolism, redox balance, and lactate utilization; these metabolic interactions support tumor survival in nutrient-poor environments. Biofilm communities produce metabolites like short-chain fatty acids (SCFAs), polyamines, and secondary bile acids, activating β-catenin signaling, MAPK/ERK pathways, PI3K-Akt signaling to enhance proliferation and stemness properties. Certain intratumoral bacteria may exert antitumor effects by stimulating antitumor immunity or producing metabolites that induce tumor cell apoptosis, although these protective roles are less well defined in CRC than the pro-tumor effects (Figure 14.9).

CRC tumors also harbor fungi, including *Malassezia restricta, Candida*, and *Saccharomyces* sp. being most prominent. *M. restricta* which correlates with the innate and the inflammatory pathways by activating the complement cascade, particularly the C3–C3a axis, which drives chronic inflammation and supports tumor progression (Aykut et al., 2019). *M. restricta* fungal cell wall components (β-glucans and mannans), activate PRRs such as Dectin-2 and TLR2, leading to NF-κB-mediated inflammatory signaling activation, resulting in the increased transcription of IL-6, TNF-α, and IL-1β, a central pathway in CRC progression, and other pro-tumorigenic cytokines activate the IL-6/STAT3 inflammatory axis activation, which is a hallmark of inflammation-driven CRC (Iliev et al., 2017, Grivennikov et al., 2010). Tumors enriched with *M. restricta* display increased expression of IL-17A, IL-22, and other Th17-associated cytokines, ultimately promoting angiogenesis, neutrophil recruitment, and tumor growth in CRC (Figure 14.9).

Tumor-resident viral components in CRC contribute to inflammation through several interconnected pathways. HPV, although detected at low prevalence in CRC tumors, can activate NF-κB signaling and the IL-6/STAT3 axis, and HPV DNA integration can further trigger cGAS-STING-mediated type I interferon signaling, adding another layer of inflammatory stress within tumor tissue (Galasso et al., 2025). Bacteriophages, which are abundant in CRC tumor tissue, contribute indirectly by reshaping bacterial communities and directly by activating innate immune receptors. Phage-induced bacterial lysis releases microbial components such as LPS, peptidoglycan, and flagellin,

which activate TLR2, TLR4, TLR5, and NOD1/NOD2, amplifying MyD88-dependent inflammatory signaling. These processes enrich pro-tumorigenic bacteria like *Fusobacterium* and *Bacteroides*, further reinforcing inflammatory pathways such as β-catenin, NF-κB, and IL-6/STAT3 within CRC tumors (Strathdee et al., 2023, Sepich-Poore et al., 2021).

14.5.6.4 Clinical Significance and Translational Potential

Tumor biopsies, circulating tumor DNA and exosomes were detected with microbial DNA/RNA that could make microbial signatures serve as specific diagnostic biomarkers. High *F. nucleatum* levels correlate with tumor stage and molecular features, including the unstable genetic microsatellite instability (MSI) status of CRC (Song et al., 2016). It has also been associated with CIMP, the CpG island methylator phenotype with abnormal DNA hypermethylation silencing tumor suppressor and DNA repair genes (Weisenberger et al., 2006). High intratumoral *F. nucleatum* levels correlate with increased recurrence, reduced survival, and increased metastatic potential in CRC (Song et al., 2016). Therapeutic manipulation of the tumor microbiome in CRC is rapidly emerging as a transformative avenue in precision oncology. Therapeutic strategies such as targeted antibiotic modulation selectively aim to eliminate tumor-resident bacteria that drive oncogenic signaling and contribute to therapeutic resistance. Bacteriophage-based approaches offer an even higher level of specificity in eradicating pathogenic intratumoral species while sparing surrounding tissues. In parallel, microbiome-targeted immunotherapy uses tumor-associated microbial signatures to better stratify patients, boost antitumor immune activity, and reduce microbe-driven immune suppression supported by key studies showing that microbial composition can influence immunotherapy response (Gopalakrishnan et al., 2018). Complementing these modalities, microbial enzyme inhibitors target bacterial toxins and metabolic enzymes that promote DNA damage, inflammation, or chemoresistance. Collectively, these therapeutic strategies highlight how deliberate modulation of the tumor microbiome can reprogram the TME, enhance treatment efficacy, and open new pathways for individualized cancer therapy. Despite significant progress, several fundamental questions remain unresolved, particularly how microbes enter tumor cells, how they persist intracellularly, whether they co-evolve alongside cancer clones, and how their spatially defined niches shape therapeutic responsiveness. Addressing these questions will be essential for determining whether targeted microbial modulation can meaningfully improve patient survival. Validating these therapy-associated microbial effects will require deeper mechanistic studies, and the integration of single-cell sequencing, spatial multi-omics, and culturomics will be essential for resolving the functional roles of specific tumor-resident microbes

The tumor microbiome has emerged as a distinct, functionally active, and clinically meaningful dimension of CRC biology. Rather than passive bystanders, tumor-resident microbes directly evolve to shape oncogenic signaling networks, sculpt immune evasion programs, rewire metabolic pathways, and promote metastatic progression. Their influence provides mounting evidence that microbial composition within tumors can modulate sensitivity or resistance to chemotherapy, targeted agents, and immunotherapies. As new insights emerge, it is becoming evident that the tumor microbiome represents a hidden reservoir of diagnostic, prognostic, and therapeutic potential. Exploring this mysterious microbial world through targeted depletion, precision modulation, or integration into biomarker-associated treatment strategies could unearth new arenas for specialized cancer care and fundamentally reshape the future landscape of CRC management.

14.5.7 Gastric Cancer

Gastric cancer (GC) arises in a chronically damaged mucosal environment where epithelial, stromal, immune, and microbial elements are intricately connected. Traditionally, the main etiologic model focused on *H. pylori*-induced chronic gastritis and the Correa cascade (non-atrophic gastritis → atrophic gastritis → intestinal metaplasia → dysplasia → carcinoma). Recent advances in high-depth 16S rRNA sequencing, metagenomics, and metatranscriptomics have shown that the gastric mucosa, and specifically gastric tumors, embrace a complex microbiome that extends beyond *H. pylori* and persists even as *H. pylori* levels decrease in advanced disease (Sgamato et al., 2024). The tumor-associated microbiome aggressively contributes to carcinogenesis via modulating epithelial signaling, shaping the TIME, causing genotoxic and epigenetic changes, and affecting therapeutic outcomes. Spatial analyses show that bacteria are present in mucus layers, glandular lumina, necrotic areas, and even inside cells, where they interact directly with epithelial and immune cells (Zeng et al., 2024). Current evidence suggests that GC is partly driven by microbial influences, with early *H. pylori*-driven changes later reinforced or modified by a broader polymicrobial community (Bautista et al., 2025c, Bakhti et al., 2021).

14.5.7.1 Composition and Spatial Ecology

The gastric tumor microbiome shifts from an acidic, low-diversity environment to one with lower acid levels, increased inflammation, and shifted metabolism, allowing more potentially harmful anaerobic bacteria to grow. *H. pylori* is often the most common microbe, accounting for 40–90% of the microbial population in non-atrophic and atrophic gastritis. Its numbers drop sharply in intestinal metaplasia and cancer,

signifying changes in the stomach environment (Zeng et al., 2024). *H. pylori* can trigger cancer development. Its type IV secretion system injects CagA into gastric epithelial cells, where it can activate signaling pathways such as SHP2, ERK/MAPK, and β-catenin. This process can increase cell growth, disturb normal cell structure, and activate genes linked to cancer (Sgamato et al., 2024). VacA, another *H. pylori* factor, can damage mitochondria, help cells avoid programmed death, and weaken T-cell responses. Ongoing infection triggers a strong inflammatory response, with elevated levels of IL-1β, IL-8, and TNF-α, leading to the production of molecules that can damage DNA. Over time, a long-term infection can cause persistent changes in gene regulation, such as increased methylation of the *CDH1*, *p16*, and *RUNX3* promoters, even after *H. pylori* levels fall (Bakhti et al., 2021).

Changes in the gastric environment, such as hypochlorhydria, mucin depletion, and increased oxygen levels, promote the growth of oral-derived anaerobes that affect tumor biology. *F. nucleatum*, usually uncommon in healthy stomachs, is 3–10 times more abundant in GC tissues (Yang et al., 2025a). Its FadA adhesin binds E-cadherin, activating β-catenin signaling and increasing transcription of *MYC* and *CCND1*. Fap2 interacts with TIGIT on natural killer and T-cells, reducing cytotoxicity and enhancing immune evasion (Zeng et al., 2024). *F. nucleatum* also increases IL-6 and IL-8 production, induces EMT, and raises MMP-2 and MMP-9 expression, all of which enhance tumor invasiveness and worsen prognosis. *Peptostreptococcus stomatis* shows similar GC content and is 2–6 times more abundant in tumor tissues (Zeng et al., 2024). In hypoxic tumor areas, *P. stomatis* ferments amino acids and carbohydrates to produce lactate and short-chain fatty acids, which regulate histone acetylation and support tumor metabolism (Bautista et al., 2025c). *P. stomatis* activates IL-6/STAT3 signaling, promoting cell proliferation, survival, and stemness. This species frequently co-occurs with *Fusobacterium* and *Prevotella*, forming an inflammatory network that increases genotoxic stress. The *Streptococcus anginosus* group (SAG) is also more common in GC, with tumor tissues 2–4 times richer in SAG (Bakhti et al., 2021). SAG induces ROS-mediated DNA damage and activates TLR2-dependent NF-κB signaling, producing increased secretion of IL-6, IL-8, and TNF-α. Tumors with SAG often show neutrophil-dominant inflammation, stromal activation, and MMP-driven ECM degradation, which support invasion and metastasis (Zeng et al., 2024). The frequent incidence of SAG with *Fusobacterium* suggests cooperative pathogenicity in a polymicrobial TME.

Prevotella species, usually rare in healthy stomachs, become more prevalent in atrophic gastritis and GC, frequently reaching 5–15% relative abundance (Bakhti et al., 2021). Their mucin-degrading enzymes compromise the gastric mucus barrier, exposing epithelial cells to acid, bile, and microbial toxins. *Prevotella* prompts strong IL-1β, IL-6,

and IL-8 responses, which increase neutrophil recruitment and promote chronic inflammation. Through cross-feeding, *Prevotella* produces succinate and lactate that support the growth of other anaerobes, forming a constant inflammatory and metabolic cycle (Zeng et al., 2024). *Actinomyces* species, which are moderately enriched in GC, contribute to chronic suppurative inflammation and stimulate IL-6 and TNF-α secretion. Their filamentous growth facilitates deep mucosal penetration, supporting chronic inflammation, fibroblast activation, and desmoplastic remodeling. This process alters tissue stiffness and immune cell infiltration (Zeng et al., 2024). *Veillonella* species, typically low-abundance commensals, increase by threefold to fivefold in GC (Bautista et al., 2025c). They metabolize lactate into propionate and acetate, which in turn influence epithelial proliferation and immunity. Their enrichment indicates a metabolic biota in which lactate-rich tumor niches support anaerobic microbial expansion, while changes in short-chain fatty acids contribute to inflammation. *Campylobacter* species, uncommon in the healthy stomach, are detected more frequently in GC, especially in atrophic and metaplastic mucosa (Bakhti et al., 2021). They activate TLR4 and TLR5, leading to NF-κB-driven cytokine secretion, epithelial injury, ROS generation, and DNA damage. Certain pathogenic *Lactobacillus* strains become abundant in the hypochlorhydric stomach, reaching 10–20% relative abundance in GC tissues (Bakhti et al., 2021). These strains produce acetaldehyde from ethanol and generate ROS, both of which add to DNA damage. Also, they regulate bile acid metabolism and epithelial proliferation, indicating context-dependent pathogenicity in GC. Together, these microbial shifts demonstrate a synchronized ecological and mechanistic progression in early *H. pylori*-driven inflammation, DNA damage, and epigenetic remodeling, establishing a carcinogenic foundation that is later advanced by anaerobic, mucin-degrading, pro-inflammatory, and immunomodulatory taxa. The resulting polymicrobial ecosystem highlights chronic inflammation, metabolic reprogramming, immune evasion, EMT, and invasive behavior, collectively regulating gastric tumorigenesis.

14.5.7.2 Mechanisms

Gastric carcinogenesis derives from a dynamic crosstalk between epithelial injury, chronic inflammation, and a dysbiotic microbial ecosystem that remodels the gastric mucosa toward malignancy. Persistent colonization by *H. pylori* and other taxa such as *F. nucleatum, Peptostreptococcus stomatis, Prevotella*, and *S. anginosus* activates an inflammatory mesh driven by TLR2, TLR4, TLR5, and NOD1/2 signaling. This results in sustained NF-κB, STAT3, and AP-1 activation and increased secretion of IL-1β, IL-6, IL-8, TNF-α, and CXCL chemokines. These cytokines promote epithelial proliferation, inhibit apoptosis, and recruit neutrophils and macrophages that generate abundant ROS/reactive nitrogen species (RNS), leading to a mutagenic TME that accelerates

DNA damage and clonal evolution (Sgamato et al., 2024, Zeng et al., 2024). Microbial genotoxicity increases through bacterial nitrate reductase-mediated formation of N-nitroso compounds in hypochlorhydric stomachs and by microbial metabolites such as acetaldehyde and secondary amines, which induce mutagenic DNA adducts. These changes cause recurrent mutations in *tumor protein p53* (*TP53), CDH1, ARID1A*, and other key regulators of gastric epithelial integrity, shaping distinct mutational signatures linked with microbial exposure (Bautista et al., 2025c). Chronic microbial activation also causes substantial epigenetic remodeling. *H. pylori*-driven inflammation accelerates promoter hypermethylation of *CDH1, p16, RUNX3*, and other tumor suppressors, while other taxa withstand these chromatin changes by producing short-chain fatty acids, polyamines, and inflammatory mediators that modulate DNA methyltransferases and histone-modifying enzymes, strengthening lineage plasticity and metaplastic transitions (Sgamato et al., 2024, Zeng et al., 2024). Microbial adhesins and virulence factors, including *H. pylori* CagA and *Fusobacterium* FadA, directly engage epithelial receptors to activate β-catenin, TGF-β/SMAD, and PI3K/Akt pathways. This drives EMT, downregulation of E-cad, upregulation of vimentin and N-cadherin, and induction of MMPs that facilitate invasion and metastasis (Bautista et al., 2025c, Wang et al., 2023). At the same time, tumor-resident microbes shape the TIME by skewing macrophages toward an M2-like phenotype, promoting regulatory T-cell accumulation, inducing exhaustion markers (PD-1, TIM-3, LAG-3) on $CD8^+$ T-cells, and reducing dendritic cell maturation. *Fusobacterium* also directly inhibits NK-cell cytotoxicity via Fap2-TIGIT interactions. These immunosuppressive effects reduce antitumor immunity and influence responsiveness to immune checkpoint blockade (Zeng et al., 2024). Microbes also rewire tumor metabolism: fermentation of carbohydrates and amino acids yields lactate and short-chain fatty acids that fuel tumor bioenergetics and modulate histone acetylation. Microbial polyamines such as putrescine and spermidine enhance proliferation and DNA stability, while changes in bile acid and cholesterol metabolism alter membrane composition and signaling. This cellular crosstalk reinforces the Warburg phenotype, supports survival in hypoxic tumor niches, and creates a biochemical environment that favors both microbial persistence and malignant progression (Bautista et al., 2025c). Together, these inflammatory, genotoxic, epigenetic, immunologic, and metabolic mechanisms show that GC is not simply a consequence of chronic infection, but a malignancy orchestrated by a complex, evolving tumor-associated microbiome.

14.5.7.3 Impact of the Loss of Protective Commensals

Gastric tumorigenesis is now recognized as a dual process involving the expansion of pro-inflammatory, oncogenic bacteria and the loss of protective commensal

microorganisms that maintain epithelial integrity, colonization resistance, and immune balance. In a healthy stomach, a limited group of acid-tolerant bacteria, such as certain *Lactobacillus* and *Streptococcus* sp., supports mucus barrier stability, antimicrobial peptide production, and immune tolerance (Zeng et al., 2024). Chronic *H. pylori* infection causes atrophy and hypochlorhydria, resulting in a decline in these commensals due to increased pH, loss of parietal cells, changes in mucus composition, and ongoing inflammation (Sgamato et al., 2024). This loss weakens antimicrobial defenses, reduces competitive exclusion, and allows persistent colonization by oral-derived pathobionts, including *Fusobacterium, Peptostreptococcus, Prevotella*, and *S. anginosus* (Zeng et al., 2024, Bakhti et al., 2021). Without commensal-mediated immunoregulation, inflammation worsens, regulatory T-cell induction is impaired, and inflammasome activation increases. The ecological gap is then filled by metabolically active, pro-inflammatory bacteria that increase ROS/RNS, promote the formation of *N*-nitroso compounds, and drive cytokine-mediated epigenetic changes. These processes activate *β*-catenin, TGF-β/SMAD, and PI3K/Akt signaling pathways, which promote EMT and invasion (Zeng et al., 2024, Bautista et al., 2025c). Therefore, gastric carcinogenesis results from both the growth of pathogenic species and the significant loss of a protective microbial barrier that normally prevents malignant transformation (Zeng et al., 2024).

14.5.7.4 Clinical Significance and Translational Implications

Distinct microbial patterns support early detection of high-risk precursor lesions, including atrophic gastritis and intestinal metaplasia. Changes in diversity and increased oral-derived anaerobes often precede neoplastic transformation (Zeng et al., 2024). In *H. pylori*-infected patients, microbial co-signatures enhance risk stratification by distinguishing those at risk of progressing to dysplasia or carcinoma from those with stable chronic gastritis. In established GC, specific microbial profiles, such as *Fusobacterium*-high versus *Fusobacterium*-low tumors, correlate with differences in tumor biology, immune infiltration, and clinical outcomes, underscoring their prognostic value (Bautista et al., 2025c). Integrating microbial data with host genomics, epigenomics, and histopathology permits precise surveillance and the design of personalized risk models. The GC microbiome also influences therapeutic response, affecting the efficacy of chemotherapy and immunotherapy. Microbes in the TME can metabolize chemotherapeutic agents, alter drug concentrations, and modulate DNA damage responses, thus affecting treatment outcomes. In immunotherapy, certain microbial configurations are associated with varied responses to PD-1/PD-L1 blockade: some taxa promote antigen presentation, dendritic cell activation, and effector T-cell recruitment, while others drive Treg accumulation, immune exhaustion, or the

expression of inhibitory ligands, resulting in resistance. Understanding these interplays supports microbiome-informed patient selection, where microbial signatures guide therapies and microbiome-modulating co-therapies could improve outcomes. This evidence has increased interest in microbiome-targeted interventions as adjuncts to standard GC therapies. Investigational strategies include eliminating or suppressing tumor-promoting taxa such as *Fusobacterium* and *Peptostreptococcus* with targeted antibiotics or bacteriophages, restoring protective commensal communities using probiotics, next-generation live biotherapeutics, or defined fecal microbiota-derived consortia; and targeting microbial pathways involved in polyamine synthesis, N-nitroso compound formation, and other genotoxic or pro-proliferative processes (Bautista et al., 2025c). These approaches must be balanced against the risk of disrupting beneficial microbes, causing ecological instability, or enabling colonization by opportunistic taxa. Despite progress, several conceptual and technical obstacles limit clinical translation. Establishing causality rather than correlation is still a major obstacle, as most links between microbes and GC are based on observational studies. Addressing this requires longitudinal cohort studies, interventional trials, and mechanistic validation using organoids and gnotobiotic models. The low-biomass environment of the stomach complicates analysis, making microbial signals susceptible to contamination from reagents and surrounding factors. This necessitates gold-standard controls, decontamination protocols, and orthogonal validation methods such as FISH, qPCR, or culture-based techniques (Zeng et al., 2024). Bulk sequencing methods obscure spatial differences, demonstrating the need for spatial transcriptomics, imaging mass cytometry, and spatial metagenomics to map microbe-cell interactions *in situ*. GC also contains polymicrobial consortia with developing behaviors that cannot be inferred from single-species studies. Systems biology and network-based approaches are needed to clarify cooperative and rivalrous interactions occurring within these communities. Clinical implementation depends on standardizing sampling methods, sequencing platforms, and bioinformatic pipelines to ensure reliable integration of microbial biomarkers. Future research needs to focus on multi-omics integration, spatially characterized microbial mapping, and interventional trials evaluating microbiome-modulating strategies in defined patient subsets. As mechanistic understanding advances, the GC microbiome is expected to play a central role in precision oncology, informing prevention, diagnosis, and therapy selection, and enabling innovative microbial- or metabolite-targeted interventions.

14.5.8 Pancreatic Ductal Adenocarcinoma

PDAC is characterized by dense desmoplasia, profound immune suppression, and marked therapeutic resistance. Over the past several years, contamination-aware studies have overturned the long-standing assumption that PDAC tissue is sterile, revealing instead a low-biomass intratumoral microbiome composed of both bacteria and fungi embedded within the TME. These tumor-resident microorganisms are increasingly implicated in immune-relevant processes such as antigen presentation, innate pattern-recognition signaling, myeloid polarization, immune checkpoint regulation, and patient outcomes. Studies now recognize the intratumoral microbiota as a bona fide component of the TME capable of driving oncogenesis, reshaping antitumor immunity, and modulating therapeutic responsiveness, even in PDAC tissues where microbial biomass is extremely low.

14.5.8.1 Composition

Multiple human studies using FISH, qPCR, and 16S rRNA sequencing consistently confirm the presence of bacterial DNA within resected PDAC tissue. These bacteria often belong to *Proteobacteria* and *Firmicutes*, while anaerobic genera such as *Bacteroides*, *Lactobacillus*, and *Peptoniphilus* have been linked to reduced intratumoral T-cell infiltration and poorer patient survival (Figure 14.10). Earlier landmark studies demonstrated that long-term PDAC survivors possess significantly higher intratumoral microbial diversity, including a four-taxon signature, *Pseudoxanthomonas, Streptomyces, Saccharopolyspora*, and *Bacillus clausii*, associated with immune activation within the tumor (Riquelme et al., 2019). More recent integrated microbiome–metabolome mapping comparing tumors with adjacent tissue (n~105) has identified distinct tumor-enriched species such as *Staphylococcus aureus* and *C. acnes/granulosum*, as well as the presence of *Ralstonia pickettii*, a species associated with worse survival. These studies further highlight microbe-linked metabolic reactions involving molecules such as glycerol-3-phosphate, succinate, and β-alanine that shape the biochemical environment of PDAC tumors (Fang et al., 2024). Among specific candidates of interest, *F. nucleatum*, a well-known oncobacterial species, has been detected within PDAC tumors and linked to poor prognosis, chemoresistance, and immunomodulatory activity driven by virulence factors such as FadA, Fap2, and TIGIT-binding adhesins (Xie et al., 2025a, D'Antonio et al., 2024).

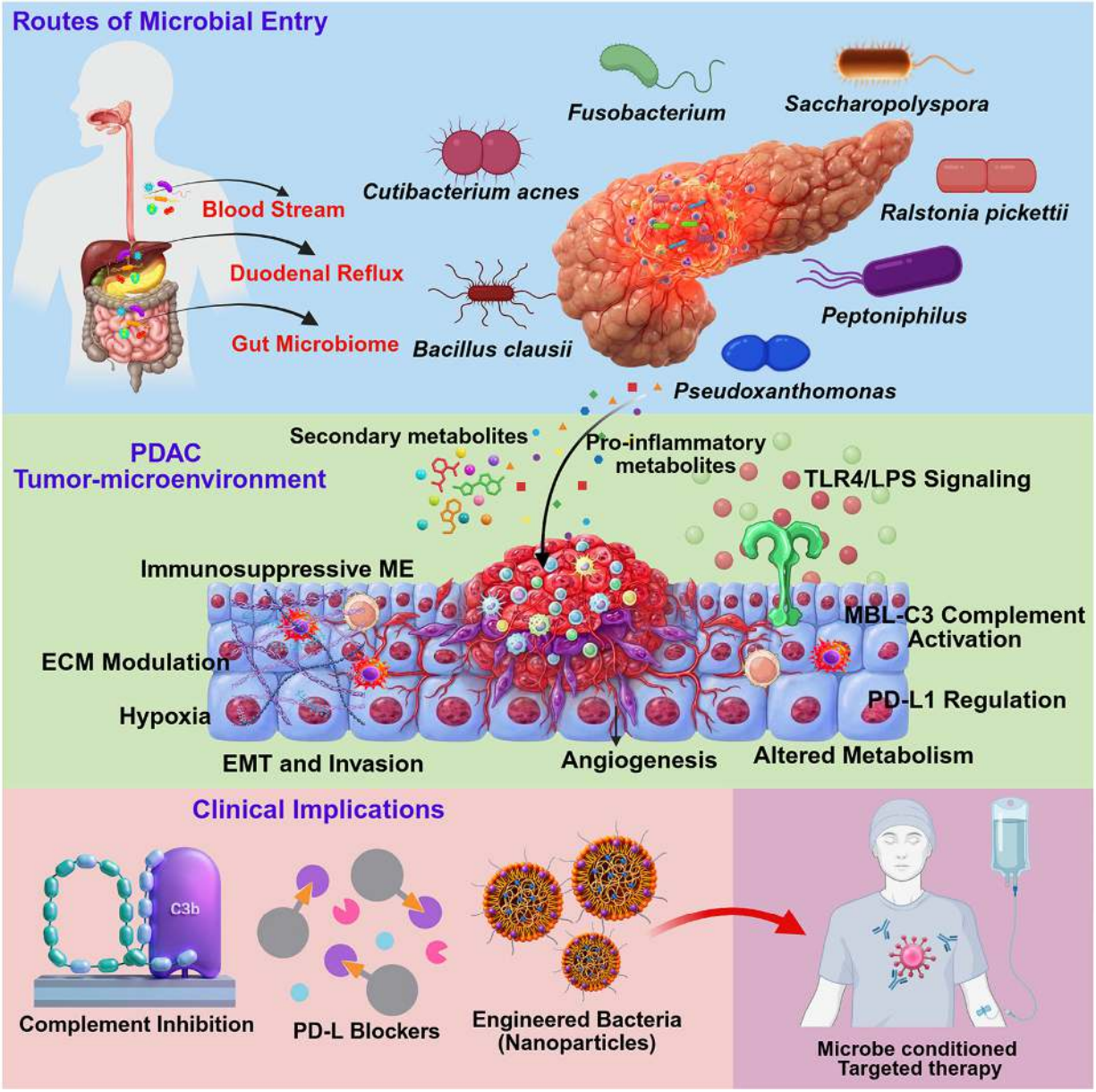

FIGURE 14.10 Intratumoral microbiome trafficking and functional remodeling in PDAC. ⏎

In addition, the intratumoral mycobiome has emerged as a potent regulator of PDAC progression. Mechanistic studies show that *Malassezia* species can accelerate tumorigenesis through activation of the mannose-binding lectin (MBL) pathway, which triggers complement protein C3 and leads to pro-tumor inflammatory cascades. Antifungal treatments or genetic knockout of MBL or C3 suppress tumor growth in preclinical models, demonstrating a direct functional role for intratumoral fungi (Dambuza et al., 2019). Clinically, higher intratumoral abundance of *Malassezia globosa* has been correlated with differences in survival and response to adjuvant

chemotherapy, suggesting a prognostic/theranostic role for fungal load in PDAC tissue itself (Okuno et al., 2023) (Figure 14.10).

14.5.8.2 Mechanisms

Microbial molecules within PDAC engage innate immune signaling pathways, notably TLR–NF-κB cascades, in both immune and malignant cells. LPS acting through TLR4 has been shown to increase PD-L1 expression via MyD88/Akt/NF-κB signaling, paradoxically promoting T-cell infiltration while driving T-cell exhaustion. Blocking PD-L1 reverses this escape mechanism (Yin et al., 2021). On the fungal side, complement activation through the MBL-C3 pathway contributes to tumor proliferation, angiogenesis, and local immunosuppression, positioning fungal dysbiosis as an active participant in PDAC progression (2019, Speth et al., 2022). Single-cell and spatial transcriptomic profiling of primary and metastatic PDAC reveals distinct spatial "ecotypes" characterized by varying degrees of macrophage polarization, complement activation, and T-cell exclusion, particularly at invasive borders and metastatic sites (Khaliq et al., 2024). These spatial maps also identify tumor-promoting stromal programs, such as POSTN$^+$ fibroblasts and SPP1$^+$ macrophages, in contrast to immune-active niches enriched in CCL4$^+$ CD8$^+$ T-cells and IGHG1$^+$ plasma cells, suggesting that local microbial cues could tip these equilibria (Wu et al., 2025). Earlier single-cell atlases of PDAC additionally show dynamic immune remodeling, rising myeloid infiltration, specialized CAF subsets, and NK/T-cell dysfunction, which can be mechanistically linked to microbial PRR and complement signals (Chen et al., 2021, Fu et al., 2024). Across multiple human PDAC cohorts, greater intratumoral bacterial diversity correlates with more robust antitumor immune activation and longer survival, while anaerobe-dominated microbial patterns align with reduced CD4$^+$/CD8$^+$/CD45RO$^+$ effector T-cell infiltration and worse outcomes (Riquelme et al., 2019, Abe et al., 2024). Pan-cancer computational models using intratumoral microbiome survival indices also reveal relationships between microbial signatures, immune enrichment patterns, and predicted drug sensitivity, highlighting the broader immunologic significance of tumor-resident microbes. However, as PDAC tissues contain extremely low microbial biomass, and as small amounts of contamination can produce misleading results, improved and all-inclusive methodological measures (contamination control, sterile surgical procurement, extraction blanks, spike-ins, computational decontamination, orthogonal validation using FISH or culturomics, spatial localization) are required to confirm that microbes truly reside within tumor compartments. To that end, modern multi-omics and high-resolution imaging have affirmatively exposed the genuine presence and biological activity of tumor-resident microbes in PDAC (Figure 14.10).

Within PDAC tissue, LPS signaling through TLR4 can upregulate PD-L1 via MyD88/Akt/NF-κB, and foster resistance to cytotoxic T-cells. PD-L1 blockade restores cytotoxicity, suggesting that even trace amounts of intratumoral LPS from bacterial fragments or dying bacteria may reinforce local immune evasion (Yin et al., 2021). Fungal species such as *Malassezia* activate MBL and subsequently C3 to drive PDAC progression. Antifungal approaches or MBL/C3 inhibition disrupt this pathway in preclinical models, and higher *Malassezia globosa* levels in PDAC tumors correlate with survival and adjuvant chemotherapy benefit, highlighting the clinical relevance of this axis (Dambuza et al., 2019, Okuno et al., 2023). Intratumoral bacteria correlate with distinctive metabolic patterns in PDAC tissue such as altered succinate or glycerol-3-phosphate levels, suggesting functional metabolic cooperation between microbes and tumor cells. These metabolites can modulate macrophage polarization, redox balance, and T-cell fitness, forming a triangular relationship between microbes, metabolism, and immunity (Fang et al., 2024). Intratumoral diversity and specific taxa can stratify patients based on survival and immune infiltration, providing biomarkers potential independent of gut or oral microbiomes. To that end, antifungal and complement-modulating strategies may suppress MBL-C3-driven tumorigenesis; clinical correlations between fungal load and chemotherapy response supporting biopsy-guided trials. Likewise, if PD-L1 is microbially induced, TLR inhibitors or downstream signaling antagonists could be paired with immune checkpoint blockade in PD-L1 high tumor regions. Owing to the identification of microbe-conditioned immune ecotypes at PDAC invasive fronts with spatial transcriptomics, targeted immunotherapies are plausible and most effective. Furthermore, emerging concepts involve engineered bacteria or nanoparticles delivering immune-stimulatory molecules directly into PDAC tumors to overcome stromal barriers. However, the viability and spatial localization of intratumoral microbes, their causal impact on human antitumor immunity, their signaling breadth beyond TLR4 and complement, and their metabolic interactions with cancer and immune cells require immediate focus. Mechanistic and spatial multi-omics approaches are needed to resolve whether microbe-derived metabolites or signaling molecules can be harnessed therapeutically inside the tumor itself (Figure 14.10).

14.5.8.3 Clinical Correlations and Translational Levers

The clinical relevance and translational benefit of intratumoral microbes' presence in PDAC is multifold, including: (1) p*rognostics from tumor microbiome signatures*—tumor-based diversity/abundance profiles and specific taxa (e.g., anaerobe-enriched vs. "survivor" taxa) stratify outcomes and T-cell infiltration, offering tissue-level biomarkers independent of extratumoral niches (Riquelme et al., 2019, Abe et al., 2024); (2) *targeting complement/mycobiome in tissue*—data support antifungal strategies (or

complement modulation) aimed at the tumor mycobiome to blunt MBL-C3 signaling; early human correlative evidence around *Malassezia* abundance and therapy response motivates biopsy-guided trials (Okuno et al., 2023, Speth et al., 2022); (3) l*ocal checkpoint rewiring via TLRs*—if TLR4-PD-L1 upregulation is driven by in situ microbial products, combining TLR antagonists or downstream pathway inhibitors (e.g., Akt/NF-κB modulators) with anti-PD-L1 could be rational, particularly in PD-L1-high, LPS-rich tumor regions (Yin et al., 2021); (4) s*patially resolved therapeutic niches*—spatial atlases suggest immunotherapy-amenable ecotypes at invasive borders and distinct metastatic habitats; overlaying microbial distributions could identify microbe-conditioned niches most likely to respond to local immunomodulation (Khaliq et al., 2024); and (5) engineered intratumoral microbes/nanocarriers—studies propose engineered bacteria or nanoparticles that home to tumors and deliver immune agonists or drug-activating enzymes; with PDAC's stromal barriers, tumor-tropic microbe platforms may help locoregional immunostimulation (Liu et al., 2025b) (Figure 14.10).

14.5.8.4 Future Directions

With such compelling foreseen benefits of intratumoral microbiome in PDAC, addressing the following critical gaps would immensely impact PDAC biology and treatment. The gaps include:

- What fraction of PDACs harbor *viable* intratumoral microbes vs. DNA remnants, and how are they distributed across CAF-rich vs. immune-active niches?
- Can tumor-directed antifungals or complement inhibitors shift infiltrating T-cell phenotypes and outcomes in PDAC patients?
- Beyond TLR4 and complement, do TLR2/1, STING, or C-type lectin pathways mediate tumor-localized microbial effects on antigen presentation and exhaustion in PDAC?
- Which microbial enzymes and metabolites inside PDAC best predict macrophage and T-cell states, and can metabolite scavengers/supplements be delivered intratumorally to rewire immunity?

Altogether, the tumor-immune microbiome of PDAC, distinct from extratumoral communities, has moved from a curiosity to a candidate driver of immune contexture and outcome. Within PDAC tissue, bacteria and fungi engage TLR and complement pathways, sculpting myeloid polarization, checkpoint expression, and spatially patterned T-cell activity. Prognostic links (diversity, specific taxa), fungal-complement axes, and metabolite correlations underscore opportunities for biopsy-guided interventions that act inside the tumor. The field's next phase demands contamination-aware spatial multi-

omics, mechanistic validation in human tissue, and intratumorally-targeted trials, with the promise of finally cracking PDAC's immune fortress from within.

14.5.9 Liver Cancer

For decades, primary liver tumors, especially hepatocellular carcinoma (HCC), were treated as microbially "sterile" spaces, with microbes considered relevant only as etiologic viruses (HBV/HCV) or as systemic infectious complications. That assumption has shifted. A growing body of work now supports the finding that low-biomass microbial communities are detectable within liver tumor tissues (and often in paired non-tumor liver), and that these organisms can correlate with tumor metabolism, epigenetic and transcriptional programs, immune phenotypes, invasion/metastasis, and prognosis (Komiyama et al., 2021, Xue et al., 2022, Chen and Bie, 2025). While the landmark pan-cancer study focused on several tumor types not including HCC, it established key conceptual and methodological foundations: distinct tumor-type microbial signatures, substantial attention to contamination controls, and evidence that bacteria can localize within cancer and immune cells (Nejman et al., 2020, Xue et al., 2022). Most intratumoral microbiome data in "liver cancer" are currently concentrated in HCC, and smaller datasets in other primary liver tumors (e.g., cholangiocarcinoma) or metastatic lesions sampled in the liver (Komiyama et al., 2021, Chen and Bie, 2025).

14.5.9.1 Composition

Multiple 16S rRNA gene sequencing studies on resected specimens report that tumor regions differ from neighboring non-tumor liver in diversity and composition (Komiyama et al., 2021, Xue et al., 2022). A paired liver tumor and adjacent normal liver study across primary and metastatic liver cancers found that tumor regions exhibited higher amplicon sequence variant richness than non-tumor regions in primary liver cancers, along with significant beta-diversity separation between tumor and non-tumor compartments (Komiyama et al., 2021). Likewise, another similar paired HCC and control liver tissues study reported distinct microbial population structures between groups and significant compositional deviation (Xue et al., 2022). These studies, designed for low-biomass and higher resolution (e.g., specialized microbial profiling strategies and integrative multi-omics), emphasized that detection sensitivity, contamination control, and validation orthogonality are decisive for tumor microbiome claims in HCC (Xue et al., 2023, Chen and Bie, 2025). To that end, a study of intratumoral bacteria and metabolism in HCC directly reported detecting bacterial 16S rRNA by FISH and LPS by IHC in patient HCC tissues, strengthening the argument that signals are not purely computational artifacts (Xue et al., 2024). Similarly, multi-omic

work in multifocal HCC has used histologic and ultrastructural methods (e.g., Gram staining, LPS/LTA staining, TEM) to support the presence of bacteria within tumor nodules, while also demonstrating that microbial communities can differ between nodules in the same liver (Lu et al., 2025c).

Across multiple cohorts, liver tumor-associated microbial communities are frequently dominated by phyla commonly detected in human-associated microbial surveys, especially *Proteobacteria, Firmicutes*, and *Bacteroidetes*, with variability across studies, cohorts, etiologies, and sampling/processing pipelines (Komiyama et al., 2021, Xue et al., 2022). Likewise, an independent study reported dominance of these phyla and identified candidate signature taxa distinguishing primary versus metastatic liver cancers including *Bacteroides*, *Romboutsia*, and *Lachnospiraceae* (Komiyama et al., 2021). A notable theme is that liver tumor microbial patterns can correlate with underlying liver disease etiology. A taxon mapping study identified *Ruminococcus gnavus* as a signature taxon for viral-associated HCC (HBV/HCV), suggesting that viral status may stratify tumor microbial features (Komiyama et al., 2021). Mechanistically, the authors highlight prior work showing that *R. gnavus* can produce a pro-inflammatory polysaccharide ligand for TLR4 and can stimulate TNF-α production in immune contexts providing a biologically plausible bridge to inflammation-associated carcinogenesis, though direct causality in HCC remains to be proven (Komiyama et al., 2021). HCC commonly arises on chronically diseased liver and may be multifocal. A large multi-omics analysis of 242 tumor nodules from 58 multifocal HCC patients demonstrated that microbial communities could vary between nodules and correlate with genomic/transcriptomic differences and clinical patterns consistent with intrahepatic metastasis vs. multicentric occurrence (Lu et al., 2025c). Particularly, a bacterial biomarker panel distinguished these biologic categories with reasonable discrimination, and specific bacteria enriched in intrahepatic metastasis nodules (e.g., *Enterococcus faecalis* and *S. anginosus*) were linked to phenotypes such as migration/invasion and immunosuppressive programs in experimental models (Lu et al., 2025c).

14.5.9.2 Mechanisms

Mechanistic work in HCC tumor microbiome research is still emerging and faces classic "chicken-or-egg" uncertainty: do microbes drive phenotypes, or do tumor niches select microbes? Nevertheless, several plausible and partially supported mechanisms have been described. Intratumoral microbes may modulate the immune microenvironment through PRR signaling (e.g., TLR pathways), myeloid skewing and immunosuppressive states, and cytokine/chemokine modulation that affects T-cell infiltration and function. In multifocal HCC, bacteria enriched in intrahepatic-metastasis-like nodules were reported to promote tumor progression in orthotopic mouse models by inducing an

immunosuppressive microenvironment and enhancing EMT-associated programs (Lu et al., 2025c). In addition, a compelling line of evidence connects intratumoral bacteria to metabolic differences between tumors and non-tumor tissues. Paired HCC and normal liver samples showed differential microbial taxa along with distinct metabolic profiles, and displayed correlations between bacterial classes and differentially abundant metabolites (Xue et al., 2022). Building on this, another study reported direct detection of bacterial signals (16S rRNA FISH, LPS staining) in HCC tissues, used 2bRAD-seq for intratumoral microbiome profiling and LC-MS for metabolites, and reported associations between certain bacteria (e.g., *Pseudomonas koreensis*) and metabolites (e.g., N-acetyl-D-glucosamine), suggesting that intratumoral bacteria may participate in, or respond to, metabolic niche features (Xue et al., 2024). While correlation is not causation, these studies provide a rational basis to hypothesize that intratumoral microbes could affect nutrient availability (e.g., amino acids, sugars), redox and hypoxia adaptation, and metabolite signaling that feeds back on immune and cancer cell states (Xue et al., 2024).

Next, a HCC study integrated microbial signals with host transcriptome and DNA methylation profiling in paired samples and reported that microbial taxa correlated with differentially methylated genes and differentially expressed genes, raising the possibility that microbial presence or products might couple to epigenetic regulation in tumor cells or infiltrating immune/stromal cells (Xue et al., 2022). Lastly, the multifocal HCC analysis provided the strongest functional evidence of specific bacteria enriched in metastasis-like nodules that enhance migration/invasion and correlate with EMT-pathway upregulation, with experimental support in orthotopic models (Lu et al., 2025c). This dovetails with the general concept in tumor microbiome research that microbes can influence metastatic potential, either indirectly (via immune suppression) or directly (via genotoxicity, metabolic support, or signaling pathway modulation), although the precise mechanisms in HCC remain an active frontier (Chen and Bie, 2025, Lu et al., 2025c).

14.5.9.3 Clinical and Translational Relevance

HCC tumors possess a distinct intratumoral microbiome compared with adjacent tissues, exhibit heterogeneity, and clustering of microbial profiles has enabled the definition of "hepatotypes" that predicted prognosis after surgery (Sun et al., 2023). This represents an important translational direction: intratumoral microbial signatures could become risk stratifiers for recurrence or survival, especially as they add information beyond established clinicopathologic predictors. Exploratory analyses in HCC cohorts have attempted to associate intratumoral microbial diversity and composition with survival and tumor burden. For example, a gut pathogens study analyzing archival tissues from

the SORAMIC trial reported detectable bacteria in tumoral and non-tumoral tissues and described associations between diversity and survival within subgroups (Schulz et al., 2025). In multifocal HCC, a microbial panel was reported to discriminate intrahepatic-metastasis-like versus multicentric-occurrence-like nodules (AUROC ~0.795), linking microbial patterns to clinically relevant evolutionary trajectories of disease within the liver (Lu et al., 2025c). Collectively, these studies indicate that intratumoral microbiome signals may align with clinically meaningful phenotypes (prognosis, recurrence, metastatic behavior), but the field still needs multicenter validation, standardized pipelines, and prospective studies embedded in clinical workflows. Intratumoral microbial signatures have been proposed as potential diagnostic, prognostic, and predictive biomarkers. In HCC, studies have linked intratumoral microbiome patterns to prognosis after surgery and to multifocal evolutionary patterns (Lu et al., 2025c, Sun et al., 2023). To move from signal to clinical test, the field needs standardized sampling and processing, validated reference ranges and signatures, robust statistical modeling that addresses confounding (etiology, antibiotics, and hospital site), and a demonstration of incremental value beyond existing markers and imaging (Xue et al., 2023, Chen and Bie, 2025). Likewise, the idea of targeting intratumoral microbes could include direct antimicrobial strategies, microbe-informed personalization of systemic therapies, manipulation of microbial metabolites or sensing pathways (e.g., blocking pro-tumor microbial products), and engineered bacterial platforms (longer-term and currently outside routine liver cancer care). While therapeutic targeting is conceptually attractive, clinical translation requires far stronger causal evidence and careful attention to safety in cirrhotic patients, who are already infection-prone (Xue et al., 2023, Chen and Bie, 2025).

14.5.9.4 Future Directions

The tumor microbiome in liver cancer is transitioning from an “interesting observation” to a biologically and clinically relevant variable, supported by repeated detection of microbial signals in tumor tissue, tumor-versus-non-tumor compositional differences, associations with metabolites and host gene regulation, and emerging functional evidence linking specific bacteria to invasion and immunosuppression in HCC models (Komiyama et al., 2021, Xue et al., 2022, Xue et al., 2024, Lu et al., 2025c). However, the strongest next steps are methodological and causal, including defining spatial localization, viability assessment, perturbation experiments, and prospective clinical cohorts. If these challenges are met, intratumoral microbiome features could become part of how we stratify HCC risk and recurrence, understand multifocal tumor evolution, and personalize therapy through microbe-aware TME profiling.

14.5.10 Kidney Cancer

Kidney cancers, most commonly renal cell carcinoma (RCC) and particularly clear cell RCC (ccRCC), are increasingly recognized as tumors that can harbor low-biomass, tumor-associated microorganisms detectable within tumor tissue (Table 14.3), and in adjacent kidney. Rather than focusing on the intestinal microbiome, this chapter provides evidence for intratumoral bacteria and fungi (mycobiome) in kidney cancer (Table 14.3), how these communities are measured and validated, what taxa and ecological patterns have been reported, and the mechanistic hypotheses linking tumor microbes to metabolism, immune remodeling, progression, and therapy outcomes, including immunotherapy (Wang et al., 2021, Xu et al., 2024, Poore et al., 2020, Mou et al., 2025, Wu et al., 2024). RCC is a highly vascular and immunologically complex malignancy, where immune infiltration and TME programs strongly shape disease course and response to systemic therapies. Within this context, the idea that microorganisms reside inside tumors, often intracellularly and at low-biomass, has gained traction across oncology, shifting older assumptions that deep tissues (including kidney) are sterile under noninfectious conditions (Wang et al., 2021, Poore et al., 2020, Vuong et al., 2019, Wen et al., 2025). Tumor microbiome research in kidney cancer has progressed along two complementary tracks. First are direct tissue studies using 16S rRNA gene sequencing in paired tumor and adjacent tissue, supported by diversity and compositional analyses. Second are computational "tumor microbiome" reconstructions from large cancer genomics resources (e.g., RNA-seq), where microbial reads are extracted and then filtered through decontamination pipelines to reduce reagent and batch artifacts. Together, these approaches suggest that kidney tumors can support distinct microbial signatures and that these signatures may align with tumor grade, prognosis, immune state, and immunotherapy outcomes, although causality is still being established (Wang et al., 2021, Xu et al., 2024, Poore et al., 2020, Mou et al., 2025, Wu et al., 2024). Intratumoral microbial biomass in RCC is typically low, which elevates the risk that reagent contaminants ("kitome"), environmental DNA, and handling artifacts dominate the signal. This is why modern tumor microbiome work emphasizes negative controls, batch correction, and explicit decontamination steps (Xu et al., 2024, Poore et al., 2020). Most RCC tissue studies have used 16S rRNA gene amplicon sequencing (commonly V3–V4 regions) to compare paired tumor and adjacent non-tumor tissue, using pipelines such as QIIME and differential abundance tools (e.g., LEfSe), sometimes complemented by functional inference (e.g., PICRUSt). In parallel, computational studies have mined RNA-seq for microbial reads and then applied stringent decontamination to produce tissue- and cancer-type microbial signatures (Wang et al., 2021, Xu et al., 2024, Poore et al., 2020). A newer line of work in renal tumors has adopted orthogonal validation, including 16S rRNA FISH, IHC, TEM, and

even culture, to establish that microbes are not merely sequencing artifacts and may be viable. While some of the most detailed mechanistic work is still emerging, these studies are important because they address the key critique of low-biomass tumor microbiome research: *detection does not equal living intratumoral residency* (Wang et al., 2025b, Kovaleva et al., 2024).

TABLE 14.3

Composition, Expression, and Spatial Localization of Intratumoral Microbiome in Kidney Cancer (RCC) ⏎

Genus/Species	Detection Context	Localization	Reported Association in RCC	Evidence Level
A. Intratumoral Bacterial Microbiome				
Proteobacteria	Tumor vs. adjacent kidney tissue (16S, RNA-seq)	Predominantly intracellular	Dominant phylum in RCC tumors; reduced diversity but altered composition	High (Nejman et al., 2020, Xue et al., 2024)
Burkholderia vietnamiensis	ccRCC tumor tissue (2bRAD-M, metagenomics)	Intracellular within tumor cells	Enriched in high-grade ccRCC; promotes tumor progression in models	Emerging/mechanistic (Nejman et al., 2020, Xue et al., 2024)
Ralstonia sp.	RCC tumor cores (16S)	Intracellular / tissue-resident	Associated with inflammatory signaling pathways	Moderate (Nejman et al., 2020, Xue et al., 2024)
Pseudomonas sp.	RCC tumor tissue (16S)	Intracellular	Stress adaptation; metabolic flexibility	Moderate (Nejman et al., 2020, Xue et al., 2024)
Acinetobacter sp.	Tumor tissue (16S, RNA-seq)	Intracellular	Oxidative stress tolerance; poor prognosis clusters	Moderate (Xue et al., 2024)
Streptococcus sp.	Tumor and adjacent tissue	Immune-associated	Pro-inflammatory TME	Low–moderate (Xue et al., 2024)
Staphylococcus sp.	RCC tumor tissue	Intracellular / macrophages	Immune activation signatures	Low–moderate (Xue et al., 2024)
Clostridium sp.	RCC DNA profiles	Intratumoral	Metabolic modulation	Low (Nejman et al., 2020)
Corynebacterium sp.	Tumor tissue	Stroma / intracellular	Core solid-tumor taxa	Moderate (Xue et al., 2022)

Genus/Species	Detection Context	Localization	Reported Association in RCC	Evidence Level
C. acnes	Tumor stroma	Stromal / intracellular	Ubiquitous tumor-resident bacterium	Moderate (Xue et al., 2022)
B. Intratumoral Fungal Microbiome (Mycobiome)				
Mycobiome (overall fungal load)	Tumor tissue, multi-cohort WGS/ITS	Intratumoral	High fungal ecology → worse prognosis, T-cell exhaustion	High (Schulz et al., 2025)
Aspergillus tanneri	ccRCC tumor (ITS, WGS)	Tumor-resident	Key species linked to poor survival, immunotherapy resistance	High (Schulz et al., 2025)
Rhizopus delemar	ccRCC tumor models	Intratumoral	Promotes ccRCC growth via neutrophil-mediated suppression	Emerging/experimental (Komiyama et al., 2021)
Candida sp.	Tumor tissue	Tumor stroma	Chronic inflammation	Moderate (pan-cancer extrapolated) (Schulz et al., 2025)
Malassezia sp.	Tumor-associated immune cells	Macrophages	Immunosuppressive signaling	Moderate (Schulz et al., 2025)
Cladosporium sp.	Tumor tissue	Tumor-resident	Part of prognostic fungal signatures	Moderate (Schulz et al., 2025)
Aspergillus sp.	Tumor tissue	Intratumoral	Metabolic rewiring	Low-moderate (Xie et al., 2023)
C. Viral Components of the Kidney Tumor Microbiome				
Epstein–Barr virus	Tumor RNA-seq / immune cells	Tumor-infiltrating immune cells	Immune modulation	Moderate (Li et al., 2024)
Cytomegalovirus	Tumor transcriptomics	Tumor / immune cells	Immunoregulation	Moderate (Li et al., 2024)
Torque teno virus	Tumor-derived sequencing	Tumor-resident	Marker of immune dysfunction	Low-moderate (Li et al., 2024)
HPV (rare, incidental)	Tumor DNA			

14.5.10.1 Composition

One of the earliest paired-tissue RCC studies profiled 24 RCC tumors and adjacent normal tissues reported reduced bacterial diversity in RCC tissue (Simpson index) and significant differences in community composition between tumor and adjacent tissue based on UniFrac distance metrics. The same study identified numerous taxa that differed between tumor and normal tissue and proposed that certain features could discriminate RCC from adjacent tissue with high AUC in their dataset (noting the need for validation) (Wang et al., 2021). Independent evidence for tumor-specific microbial patterns in kidney cancer also comes from TCGA-based analyses. A study focusing on ccRCC applied microbial decontamination to RNA-seq data and reported that tumor samples exhibited reduced diversity and distinct phylum-level communities compared with adjacent non-tumorous samples. While RNA-seq microbial extraction has unique limitations, it offers scale and clinical annotation that paired 16S cohorts often lack (Xu et al., 2024, Poore et al., 2020). Across renal tumor microbiome studies, recurring themes include the presence of *Proteobacteria and Firmicutes*, although the exact taxa vary by cohort, platform, and decontamination stringency. The RCC 16S paired-tissue study reported dozens of taxa with differential abundance (some increased and others reduced in tumors), highlighting that the renal TME is associated with a reshaped microbial community rather than a simple "presence/absence" phenomenon (Wang et al., 2021, Wu et al., 2024, Kovaleva et al., 2024). At the large-cohort computational end, the ccRCC TCGA analysis identified a set of genera associated with survival groups, with some correlating with better prognosis and others with worse prognosis. Such findings are hypothesis-generating and underscore that tumor microbial patterns may reflect, or potentially contribute to, immune and metabolic states linked to outcomes (Xu et al., 2024, Wu et al., 2024). A study on kidney tumor resident microbiome summarizes accumulating evidence that kidney tissues can harbor bacteria and that differences between normal and tumor tissues have been reported. More strikingly, an EAU-linked report (UROONCO) describes a multi-method approach in renal tumors including 16S rRNA FISH, IHC, TEM, and tissue culture, reported viable bacteria within renal tumor tissues, frequently located inside tumor cells, and proposing functional linkage between intratumoral microbiota and ccRCC progression using patient-derived xenograft models and antibiotic perturbation. This source illustrated the direction of the field toward causal and spatial validation rather than taxonomic cataloging alone (Nejman et al., 2020, Kovaleva et al., 2024). Pan-cancer work has made it clear that tumors can carry multi-kingdom communities (bacteria, fungi, and viruses), and kidney cancer is becoming one of the most data-rich settings for tumor mycobiome (Poore et al., 2020, Mou et al., 2025). A study profiled intratumoral mycobiome data from 1,044 RCC patients across four cohorts, including 466 immunotherapy-treated patients, and

stratified tumors into mycobiota ecology "depauperate" versus "flourishing" groups based on fungal abundance. The study reported that the high-fungal ("flourishing") group had worse prognosis, higher fungal diversity, downregulated lipid catabolism, and exhausted $CD8^+$ T-cells, and they developed fungal and fungal-gene expression signatures that predicted prognosis and immunotherapy outcomes in RCC and other cancers. They highlighted *Aspergillus tanneri* as a potential key fungal species associated with RCC prognosis and proposed a mechanistic model in which intratumoral fungi influence tumor metabolism and T-cell exhaustion (Mou et al., 2025). This work is notable because it connects tumor mycobiome patterns to RCC's defining clinical feature: variable benefit from ICIs. It also aligns with broader RCC TME literature showing that exhaustion trajectories and metabolic states are central to therapy response heterogeneity (Mou et al., 2025, Vuong et al., 2019, Wen et al., 2025, Gok Yavuz et al., 2025) (Figure 14.11).

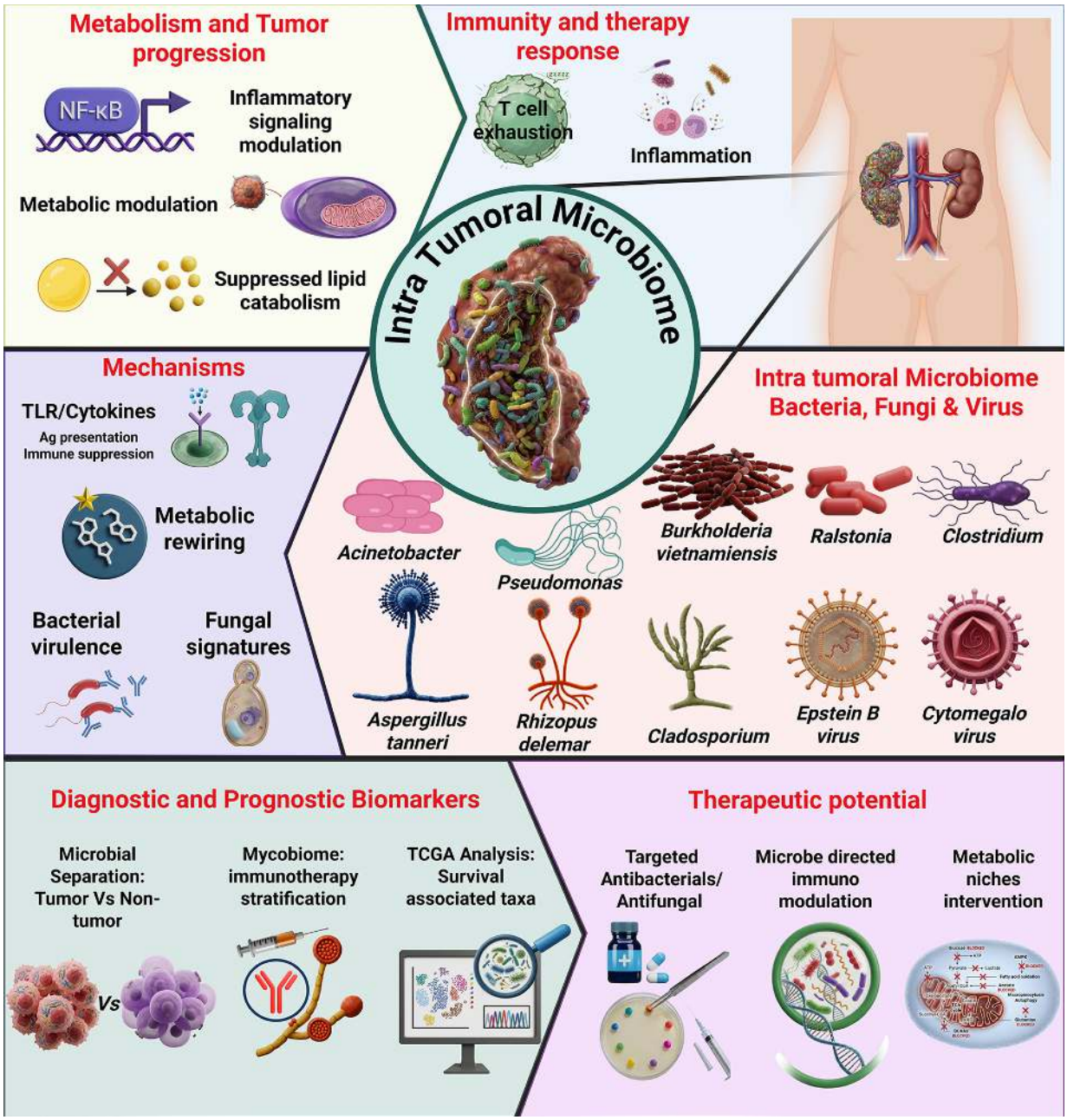

FIGURE 14.11 Functional landscape of intratumoral microbiome in kidney cancer. ⏎

14.5.10.2 Mechanisms

ccRCC is widely recognized as one of the most immune-infiltrated tumors, and immune composition strongly affects systemic therapy response. Within this environment, intratumoral microbes may shape immunity through PRR activation, altered cytokine tone, and remodeling of antigen presentation pathways, general mechanisms emphasized in RCC microbiota reviews and in broader tumor microbiome frameworks (Xue et al., 2023, Wu et al., 2024, Vuong et al., 2019, Gok Yavuz et al., 2025). The RCC

mycobiome study provides kidney-specific evidence for this principle, associating higher fungal ecology with exhausted $CD8^+$ T-cells and poorer immunotherapy outcomes. While causal inference remains difficult in observational cohorts, the consistent linkage between intratumoral fungal burden, immune exhaustion, and outcome supports a biologically plausible route for microbes to function as TME modulators in RCC (Mou et al., 2025, Wen et al., 2025). RCC, especially ccRCC, is characterized by major metabolic rewiring (including lipid and oxygen-sensing programs). Intratumoral organisms could exploit and reinforce these niches by consuming or producing metabolites that influence tumor and immune cell states. The RCC mycobiome study explicitly links fungal ecology to suppressed lipid catabolism, connecting tumor fungi to a metabolic axis central to RCC biology. Reviews of microbiota in RCC similarly highlight microbial effects on host metabolism and drug reactions as plausible mechanisms that warrant experimental validation (Mou et al., 2025, Wu et al., 2024, Vuong et al., 2019, Kovaleva et al., 2024). Mechanistic claims are strongest when supported by perturbation. The EAU-linked renal tumor microbiota report describes a workflow combining PDX models that retain tumor microbiota with antibiotic perturbation to test whether removing intratumoral microbiota affects tumor progression, and it identifies a candidate bacterium (*Burkholderia vietnamiensis*) enriched in high-grade malignant ccRCC that promoted progression in experimental systems. This is promising but should be treated as preliminary until full peer-reviewed publication; nevertheless, it exemplifies the next stage of kidney tumor microbiome science: moving from "what's there" to "what it does" (Wu et al., 2024, Kovaleva et al., 2024) (Figure 14.11).

14.5.10.3 Clinical and Translational Implications

Large cancer-genomics re-analyses have shown that microbial signatures can be found in tissue datasets and may have diagnostic potential across cancers, motivating tumor-resident microbiome biomarker work (with stringent decontamination). In kidney cancer specifically, TCGA-based analyses have reported microbial genera associated with survival groups, suggesting potential prognostic utility if validated prospectively and across platforms. Tissue-based 16S studies also report tumor-versus-normal separation by diversity and composition metrics, supporting the feasibility of tumor microbiome features as classifiers, again with the important caveat that low-biomass signals require rigorous replication (Wang et al., 2021, Xu et al., 2024, Poore et al., 2020). RCC is a flagship disease for ICI, yet outcomes are heterogeneous and biomarkers remain imperfect. The intratumoral mycobiome work directly addresses this gap by developing mycobiome-based signatures that predicted immunotherapy outcomes in RCC cohorts. If validated clinically, tumor mycobiome features could complement TME-based

stratification approaches (immune-enriched vs. stroma-enriched states) that are already known to correlate with therapy response (Mou et al., 2025, Wen et al., 2025, Gok Yavuz et al., 2025). The idea of modulating tumor-resident microbes through targeted antimicrobials, microbe-directed immunomodulation, or metabolic intervention, remains early in RCC. RCC microbiota studies emphasize both opportunity and risk: altering microbial components might improve therapy response, but intervention must avoid collateral harm and must be grounded in causal evidence. Preliminary perturbation strategies (e.g., antibiotic elimination of intratumoral microbes in models) have been described in emerging work, illustrating possible experimental paths toward therapeutic translation (Wang et al., 2025b, Wu et al., 2024, Kovaleva et al., 2024) (Figure 14.11).

Moving forward, the most promising advances in kidney tumor microbiome research are likely to come from spatially resolved, multi-omic, and perturbation-based approaches. RCC TME heterogeneity is now routinely mapped by single-cell and spatial technologies, and integrating these with microbial localization (e.g., multiplex FISH/IHC) could reveal where microbes reside (tumor cells, macrophages, and endothelial niches) and how they align with immune exhaustion and metabolic programs (Mou et al., 2025, Gok Yavuz et al., 2025). Second, the prospective clinical validation is essential, particularly for mycobiome-based immunotherapy predictors, because retrospective cohort signatures often fail when sampling and processing differ across institutions. Third, mechanistic work should prioritize defined organisms and pathways (e.g., candidate bacteria linked to grade or candidate fungi linked to exhaustion), tested in models that preserve tumor architecture and immune context (organoids, ex vivo slices, humanized systems) (Ding et al., 2025, Mou et al., 2025, Wu et al., 2024).

14.5.11 Bladder Cancer

The discovery of a resident bladder microbiome has transformed our understanding of urothelial biology and bladder cancer, shifting the view from sterility to a dynamic microbial environment. The bladder microbiome is now recognized as a key factor in disease development and treatment, as changes in microbial abundance can influence immune responses, epithelial signaling, and carcinogenesis (Bucevic Popovic et al., 2018). In healthy individuals, the microbiome consists of *Lactobacillus*, *Gardnerella*, *Streptococcus*, *Corynebacterium*, and *Staphylococcus*, which help maintain bladder health and support immune function. Cancer disrupts this balance, causing significant shifts in microbial composition. Some bacteria become more common while others decrease, actively modulating the TME by affecting inflammation, host-microbe interactions, and immune surveillance. Consequently, the composition of the bladder microbiome may influence disease progression and treatment outcomes.

14.5.11.1 Bladder Physiology and Its Relevance to Microbial Ecology

The physiology of the bladder creates a distinct ecological niche that tightly regulates microbial colonization and persistence. The urothelium is a stratified epithelium composed of basal, intermediate, and umbrella cell layers, the latter forming a highly impermeable barrier *via* dense tight junctions, uroplakin plaques, and a surface glycosaminoglycan (GAG) coating. This combined barrier reduces permeability and defends the underlying stroma from urine-borne toxins, solutes, and potential pathogens (Birder et al., 2013). Recurrent bladder expansion and compression during filling and emptying mechanically stress the urothelium, transiently altering membrane tension and receptor accessibility, potentially influencing microbial adherence, detachment, or invasion dynamics. Moreover, the urine-clearing associated with urination imposes strong selective pressure favoring microorganisms capable of biofilm formation, epithelial adhesion, or intracellular localization as survival strategies (Mysorekar et al., 2006). Immune surveillance within the bladder further contributes to this selective landscape. Unlike the gut, the bladder lacks organized mucosal lymphoid structures and instead relies on rapid, innate responses coordinated by urothelial and local immune cells. TLRs (TLR2, TLR4, TLR5) expressed on urothelial cells detect conserved microbial ligands, initiating cascades that drive cytokine release and antimicrobial peptide production (Song et al., 2008) (Figure 14.12). While acute activation of these pathways provides effective protection, chronic or dysregulated signaling can aggravate sustained inflammation, epithelial remodeling, and oxidative or genotoxic stress, which could contribute to malignant transformation. In this way, bladder physiology and immunity jointly define not only which microbes can establish a habitat but also shape host tissue adaptation and responses to microbial molecular signatures.

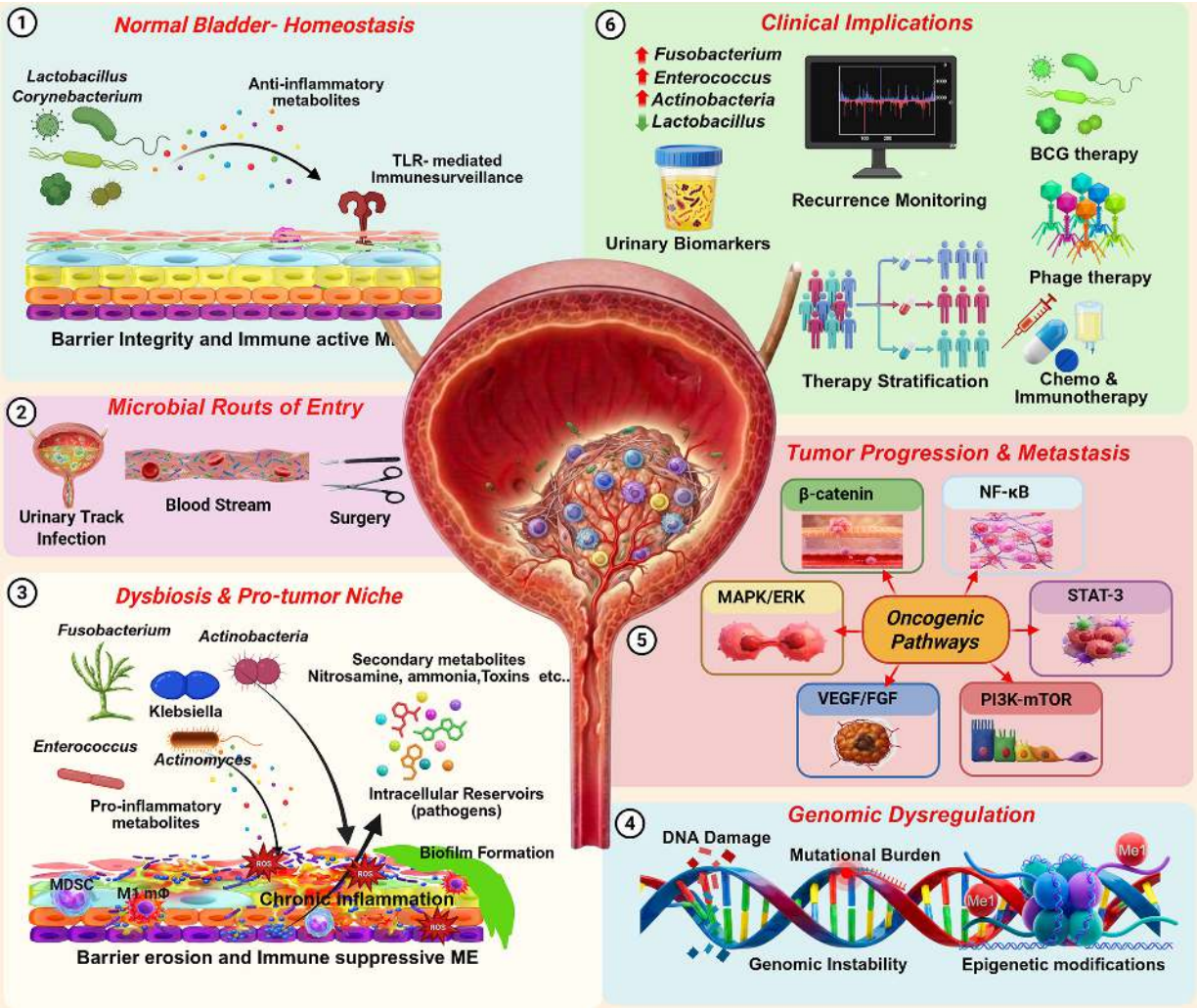

FIGURE 14.12 Urothelial dysbiosis and microbiome-associated bladder cancer progression. ⏎

14.5.11.2 Composition and Spatial Localization

Bladder cancer (BLCA) is relatively tumor microbiome-active and is known to harbor *F. nucleatum, Acinetobacter* sp., *Actinomyces* sp., *E. faecalis, Klebsiella pneumoniae, Streptococcus* sp., etc. *F. nucleatum* is rarely detected in healthy urine, yet its relative abundance rises to 2–8% in high-grade or muscle-invasive BLCA and correlates with higher-grade, more aggressive disease (Mansour et al., 2020). It activates β-catenin and NF-κB signaling to drive epithelial proliferation and inflammatory gene expression (Kostic et al., 2013). *F. nucleatum* dampens antitumor immunity by recruiting myeloid-derived suppressor cells (MDSCs) and curbing cytotoxic T-cell function (Kostic et al., 2013). The FadA adhesin binds to E-cadherin, destabilizing epithelial junctions and promoting invasion (Rubinstein et al., 2013). It also generates SCFAs and formate that

reshape epigenetics in urothelial cells, while ROS production induces DNA damage and impairs repair, fueling genomic instability (Gherasim et al., 2025). *Acinetobacter* sp. is rarely present in healthy bladders however, their abundance increases in BLCA, frequently comprising 3–10% of tumor-associated microbial communities and reaching higher levels in Bacillus Calmette-Guérin (BCG) non-responders (Miyake et al., 2020, Ginwala et al., 2025). *Acinetobacter* sp. drives BLCA inflammation by triggering robust secretion of IL-6, IL-8, and TNF-α, creating a milieu that spurs urothelial growth and remodeling (Ginwala et al., 2025). Their enrichment in BCG non-responders suggests interference with BCG adherence or antigen presentation, which are critical for antitumor immunity (Miyake et al., 2020). *A. baumannii* outer membrane vesicles (OMVs), laden with lipo-oligosaccharides (LOS) and proteases, skew macrophage polarization and blunt adaptive responses (Weng et al., 2024). These OMVs erode epithelial barriers, aiding microbial ingress and carcinogen exposure, while biofilms shield bacteria from clearance and sustain pro-carcinogenic inflammation (Ginwala et al., 2025, Weng et al., 2024). Likewise, *Actinomyces* sp. constitutes less than 1% of the microbial population in healthy urothelium, but its abundance increases to 2–6% in inflamed or dysplastic BLCA (Gherasim et al., 2025, Valour et al., 2014). *Actinomyces* sp. inflicts urothelial damage via the production of nitrosamines and ammonia derivatives, compounds that trigger DNA lesions and epithelial hyperplasia (Gherasim et al., 2025). Its filamentous growth enables stromal invasion, disrupting architecture to favor tumor aggressiveness and persistence (Valour et al., 2014). Metabolic shifts in urinary pH further sensitize the urothelium to carcinogens, amplifying mutagenic pressure (Gherasim et al., 2025). Again, *E. faecalis* is infrequently observed in healthy urine, but its abundance increases to 1–4% in BLCA, particularly within ROS-rich TMEs (Horsley et al., 2013, Huycke et al., 2002). *E. faecalis* initiates destruction through genotoxic ROS-extracellular superoxide (O_2^-) and H_2O_2, which shatter DNA strands and destabilize chromosomes (Huycke et al., 2002). It ignites TLR2/4-NFκB pathways, kindling inflammation that synergizes with carcinogens to hasten tumorigenesis (Horsley et al., 2013). Intracellular persistence prolongs injury, while gelatinase and cytolysin digest ECM to boost invasion and progression (Horsley et al., 2013, Huycke et al., 2002). *K. pneumoniae* is present at low levels in healthy bladders but is consistently elevated in BLCA, often accounting for 2–7% of the microbial community, particularly in biofilm-dominant niches. *K. pneumoniae* unleashes siderophores and colibactin-like genotoxins that scar DNA and kindle tumor initiation (Lawlor et al., 2007, Vuotto et al., 2014). Biofilms lock in urothelial colonization, while iron chelation via siderophores starves or feeds proliferating cells as needed (Lawlor et al., 2007, Vuotto et al., 2014). Urease increases NH_3 production, irritating the epithelium and cementing an inflammatory niche. In addition, *Streptococcus* sp. represents a minor

constituent in healthy bladders, yet its abundance increases to 3–9% in BLCA characterized by IL-6- and IL-8-mediated inflammation. *Streptococcus* sp. provokes IL-6/IL-8 release from urothelial cells, feeding chronic inflammation and hyperplasia (Leclercq et al., 2016). Tumor enrichment implicates this genus in pro-TMEs, with H_2O_2 adding oxidative DNA hits and TLR2 crosstalk amplifying signals for niche remodeling (Leclercq et al., 2016) (Figure 14.12).

14.5.11.3 Mechanisms

BLCA develops through two primary molecular pathways: the papillary pathway, which is driven by *fibroblast growth factor receptor 3* (*FGFR3)* and *HRAS* mutations and results in low-grade, non-invasive tumors, and the carcinoma in situ pathway, which is marked by early loss of *TP53* and *retinoblastoma 1*(*RB1)*, leading to genomic instability and progression to muscle-invasive disease (Knowles et al., 2015, Al-Obaidy et al., 2021). Microbial dysbiosis contributes to both pathways by increasing DNA damage, compromising urothelial barrier integrity, and creating an immune environment that favors malignant transformation. Sustained microbial activation of urothelial TLRs maintains NF-κB signaling, which promotes epithelial hyperproliferation, cytokine release, and ROS-mediated injury. This mutagenic environment closely resembles the tissue damage caused by tobacco-derived nitrosamines and industrial arylamines (Stanfill et al., 2023, Skipper et al., 2003). Many bladder-associated microbes produce genotoxic intermediates, such as nitrosamines, ammonia derivatives, and ROS, which induce DNA adducts and chromosomal instability, mirroring the mutational signatures of chemical carcinogens (Liu et al., 2020, 2024). Disruption of tight junctions by microbes further increases urothelial permeability, allowing deeper penetration of environmental toxins and intensifying interactions between carcinogens and the epithelium. Persistent dysbiosis allows microbial communities to increasingly influence oncogenic signaling networks. β-catenin promotes stem-like properties and cellular invasion; STAT3 facilitates survival during inflammation; PI3K/Akt/mTOR drives unchecked proliferation; and MAPK/ERK accelerates cell-cycle progression, collectively supporting the transition from dysplasia to carcinoma (Dinney et al., 2004, Lou et al., 2024). Biofilm formation and intracellular persistence let certain microbes evade immune surveillance, creating protected niches that sustain chronic inflammation and promote tumor initiation. Microbial metabolites, such as SCFAs, polyamines, and other bioactive intermediates, modulate histone acetylation and DNA methylation, often silencing tumor-suppressive pathways (Thursby et al., 2017). Some microbial species also promote angiogenesis by inducing VEGF or IL-8. In advanced disease, they upregulate PD-L1 expression, suppressing antitumor immunity and facilitating metastasis (Yu et al., 2023). Microbial influences become more pronounced as the

BLCA TME evolves. Hypoxia, metabolic changes, and immune cell infiltration create conditions that selectively favor anaerobic and facultative anaerobic microbes. Species such as *Fusobacterium*, *Enterococcus*, and *Actinomyces* exploit tumor-associated gradients of oxygen, lactate, and glucose to establish persistent colonization (Ginwala et al., 2021). These microbes promote malignant progression by inducing epithelial-mesenchymal transition, angiogenesis, and stromal remodeling (Lou et al., 2024). Activation of NF-κB, STAT3, and β-catenin by microbes stimulates proliferation, inhibits apoptosis, and fosters an immunosuppressive microenvironment enriched in regulatory T-cells, M2-polarized macrophages, and MDSCs (Kostic et al., 2013). These immune changes mirror mechanisms that drive BLCA progression and therapeutic resistance (Knowles et al., 2015). Microbial metabolites also modulate tumor metabolism by promoting glycolysis in urothelial cells, altering mitochondrial function, and affecting redox balance, thereby enhancing tumor survival under hypoxic conditions. Overall, microbial activities induce genomic injury, amplify oncogenic signaling, remodel the immune system, and reinforce metabolic reprogramming, collectively driving the progression to aggressive BLCA (Figure 14.12).

14.5.11.4 Impact of the Loss of Protective Commensal Microorganisms

The exhaustion of protective commensal microorganisms, such as *Lactobacillus* and *Corynebacterium*, interrupts mechanisms critical for maintaining urothelial homeostasis. *Lactobacillus* species contribute to epithelial integrity by synthesizing lactic acid, bacteriocins, and immunomodulatory metabolites that suppress inflammation and inhibit the proliferation of pathogenic organisms (Littman et al., 2011). Similarly, a reduction in *Corynebacterium* populations compromises mucosal defenses, diminishes competitive exclusion, and impairs innate immune barriers (Ginwala et al., 2025). These alterations create ecological niches that facilitate the expansion of opportunistic, pro-tumorigenic taxa. The loss of these beneficial microorganisms also reduces the availability of anti-inflammatory metabolites, such as indole derivatives, which modulate epithelial signaling and protect against carcinogen-induced stress. Overall, reductions in commensal populations advance chronic inflammation, microbial dysbiosis, and carcinogenic progression.

14.5.11.5 Clinical Significance and Translational Implications

Microbial signatures are emerging as valuable biomarkers for BLCA detection, risk assessment, and surveillance. Increased levels of *Fusobacterium*, *Acinetobacter*, and *Enterococcus*, together with reduced *Lactobacillus*, are associated with high-grade disease, while diminished microbial diversity is linked to a higher risk of recurrence (Leclercq et al., 2016, Bučević Popović et al., 2018). Urinary microbiome profiling has

the potential to complement cytology and molecular assays, offering a non-invasive method to detect early disease, monitor recurrence, and predict therapeutic response. Distinct microbial profiles may also enable more precise patient stratification for BCG or immunotherapy, supporting individualized treatment planning. The bladder microbiome is increasingly recognized as a critical determinant of responses to BCG immunotherapy, intravesical chemotherapy, and immune checkpoint blockade. Elevated levels of *Lactobacillus* or *Streptococcus* are associated with enhanced immune activation following BCG treatment, suggesting that commensal-supported mucosal immunity augments BCG's antitumor efficacy (Miyake et al., 2020). Conversely, increased abundance of *Acinetobacter* or *Klebsiella* correlates with BCG treatment failure, likely due to microbial interference with BCG internalization, antigen presentation, or local cytokine signaling (Keith et al., 2019, Jiang et al., 2022). Microbial metabolism can further influence the pharmacodynamics of intravesical chemotherapeutics by modifying drug activation or degradation within the bladder (Ginwala et al., 2025). Bladder-resident microbes may also modulate responses to ICIs by affecting antigen presentation, interferon signaling, and the recruitment or exclusion of effector T-cells within the TME (Okobi et al., 2023). Collectively, these findings indicate that microbiome profiling may facilitate personalized therapeutic strategies and help identify patients most likely to benefit. Despite these advances, several challenges persist. The low microbial biomass of the bladder increases the risk of contamination in sequencing studies, underscoring the necessity for gold-standard standardization protocols (Hourigan et al., 2020). Further progress will depend on mechanistic studies employing bladder organoids, ex vivo urothelial tissues, and gnotobiotic animal models to elucidate causal relationships between microbes and tumor biology. The integration of metagenomics, metabolomics, spatial transcriptomics, and immune profiling will be essential to map microbe–tumor–immune interactions with high spatial and functional resolution. As these technologies evolve, the bladder tumor microbiome is anticipated to become central to BLCA research, providing new opportunities to define carcinogenesis, refine biomarkers, and develop microbiome-informed therapies (Figure 14.12).

14.5.12 Ovarian Cancer

Ovarian cancer (OVAC) has always been investigated through the face of genetics, stromal biology and immune TME. Recent studies have explored the hidden complexity in OVAC, stating the presence of tumor resident microbiome which is one of the functional components of the tumor ecosystem. This intratumoral microbiome has the capability in shaping the immune responses, cancer behavior, and more specifically the

tumor outcomes (Wang et al., 2025b, Leonov et al., 2025). Being often presented at an advanced stage and having a high mortality rate, deeper understanding of the OVAC tumor microbiome is inevitable in planning the therapeutic strategies. OVAC is not localized and often extends beyond its localization into the peritoneal cavity, and spreads to ovaries, fallopian tubes, omentum and even the peritoneal surfaces. These intratumoral microbes are not mere commensals/contamination rather they interact with tumor, immune cells and ECM, and show reproducible pattern across each patient and tumor types (Wang et al., 2025b, Asgharzadeh et al., 2025). They are also functionally active in enhancing the tumor progression, metastasis, and, in turn, modulating the response to chemotherapy (Tingting et al., 2025, Sheng et al., 2023). These microbes can be intracellular, interstitial within the ECM and stromal spaces and could even be associated with the vasculature or lymphatics. Interestingly even though each site, such as primary ovarian or fallopian tube tumor, peritoneal and omental metastatic tumor, even the cluster of tumor cells in malignant ascites, contain microbial communities that are overlapping, localized conditions like oxygen tension, nutrient availability, and immune pressure shape microbial residency to vary across anatomical sites, resulting in distinct community compositions at each location. Because these procedures are performed primarily in the operating room, sterile samples should be collected with utmost care along with parallel blank controls are essential to differentiate between actual tumor microbiome and environmental, skin, and surgical contaminants like *C. acnes* and *Staphylococcus epidermidis*.

14.5.12.1 Composition and Spatial Localization

Pan-cancer tumor microbiome analyses have established that OVAC harbors a distinct intratumoral bacterial landscape, clearly separable from background contamination and from microbiomes associated with mucosal organs. In particular, OVAC contains low-biomass but reproducible bacterial communities that are predominantly intracellular rather than extracellular or vascular in origin (Nejman et al., 2020). Importantly, bacterial signals are consistently detected within malignant epithelial cells and tumor-associated immune cells, particularly macrophages, supporting the concept that bacteria are integral components of the OVAC microenvironment rather than incidental contaminants. Across independent cohorts, the OVAC bacteriome shows high reproducibility, despite inter-patient heterogeneity. The microbial profiles are tumor-type specific and do not resemble gut-derived microbial communities, reinforcing the conclusion that ovarian cancer supports its own localized microbial ecosystem. Collectively, these features, low total biomass, intracellular localization, reproducibility across datasets, and tumor specificity, define the global intratumoral bacterial landscape of ovarian cancer (Nejman et al., 2020).

Proteobacteria constitute the dominant bacterial phylum detected within ovarian tumors and represent the most abundant and consistently observed intratumoral taxa. To that end, genera such as *Pseudomonas*, *Acinetobacter*, and *Ralstonia* have been repeatedly identified in ovarian tumor tissue, with careful exclusion of reagent and environmental contaminants (Nejman et al., 2020, Sepich-Poore et al., 2021) (Figure 14.13). These bacteria are frequently localized intracellularly and are enriched in tumors with adverse clinical features. Proteobacteria are notable for their metabolic versatility. *Pseudomonas* species are associated with stress-response pathways and xenobiotic metabolism, while *Acinetobacter* species have been linked to redox tolerance and drug-resistance phenotypes. *Ralstonia* species, detected within OVAC cores, have been associated with inflammatory signaling pathways. Importantly, *Proteobacteria*-rich tumor microbiomes are enriched in poor-prognosis ovarian cancer subtypes, suggesting a potential relationship between bacterial composition and disease aggressiveness (Nejman et al., 2020, Sepich-Poore et al., 2021). *Firmicutes* represent another recurrent component of the ovarian tumor microbiome, albeit at lower relative abundance than *Proteobacteria*. Members of this phylum, including *Streptococcus* and *Staphylococcus* species, have been detected in both ovarian tumor tissue and tumor-associated ascites. These organisms are primarily localized intracellularly or within immune cell populations and are thought to contribute to the inflammatory signaling within the TME (Nejman et al., 2020). Low-abundance *Clostridium* species have also been identified through tumor DNA analyses, where they may participate in metabolic modulation of the local tumor niche. Overall, *Firmicutes* in OVAC appear to be immune-associated and intracellular, reinforcing the concept that tumor-resident bacteria interact closely with host immune pathways rather than existing as free-living extracellular organisms (Nejman et al., 2020). *Actinobacteria*, including *Corynebacterium* species and *C. acnes*, are consistently detected within OVAC tissues and stromal compartments. These taxa are among the most stringently validated microbial signals in tumor microbiome research (Nejman et al., 2020). In OVAC, *Actinobacteria* are not uniquely enriched but instead represent part of a conserved, tumor-associated bacterial core shared across solid malignancies. Their presence supports the notion that certain bacterial taxa are broadly adapted to the TME, regardless of the tissue of origin.

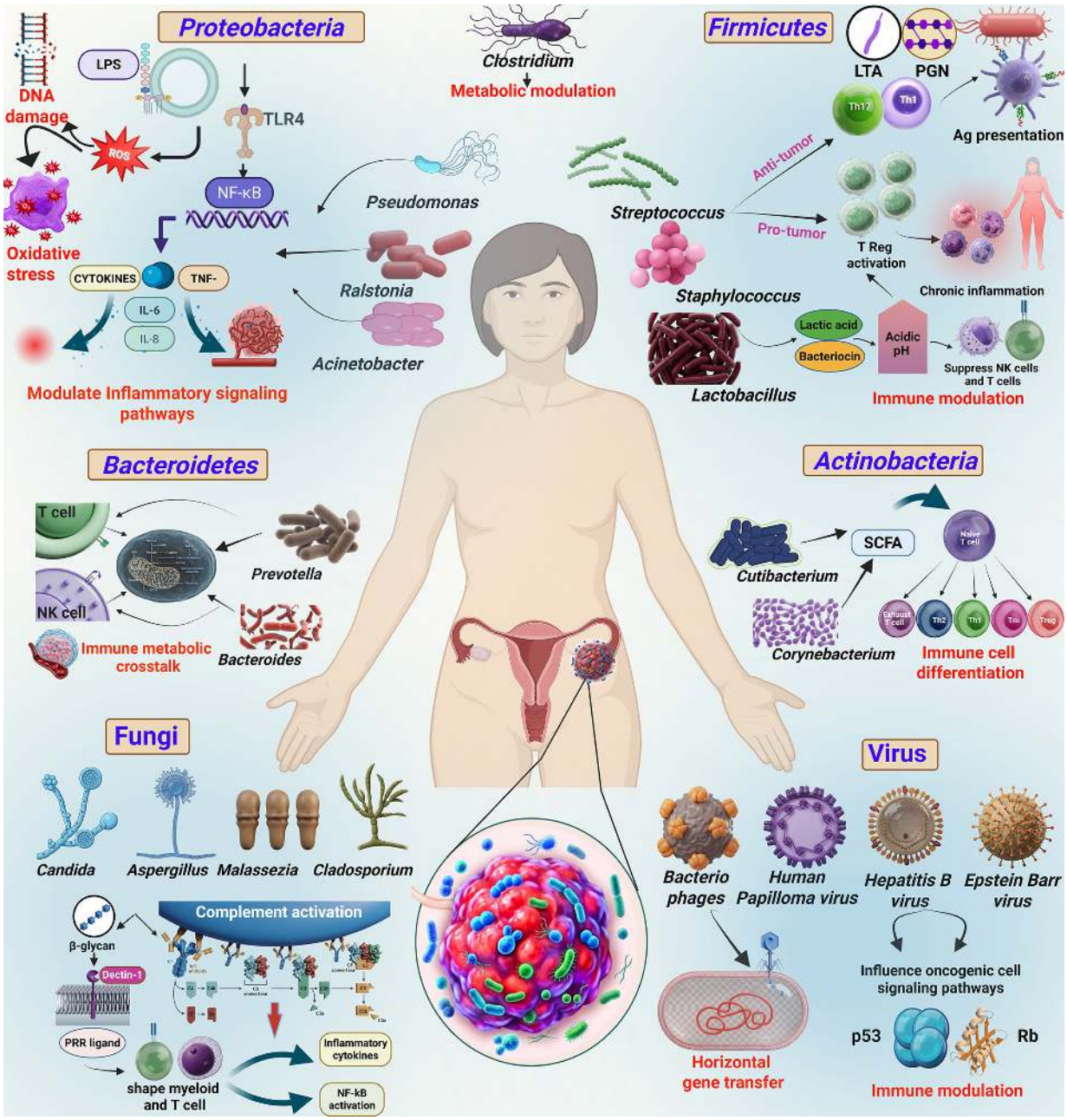

FIGURE 14.13 Tumor–microbial interactions in ovarian cancer evolution. ⏎

Large-scale analyses using ITS sequencing and WGS across more than 17,000 tumor samples have conclusively demonstrated that fungi are ubiquitous components of solid tumors, including OVAC (Narunsky-Haziza et al., 2022). Similar to bacteria, tumor-associated fungal communities are low in abundance but tumor-type specific, with distinct ecological patterns across cancer types. In OVAC, several fungal genera have been repeatedly detected within tumor tissue and associated immune compartments. *Candida* species are commonly localized within the tumor stroma and are linked to

inflammatory signaling. *Malassezia* species are enriched in tumor-associated immune cells and have been associated with poor survival outcomes in pan-cancer analyses. *Cladosporium* species have emerged as part of a prognostic fungal signature, while low-abundance *Aspergillus* species have been detected and may contribute to metabolic activity within the TME (Narunsky-Haziza et al., 2022, Sepich-Poore et al., 2021) (Figure 14.13).

Viral nucleic acids represent a smaller but detectable component of the OVAC microbiome. HPV DNA has been identified at a low prevalence in ovarian tumor tissue, though it is considered rare and unlikely to play a dominant oncogenic role. In contrast, latent EBV DNA has been detected within tumor cells and infiltrating immune cells, where it may contribute to immune modulation. Cytomegalovirus (CMV) transcripts have also been identified in ovarian tumor transcriptomic datasets, suggesting a role in immunoregulation rather than direct oncogenesis (Nejman et al., 2020, Sepich-Poore et al., 2021). Overall, viral components are less dominant than bacterial or fungal populations but may exert disproportionate effects on tumor immune states (Figure 14.13).

Spatial analyses have revealed that more than 70%–90% of bacteria detected in OVAC are intracellular, with minimal extracellular signal. Bacterial DNA and structures are found predominantly within malignant epithelial cells and tumor-associated macrophages, rather than within stromal spaces or the vasculature (Nejman et al., 2020). This intracellular localization has been confirmed using multiple orthogonal approaches. Malignant ascites associated with OVAC contains a complex tumor-associated microbiome that includes bacterial taxa dominated by *Proteobacteria* and *Firmicutes*, fungal species such as *Candida* and *Malassezia*, and detectable viral DNA. Importantly, the ascites microbiome more closely resembles tumor tissue microbiota than circulating microbial profiles, supporting the concept of local microbial exchange within the peritoneal tumor ecosystem rather than systemic microbial spillover (Deng et al., 2025b).

14.5.12.2 Mechanisms

Bacterial communities which are specifically linked to immune-related gene expression influence the tumor immune landscape and the immune filtration patterns, which correlates with the patient outcomes in OVAC (Sheng et al., 2023). Gram-negative species like *Proteobacteria* (e.g., *Pseudomonas*, *Acinetobacter*, and *Escherichia/Shigella*) are often metabolically versatile and stress tolerant and LPS from these bacterial communities play a very important role in activating TLR4 on tumor cells, macrophages and even myeloid cells, which in turn trigger TLR4-NF-κB inflammatory signaling pathway leading to the secretion of IL-6, IL-8, TNFα, and

cytokines. Thus, in OVAC both IL-6/STAT3 and NF-κB signaling pathways play a vital role in promoting tumor proliferation, EMT, angiogenesis and resistance to chemotherapy, thereby amplifying the pro-tumor inflammation (Umemoto et al., 2009, Liang et al., 2020, Alberti et al., 2012). Chronic LPS exposure shifts the tumor associated macrophages (TAM) toward either pro-inflammatory (M1-like) or anti-inflammatory (M2-like) phenotype. These TAMs secrete cytokines like IL-10 and TGF-β that, in turn, inhibit cytotoxic T-cell activation and proliferation. Immature MDSCs suppress T-cell responses by depleting certain key amino acids like arginine which directly associates with poor OVAC prognosis. Together they create a cold TME that evades the immune response (Sheng et al., 2023). Certain *Proteobacteria* also produce ROS, nitric oxide (NO), and other metabolites that induce DNA damage and oxidative stress. Earlier studies state that these *Proteobacteria* can also inactivate drugs and alter drug penetration enzymatically, leading to chemoresistance (Figure 14.13).

Gram positive *Firmicutes*, including *Streptococcus*, *Staphylococcus*, *Lactobacillus* produces lipoteichoic acid and peptidoglycan that activate TLR2 and associated PRR-mediated signaling driving a pro-inflammatory state, enhancing tumor cell growth and survival, while diminishing their susceptibility to apoptosis (Wang et al., 2025a, Asgharzadeh et al., 2025). Certain *Streptococcus* species play a dual role in promoting antitumor immunity by inducing strong Th1/Th17 responses and enhancing antigen presentation, yet also facilitating immune evasion (pro-tumor) through chronic low-grade inflammation and expansion of regulatory T-cells. The presence of this *streptococcus* sp. helps define a tumor whether it is an immune-hot or immune-cold tumor (Sheng et al., 2023). *Lactobacillus* sp. produce metabolites like lactic acid and bacteriocins that could alter the pH, immune cell function, and even tumor cell metabolism. This acidic environment favors the Treg activation by suppressing the effector T-cell and NK cells (Zhang et al., 2024c). The localization of this *Lactobacillus* sp. in peritoneal metastatic tumor create a metabolically immunosuppressive niche, contributing to overall immune evasion. Even though in most cases they are beneficial, their role in OVAC is entirely context dependent. As *Firmicutes* are diverse in nature, their effects are entirely dependent on the species or strains and their interaction with the tumor and immune cells (Figure 14.13).

Actinobacteria (e.g., *Corynebacterium* and *Cutibacterium/Propionibacterium*) should be interpreted carefully as they are often present as contaminants. Certain cell wall components of *Actinobacteria* sp. activate PRRs and maintain an indolent inflammatory state, supporting angiogenesis and tumor growth (Chefetz et al., 2013). They are also involved in lipid metabolism and produce SCFA, influencing tumor cell signaling and immune cell differentiation. Persistent low levels of antigen exposure cause immune dysfunction in OVAC by driving T-cell exhaustion and Treg expansion. Complex

polysaccharides from Bacteroidetes (e.g., *Bacteroides* and *Prevotella*) modulate the immune system by shaping T-cell differentiation (likely Th1 vs. Treg) and dendritic cell function, which could shift the balance either toward immune activation or tolerance based on the involvement of specific molecules (Wherry et al., 2015). This *Bacteroidetes* sp. are also involved in the production of SCFA, influencing histone acetylation and gene expression in both tumor and immune cells, thereby affecting tumor proliferation. Thus, even though Bacteroidetes are less consistently found in OVAC, they play a prominent role in immune-metabolic crosstalk (Wang et al., 2022, Schratz et al., 2023). Microbial enzymes like proteases and hyaluronidases, as well as microbe-induced host enzymes like MMP, can degrade or remodel ECM, facilitating peritoneal dissemination and omental colonization. Microbial antigens from OVAC drive local B-cell activation and antibody production. In certain tumors, this contributes to the formation of tertiary lymphoid structures (TLS), which are often associated with better prognosis and improved response to immunotherapy. This is still being investigated, but the idea that local antigens shape adaptive immunity is highly relevant. Microbial stimulation of inflammatory cytokines (IL-6, IL-1β, and TNFα) upregulates VEGF and other pro-angiogenic factors, supporting angiogenesis and lymphangiogenesis (Wang et al., 2025b, Tingting et al., 2025) (Figure 14.13).

Besides bacterial communities, intratumoral niches have resident fungi and viruses, such as bacteriophages and oncogenic viruses. Even though less extensively mapped, genera such as *Candida*, *Malassezia*, and *Aspergillus* have been identified and increasingly recognized. Fungi produce metabolites that could influence tumor behavior and modulate the immune responses (Wang et al., 2025b, Leonov et al., 2025). Adding to that, the mycobiome in OVAC is an emerging field, but mechanistically, fungi shape myeloid and T-cell responses by activating β-glucan mediated PPR ligands and the complement system. Whereas human oncogenic viruses like HPV, EBV, and HBV are not classical drivers of OVAC, viral sequences and bacteriophages present within tumor tissue play a key determinant role in shaping bacterial communities and immune responses. These viral proteins interfere with oncogenic signaling pathways, like classical p53, Rb, and other pathways, and modulate the antigen presentation and interferon responses (Moore et al., 2010). Chronic viral infection consistently maintains type I interferon signaling and inflammation, which either promotes or suppresses tumor growth depending on the TME (Balkwill et al., 2001). Bacteriophages can selectively lyse specific bacteria, which alters the presence of certain metabolites and PPR ligands, resulting in the shaping of intratumoral immunity (Hsu et al., 2019). Phages have the capability of moving toxic genes, metabolic genes, and antibiotic-resistant genes between the bacterial communities, resulting in changing the behavior of the TME (Figure 14.13). Thus, even though phages in OVAC do not directly interact with tumor

cells, they could potentially reprogram the intratumoral microbial components (Alkhalil, 2023).

14.5.12.3 Clinical Implications

Standard first-line treatment for high-grade serous OVAC includes cytoreductive surgery followed by platinum-based chemotherapy consisting of carboplatin and paclitaxel. Although the direct mechanisms in OVAC are yet to be explored, evidence supports the idea that the tumor microbiome may influence both chemosensitivity and chemoresistance. Certain microbial communities modulate the response to immunotherapy by regulating T-cell infiltration and exhaustion, influencing PD-L1 expression, and even promoting an inflamed, antigen-rich TME. In OVAC, microbes play a very important role as biomarkers of prognostic relevance (Sheng et al., 2023), integrating T-cell infiltration or gene expression modules to assess immune scores, as well as predicting chemotherapy response or resistance by analyzing their metabolic profiles. Microbial targeted therapies include the use of phages specifically targeting tumor-associated bacteria. Usage of microbial components, such as TLR agonists, to enhance antitumor immunity, and synthesis of engineered bacteria that specifically colonize tissues and deliver certain cytokines, checkpoint inhibitors, and prodrug-converting enzymes. The ability to deliver therapeutics intraperitoneally either through implanted catheters or during surgical procedures makes localized microbiome-modulating strategies particularly feasible in OVAC. Due to the disease's heterogeneous nature at the genomic, histologic, and TME levels, it is a challenging task to design a universal microbial signature for therapeutic applications in OVAC. Future mechanistic studies using co-culture systems and organoids, along with the proper execution of early-phase trials exploring the efficacy of microbiome-modulating strategies in combination with other treatment modalities, will add a new dimension to understanding OVAC biology. Even though many questions remain unanswered on how the tumor microbiome directly influences the tumor progression, survival, and therapy response, with further understanding, the intratumoral microbiome will likely become a promising factor and also an integral part of classifying, predicting, and even treating OVAC patients in combination with traditional treatment modalities.

14.5.13 Cervical Cancer

Over a long period, cervical cancer (CC) has been primarily understood as a malignancy driven by a persistent infection with hrHPV. Despite the availability of HPV screening and vaccination, CC remains one of the most common cancers in women worldwide, specifically in low- and middle-income countries. Types of hrHPV, specifically HPV16

and HPV18 has been detected in 99% of the women. HPV16 and HPV18, have been detected in ~99% of cervical cancer cases; however, in the general population, most hrHPV infections are cleared spontaneously. Only a small fraction of infected women progress to high-grade lesions or invasive carcinoma, indicating that hrHPV infection alone is insufficient for malignant transformation and that additional cofactors modulate the disease trajectory. Over the last decade, the local microbiome in the cervicovaginal region and the tumor adjacent microbiome, which were inadequately explored, have now been discovered to be among the key modulators influencing the persistence of HPV viral determinants, EMT, and tumor evolution. Rather than being a passive resident, the local microbial ecosystem plays a critical role in influencing epithelial barrier integrity, mucosal immunity, the inflammatory milieu, and molecular TME in which viral oncoproteins execute their functions.

14.5.13.1 Composition and Spatial Localization

Although it was traditionally assumed that the upper genital tract was sterile, advances in high-resolution sequencing have overturned this idea, revealing a complex microbial ecosystem in the cervical tumor, which is a transformation zone harboring specific microbial communities that distinctly differ from those of healthy women (Table 14.4). *Lactobacillus crispatus* and *L. jensenii*, which are protective species normally enriched in the cervical area to help clear HPV by maintaining a low pH and an anti-inflammatory niche, are drastically lowered in dysbiotic tumor states (Bautista et al., 2025c). These dysbiotic states are enriched in aerobes like *Gardnerella, Fannyhessea, Sneathia, Prevotella*, and *Megasphaera*, which dominate the high-diversity community state type (CST) IV microbiomes associated with cervical carcinogenesis (Bautista et al., 2025c). These specific microbial taxa trigger chronic inflammation, epithelial micro-abrasions, mucin degradation, and ROS, which act synergistically with the genomic instability driven by HPV. Intratumoral taxa in advanced lesions, including *Fusobacterium*, *Streptococcus agalactiae*, and *Pseudomonas oleovorans* (Audirac-Chalifour et al., 2016), further influence the TME by facilitating angiogenesis, immune suppression, and invasive behavior. HPV therapeutic vaccines and microbiome-informed strategies, such as probiotics, vaginal microbiota transplantation, and targeted antimicrobials, are shifting from a conceptual paradigm to translational execution. Thus, it is fundamental to understand the role of microbial taxa linking to cervical carcinogenesis, their functional impact, and their clinical relevance. Understanding the mechanistic function of these intratumoral microbes is no longer a theoretical discussion; it has shifted from theory to real-world application in re-evaluating early detection, CC prevention, and in designing proper therapeutic strategies.

TABLE 14.4

Composition, Expression, and Spatial Localization of Intratumoral Microbiome in Cervical Cancer

Site	Dominant taxa	Localization	Functional impact
Cervicovaginal lumen	*Lactobacillus crispatus*, *L. iners Gardnerella*—healthy states *Fannyhessea*, *Prevotella*, *Megasphaera, Sneathia*—dysbiosis	Mucus layer, squamous epithelium, vaginal–cervical interface. Dysbiotic taxa form biofilms on ecto-cervical suface	pH regulation, inflammation, HPV persistence (Bautista et al., 2025c)
Ectocervical epithelium	*Gardnerella*, *Sneathia*, *Prevotella*	Stratified squamous epithelial surface of the ectocervix	Biofilm formation, mucin degradation, epithelial disruption (Bautista et al., 2025c)
Transformation zone	*Gardnerella, Fannyhessea, Sneathia, Provotella*	Primary site of HPV entry, micro abrasions, and areas of epithelial turnover	Facilitates HPV entry, chronic inflammation (Bautista et al., 2025c)
Tumor surface and tumor adjacent mucosa	*Fusobacterium*, *Sneathia*, *Streptococcus agalactiae*	Inflamed epithelial clusters, stromal regions, and immune-suppressed niches. *Fusobacterium:* tumor-adjacent mucosa and necrotic tumor pockets. *Sneathia and Prevotella:* pro-inflammatory cytokine expression zones	Immune suppression, NF-κB activation (Audirac-Chalifour et al., 2016)
Intratumoral regions	*Fusobacterium*, *Pseudomonas oleovorans*	Immune exhausted T-cell neighborhoods, myeloid-rich suppressive niches, and hypoxic tumor regions	Pro-tumorigenesis signaling, angiogenesis, hypoxia-linked colonization, immune evasion (Xia et al., 2025)

Site	Dominant taxa	Localization	Functional impact
Immune cell neighborhoods	*Fusobacterium*, *Sneathia*, *Prevotella*	Myeloid-dominant clusters, Treg-rich zones, and areas of cytokine dysregulation	Cytokine dysregulation, T-cell exhaustion (Xia et al., 2025)

14.5.13.2 Mechanisms

Certain species like *Lactobacillus crispatus* are dominant in healthy CST I communities, lowering the risk of Cervical intraepithelial neoplasia (CIN) 2^+ risk and strongly associated with HPV negativity. They produce D-lactic acid that maintains a low pH (~3.5–4.5) that potentially inhibit the pathogen growth and limits the access to HPV by strengthening the epithelial barrier integrity. They also suppress the growth of anaerobes like *Gardnerella* and *Sneathia* by producing antimicrobial factors such as H_2O_2, which reduces chronic inflammation. Thus, this *L. crispatus* species favors an anti-inflammatory, interferon-competent environment that plays a vital role in HPV clearance (Bautista et al., 2025a, Alizhan et al., 2025). Compared to *L. crispatus*, *Lactobacillus iners* is less protective as it is associated with a higher pH and the transition to dysbiosis will be easier. Functionally, they are ambivalent or pro-dysbiotic, as they are often enriched in HPV-positive CC and in CIN lesions. They produce the pore-forming toxin inerolysin, which disturbs epithelial integrity and mucus, facilitating access for anaerobes and HPV strains. They are referred to as "Gateway species" because they predominantly co-exist with the CST IV high-diversity microbial communities associated with highly invasive lesions and CIN (Bautista et al., 2025a). Commensal *Lactobacillus* species will compete with the pathogenic or pro-inflammatory bacteria for nutrients and adhesion sites, indirectly influencing HPV persistence. *Gardnerella vaginalis* plays a prominent role in maintaining HPV persistence as it forms protective biofilms that protect co-colonizers, leading to chronic inflammation in the cervicovaginal epithelium (Di Paola et al., 2017). They produce sialidases, which degrade the mucosal barrier, giving HPV and inflammatory mediators access to basal epithelial cells (Hardy et al., 2017). They also mediate pro-inflammatory signaling by inducing IL-1β, IL-6, TNFα, and chemokines, while producing ROS and RNS, thereby causing genotoxic stress that synergizes with the HPV E6/E7-associated genomic instability (Bautista et al., 2025c). Species like *Atopobium vaginae* and *Fannyhessea vaginae* often co-exist with the *Gardnerella* sp. in women with highly invasive CIN and CST IV communities (Castro et al., 2020). They typically stabilize *Gardnerella* sp. biofilms, thereby increasing resistance to host defenses and antibiotics. They facilitate the loss of the acidic environment, indirectly linking to HPV persistence and anaerobic

species invasion (Muzny et al., 2016). They play a key role in pro-inflammatory signaling by enhancing the production of pro-inflammatory cytokines and elevated levels of IL-1β in cervicovaginal fluid lead to chronic epithelial injury that favors transformation to a malignant state (Bautista et al., 2025c, Gajer et al., 2012). *Sneathia* sp. (*Sneathia amnii*) has been consistently associated with highly invasive CC, CIN 2^+, and hrHPV infection. It is a potent pro-inflammatory driver as it enhances the production of IL-1β, IL-6, IL-8, and TNFα, driving a highly inflamed TME and inducing DNA damage with enhanced production of ROS. Similarly, like other anaerobes, they play a vital role in facilitating HPV entry by causing micro-abrasions in the cervical epithelium, which in turn increases permeability to invaders. It reduces local antiviral responses and promotes tumor formation (Bautista et al., 2025c). *Prevotella* sp. are enriched in CC compared with healthy women. LPS from this species specifically mediates the NF-κB signaling pathway-driven cytokine production (IL-6 and IL-8) by activating TLR4 receptors on epithelial and immune cells, thereby promoting tumor cell proliferation, angiogenesis, and immune evasion (Dong et al., 2022, So et al., 2020). *Megasphaera* sp. synthesizes SCFA like acetate and propionate, which alter epithelial proliferation and immune responses, causing persistent low-grade inflammation in dysbiotic states (Chen et al., 2020). There are some taxa that are invasive and associated with tumor, such as *F. nucleatum;* they are often present in the advanced stages of high-risk tumors, and they activate adhesin-mediated and β-catenin signaling that promote tumor proliferation and EMT. They also foster immune tolerance by inducing myeloid-derived suppressor cells and T-cell exhaustion markers. LPS and PAMPs from *F. nucleatum* result in sustained NF-κB activation, supporting tumor growth and invasion. Other bacterial vaginosis-associated genera like *Mobiluncus* and *Peptostreptococcus* are often enriched with *Gardnerella*, *Prevotella*, and *Sneathia* in CIN and cancer-causing synergistic inflammation, contributing to the additional PAMPs, such as LPS, lipoteichoic acid, and peptidoglycan amplifies TLR-mediated cytokine secretion. They also alter the tumor metabolism by releasing proteases, phospholipases, and other enzymes that potentially damage the epithelium, ECM that correlates with HPV invasion and metastasis (Mitra et al., 2015). Emerging studies state that *lactobacillus*-depleted microbiomes and the enrichment of anaerobic genera correlate with advanced-stage and high-grade lesions. Even though analysis involving large cohorts is needed for validation, available studies state specifically that microbial signatures are associated with lymph node involvement and response to chemoradiation, meaning they could serve as non-invasive biomarkers for risk stratification and therapeutic planning.

Tumor-associated fungi, such as *Candida, Malassezia*, and other opportunistic yeasts, activate the Dectin-1/Syk, TLR2, and TLR4 pathways, which in turn activate IL-6, IL-1β, and TNFα production, resulting in chronic inflammation that leads to HPV

persistence and tumor progression (Awad et al., 2022). These fungal taxa produce β-glucans and mannans that cause immunosuppression and shift immunity toward tolerogenic macrophages, regulatory T-cell expansion, and reduced antiviral responses, thereby weakening the clearance of HPV infection (Xu et al., 2016). Fungal enzymes, such as proteases, lipases, and phospholipases, break the epithelial barrier, junctions, and ECM, promoting the invasion of HPV (Somboonna et al., 2011). They also produce metabolites, such as acetaldehyde and prostaglandins that can potentially damage the DNA, enhance epithelial proliferation, and promote immune evasion that synergistically correlates with HPV oncogenesis.

Viral components play a major role in CC proliferation and metastasis. HPV drives the oncogenic transformation through integration into the host DNA that leads to the E6/E7-mediated degradation of p53 and Rb that in turn causes the genomic instability and malignant transformation (Zapatka et al., 2020). HPV often coexists with the BV-like anaerobes (*Prevotella, Gardnerella*, and *Sneathia*), showing a potentiating activation of NF-κB with an increased production of IL-6 and IL-8, along with impaired antiviral immunity that promotes CIN progression and immune evasion (Molina et al., 2022). Tumor-associated viruses, like *Anelloviruses*, co-exist with HPV, modulate interferon pathways, reduce antigen presentation, and promote immunosuppression (Arze et al., 2021). Viral particles and viral-derived nucleic acids, such as DNA or RNA, activate the TLR9, cGAS-STING, and RIG-I pathways, causing persistent chronic inflammation that promotes tumor growth and angiogenesis (Chen et al., 2016).

14.5.13.3 Clinical Implications: Microbiome as Biomarker and Therapeutic Target

Newly emerging microbiome-modulating therapies, including the reacidification of the cervicovaginal environment by the administration of oral or intravaginal *L. crispatus* or *L. rhamnosus* formulations, could inhibit the anaerobic dysbiosis, strengthen the epithelial barrier to reduce HPV entry, and enhance tlocal antiviral immunity (IFN-α/β) (Bautista et al., 2025c, Shen et al., 2024). Another innovation in therapeutic modalities is vaginal microbiota transplantation (VMT) for the cervicovaginal microbiota, which results in the rapid restoration of a *Lactobacillus* sp. dominant CST I. This technique could displace biofilm-forming anaerobes, thereby reducing the ROS-mediated DNA damage and the persistent harmful effects of chronic inflammation (Zhang et al., 2024c). Targeted antimicrobials, including short-course antibiotics like metronidazole are administered followed by the probiotic restoration could prevent the rebound dysbiosis in CC sites. ICIs, such as pembrolizumab, nivolumab restores T-cell activity, and as the HPV oncoproteins interferes with the antigen presentation and activate the immune checkpoints, which makes the ICIs effective (Zhou et al., 2026). Previous studies approve pembrolizumab for patients with advanced-stage, PD-L1 positive CC patients

with progression irrespective of chemotherapy administration (Chung et al., 2019). Preventive and therapeutic HPV-targeted vaccines are gaining recognition as a promising strategy for managing HPV-associated lesions and cancers. Because chronic hrHPV infection is the major driver of cervical cancer and preventing it is the strongest protective measure (Shen et al., 2024). The preventive HPV vaccines available, such as Gardasil-9, Cervarix, and Gardasil, induce neutralizing antibodies against HPV16/18, which prevent initial infection and, as a downstream effect, prevent HPV-mediated microbial dysbiosis (Roy et al., 2023). E6/E7-targeted therapeutic HPV vaccines, including DNA vaccines (such as VGX-3100), peptide vaccines, viral vector vaccines, and mRNA-based therapeutic vaccines, are designed specifically to target existing HPV infection by inducing cytotoxic T-cell responses against HPV-infected cells. They activate $CD8^+$ T-cells against HPV E6/E7 oncoproteins and reduce the lesion size and viral load (Zhou et al., 2026). Studies have shown that VGX-3100 induces the regression of CIN2/3 in phase III trials. Microbial-targeted adjunct therapies, including topical anti-inflammatory agents, mucin-enhancing therapies, and antioxidants reducing ROS-mediated mutagenesis, are being explored. Genetically engineered *Lactobacillus* strains delivering anti-HPV peptides, clustered regularly interspaced short palindromic repeats (CRISPR)-based antiviral constructs, and immune-modulating cytokines are generated. The cervicovaginal microbiome has emerged as a critical, newly explored arena in cervical cancer. Dysbiosis in the microbial communities is consistently associated with hrHPV persistence, high-grade lesions, and invasive disease. Deep exploration of this field could help shape the trajectory from infection to malignancy that could influence the behavior of high-risk cervical tumors.

14.5.14 Endometrial Cancer

Intracellular bacteria are enriched in tumor and immune cells across multiple cancer types, including endometrial cancer (EC) (Nejman et al., 2020). EC is one of the most common gynecologic malignancies, which has always been studied in connection with metabolic dysfunction, obesity, and hormonal imbalance. Though the common classical risk factors remain constant, recent advances in cancer have discovered that tumors harbor their own specific microbial ecosystems. Microsatellite instability high (MSI-H) tumors harbor pro-inflammatory taxa, such as *Fusobacterium* and *Prevotella* (Mima et al., 2016), while POLE-mutated ECs show potentially less dominant immunosuppressive taxa, consistent with their distinct T-cell infiltrates (Cancer Genome Atlas Research, 2013). Conversely, copy number serous-like high-grade EC frequently associates with *Gardnerella* and *Atopobium*, which are non-*Lactobacillus*, anaerobe-rich, bacterial communities (Walther-Antonio et al., 2016). These diverse microbial

community, including bacteria, fungi, viruses, and other microeukaryotes, are increasingly recognized as regulators of tumor biology, immune responses, and therapeutic strategies. The tumor microbiome and its extensive mechanistic roles in tumor progression have been extensively studied and explored in breast, g, and pancreatic tumors, but their role in EC is yet to be investigated more. Emerging multi-omics studies demonstrate that ECs harbor low-biomass but biologically active microbial communities that differ from adjacent normal tissue, associating with immune infiltration and clinical outcomes (Walther-Antonio et al., 2016, Ravel et al., 2016). Spatial profiling shows that microbes are localized to necrotic cores providing nutrients to microbial communities, hypoxic regions supporting specific anaerobic bacteria, tumor glands and stroma, perivascular niches, and immune cell aggregates. They also interact with epithelial cells, stromal fibroblasts, and endothelial cells, suggesting that EC may harbor intracellular microbial reservoirs that influence signaling pathways, immune evasion, tumor progression, and therapeutic response in EC.

14.5.14.1 Composition

Studies revealed that endometrial tumors harbor distinct bacterial taxa compared to benign endometrium (Table 14.5). Ethnic disparities in microbiota composition have also been observed, with increased microbial diversity in black endometrial cancer patients than in White patients (Galasso et al., 2025). Microbial signatures correlate with molecular subtypes, including POLE-mutated and MSI-H tumors, suggesting that intratumoral bacterial dysbiosis may contribute to EC pathogenesis and progression (Blazheva, 2022). The depletion of *Lactobacillus* species in tumor tissue relative to normal endometrium was identified (Semertzidou et al., 2024). *Atopobium vaginae*, *Porphyromonas somerae*, and *Gardnerella* species were enriched in EC tissues, with strong associations between *A. vaginae* and high-grade disease (Walther-Antonio et al., 2016). *Gardnerella* and *Atopobium* are hallmark taxa of bacterial vaginosis-like communities, and several studies report their enrichment in women with endometrial hyperplasia and carcinoma, suggesting an ascending or shared dysbiotic niche (Semertzidou et al., 2024). Beyond specific genera, EC is often characterized by a shift from low-diversity, *Lactobacillus*-dominant communities to high-diversity, anaerobe-rich communities including *Prevotella, Porphyromonas, Sneathia, Dialister, Peptoniphilus*, and *Anaerococcus* (Semertzidou et al., 2024, Lu et al., 2021). Even though historically the uterus was considered sterile, modern sequencing has overturned this assumption by identifying the microbial entry into the endometrium through ascending migration from the lower reproductive tract, hematogenous spread from distant sites, and lymphatic trafficking, as the two major possible routes of microbial entry displays the fact that tumor-resident microbes not just simply reflect the local

dysbiosis but inflicts the systemic microbial trafficking into the tumor tissue (Figure 14.14).

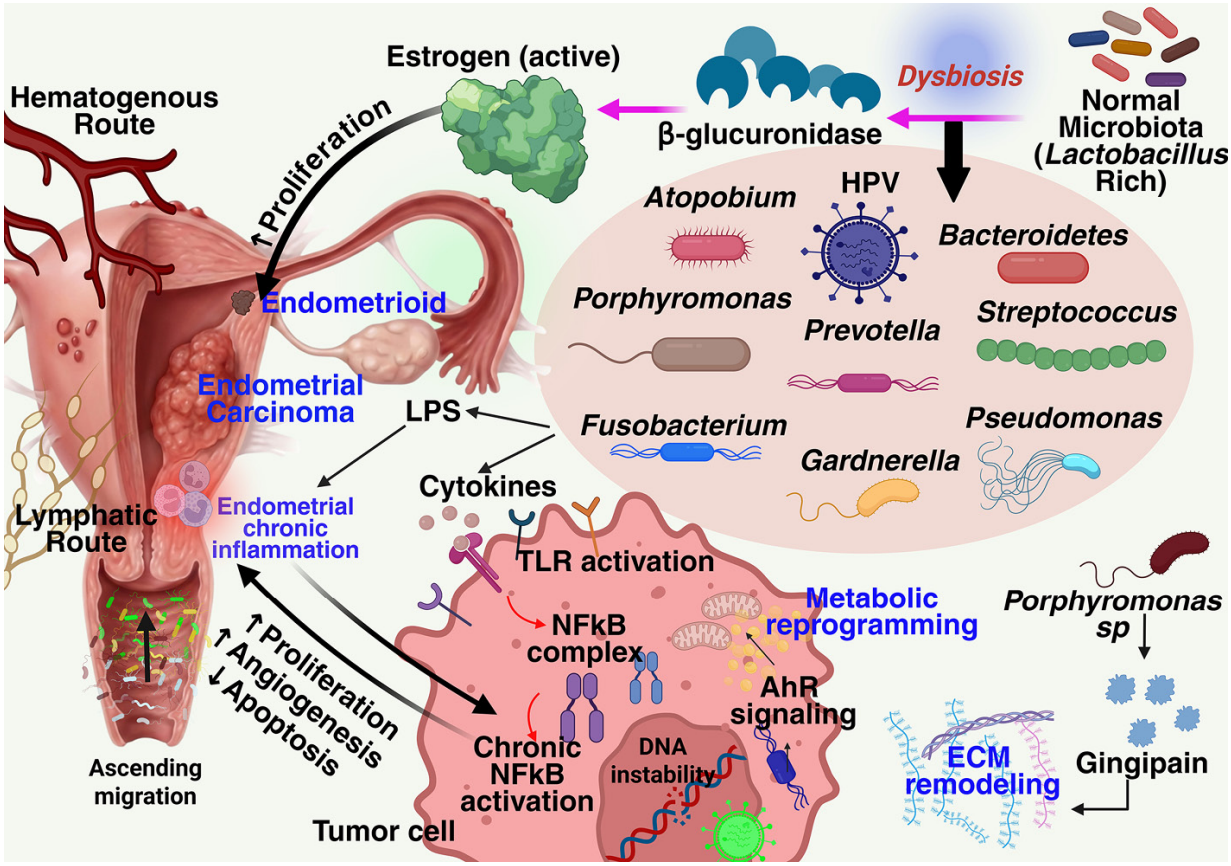

FIGURE 14.14 Tumor–microbe interaction and function in endometrial cancer.

TABLE 14.5
Microbial Taxa Reported in Endometrial Cancer

Genus/Species	Association	Source/Notes
Porphyromonas somerae	Enriched in EC uterine/endometrial tissue; potential invasion into cancer cells	Linked with pathogenic behavior in uterine microbiome of EC patients
Porphyromonas (other sp.)	Enriched in lower genital tract and endometrium in EC	Reported consistently as EC-associated anaerobic bacteria
Prevotella (incl. *P. timonensis*)	Enriched in endometrial/vaginal sites; associated with EC and microbe transfer	Prevotella enriched and shared across niches

Genus/Species	Association	Source/Notes
Peptoniphilus (various)	Enriched in EC vaginal/rectal microbiome	Observed in EC samples; microbe transfer noted
Anaerococcus	Enriched in EC lower genital tract	Reported in EC microbiota studies
Atopobium	Enriched in EC endometrium/vagina	Identified as associated with EC
Dialister	Enriched in EC	Reported increases in EC microbiome
Ruminococcus	Enriched in some EC studies	Linked with metabolic and inflammatory profiles in EC
***Bacteroides* (incl. *B. vulgatus/B. caccae*)**	Enriched in EC	Gut and endometrial microbiome studies link *Bacteroides* sp. to EC; metabolic associations reported
Collinsella aerofaciens	Reported as enriched in adjacent normal (possible tumor-suppressive) tissue	Functional study suggests antitumor activity via p53 signaling
Delftia tsuruhatensis	Enriched in EC tumors (some reports)	Identified in intratumoral studies
***Lactobacillus* (incl. *L. crispatus*)**	Depleted in EC compared with controls	Usually protective; depletion associated with EC
F. nucleatum	Reported in vaginal microbiome of EC and other gynecologic pathologies	Seen in higher-grade EC associations
Mobiluncus curtisii	Reported in EC vaginal microbiome	Part of dysbiotic profiles in EC
Akkermansia muciniphila	Enriched in EC vaginal samples	Reported in regional microbiome studies
Acinetobacter	Enriched in EC vaginal samples	Identified in microbiome differences
Pseudomonas	Enriched in EC endometrium and vagina	Found in multiple studies of genital tract microbiome
Bacillus	Enriched in EC endometrium in some cohorts	Detected via 16S studies
Streptomyces	Enriched in EC endometrium	Reported in microbiome profiling

Genus/Species	Association	Source/Notes
Burkholderia-Caballeronia-Paraburkholderia	Enriched in EC endometrium	Reported in Chinese cohort
Actinobacteria (incl. *Atopobium*, etc.)	Frequently enriched phylum in EC microbiome	Identified in multiple studies
Firmicutes (phylum)	Mixed changes; overall often more diverse anaerobes	Observed in EC microbiome
Proteobacteria (phylum)	Enriched taxa, including Gammaproteobacteria	Found in EC microbiota differences
Bacteroidetes (phylum)	Often enriched in EC	Phylum containing multiple associated taxa

14.5.14.2 Mechanism and Functions

Certain tumor-resident microbes modulate estrogen and hormonal signaling by harboring enzymes capable of modifying steroid hormones. *Atopobium* and *Gardnerella* species produce β-glucuronidase, which can reactivate estrogen metabolites within the TME, promoting endometrial proliferation, DNA damage *via* estrogen quinones, and hormone-driven tumor aggressiveness relevant for type I (endometrioid) EC, which is estrogen-responsive (Walther-Antonio et al., 2016, Ervin et al., 2019). The loss of *Lactobacilli* is associated with higher pro-inflammatory cytokines (IL-6, IL-8, and TNFα) and the recruitment of myeloid cells that can become tumor-promoting (M2-like macrophages and MDSCs). It also leads to the decreased production of lactic acid and antimicrobial peptides that suppress the overgrowth of pro-inflammatory and genotoxic bacteria (Abdalla et al., 2025). *Lactobacilli* help maintain epithelial tight junctions and the quality of mucus. Tumor-resident microbes modulate immune and inflammatory signaling mechanisms, activating TLR2, TLR4, and NOD-like receptors, leading to chronic inflammation and cytokine production. Especially *Lactobacilli* depletion facilitates this bacterial translocation, the exposure of pattern-recognition receptors (TLR2/4/9), and chronic NFκB activation, which supports proliferation, angiogenesis, and resistance to apoptosis in the endometrial epithelium (Abdalla et al., 2025). Their reduction also allows the colonization by taxa that secrete LPS, proteases, and other virulence factors, increasing epithelial stress and inflammation (Abdalla et al., 2025). *Porphyromonas* and *Prevotella* are frequently enriched in non-*Lactobacillus*-dominant vaginal and uterine microbial communities and have been linked to the development of endometrial hyperplasia and carcinoma (Semertzidou et al., 2024). *Porphyromonas*

species generate ROS and secrete gingipains along with other proteolytic enzymes that degrade ECM components and disrupt tight-junction integrity. These activities enhance epithelial invasion and promote stromal remodeling within the endometrium (Abdalla et al., 2025). Microbial LPS enhances STAT3 and NFκB signaling, enhancing proliferation and angiogenesis (Lu et al., 2021) (Figure 14.14). Microbial antigens in dysbiotic tumors modulate the infiltration of T-cells, showing depleted $CD8^+$ T-cell activity (Yu et al., 2020), and produce genotoxins or metabolites that potentially damage the DNA.

While direct genotoxicity in EC remains under investigation, parallels from other cancers suggest plausible mechanisms of DNA damage by *F. nucleatum*. Microbial metabolites can influence tumor metabolism, such as SCFAs modulating histone acetylation, polyamines promoting proliferation, indole derivatives influencing oxidative stress, and lactic acid supporting angiogenesis, and shift the hallmarks of EC by reprogramming tumor metabolism. Tumor microbes could convert tryptophan into indole derivatives and kynurenine-pathway-associated metabolites that activate the aryl hydrocarbon receptor (AhR) (Zelante et al., 2013) (Figure 14.14). This AhR signaling in endometrial cells and immune cells promotes EMT, Treg induction, proliferation, and immune suppression (Willing et al., 2011). Dysbiotic EC tumors show altered expression of metabolic genes involved in certain metabolic processes, which leads to enhanced glycolysis, lipid synthesis, and oxidative phosphorylation, suggesting that an altered EC microbiome rewires the bioenergetic EC networks (Lu et al., 2021). The EC intratumoral microbiome alters estrogen metabolism, modulates immune responses, and affects microbial drug metabolism, collectively impacting hormonal therapy, immunotherapy efficacy, and resistance (Geller et al., 2017) (Figure 14.14).

Although understudied, fungal DNA—including *Candida, Malassezia*, and *Cladosporium*—is detectable in EC tumors, with stronger signatures in advanced high-grade disease, mirroring patterns seen in other cancers (Nejman et al., 2020). Fungi can modulate Th17 responses, IL-17/IL-22 signaling, and barrier integrity, potentially synergizing with bacterial dysbiosis to drive chronic inflammation and ECM remodeling (Cho et al., 2022). Viral components, specifically HPV, polyomaviruses, and herpesviruses, have been sporadically detected in EC tissues. While HPV is not a classical driver of EC, viral presence influences the immune TME or genomic instability (Bichri et al., 2025, Marci et al., 2016) (Figure 14.14). Studies have shown that polyomavirus integration disrupts host genomic stability. Endogenous retroviruses can be reactivated in EC through inflammation-driven and epigenetic mechanisms (Chiappinelli et al., 2015), and the resulting cytosolic ERV-derived nucleic acids activate cGAS–STING signaling to shape innate and antitumor immunity (Liu et al., 2019).

14.5.14.3 Clinical Implications

Earlier studies propose microbial signatures as non-invasive biomarkers for EC by correlating microbial diversity indices with tumor grade and stage, and by associating microbial gene expression signatures for distinguishing EC from benign endometrium, supporting the development of microbiome-based diagnostic assays (Semertzidou et al., 2024, Hakimjavadi et al., 2022). Interestingly, the enrichment of anaerobic bacteria is associated with poorer prognosis and reduced immune activation. Microbial profiles are also associated with lymphovascular invasion and myometrial infiltration, and they even correlate with relapse status.

Antimicrobial peptides and targeted antibiotics are administered intratumorally or intrauterinely to influence the tumor-resident microbes. Microbiome-targeted immunotherapy, including PD-1/PD-L1 inhibitors, adoptive T-cell therapies, cancer vaccines, has been developed. Synthetic biology approaches aim to engineer certain types of bacteria that could play a role in delivering therapeutic molecules and cytokines, activating immune responses, and helping to disrupt tumor metabolism; furthermore, in some cases, there are emerging microbial "biosensors" to detect the tumor metabolites. Even though these strategies are in the experimental stages, they hold promise as a treatment strategy for EC. In the future, functional studies to discover the underlying mechanistic pathways and to integrate microbial, genomic, and immunologic data will play a vital role in the development of tumor microbiome-based diagnostics and therapeutics. The tumor microbiome in EC represents a rapidly evolving field with profound implications for understanding tumor biology, improving diagnostics, and developing novel therapeutics. Intratumoral microbial communities interact with hormonal, immune, and metabolic pathways to shape disease progression. A deeper understanding and continued research are required to clarify their mechanistic roles and unlock new opportunities for clinical translation in EC (Wang et al., 2025b).

14.5.15 Prostate Cancer

Prostate cancer (CaP) is increasingly understood not only as a disease of malignant epithelial transformation but as a complex ecological system shaped by host-microbe interactions. Although microbial influences on prostate pathology were historically attributed to chronic prostatitis or systemic infections, advances in high-resolution sequencing, spatial transcriptomics, and metagenomic profiling have overturned the long-held assumption that the prostate is a sterile organ. These technologies now reveal that prostate tumors harbor a distinct, localized microbiome embedded within the TME, with microbial DNA, RNA, and in some cases intact organisms detectable directly within malignant tissue (Du et al., 2026). Emerging evidence demonstrates that CaP tumors contain microbial signatures enriched for taxa associated with inflammation,

oxidative stress, and DNA damage, features tightly linked to prostate carcinogenesis and progression. These tumor-specific microbial communities are increasingly implicated in shaping key biological processes that drive disease behavior, including epithelial injury, stromal remodeling, immune modulation, and therapeutic resistance (2019, Papavassiliou et al., 2024, Podlipnik et al., 2024, Filomena et al., 2024). Rather than functioning as passive bystanders, intratumoral microbes actively participate in remodeling the ecological dynamics of the tumor. They alter the redox balance, modulate androgen receptor activity, and contribute to genomic instability. Within this framework, the prostate TME emerges as a multi-kingdom ecosystem in which microbial, epithelial, stromal, and immune components interact to shape tumor evolution. The presence and activity of these microbes introduce an additional layer of biological complexity, with implications for tumor initiation, progression, and response to therapy.

14.5.15.1 Composition and Spatial Localization

The microbial landscape of prostate tumors is diverse, encompassing bacteria, fungi, and viruses that collectively form a localized ecological network. Bacterial taxa, such as *Escherichia*, *Acinetobacter*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Cutibacterium*, and anaerobic genera associated with the urogenital tract have been consistently detected in CaP (Fu et al., 2025a). Fungal species and viral signatures have also been identified, suggesting dynamic interactions between microbes and host cells (Hill et al., 2024). Fungal DNA, particularly from *Candida* species, has been detected in prostate tissue, and these organisms influence epithelial proliferation and immune responses (Aykut et al., 2019)). Viral signatures, including bacteriophages and human viruses, have been identified. Bacteriophages modulate bacterial community structure, influencing microbial competition and metabolic output. Human viruses, including HPV and polyomaviruses, have been sporadically detected in prostate tissue, although their causal role remains controversial (Sheraz et al., 2025). Spatial profiling has revealed that microbes within CaP are not randomly dispersed but instead exhibit distinct patterns of localization. Microbial signals are frequently detected in periglandular stromal regions, hypoxic tumor cores, and immune-cell-rich niches. These spatial patterns suggest that microbes exploit microenvironmental gradients such as oxygen tension, nutrient availability, and immune accessibility. It is also demonstrated that microbial RNA colocalizes with specific host cell populations, influencing gene expression and cellular behavior (Poore et al., 2020).

14.5.15.2 Mechanisms

Microbial influences on CaP extend far beyond incidental colonization; accumulating evidence shows that bacteria, fungi, and their metabolites can directly shape the molecular and cellular landscape of prostate tumors. One of the most well-established mechanisms by which microbes influence CaP is through chronic inflammation. Microbial components, such as LPS, peptidoglycan, and lipoteichoic acid, activate innate immune receptors, including TLR2 and TLR4, triggering downstream NF-κB and STAT3 signaling. These pathways drive the production of pro-inflammatory cytokines, chemokines, and growth factors that promote epithelial proliferation, inhibit apoptosis, and recruit immunosuppressive myeloid cells. Chronic inflammation also generates ROS and RNS that induce DNA damage, contributing to mutational burden and genomic instability. The second mechanism involves microbial modulation of androgen metabolism. Some prostate-associated microbes possess enzymes capable of converting steroid precursors into androgenic metabolites, thereby sustaining androgen receptor (AR) signaling even under androgen-deprivation therapy (Figure 14.15). Microbial steroid metabolism has been documented in gut and tumor-associated bacteria (Ridlon et al., 2006) and emerging evidence suggests similar pathways may exist in CaP. This microbial contribution to intratumoral androgen synthesis helps explain the persistence of AR activity in castration-resistant CaP (CRPC). Further, the tumor microbiome exerts profound effects on the immune landscape of prostate cancer. Microbial antigens and metabolites shape the recruitment, differentiation, and function of immune cells within the tumor. Several studies describe increased infiltration of M2-polarized macrophages, the expansion of regulatory T-cell, and the suppression of cytotoxic T-cell activity in microbially enriched tumor regions. These immunosuppressive niches reduce antigen presentation and blunt responses to immunotherapy, contributing to the immune-cold phenotype characteristic of CaP. Microbial-immune crosstalk is now recognized as a major determinant of tumor progression and therapeutic response (Gopalakrishnan et al., 2018). Microbes within the CaP TME produce metabolites that influence host cell metabolism. SCFAs, polyamines, and other microbial metabolites modulate epithelial proliferation, immune cell function, and stromal remodeling. Some metabolites enhance glycolysis or lipid synthesis in tumor cells, supporting rapid growth and survival. Others modulate redox balance, influencing sensitivity to RT and chemotherapy. Emerging evidence suggests that microbial metabolites can influence host epigenetic regulation. SCFAs, such as butyrate, act as histone deacetylase inhibitors, altering chromatin accessibility and gene expression (Davie, 2003). Although prostate-specific data are limited, the presence of microbial metabolites within the TME raises the possibility of microbiome-driven epigenetic reprogramming (Figure 14.15).

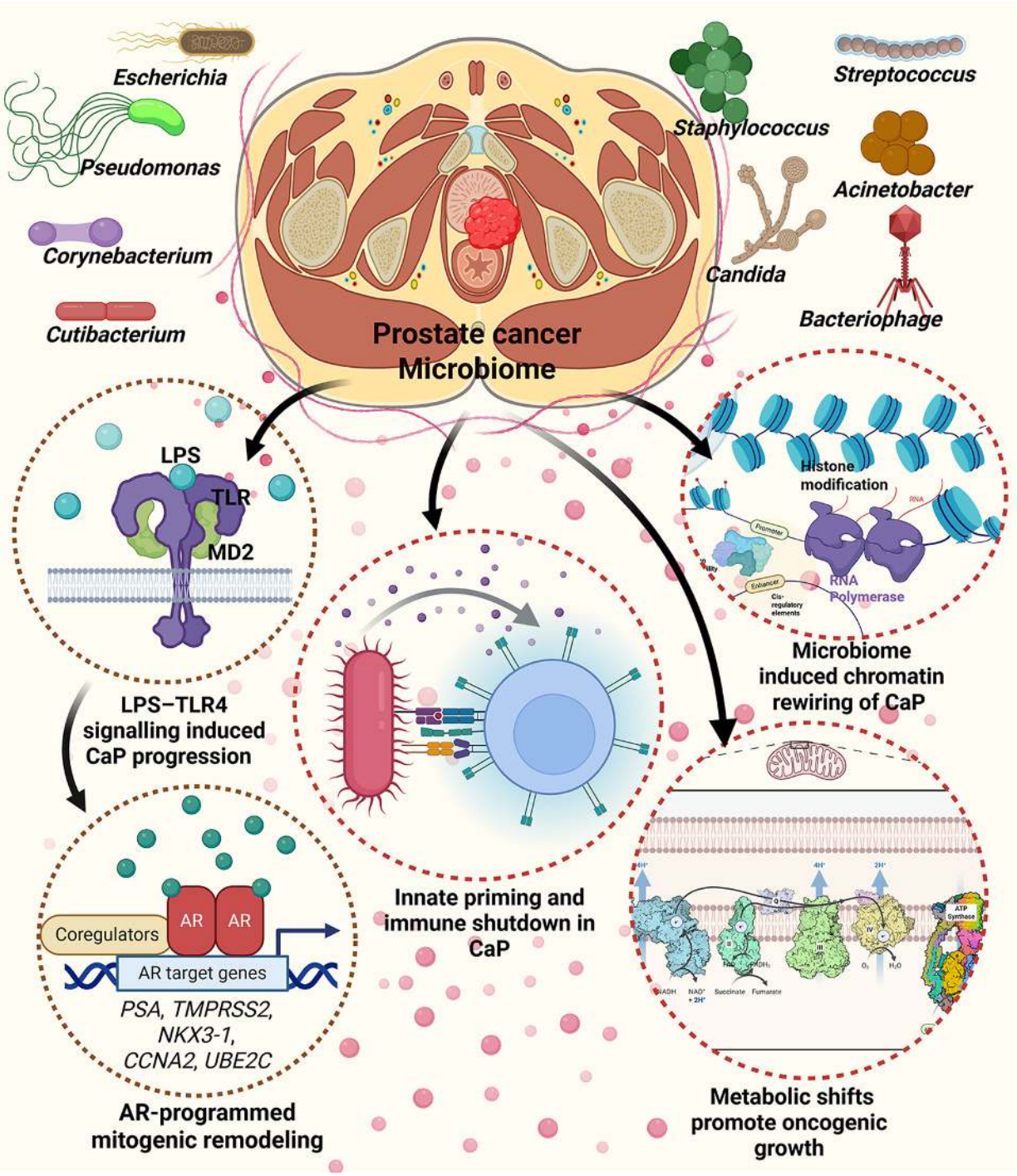

FIGURE 14.15 Microbiome-mediated oncogenic reprogramming in prostate cancer. ⏎

14.5.15.3 Clinical Implications and Translational Relevance

The expanding recognition that CaP harbors distinct microbial communities has opened new avenues for biomarker discovery and therapeutic stratification. Microbial signatures detected in tumor tissue, prostatic secretions, urine, and seminal fluid increasingly

demonstrate diagnostic and prognostic value, with several studies showing that microbial DNA and RNA profiles differ between indolent and aggressive disease. These tumor-associated microbial patterns mirror underlying biological processes, such as inflammation, oxidative stress, and genomic instability, that drive tumor progression (Podlipnik et al., 2024). Integrating these microbial features into existing diagnostic frameworks could enhance early detection and risk stratification. Traditional biomarkers like prostate-specific antigen (PSA) lack specificity, contributing to overdiagnosis and overtreatment, whereas multi-omics models that incorporate microbial signatures alongside PSA, imaging, and host transcriptomics show improved performance in distinguishing clinically significant from indolent CaP (Deng et al., 2025b). Beyond nucleic acid-based signatures, microbially derived metabolites represent an emerging class of non-invasive biomarkers. Polyamines, SCFAs, and steroid derivatives produced by tumor-associated microbes can be detected in urine or prostatic secretions and may correlate with tumor grade, metabolic state, or treatment response. These metabolites serve as functional readouts of host-microbe metabolic interactions, offering insight into how microbial activity shapes tumor behavior. Microbial signatures also hold prognostic value. Although prostate-specific data remain limited, early findings suggest that microbial biomarkers could complement genomic classifiers, such as Decipher and Prolaris, refining postoperative risk prediction and informing adjuvant therapy decisions. In RT, microbes capable of scavenging ROS attenuate radiation-induced DNA damage, promoting radioresistance, whereas other microbial metabolites may sensitize tumor cells by altering redox balance or impairing DNA repair pathways. In chemotherapy, microbes can metabolize or inactivate drugs, alter drug transport, or modulate host metabolic pathways that influence drug sensitivity. Microbial steroid-transforming enzymes sustain AR signaling during androgen deprivation therapy, contributing to the emergence of CRPC. Such steroid-modifying pathways are well characterized in other settings (Ridlon et al., 2006), and emerging evidence suggests analogous mechanisms may operate within the CaP TME. Immunotherapy represents another domain in which microbial influences are increasingly recognized. CaP is classically considered an immune-cold tumor, characterized by sparse T-cell infiltration and abundant immunosuppressive myeloid populations. Microbial antigens and metabolites can reinforce this immunosuppressive milieu by promoting regulatory T-cell expansion, inhibiting cytotoxic T-cell activity, and recruiting M2-polarized macrophages (Zhang et al., 2024c). These effects blunt ICI responses, paralleling microbiome-driven immunomodulation observed in other cancers (Gopalakrishnan et al., 2018). Together, these findings position the prostate tumor microbiome as a powerful, multi-dimensional biomarker and a functional determinant of therapeutic response. Its integration into

clinical frameworks has the potential to transform early detection, risk stratification, and precision treatment in prostate cancer.

14.5.15.4 Microbiome-Targeted Therapeutic Strategies

Given the influence of the tumor microbiome on CaP biology and treatment response, microbiome-targeted therapies represent a promising frontier. Potential strategies include localized antimicrobial therapy to disrupt biofilms or eliminate specific pathogenic taxa, bacteriophage-based approaches to selectively target microbial populations, and inhibitors of microbial steroid metabolism to prevent microbe-driven androgen synthesis. Immunomodulatory therapies that reprogram microbe-shaped immune niches may enhance the efficacy of immunotherapy. Synthetic biology offers additional opportunities for therapeutic innovation. Engineered bacteria capable of delivering therapeutic payloads, producing immunostimulatory molecules, or modulating metabolic pathways, which could be deployed within the TME. Microbial CRISPR systems may also be harnessed to target oncogenic pathways or disrupt microbial genes that contribute to CaP progression. Overall, the field of CaP microbiome research is poised for rapid expansion as modern technologies and analytical frameworks emerge. Spatial metagenomics and spatial metabolomics will enable precise mapping of microbe-host interactions at single-cell resolution, revealing how microbial signals influence epithelial, stromal, and immune compartments. High-resolution imaging techniques will further elucidate the spatial organization of microbial communities and their interactions with tumor architecture. Another major direction involves determining whether microbial signatures influence metastatic tropism. CaP metastasizes predominantly to bone, and it is unknown whether microbial metabolites, inflammatory mediators, or microbial-immune interactions contribute to this tropism. Finally, the integration of microbial data into precision oncology frameworks could refine risk stratification, improve treatment planning, and enhance therapeutic outcomes. Microbiome engineering, synthetic biology, and microbial CRISPR systems represent exciting avenues for therapeutic innovation, although significant challenges remain in terms of safety, specificity, and regulatory approval (Nazir et al., 2024)

The CaP microbiome represents a biologically meaningful and clinically relevant dimension of CaP. Far from being a passive bystander, the tumor-resident microbial community actively shapes inflammation, androgen signaling, immune evasion, genomic instability, and therapeutic response. Recent studies have demonstrated that CaP tumors harbor distinct microbial ecosystems that differ from benign prostate tissue and influence key aspects of tumor biology. These microbial communities interact with epithelial, stromal, and immune compartments, forming a complex ecological network that contributes to tumor progression and therapeutic resistance. Clinically, microbial

signatures hold promise as diagnostic and prognostic biomarkers, and microbial metabolites may serve as non-invasive indicators of tumor behavior. The tumor microbiome also influences therapeutic response, affecting radiation sensitivity, chemotherapy metabolism, and immunotherapy efficacy. Microbiome-targeted therapies, including antimicrobial agents, bacteriophages, metabolic inhibitors, and engineered microbes, represent emerging strategies for precision oncology (Bautista et al., 2025c).

14.5.16 Testicular Cancer

Testicular cancer, particularly testicular germ cell tumors (TGCTs), is the most common solid malignancy in young adult men. Despite high cure rates, the biological mechanisms underlying TGCT initiation, progression, and therapeutic response remain incompletely understood. In recent years, the testicular microbiome, a resident microbial community within the testis, has emerged as a provocative and biologically plausible contributor to testicular physiology and pathology. Although the evidence for a tumor-resident microbiome in testicular cancer is far more limited, recent studies have demonstrated that the testis is not sterile, as long believed. Instead, it harbors a low-biomass but detectable microbial community that interacts with sertoli cells, leydig cells, germ cells, and the immune-privileged microenvironment of the testis (Coticchio et al., 2022). The testis is a uniquely immune-privileged organ, protected by the blood testis barrier (BTB), specialized sertoli cell junctions, and immunosuppressive cytokine networks. Microbial interactions within this environment may influence inflammation, DNA damage, endocrine signaling, and germ cell transformation. Moreover, infections such as mumps orchitis, epididymo-orchitis, and sexually transmitted infections have been associated with altered testicular function and, in some studies, increased TGCT risk (Trabert et al., 2012). These observations raise the possibility that microbial dysbiosis or infection-driven inflammation may contribute to TGCT pathogenesis.

14.5.16.1 Composition

Recent sequencing studies have identified bacterial DNA and RNA within healthy testicular tissue, semen, and epididymis. Common taxa include *Corynebacterium*, *Staphylococcus*, *Cutibacterium*, *Lactobacillus*, and anaerobic genera associated with the urogenital tract. These microbes likely originate from ascending urogenital colonization, hematogenous spread, or early-life microbial seeding (European Academy of Andrology et al., 2022). Sertoli cells form the BTB, which restricts immune cell infiltration and antigen exposure. This environment is characterized by elevated levels of TGF-β, IL-10, and other immunosuppressive cytokines, regulatory T-cell enrichment, low MHC class I expression on germ cells, and specialized macrophage populations with anti-

inflammatory phenotypes. Microbial communities within this environment may exploit immune privilege to persist, mirroring microbial persistence in tumors with immunosuppressive niches (Gopalakrishnan et al., 2018). Therefore, the microbes or microbial metabolites within TGCTs contribute to immune evasion, reduced antigen presentation, the suppression of cytotoxic T-cell activity, and the recruitment of tolerogenic macrophages.

14.5.16.2 Mechanisms

The testicular microbiome—whether resident, transient, or inflammation-induced—influences TGCT initiation, evolution, and therapeutic response through a network of immunologic, metabolic, endocrine, and genomic mechanisms. Although TGCTs are not classically categorized as inflammation-driven malignancies, accumulating evidence suggests that infection-associated inflammation can contribute to germ cell malignant transformation. Mumps orchitis, for example, induces severe testicular inflammation, germ cell loss, and long-term impairment of testicular function, while chronic epididymo-orchitis has been linked to increased TGCT risk in epidemiological studies. Sexually transmitted infections similarly generate oxidative stress and DNA damage within testicular tissue, creating a microenvironment permissive to oncogenic change. Microbial components, such as LPS, peptidoglycan, and lipoteichoic acid, activate TLR2, TLR4, and NOD-like receptors on sertoli and leydig cells, triggering NF-κB and STAT3 signaling cascades. These pathways amplify cytokine production, oxidative stress, and DNA damage while disrupting spermatogonial stem cell niches. Collectively, these inflammatory perturbations facilitate the transition from normal germ cells to germ cell neoplasia in situ (GCNIS), the obligatory precursor to TGCTs. Building on this inflammatory foundation, a second mechanistic axis emerges from the endocrine nature of the testis. Because testicular function depends on tightly regulated steroidogenic pathways, even subtle microbial influences on hormone metabolism can have disproportionate biological consequences. Leydig cells synthesize testosterone through a multistep enzymatic cascade involving CYP11A1, HSD3B1/2, CYP17A1, and HSD17B3, and each of these nodes is exquisitely sensitive to inflammatory cytokines, microbial metabolites, and PAMPs. Microbes are known to express steroid-transforming enzymes, including hydroxysteroid dehydrogenases, ketosteroid isomerases, and reductases, that convert host-derived precursors into bioactive androgens or estrogens (Ridlon et al., 2006). If similar enzymatic activities occur within the testicular microenvironment, microbial communities could directly reshape intratesticular androgen pools, altering the hormonal milieu that governs germ cell differentiation, survival, and tumor susceptibility. Thus, endocrine disruption represents a plausible and underexplored route through which microbial dysbiosis may influence TGCT biology. A

third mechanistic layer involves genomic instability, a hallmark of TGCTs characterized by isochromosome 12p formation and widespread aneuploidy. Microbial metabolites and toxins are well-established contributors to genomic instability in other cancers. Although colibactin-producing strains have not yet been identified in TGCTs, the detection of *Enterobacteriaceae* in testicular tissue raises the possibility of genotoxic interactions analogous to those observed in CRC. Chronic inflammation further exacerbates DNA damage through ROS and RNS production, compounding the genomic stress that drives germ cell transformation and tumor evolution. Epigenetic dysregulation also represents a mechanistic axis linking microbial activity to TGCT pathogenesis. TGCTs exhibit global DNA hypomethylation, altered histone modification patterns, and that reactivation of pluripotency programs. Microbial metabolites, such as butyrate, function as histone deacetylase inhibitors (Davie, 2003); if present within the testicular microenvironment, these metabolites can influence chromatin accessibility, germ cell differentiation, pluripotency gene expression, and the progression of GCNIS. This provides a biologically coherent link between microbial dysbiosis and the epigenetic reprogramming that characterizes TGCTs. Importantly, the same microbial signals that reshape chromatin architecture and germ cell identity also intersect with pathways that determine how these tumors respond to therapy. Cisplatin-based chemotherapy remains the cornerstone of TGCT treatment, yet cisplatin resistance poses a significant challenge in a subset of patients with refractory disease. Insights from other cancers demonstrate that the microbiome can profoundly influence chemotherapy response. Intratumoral bacteria metabolize gemcitabine into inactive forms (Geller et al., 2017), while gut microbes modulate host responses to cyclophosphamide and oxaliplatin (Viaud et al., 2013). Although direct evidence in TGCTs is lacking, several plausible pathways exist: microbial metabolites may alter redox balance and thereby modulate ROS-dependent cisplatin cytotoxicity; inflammation-induced activation of DNA repair pathways, such as poly (ADP-ribose) polymerase (PARP) and NER, may reduce cisplatin efficacy; and microbiome-driven immune suppression may blunt immunogenic cell death. Given the extraordinary chemosensitivity of TGCTs, even subtle microbial influences could meaningfully affect therapeutic outcomes.

The testicular microbiome may shape fertility outcomes in TGCT survivors, a population for whom long-term reproductive health is a major concern. Microbial inflammation can disrupt spermatogonial stem cell niches by damaging Sertoli cell tight junctions and compromising BTB integrity. Dysbiosis has been associated with increased sperm DNA fragmentation, while chemotherapy and orchiectomy may shift seminal microbial composition, influencing sperm motility, morphology, and overall reproductive potential. These interactions underscore the importance of understanding

microbiome-testis crosstalk not only for tumor biology but also for survivorship and quality of life.

14.5.16.3 Translational Relevance

Although TGCT tissue is available after orchiectomy, repeat tissue sampling is uncommon; therefore, seminal plasma and other local urogenital samples could serve as practical biomarker sources. The study of the seminal plasma microbiome demonstrates the feasibility of detecting microbial reads and comparing TGCT and GCNIS signatures to controls. Such signatures could be tested prospectively for correlation with tumor subtype, stage, relapse risk, and treatment toxicity while controlling for confounders, including infection, antibiotics, and semen quality (Morup et al., 2023, Porto et al., 2023, Cao et al., 2023). However, the most promising translational path is not taxonomy alone, but microbe-immune coupling, i.e., correlating microbial features with TGCT immune cell programs (T-cell subsets, antigen presentation markers, and myeloid states) that are now measurable by scRNA-seq and multiplex imaging. This is consistent with broader tumor microbiome work linking microbial diversity to immune features and therapy response (Battaglia et al., 2024, Lu et al., 2024, Wang et al., 2025b, Islam et al., 2024).

Overall, the emerging concept of a testicular microbiome challenges long-standing assumptions about testicular sterility and opens new avenues for understanding testicular cancer biology. Although direct evidence for a tumor-resident microbiome in TGCTs remains limited, the testis clearly harbors a low-biomass microbial community that interacts with the immune-privileged microenvironment, endocrine signaling pathways, and germ cell biology. These interactions may influence inflammation, DNA damage, epigenetic regulation, and spermatogenesis, all processes relevant to TGCT initiation and progression. As the field matures, the testicular microbiome may emerge as a novel dimension of TGCT precision medicine, offering new biomarkers, therapeutic targets, and strategies to improve both oncologic and reproductive outcomes.

14.5.17 Skin Cancer

and comprises a diverse set of malignancies originating from distinct cellular lineages, including epidermal keratinocytes giving rise to cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC); melanocytes in melanoma; and neuroendocrine cells in Merkel cell carcinoma (MCC) (Plachta et al., 2021, Chen and Liu, 2025). Beyond genetic and environmental drivers, the skin's role as the body's largest barrier organ places it at the interface of host-microbe interactions, rendering it uniquely susceptible to microbiome-mediated influence on carcinogenesis. The cutaneous surface

is continuously colonized by complex microbial communities whose composition is shaped by site-specific ecological niches (sebaceous, moist, or dry microenvironments) as well as extrinsic factors such as UV radiation, topical antimicrobials, occlusion, and immune status. These features make the study of microbiome-cancer interactions important in skin malignancies. Recent investigations on skin cancer microbiome distinguish topical microorganisms from those present within the tumor tissue itself. Surface-associated communities are typically sampled using swabs or tape strips, whereas intratumoral and tissue-resident microbiota are detected using rigorously decontaminated DNA or RNA sequencing approaches and, more recently, spatially resolved profiling techniques. This distinction is critical, as surface colonizers and tumor-associated microbes may exert fundamentally different biological effects in the context of cancer initiation, promotion, and immune modulation. Among keratinocyte-derived tumors, cSCC and its precursor lesion, actinic keratosis (AK), exhibit a markedly altered bacterial landscape compared with adjacent patient-matched normal skin (Voigt et al., 2022, Krueger et al., 2022, Zhang et al., 2025b).

14.5.17.1 Composition

Skin lesions are characterized by increased bacterial burden, reduced α-diversity, and a reproducible enrichment of *Staphylococcus* species, accompanied by a depletion of commensal genera such as *Cutibacterium* and *Corynebacterium*. Notably, this *Staphylococcus*-dominant signature persists across clinical contexts, with *Staphylococcus epidermidis* enriched in immunosuppressed populations and *S. aureus* in immunocompetent tumors. In addition to bacterial dysbiosis, the cutaneous virome plays a critical role in cSCC pathobiology. β-HPVs are epidemiologically associated with cSCC and, more importantly, are documented for their integration into host genome with sustained oncoprotein expression in aggressive cSCC, providing proof that β-HPVs can function as direct oncogenic drivers in skin rather than as passenger viruses or inflammation-enhancing cofactors (Sandoval-Clavijo et al., 2025, Ye et al., 2025). Melanoma and BCC, while far less explored from a microbiome perspective, appear to exhibit subtler but distinct alterations. Early paired analyses comparing BCC tumors and adjacent normal skin suggest a depletion of *C. acnes* within BCC lesions, a species of interest given its antioxidant capacity and potential role in maintaining cutaneous redox homeostasis (Ding et al., 2024). These observations raise the possibility that the loss of protective commensals may contribute to oxidative stress–driven tumorigenesis in BCC. MCC stands in contrast and exemplifies a viral-dominated model of skin cancer. Global meta-analyses estimate that approximately 80% of MCCs are positive for Merkel cell polyomavirus (MCPyV), with viral integration and oncoprotein expression representing a central oncogenic mechanism. MCC thus provides one of the most compelling

examples of a virome-driven human cancer and underscores the broader relevance of host-microbe interactions across the skin cancer spectrum.

14.5.17.2 Spatial Localization

Skin tumors arise within a tissue that is already densely colonized by resident microbial communities, making microbial redistribution and relocalization more likely than de novo invasion. Consequently, the spatial organization of microbes in skin cancer reflects changes in epithelial integrity and local microenvironmental remodeling rather than the simple acquisition of new organisms. Across multiple skin cancer types, microbial localization is tightly linked to the disruption of the epidermal barrier, enabling epithelial ingress and altered retention within lesions. In AK and cSCC, barrier disruption induced by chronic UV exposure, inflammation, and keratinocyte dysplasia precedes malignant transformation and coincides with shifts in microbial distribution. Using both swab-based methods and biopsies, recent studies (Woo et al., 2022) reported a significant enrichment of *Staphylococcus aureus* DNA in AK and cSCC compared with matched healthy skin, with the microbial signal detected within the tumor tissue rather than being confined to the superficial surface, consistent with inward redistribution (Woo et al., 2022). Tumor-associated alterations in the cutaneous microenvironment further shape microbial localization. Reductions in sebum availability, the elevation of skin pH, and increased transepidermal water loss selectively deplete lipophilic commensals such as *Cutibacterium* and *Malassezia* while favoring the expansion of *Staphylococcus* species (Woo et al., 2022). These changes suggest that microbes are not uniformly distributed across the tumor surface but are selectively retained or excluded according to metabolic and physicochemical constraints imposed by the tumor niche. More importantly, microbial localization in skin cancer is also strongly influenced by anatomical skin microenvironments with the strongest causal signals arising from moist and sebaceous regions (Zhu et al., 2024). Together, these studies support a model in which skin cancer-associated microbes originate from resident communities and become redistributed across disrupted epidermal, adnexal, and tumor-associated niches as the TME evolves.

14.5.17.3 Mechanisms

Within the skin TME, cutaneous and intratumoral microbes act as integral ecological components that reprogram carcinogenic trajectories by jointly modulating epithelial stress responses, immune calibration, and stromal remodeling. Chronic UV exposure induces durable shifts in microbial composition, establishing a dysbiotic state that reshapes microbe-keratinocyte and microbe-melanocyte interactions, amplifies local oxidative stress, perturbs DNA damage resolution, and reinforces pro-oncogenic

inflammatory signaling pathways, including NF-κB and MAPK, within nascent lesions (Woo et al., 2022, L'Orphelin et al., 2025). UV irradiation preferentially selects for stress-resilient taxa, whose metabolites directly influence melanocyte biology and local antitumor immunity; notably, the protective commensal *Staphylococcus epidermidis* suppresses DNA synthesis in transformed cells through the microbe-derived antimetabolite 6-N-hydroxyaminopurine, thereby limiting UV-induced tumor formation (L'Orphelin et al., 2025, Nakatsuji et al., 2018). Specifically in melanoma, intracellular bacteria introduce an additional layer of immune modulation by providing HLA-presented antigens on tumor cells and antigen-presenting cells that can be recognized by autologous TILs, revealing a direct antigenic axis by which intratumoral bacteria modulate anti-tumor immunity (Kalaora et al., 2021). In contrast, skin cancer-associated *Staphylococcus aureus* strains secrete distinct toxin repertoires that drive IL-6, IL-8, and TNF cytokine amplification programs, suppress DNA repair machinery, and elevate oxidative stress, collectively generating a pro-tumorigenic inflammatory and genotoxic field across AK and cSCC (Krueger et al., 2021, 2022). Beyond staphylococci, dysbiotic enrichment of tumor-resident taxa broadly reprograms immune dynamics through TLR and NOD signaling, diminishing dendritic cell maturation, restraining $CD8^+$ T-cell and NK-cell cytotoxicity, and promoting the expansion of regulatory T-cell and M2-polarized macrophages (Woo et al., 2022, L'Orphelin et al., 2025). At the cellular interface, microbe-associated molecular patterns and secreted metabolites engage pattern-recognition receptors on tumor cells, Langerhans cells, and dermal myeloid populations, reshaping antigen presentation fidelity and biasing immune surveillance toward tolerance rather than elimination (Woo et al., 2022). This immune reprogramming is further reinforced by intratumoral microbial communities that entrench immune escape through impaired dendritic cell maturation, progressive attenuation of cytotoxic $CD8^+$ T-cell and NK-cell effector functions, and sustained enrichment of regulatory T-cell and pro-tumorigenic macrophages (Woo et al., 2022). Notably, in cSCC, *Fusobacterium*-dominated communities are repeatedly linked to NF-κB activation, myeloid-rich inflammation, immune exclusion, and CAF enrichment, together accelerating tumor growth and phenotypic plasticity (Tanaka et al., 2025, Mekadim et al., 2022). Beyond immune modulation, intratumoral microbes influence core oncogenic programs by altering host-cell metabolism, generating ROS, and perturbing β-catenin, PI3K-AKT, and stress-adaptive signaling pathways, thereby coupling microbial persistence to increased invasiveness and therapeutic resistance (Woo et al., 2022, L'Orphelin et al., 2025). Oncoviral constituents of the cutaneous microbiota introduce an additional oncogenic axis, as β-HPV associate with cSCC even in immunocompetent hosts and, under conditions of impaired T-cell control, can directly drive carcinogenesis through p53 and pRB inactivation and viral integration into tumor

genomes (Chahoud et al., 2016, Lambert et al., 2020). Accordingly, both melanoma and cSCC lesions display pronounced taxonomic and functional divergence from adjacent healthy skin, with enrichment of pro-inflammatory genera such as *Staphylococcus*, *Streptococcus*, *Fusobacterium*, and *Bacteroides*, alongside loss of commensal signatures associated with wound repair, collectively mirroring the immune-suppressive and EMT-permissive niche that supports tumor progression (Voigt et al., 2022, Mekadim et al., 2022). Importantly, this microbial influence is not uniformly tumor-promoting, as select commensals retain protective functions by producing antimicrobial peptides and bioactive metabolites that constrain UV-induced mutagenesis, facilitate apoptotic clearance of damaged cells, and maintain immune vigilance, underscoring a context-dependent microbial governance of tumor fate (L'Orphelin et al., 2025). Together, these mechanisms position skin cancer-associated microbiota as dynamic regulators of disease progression, operating through tightly coupled epithelial-immune-stromal networks that shape both tumor evolution and therapeutic response (Woo et al., 2022, L'Orphelin et al., 2025, Mekadim et al., 2022).

14.5.17.4 Clinical Relevance, Biomarker Utility, and Therapeutic Significance

Across the spectrum of skin cancers, the cutaneous microbiome is emerging as a clinically relevant modifier of tumor behavior, immune surveillance, and therapeutic responsiveness (Woo et al., 2022, MacGibeny et al., 2025). In AK, cSCC and BCC, dysbiosis appears early, coupled with immune exhaustion and barrier defects that favor malignant progression (Zhang et al., 2025b). These microbial signatures distinguish premalignant from normal skin and align with more aggressive disease, positioning microbial patterns as candidate biomarkers of progression risk (Duman et al., 2025). In cutaneous T-cell lymphoma (CTCL), clonal disease evolution is tightly coupled to microbial imbalance that not only exacerbates inflammation and T-cell activation but also correlates with inferior event-free survival, functioning as a prognostic biomarker (Lee et al., 2024, Licht et al., 2024). Specifically, in melanoma, microbial relevance extends beyond correlative ecology into tumor immunogenicity. Bacterial DNA, RNA, and peptides have been detected within melanoma cells and antigen-presenting compartments, raising the possibility that microbe-derived antigens contribute to T-cell recognition, immune editing, and variability in immunotherapy outcomes (Sepich-Poore et al., 2021, Liu et al., 2025b). Unlike host neoantigens, microbial antigens represent non-self, opening a new avenue for vaccine strategies or T-cell receptor–based approaches. Notably, MCC-microbiome interactions provide a rational foundation for virus-directed immunotherapies and preventive vaccination strategies in virus-positive disease (Jani et al., 2023, Ryu et al., 2024). Collectively, these observations establish skin-resident microbes as clinically meaningful modifiers of cancer risk, course, and

treatment across diverse cutaneous malignancies. When addressed, microbial features have the potential to inform diagnosis, refine prognostication, reveal novel therapeutic targets, and shape combination strategies that couple tumor control with the restoration of a resilient cutaneous ecosystem.

14.5.18 Cancer in Young Adults and Adolescents

Osteosarcoma (OS) is the most common primary malignant bone tumor in young adults and adolescents. It has historically been approached from a predominantly genetic and molecular perspective. However, recent advances in multi-omics, sequencing, and culture-independent microbial profiling have highlighted that, like many other solid tumors, OS harbors a distinct intratumoral microbiome that persists as living, metabolically active microbes positioned throughout the tumor architecture, including within cancer cells, immune cells, the ECM, and deep necrotic cores. The OS microbiome emerges as a key functional component of tumor biology, modulating oncogenic signaling, immune evasion, metabolic tropism, chemoresistance, and bone remodeling. The microbial taxa become localized and reside within the TME, exerting their direct mechanistic effects on the tumor and stromal cells.

14.5.18.1 Composition

When investigated across multiple cohorts, OS tumors consistently contain low-biomass but high-diversity microbial taxa, predominated by *Actinobacteria* (*C. acnes, Corynebacterium kroppenstedtii*), *Firmicutes* (*Staphylococcus epidermidis, Streptococcus parasanguinis*), *Proteobacteria* (*Pseudomonas aeruginosa, Ralstonia pickettii*), fungal species (*Malassezia restricta* and *Candida albicans*), and occasional viral signatures (e.g., *Herpesvirus and Adenovirus*). These organisms are intracellular, matrix-embedded, biofilm-associated, and highly enriched in metastatic lesions (Nejman et al., 2020).

14.5.18.2 Species Level Mechanistic Functions in OS

Bacterial taxa have specific mechanistic functions that are analogous to bone biology, tumor immunology, and microbial oncology. Certain *Actinobacteria* sp., including *C. acnes*, promote inflammatory osteolysis via the induction of IL-1β and TNF-α in osteoblasts and osteoclast precursors (Qin et al., 2014). This *C. acnes* activates NF-κB and STAT3 pathways and enhances the metastatic signaling pathways, which are already hyperactive in OS. They induce DNA damage and genomic instability by producing ROS. They transform TAMs into an M2-like immunosuppressive phenotype that, in turn, causes immune evasion. *Corynebacterium kroppenstedtii* induces chronic

inflammation in the OS TME by producing lipid-rich cell wall components and activating TLR2. This organism specifically drives fibrotic remodeling through activating the fibroblasts, creating a protective niche for OS cells. Although this mechanism has not been directly validated in OS, existing literature shows that this species enhances chemoresistance by upregulating drug efflux pumps, suggesting that a similar process could plausibly occur in OS cells (Amaral et al., 2012). Microbial signatures belonging to the Firmicutes, including *Staphylococcus epidermidis*, which produces extracellular polysaccharides that form biofilm-like structures in intratumoral niches, protecting the OS cells from host immune surveillance. This species influences neutrophil recruitment, impairing cytotoxicity by secreting phenol-soluble modulins. Additionally, it promotes osteoclastogenesis via upregulating receptor activator of NF-κB ligand (RANKL), a TNF superfamily cytokine that plays a predominant role in bone destruction, tumor expansion, immune regulation, and tumor-bone interactions (Qin et al., 2014). Intratumoral *Streptococcus parasanguinis* releases hydrogen peroxide (H_2O_2), causing oxidative stress that selectively selects for aggressive OS clones. Furthermore, *S. parasanguinis* indirectly stimulates NLRP3 inflammation activation in stromal cells, enhancing IL-1β and facilitating the tumor-supportive inflammation (Huang et al., 2021). Microbial taxa from *Proteobacteria*, including *Pseudomonas aeruginosa*, are classically pathogenic, and the *P. aeruginosa* DNA and metabolites have been detected in OS tumors. They produce pyocyanin and enhance ROS, promoting OS cell proliferation and metastasis (Abdelaziz et al., 2023). It modulates the NF-κB-driven inflammatory pathway by secreting LPS and flagellin, and by activating TLR4/TLR5. In other cancers, its functional role includes promoting immune evasion through the induction of PD-L1 expression. They also act synergistically on OS cells and enhance matrix degradation by releasing enzymes such as elastases and proteases, which in turn facilitate tumor invasion (Hu et al., 2026).

Evidence for fungal mechanisms specifically in the OS tumor microbiome is very sparse. Recent case studies reporting *Malassezia restricta* as a causative organism in forming vertebral osteomyelitis infer that this skin commensal could invade bone, enhance strong local inflammation, and cause destructive bone lesions, supporting its capacity to potentially affect the bone microenvironment and OS TME (Peng et al., 2024). Viruses in OS modulate the immunosuppressive TME via inducing T-cell exhaustion and upregulating immune checkpoints. This supports the conceptual framework of how tumor-associated viruses influence the OS TME to escape host immune defense mechanisms (Luo et al., 2024). Certain oncolytic *herpesviruses* and *adenoviruses* (such as G207, Delta-24-RGD, and VCN-01) have been tested in OS models, demonstrating the capability of lysing OS cells, releasing tumor antigens, triggering immunogenic cell death, activating dendritic cells, and priming $CD8^+$ T-cell

that, in turn, reshape the TME. A recent review on oncolytic virus therapy states that there is upregulation of danger-associated molecular patterns (DAMPs) and chemokines within the OS TME, conveying the virus-driven mechanisms in OS tumors (Wang et al., 2025b).

14.5.18.3 Clinical and Therapeutic Implications

Osteosarcoma (OS) harbors distinct microbial signatures from other sarcomas with high accuracy (Cao et al., 2024). Certain genera, namely *Malassezia* and *Pseudomonas*, are associated with a higher metastatic burden, poor chemotherapy response, and increased metastasis risk. Therapeutic strategies, including microbe-targeted immunotherapy (such as Dectin-1 blockade for *Malassezia*) and microbial enzyme inhibitors (e.g., protease inhibitors), are widely used nowadays. A future vision for OS includes spatial mapping of microbes and functional validation of microbial metabolites, which are essential to characterize tumor progression, immune modulation, therapeutic response, and their cellular interactions. Advances in microbiome engineering offer promising avenues to reprogram tumor immunity and treat tumors with great efficacy. Eventually, the integration of microbial signatures into OS risk stratification models will enable accurate patient assessment and open the door to microbiome-informed precision oncology.

Overall, the OS tumor microbiome signifies a metabolically and dynamically active ecological site that interacts with genetic, immune, and stromal drivers of disease progression. The key mechanistic roles of intratumoral microbial taxa in OS included *C. acnes*- and *P. aeruginosa*-driven inflammation and ROS signaling, *Malassezia*-induced bone remodeling, and viral modulation of T-cell exhaustion. Consequently, these microbial communities actively modulate specific OS hallmarks such as immune evasion, matrix invasion, therapy resistance, and metastasis. In parallel, therapeutic strategies such as Dectin-1 blockade, microbial enzyme inhibition, and microbiome engineering lay a foundation for a new field in precision oncology that utilizes the intratumoral microbial signatures to reprogram the OS TME and to improve patient outcomes with high-risk OS.

14.5.19 Childhood Solid Tumors

Childhood solid cancers comprise a diverse group of non-hematologic malignancies that arise from developing tissues and organs in children and adolescents (Table 14.6). While pediatric solid tumors were long considered sterile entities, recent studies have displayed a definitive, yet lower, intratumoral microbial burden compared to adult epithelial malignancies. Despite the emergence of the microbiome field in adult cancers, the spatial profiling of the pediatric cancer tumor microbiome niche is still nascent and

currently lack evidence on tumor-specific composition, spatial distribution, function, and clinical significance. The limited and fragmented information available on select pediatric solid cancers is discussed below.

TABLE 14.6
Comparative Overview of Childhood Solid Cancers

Tumor Category	Cancer Type	Typical Age	Primary Tissue/Site	Key Features
CNS tumors	Medulloblastoma	3–8 years	Cerebellum	Highly malignant embryonal tumor; SHH/WNT subtypes
	Gliomas (low/high grade)	Childhood–adolescence	Brain, brainstem	Astrocytic origin; variable prognosis
	Ependymoma	<10 years	Ventricles, spinal cord	Ependymal cells; posterior fossa common
	ATRT	<3 years	CNS	SMARCB1 loss; extremely aggressive
Embryonal tumors	Neuroblastoma	<5 years	Adrenal medulla, sympathetic chain	MYCN amplification; spontaneous regression in infants
	Wilms tumor	2–5 years	Kidney	WT1-related developmental tumor
	Hepatoblastoma	<3 years	Liver	AFP elevated; embryonic liver tumor
	Retinoblastoma	<5 years	Retina	RB1 mutation; bilateral/hereditary forms

Tumor Category	Cancer Type	Typical Age	Primary Tissue/Site	Key Features
Bone tumors	Osteosarcoma	Adolescence	Long bones	Metaphyseal; associated with growth spurts
	Ewing sarcoma	Childhood–adolescence	Bone/soft tissue	EWSR1–FLI1 fusion
Soft tissue sarcomas	Rhabdomyosarcoma	<10 years	Skeletal muscle lineage	Embryonal/alveolar subtypes
	Synovial sarcoma	Adolescence	Soft tissue	SS18–SSX fusion
	Infantile fibrosarcoma	Infancy	Soft tissue	ETV6–NTRK3 fusion
Renal tumors	Clear cell sarcoma (kidney)	3–5 years	Kidney	Bone metastasis tendency
	Rhabdoid tumor (kidney)	Infancy	Kidney	SMARCB1 loss; extremely aggressive
Germ cell tumors	Teratoma	Infancy–adolescence	Gonadal/extragonadal	Can be benign or malignant
	Yolk sac tumor	<3 years	Gonadal/extragonadal	AFP elevated
	Germinoma/dysgerminoma	Adolescence	Gonadal/CNS	Radiosensitive
Liver tumors	Hepatocellular carcinoma	Adolescence	Liver	Often underlying liver disease
Endocrine tumors	Adrenocortical carcinoma	<5 years and teens	Adrenal cortex	Hormone-secreting; TP53 association
	Pheochromocytoma/paraganglioma	Adolescence	Adrenal/paraganglia	Catecholamine-secreting
	Thyroid carcinoma	Adolescence	Thyroid	Papillary most common
Other rare tumors	Pleuropulmonary blastoma	Infancy	Lung	DICER1-associated
	Desmoplastic small round cell tumor	Adolescence	Peritoneum	EWSR1–WT1 fusion
	NPC	Adolescence	Nasopharynx	EBV-associated

Tumor Category	Cancer Type	Typical Age	Primary Tissue/Site	Key Features
	Pediatric melanoma	Adolescence	Skin	Distinct biology vs. adult melanoma

14.5.19.1 Medulloblastoma

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, arising in the posterior fossa and subdivided into molecular groups (WNT, SHH, Group 3, and Group 4) with distinct biology, prognosis, and microenvironmental dependencies. Although the MB-specific intratumoral microbiome is sparse, it is often associated with tumor and immune cells. This is crucial for MB because its clinical course is strongly shaped by TME programs, including immune infiltration, astrocytic and vascular niches, macrophage states, and therapy-driven evolution at relapse (Lu et al., 2024, Wang et al., 2025b, Nejman et al., 2020, Michaud et al., 2024). Even without extensive MB-specific microbial catalogs (which typically note an enrichment of *Proteobacteria* and *Actinobacteria* signatures, along with *polyomavirus* and *herpesvirus* associations), it is known that MB has spatially organized microenvironmental niches that contribute to malignant cell states, astrocytic and vascular compartments, and macrophage populations, and that these niches differ by subgroup and expand or remodel at relapse. Microbial components may contribute to chronic neuroinflammation, influence microglial activation, and modulate developmental signaling pathways (SHH, WNT) via immune-mediated mechanisms. When microbial products are present in MB tissue, they can engage pattern-recognition pathways in resident or infiltrating myeloid cells, microglia and macrophages, leading to NF-κB-linked cytokine programs and immune remodeling. The MB spatial niche data showing abundant macrophage and astrocytic interactions at relapse provide a biologically plausible substrate for microbial-immune coupling in MB (Wang et al., 2025b, Michaud et al., 2024, Gao et al., 2022). MB is metabolically heterogeneous across subgroups and cell states, and relapse-associated niches often show altered stress responses and signaling programs. In this context, microbial metabolic coupling could influence tumor fitness, oxidative stress handling, or therapy tolerance. Critically, for MB, where radiotherapy and cytotoxic chemotherapy remain central, microbe-linked modulation of inflammatory signaling or oxidative stress could, in principle, affect radiosensitivity or post-therapy immune states, though this requires direct experimental testing in MB models (Wang et al., 2025b, Zhao et al., 2020, Walker et al., 2020). To that end, MB research uniquely benefits from comparing molecular subgroups and diagnosis versus relapse tissues, because subgroup biology and relapse evolution are major determinants of outcome. If microbial signatures or viral

elements are present in MB TME, their distribution and activity would also be expected to vary across these subgroup-specific and relapse-associated niches, potentially influencing immune tone, metabolic stress responses, or therapy sensitivity. To test whether microbes contribute to high-risk MB biology, sampling should be multi-region (tumor core vs. invasive edge; vascular niches vs. differentiated regions), and analyses should explicitly model clinical covariates (steroid exposure, perioperative antibiotics, and prior therapy). Assessing the clinical and translational opportunities of the tumor microbiome for MB, the most plausible near-term translational application is not microbial therapy, but the development of microbe-aware biomarkers integrated with spatial and immune features, for instance, microbial presence in relapse-associated vascular/astrocytic niches or in macrophage-rich regions that expand during recurrence (Lu et al., 2024, Wang et al., 2025b, Michaud et al., 2024). Tumor microbiome signatures may influence treatment toxicity, blood-brain barrier (BBB) permeability, and immune surveillance, particularly in immunotherapy-refractory disease. Likewise, in the context of microbe-targeted interventions (antimicrobials, phage, and engineered bacteria) in pediatric brain tumors, the safety and feasibility bar are high. In MB, realistic next steps are experimental and aim to determine whether microbial products can shift macrophage polarization, influence astrocyte–tumor signaling, or alter radiosensitivity in MB organoids or xenografts, using contamination-controlled inoculation and readouts linked to spatial niches (Chien et al., 2024).

14.5.19.2 Glioma

Pediatric gliomas span a biologically diverse spectrum from pediatric low-grade gliomas (pLGGs; WHO grade 1–2), often chronic diseases with long survival but substantial morbidity, to pediatric high-grade gliomas (pHGGs; grade 3–4) and diffuse midline glioma (DMG), which remain among the most lethal childhood cancers. The tumor microbiome field is early for pediatric gliomas; yet, several pediatric studies and frameworks already demonstrate that: (1) pediatric glioma tissue can contain detectable bacterial sequence signals, and (2) these signals may vary with disease stage and progression (Gozlan et al., 2024). Intratumoral bacterial DNA, often *Gammaproteobacteria*, and viral elements (e.g., CMV in subsets) have been described. A tissue-based analysis in diffuse intrinsic pontine glioma (DIPG) using RNA-seq files from initial and progressive disease reported bacterial read recoveries in DIPG samples. This analysis identified notable reads of *C. acnes* and observed reduced intratumoral bacterial read recoveries in progressive versus initial DIPG, alongside changes in immunoglobulin gene expression. Because DIPG tissues are low-biomass and RNA-seq is not optimized for microbiome discovery, bacterial signals must be interpreted with strong contamination controls and independent validation wherever possible, an issue

repeatedly emphasized in best-practice tumor microbiome guidance (Jawahar et al., 2025, Chen and Bie, 2025). Functionally, microbial metabolites may affect microglial polarization, isocitrate dehydrogenase (IDH)-related metabolic reprogramming, and epigenetic regulation. Pediatric gliomas differ substantially from adult counterparts in mutational burden, neoantigen landscape, immune contexture, and developmental trajectories, differences that influence both tumor biology and immunotherapy response. While pLGGs exhibit excellent long-term survival, they can recur or progress and cause substantial neurologic morbidity, whereas pHGG/DMG carries a dismal prognosis. These pediatric-specific immune and stromal programs create a plausible niche where tumor-resident microbial components, if present, could alter local innate immune sensing, myeloid polarization, cytokine tone, and metabolic wiring, thereby influencing progression or therapy tolerance (Xue et al., 2022, Messiaen et al., 2023, Ahamed et al., 2025, Van den Ende et al., 2025). The glioma TME highlights the dominance and heterogeneity of myeloid lineages, variable lymphocyte infiltration, and stromal/vascular programs that differ by tumor class, location, and treatment status. This is crucial as the macrophage-rich landscape of pediatric glioma may be a key host compartment for microbial signals (Morad et al., 2025, Messiaen et al., 2023, Ahamed et al., 2025, Adapa et al., 2025). However, at present, there is no widely replicated, pediatric-only, multi-institutional atlas of intratumoral bacteria or fungi across pLGGs and pHGGs, and this gap reflects constraints, such as limited tissue, treatment heterogeneity, and the need for stringent contamination control in tiny specimens. Moving forward, it is first critical to unveil the recurring bacterial species. While the DIPG study highlighted *C. acnes* reads in both initial and progressive DIPG RNA-seq datasets, whether it is a recurrent low-biomass signal (like in many cancers) remains unexplored. Likewise, pediatric glioma studies have not yet produced a robust, pediatric-only intratumoral mycobiome or virome atlas; determined how tumor-resident microbes could shape pediatric glioma biology; or clarified the function of the tumor microbiome in innate immune sensing within a myeloid-rich TME. Given limited evidence and safety considerations, the near-term clinical role of tumor microbiome analysis in pediatric glioma is most plausibly as a biomarker source for risk stratification, therapy response prediction, or monitoring microenvironmental states, rather than as a target for direct antimicrobial intervention.

14.5.19.3 Ependymoma

Pediatric ependymoma is the third most common brain tumor in children and remains a leading cause of cancer-related morbidity and mortality despite advances in molecular classification and surgical techniques. Traditionally, ependymoma has been understood primarily through the lenses of developmental neurobiology, epigenetics, and oncogenic

fusion events, such as *ZFTA–RELA* and *YAP1* fusions. While direct studies focusing exclusively on the microbiome of pediatric ependymoma are limited, the sparse microbial signatures documented are dominated by Proteobacteria. The impact of tumor-associated microbes likely varies by molecular subtype, where in PFA ependymoma, the profound immune suppression and hypoxia may allow microbial components to persist and reinforce immune exclusion; in ZFTA–RELA fusion ependymoma, the relatively higher immune activity and enhanced microbial antigen presentation may contribute to differential responsiveness to immunotherapy (Palermo et al., 2025, Cooper et al., 2026); and in YAP1-fusion ependymoma, the lower inflammatory signaling suggests reduced microbial-immune interaction. Functionally, immune-enriched subsets of PFA ependymomas demonstrate activation of interferon-γ, TNF-α, and chemokine signaling pathways, suggesting that microbial antigens may partially shape cytokine gradients within the TME (Lammers et al., 2021, Yao et al., 2023). Building on this, multi-omic studies have identified hypoxia-associated myeloid cell populations in high-risk pediatric ependymoma that exhibit features of myeloid-derived suppressor cells (MDSCs), including IL-6/STAT3 signaling and wound-healing transcriptional programs. Microbial ligands may contribute to the maintenance of this phenotype by sustaining chronic, low-grade inflammatory signaling (Griesinger et al., 2023). Microbial components within tumor tissue may activate PPRs, such as TLRs and cGAS–STING pathways, expressed by microglia and infiltrating myeloid cells. In pediatric ependymoma, activation of these pathways may paradoxically reinforce immune tolerance instead of immune activation, due to the immature or developmentally programmed immune context of the CNS (Yao et al., 2023, de Koning et al., 2023). The presence of a tumor-associated microbiome in pediatric ependymoma has several clinical implications: (1) Immunotherapy modulation: Microbial antigens may prime, but simultaneously suppress, anti-tumor immune responses, explaining the limited efficacy of ICIs in unselected pediatric ependymoma cohorts (Palermo et al., 2025); (2) Biomarker development: Microbial DNA or RNA signatures within tumor tissue could serve as biomarkers of immune state or prognosis, particularly in distinguishing immune-enriched versus immune-excluded tumors (Lammers et al., 2021); (3) Microenvironment-targeted therapies: strategies aimed at reprogramming myeloid cells or modulating innate immune sensing may indirectly alter microbial–tumor interactions and improve treatment response (Cooper et al., 2026, Griesinger et al., 2023). Although pediatric ependymoma is not classically infection-driven, accumulating evidence supports the presence of a tumor-associated microbiome within the local CNS TME. These microbial components interact with myeloid-dominant immune ecosystems, hypoxic niches, and developmental signaling pathways, subtly shaping tumor biology

and therapeutic response. Understanding these interactions represents a promising frontier in pediatric neuro-oncology.

14.5.19.4 Atypical Teratoid/Rhabdoid Tumor

Atypical teratoid/rhabdoid tumor (ATRT) is an aggressive pediatric CNS embryonal tumor defined by biallelic loss of *SMARCB1* (or rarely *SMARCA4*) and characterized by a profoundly altered epigenetic state, an immune-suppressive microenvironment, and early age of onset. Within this context, the tumor microbiome, understood strictly as tumor-resident microbial DNA, RNA, or cellular components within ATRT tissue rather than distal ecosystems, is an emerging but still sparsely mapped dimension. Direct ATRT-specific catalogs are limited, yet, extrapolation from rigorously controlled intracranial tumor studies indicates that low-biomass, predominantly intracellular bacterial components can be detected within pediatric brain tumors, localizing to tumor cells and tumor-associated myeloid populations, provided there are stringent contamination controls and orthogonal validation. This occurs in an ATRT setting, where the TME is enriched for myeloid cells, displays attenuated antigen presentation, and exhibits cytokine programs shaped by chromatin remodeling. Candidate microbial signals are most plausibly *Proteobacteria*- or *Actinobacteria*-derived low-abundance taxa or microbial cell-wall or DNA fragments that persist intracellularly rather than forming extracellular communities. Fungi and viruses are theoretically possible but technically not validated. Functionally, even non-viable microbial components can engage innate sensing pathways in myeloid and tumor cells (e.g., TLRs, cGAS–STING pathway), intersecting with ATRT's epigenetically driven transcriptional programs to modulate NF-κB–linked inflammation, interferon tone, and immune-metabolic states. Such interactions provide a biologically plausible route by which microbial signals could reinforce macrophage skewing, T-cell exclusion, and vascular niche crosstalk that typify ATRT biology, without invoking systemic microbiota effects. Clinically, the implications are correlative and translational rather than interventional at present. The integration of microbial readouts with ATRT molecular subgrouping, spatial transcriptomics, and longitudinal sampling (from diagnosis to progression) represents a realistic near-term path, while any microbiome-targeted therapy awaits causal demonstration in pediatric-appropriate models and careful safety considerations. Overall, ATRT's tumor microbiome should be viewed not as a separate ecosystem but as a low-abundance, intracellular layer of the TME with the potential to influence innate immunity and treatment response, demanding gold-standard low-biomass methods and pediatric-centric study designs (Tetens et al., 2025, Gigi et al., 2025).

14.5.19.5 Hepatoblastoma

Hepatoblastoma (HB) is the most common malignant liver tumor in children, typically diagnosed in the first three years of life, and remains a major clinical challenge in high-risk settings characterized by multifocal disease, vascular invasion, metastasis, chemoresistance, and recurrence. A pivotal study that explicitly investigated the existence and distribution of microbiome signals in HB tumor tissues and adjacent non-tumor tissues from children reported that both diversity and abundance differed significantly between tumor and adjacent tissue ($p < 0.01$), indicating that HB tumors harbor a distinct microbial profile relative to neighboring liver. At the phylum level, the tumor tissue was dominated by *Proteobacteria, Bacteroidetes, and Firmicutes*; at the genus level, *Ruminococcus* was more abundant in HB tumors than in adjacent tissue, while several genera (*Bacteroides*, *Parabacteroides*, *Alistipes*, and the *Lachnospiraceae* NK4A136 group) were lower in tumors (Cui et al., 2024). Notably, this HB study also reported clinical-correlative patterns: *Romboutsia* abundance correlated with alpha-fetoprotein (AFP), a key biomarker used in HB monitoring, and different taxa were reported as enriched in primary versus metastatic tumors (e.g., *Sphingomonas* in primary HB; *Oscillibacter* and *Pandoraea* in metastatic HB). However, the study cautions that whether these bacteria are causally associated with HB requires further evaluation, an important limitation consistent with low-biomass field guidance (Fierer et al., 2025, Cui et al., 2024). This intratumoral microbiome features in HB could become a new class of HB biomarkers and potentially informs immune-modulating strategies to overcome therapy resistance in high-risk disease.

14.5.19.6 Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is an EBV-associated epithelial malignancy arising in the nasopharyngeal mucosa, characterized by a highly immune-rich yet immunosuppressive TME that provides a unique ecological niche for tissue-resident and intratumoral microbiota. Large-scale clinical studies have definitively demonstrated the presence of intratumoral bacteria within NPC tissues, with microbial communities originating from the nasopharyngeal mucosa and persisting within tumor compartments rather than representing surface contamination. In terms of composition, NPC tumors show reduced microbial diversity compared with non-malignant nasopharyngeal tissue but harbor distinct bacterial genera such as *Corynebacterium, Staphylococcus, Bacteroides, Alloprevotella, Parvimonas*, and *Dialister*, with specific bacterial signatures stratifying patients into prognostic risk groups. Functionally, a higher intratumoral bacterial load in NPC is associated with suppression of antitumor immunity, including reduced infiltration of $CD8^+$ T lymphocytes and altered cytokine signaling, indicating that tumor-resident microbes actively modulate the immune landscape rather than acting as passive bystanders (Qiao et al., 2022, Tan et al., 2025).

Integrated multi-omics analyses further show that NPC tumors enriched with high-risk bacterial communities exhibit immune-excluded phenotypes and dysregulated interferon, NF-κB, and antigen-presentation pathways, intersecting with EBV-driven immune remodeling to promote tumor progression. Clinically, intratumoral microbiome features in NPC have emerged as independent predictors of disease-free, distant metastasis-free, and overall survival, suggesting that microbial load and composition may serve as robust prognostic biomarkers alongside established EBV DNA measurements. Moreover, given the growing use of immunotherapy in NPC, tumor-associated microbiota may influence therapeutic responsiveness by shaping immune exhaustion, NK cell activity, and checkpoint signaling, raising the possibility of microbiome-informed patient stratification or adjunctive microenvironment-modulating strategies (Yeo et al., 2025). However, because intratumoral microbial biomass in NPC remains low and detection is highly sensitive to contamination, rigorous sampling protocols, quantitative validation, and spatially resolved analyses are essential to ensure biological and clinical interpretability before microbiome-targeted interventions can be translated into practice (Liu et al., 2024, Lu et al., 2024, Liu et al., 2025b).

14.6 Synthesis and Takeaways

This chapter consolidates a profound paradigm shift in cancer biology: solid tumors are not sterile, but instead harbor low-biomass, spatially organized, and functionally active microbial ecosystems embedded within the TME. Across diverse malignancies, spanning epithelial, neural, endocrine, genitourinary, and gynecologic cancers, bacteria, fungi, and viruses have now been reproducibly detected within tumor tissue, often localized intracellularly within cancer cells and immune cells, as well as within stromal, necrotic, and hypoxic niches. These findings overturn decades of dogma and reposition the tumor microbiome as a core ecological layer of tumor biology, rather than a technical artifact or passive bystander.

1. *Tumors as Ecosystems, Not Isolated Genomes:* A unifying theme is that tumors function as complex, spatially structured ecosystems, shaped by gradients of oxygen, pH, nutrients, ECM composition, and immune pressure. These biophysical and immunologic constraints select for distinct microbial consortia with non-random, tumor-type specific patterns. Intratumoral microbes exploit protective niches, particularly intracellular compartments, and hypoxic stroma, to persist at low abundance, evade immune clearance, and interact directly with host signaling networks. This ecological framing moves the field beyond cataloging

taxa toward understanding where microbes live, why they persist, and how micro-niches dictate function.

2. *Convergent Functional Themes across Cancer Types:* Despite taxonomic heterogeneity, intratumoral microbes converge on a limited set of shared functional pathways that shape tumor behavior. Across cancers, microbial components engage pattern-recognition receptors (such as TLRs, NOD-like receptors, and C-type lectins) to drive chronic NF-κB, STAT3, β-catenin, and MAPK signaling. These pathways reinforce inflammation, EMT, immune evasion, and resistance to apoptosis. Microbial metabolites, including short-chain fatty acids, polyamines, bile acid derivatives, lactate, and redox-active molecules, further reprogram tumor and immune cell metabolism, influence epigenetic regulation, and sustain survival in metabolically hostile niches. Notably, immune modulation emerges as a cross-cutting consequence, with microbes shaping macrophage polarization, T-cell exhaustion, checkpoint expression, and tertiary lymphoid structure formation.
3. *Intracellular Residency as a Defining Feature:* One of the most consequential insights synthesized in this chapter is the prevalence of intracellular microbes within solid tumors. Multiple lines of orthogonal evidence, including spatial transcriptomics, RNA/DNA *in situ* hybridization, imaging mass cytometry, and ultrastructural analyses, demonstrate that tumor-resident bacteria and fungi frequently reside inside malignant epithelial cells and immune cells. Intracellular localization confers protection from antibiotics and humoral immunity, enables direct modulation of host signaling and cytoskeletal dynamics, and may allow microbes to "travel" with tumor cells during metastatic dissemination. This reframes microbes as cell-autonomous modifiers of cancer phenotypes, not merely extracellular inflammatory triggers.
4. *Methodology Is Destiny in Low-Biomass Biology:* The chapter underscores that methodological rigor is non-negotiable in tumor microbiome research. Low microbial biomass mandates contamination-aware experimental design, including randomized processing, multiple negative controls, dual-indexing, computational decontamination, and, critically, orthogonal spatial validation. Studies that integrate sequencing with imaging and spatial multi-omics consistently yield the most biologically credible insights. The field's maturation hinges not only on the discovery of new taxa, but on standardization, transparency, and reproducibility to distinguish true intratumoral residents from technical noise.
5. *Clinical and Translational Implications:* Collectively, the evidence positions the tumor microbiome as a previously hidden but clinically consequential variable. Microbial features, whether specific taxa, total microbial burden, or functional

signatures, associate with tumor subtype, invasion, immune contexture, prognosis, and response to chemotherapy, radiotherapy, and immunotherapy. These observations open multiple translational avenues: tumor-microbiome-informed biomarkers, patient stratification, microbiome-modulating co-therapies, bacteriophage, or enzyme-targeted interventions, complement or TLR pathway modulation, and engineered microbial platforms for intratumoral delivery. At the same time, the chapter emphasizes judicious restraint: causality, safety, and context-specificity must be established before clinical deployment.

6. *From Association to Mechanism, and Beyond:* The central challenge ahead is transformation of robust associations into mechanistic causality. Key unresolved questions include microbial viability versus the persistence of molecular remnants, routes of tumor entry, co-evolution with cancer clones, and the relative importance of specific taxa versus functional microbial burden. Addressing these gaps will require spatially resolved perturbation studies, human-relevant model systems, and prospective clinical cohorts explicitly designed around low-biomass microbiome principles.

Overall, the tumor microbiome represents a new dimension of cancer biology, one that integrates ecology, immunity, metabolism, and genomics within the tumor itself. Rather than replacing genetic or immune paradigms, it adds depth, revealing how cancer cells interact not only with stromal and immune neighbors, but also with microbial partners embedded in the same space. As the field advances from detection to mechanism and translation, the solid tumor microbiome stands poised to reshape precision oncology by exposing vulnerabilities, refining biomarkers, and uncovering novel therapeutic leverage points from within the tumor ecosystem itself.

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References

Abdalla W, Ibrahim WN, Abdallah AM, Al-Asmakh MA, Said SS. The role of the microbiome in endometrial carcinoma: pathogenesis, biomarkers, and therapeutic prospects. *J Obstet Gynaecol Res*. 2025;51(9):e70070. doi: 10.1111/jog.70070. PubMed PMID: 40922069; PubMed Central PMCID: PMC12417558. ⏎

Abdelaziz AA, Kamer AMA, Al-Monofy KB, Al-Madboly LA. *Pseudomonas aeruginosa*'s greenish-blue pigment pyocyanin: its production and biological activities. *Microb Cell Fact*. 2023;22(1):110. doi: 10.1186/s12934-023-02122-1. ⏎

Abe S, Masuda A, Matsumoto T, Inoue J, Toyama H, Sakai A, *et al.* Impact of intratumoral microbiome on tumor immunity and prognosis in human pancreatic ductal adenocarcinoma. *J Gastroenterol*. 2024;59(3):250–62. Epub 20240120. doi: 10.1007/s00535-023-02069-5. PubMed PMID: 38242997; PubMed Central PMCID: PMC10904450. ⏎

Adapa SR, Porshe S, Talada DP, Nywening TM, Anderson ML, Shaw TI, *et al.* Spatial transcriptomics reveals distinct architectures but shared vulnerabilities in primary and metastatic liver tumors. *Cancers (Basel)*. 2025;17(19). Epub 20251001. doi: 10.3390/cancers17193210. PubMed PMID: 41097737; PubMed Central PMCID: PMC12523610. ⏎

Ahamed C, Nguyen L, Brock CS, Farzamnia A, Millet P, Sato K, *et al.* Targeting the tumor microenvironment in pediatric gliomas: advances and future directions in immunotherapy. *Neurooncol Adv*. 2025a;7(1):vdaf193. Epub 20250901. doi: 10.1093/noajnl/vdaf193. PubMed PMID: 41030523; PubMed Central PMCID: PMC12477475. ⏎

Ahmad S, Jayamanne D, Bergamin S, Lawless A, Guminski A, Lee A, *et al.* Oral microbiome as a biomarker and therapeutic target in head and neck cancer: current insights and future directions. *Cancers (Basel)*. 2025b;17(16). Epub 20250815. doi: 10.3390/cancers17162667. PubMed PMID: 40867296; PubMed Central PMCID: PMC12384379. ⏎

Alberti C, Pinciroli P, Valeri B, Ferri R, Ditto A, Umezawa K, *et al.* Ligand-dependent EGFR activation induces the co-expression of IL-6 and PAI-1 via the NFkB pathway in advanced-stage epithelial ovarian cancer. *Oncogene*. 2012;31(37):4139–49. Epub 20111212. doi: 10.1038/onc.2011.572. PubMed PMID: 22158046. ⏎

Alizhan D, Ukybassova T, Bapayeva G, Aimagambetova G, Kongrtay K, Kamzayeva N, *et al.* Cervicovaginal microbiome: physiology, age-related changes, and protective role against human papillomavirus infection. *J Clin Med*. 2025;14(5). Epub 20250224. doi: 10.3390/jcm14051521. PubMed PMID: 40094958; PubMed Central PMCID: PMC11900180. ⏎

Alkhalil SS. The role of bacteriophages in shaping bacterial composition and diversity in the human gut. *Front Microbiol*. 2023;14:1232413. Epub 20230919. doi: 10.3389/fmicb.2023.1232413. PubMed PMID: 37795308; PubMed Central PMCID: PMC10546012. ⏎

Al-Obaidy KI, Cheng L. Fibroblast growth factor receptor (*FGFR*) gene: pathogenesis and treatment implications in urothelial carcinoma of the bladder. *J Clin Pathol*. 2021;74(8):491. doi: 10.1136/jclinpath-2020-207115. ⏎

Amaral L, Spengler G, Martins A, Armada A, Handzlik J, Kiec-Kononowicz K, *et al.* Inhibitors of bacterial efflux pumps that also inhibit efflux pumps of cancer cells. *Anticancer Res*. 2012;32(7):2947–57. PubMed PMID: 22753759. ⏎

European Academy of Andrology et al. Andrology centres accredited by the European Academy of Andrology. *Andrology*, 2022;10: 10–96. https://doi.org/10.1111/andr.13245 ⏎

Arze CA, Springer S, Dudas G, Patel S, Bhattacharyya A, Swaminathan H, *et al.* Global genome analysis reveals a vast and dynamic anellovirus landscape within the human virome. *Cell Host Microbe*. 2021;29(8):1305–15.e6. doi: 10.1016/j.chom.2021.07.001. ⏎

Asgharzadeh S, Pourhajibagher M, Bahador A. The microbial landscape of tumors: a deep dive into intratumoral microbiota. *Front Microbiol*. 2025;16:1542142. Epub 20250520. doi: 10.3389/fmicb.2025.1542142. PubMed PMID: 40463434; PubMed Central PMCID: PMC12129932. ⏎

Ataollahi H, Hedayati M, Zia-Jahromi N, Daneshpour M, Siadat SD. Investigating the role of the intratumoral microbiome in thyroid cancer development and progression. *Crit Rev Oncol Hematol*. 2024;204:104545. Epub 20241028. doi: 10.1016/j.critrevonc.2024.104545. PubMed PMID: 39476992. ⏎

Audirac-Chalifour A, Torres-Poveda K, Bahena-Roman M, Tellez-Sosa J, Martinez-Barnetche J, Cortina-Ceballos B, *et al.* Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. *PLoS One*. 2016;11(4):e0153274.

Epub 20160426. doi: 10.1371/journal.pone.0153274. PubMed PMID: 27115350; PubMed Central PMCID: PMC4846060. ⏎

Austin GI, Korem T. Planning and analyzing a low-biomass microbiome study: A data analysis perspective. *J Infect Dis*. 2026;233(1):76–86. doi: 10.1093/infdis/jiae378. PubMed PMID: 39189314; PubMed Central PMCID: PMC12445924. ⏎

Awad MM, Govindan R, Balogh KN, Spigel DR, Garon EB, Bushway ME, *et al.* Personalized neoantigen vaccine NEO-PV-01 with chemotherapy and anti-PD-1 as first-line treatment for non-squamous non-small cell lung cancer. *Cancer Cell*. 2022;40(9):1010–26.e11. Epub 20220825. doi: 10.1016/j.ccell.2022.08.003. PubMed PMID: 36027916. ⏎

Aykut B, Pushalkar S, Chen R, Li Q, Abengozar R, Kim JI, *et al.* The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature*. 2019;574(7777):264–7. Epub 20191002. doi: 10.1038/s41586-019-1608-2. PubMed PMID: 31578522; PubMed Central PMCID: PMC6858566. ⏎

Bailey A, Hogue S, Pierce CM, Paul S, La Fuente N, Thapa R, *et al.* Metagenomic characterization of the tracheobronchial microbiome in lung cancer. *Front Microb*. 2024;3:1457537. doi: 10.3389/frmbi.2024.1457537. ⏎

Bakhti SZ, Latifi-Navid S. Interplay and cooperation of *Helicobacter pylori* and gut microbiota in gastric carcinogenesis. *BMC Microbiol*. 2021;21(1):258. Epub 20210923. doi: 10.1186/s12866-021-02315-x. PubMed PMID: 34556055; PubMed Central PMCID: PMC8461988. ⏎

Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357(9255):539–45. doi: 10.1016/S0140-6736(00)04046-0. PubMed PMID: 11229684. ⏎

Battaglia TW, Mimpen IL, Traets JJH, van Hoeck A, Zeverijn LJ, Geurts BS, *et al.* A pan-cancer analysis of the microbiome in metastatic cancer. *Cell*. 2024;187(9):2324–35 e19. Epub 20240409. doi: 10.1016/j.cell.2024.03.021. PubMed PMID: 38599211. ⏎

Bautista J, Altamirano-Colina A, Lopez-Cortes A. The vaginal microbiome in HPV persistence and cervical cancer progression. *Front Cell Infect Microbiol*. 2025a;15:1634251. Epub 20251007. doi: 10.3389/fcimb.2025.1634251. PubMed PMID: 41127671; PubMed Central PMCID: PMC12537727. ⏎

Bautista J, Cardona-Maya WD, Gancino-Guevara K, Lopez-Cortes A. Reprogramming prostate cancer through the microbiome. *Front Med (Lausanne)*. 2025b;12:1690498. Epub 20251126. doi: 10.3389/fmed.2025.1690498. PubMed PMID: 41384118; PubMed Central PMCID: PMC12689563.

Bautista J, Maldonado-Noboa I, Maldonado-Guerrero D, Reinoso-Quinga L, Lopez-Cortes A. Microbiome influence in gastric cancer progression and therapeutic strategies. *Front Med (Lausanne)*. 2025c;12:1681824. Epub 20251020. doi: 10.3389/fmed.2025.1681824. PubMed PMID: 41189901; PubMed Central PMCID: PMC12580360. ⏎

Bayrak AF, Arayici ME, Savas O, Ozgur E, Ozkutuk A, Guneri EA. Prognostic impact of *Fusobacterium nucleatum* in head and neck cancers: a meta-analysis of critical oncological outcomes. *BMC Cancer*. 2025;26(1):35. Epub 20251129. doi: 10.1186/s12885-025-15404-1. PubMed PMID: 41318441; PubMed Central PMCID: PMC12781759. ⏎

Bichri K, El Ghanmi A, Kouhen F, Hamdi S, Fichtali K, El Mansouri F, *et al.* The sibylline relationship between human papillomavirus and endometrial cancer: scarcity of strong evidence linking both conditions. *Viruses*. 2025;17(5). Epub 20250424. doi: 10.3390/v17050607. PubMed PMID: 40431619; PubMed Central PMCID: PMC12115647. ⏎

Birder L, Andersson K-E. Urothelial signaling. *Physiol Rev*. 2013;93(2):653–80. doi: 10.1152/physrev.00030.2012. PubMed PMID: 23589830. ⏎

Blazheva S. P-415 study of the incidence of herpes viruses in the endometrium of women with evidence of chronic endometritis and reproductive problems. *Human Reproduct*. 2022;37(Suppl_1). doi: 10.1093/humrep/deac107.392. ⏎

Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, Lazarev MG, *et al.* The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin Infect Dis*. 2015;60(2):208–15. Epub 20141009. doi: 10.1093/cid/ciu787. PubMed PMID: 25305284; PubMed Central PMCID: PMC4351371. ⏎

Bucevic Popovic V, Situm M, Chow CT, Chan LS, Roje B, Terzic J. The urinary microbiome associated with bladder cancer. *Sci Rep*. 2018;8(1):12157. Epub 20180814. doi: 10.1038/s41598-018-29054-w. PubMed PMID: 30108246; PubMed Central PMCID: PMC6092344. ⏎

Bučević Popović V, Šitum M, Chow CT, Chan LS, Roje B, Terzić J. The urinary microbiome associated with bladder cancer. *Sci Rep*. 2018;8(1):12157. Epub 20180814. doi: 10.1038/s41598-018-29054-w. PubMed PMID: 30108246; PubMed Central PMCID: PMC6092344.

Bullman S. The intratumoral microbiota: from microniches to single cells. *Cell*. 2023;186(8):1532–4. doi: 10.1016/j.cell.2023.03.012. PubMed PMID: 37059062. ⏎

Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, *et al.* Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science*.

2017;358(6369):1443–8. Epub 20171123. doi: 10.1126/science.aal5240. PubMed PMID: 29170280; PubMed Central PMCID: PMC5823247. ⏎

Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, *et al.* Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67–73. doi: 10.1038/nature12113. PubMed PMID: 23636398; PubMed Central PMCID: PMC3704730. ⏎

Cao T, Wang S, Pan Y, Guo F, Wu B, Zhang Y, *et al.* Characterization of the semen, gut, and urine microbiota in patients with different semen abnormalities. *Front Microbiol*. 2023;14:1182320. Epub 20230524. doi: 10.3389/fmicb.2023.1182320. PubMed PMID: 37293215; PubMed Central PMCID: PMC10244769. ⏎

Cao Y, Xia H, Tan X, Shi C, Ma Y, Meng D, *et al.* Intratumoural microbiota: a new frontier in cancer development and therapy. *Signal Transduct Target Ther*. 2024;9(1):15. doi: 10.1038/s41392-023-01693-0.

Castro J, Rosca AS, Cools P, Vaneechoutte M, Cerca N. *Gardnerella vaginalis* enhances *Atopobium vaginae* viability in an in vitro model. *Front Cell Infect Microbiol*. 2020;10:83. Epub 20200304. doi: 10.3389/fcimb.2020.00083. PubMed PMID: 32195197; PubMed Central PMCID: PMC7064616. ⏎

Chahoud J, Semaan A, Chen Y, Cao M, Rieber AG, Rady P, *et al.* Association between beta-genus human papillomavirus and cutaneous squamous cell carcinoma in immunocompetent individuals—a meta-analysis. *JAMA Dermatol*. 2016;152(12):1354–64. doi: 10.1001/jamadermatol.2015.4530. PubMed PMID: 26720285. ⏎

Chang CM, Pekkle Lam HY. Intratumoral microbiota: unraveling their oncogenic impact on cancer progression with focus on breast cancer therapeutic outcomes. *Anticancer Res*. 2024;44(6):2271–85. doi: 10.21873/anticanres.17035. PubMed PMID: 38821615. ⏎

Chefetz I, Alvero AB, Holmberg JC, Lebowitz N, Craveiro V, Yang-Hartwich Y, *et al.* TLR2 enhances ovarian cancer stem cell self-renewal and promotes tumor repair and recurrence. *Cell Cycle*. 2013;12(3):511–21. Epub 20130116. doi: 10.4161/cc.23406. PubMed PMID: 23324344; PubMed Central PMCID: PMC3587452. ⏎

Chen K, Liu X. Global burden of skin cancer and its subtypes: a comprehensive analysis from 1990 to 2021 with projections to 2040. *Front Public Health*. 2025;13:1610661. Epub 20250904. doi: 10.3389/fpubh.2025.1610661. PubMed PMID: 40977764; PubMed Central PMCID: PMC12443545.

Chen K, Wang Q, Li M, Guo H, Liu W, Wang F, *et al.* Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal

adenocarcinoma malignant progression. *eBioMedicine*. 2021;66. doi: 10.1016/j.ebiom.2021.103315. ⏎

Chen M, Bie L. Intratumoral microbiota for hepatocellular carcinoma: from preclinical mechanisms to clinical cancer treatment. *Cancer Cell Int*. 2025;25(1):152. doi: 10.1186/s12935-025-03745-7. ⏎

Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS–STING pathway of cytosolic DNA sensing. *Nat Immunol*. 2016;17(10):1142–9. doi: 10.1038/ni.3558. ⏎

Chen Y, Qiu X, Wang W, Li D, Wu A, Hong Z, *et al.* Human papillomavirus infection and cervical intraepithelial neoplasia progression are associated with increased vaginal microbiome diversity in a Chinese cohort. *BMC Infect Dis*. 2020;20(1):629. Epub 20200826. doi: 10.1186/s12879-020-05324-9. PubMed PMID: 32842982; PubMed Central PMCID: PMC7449047. ⏎

Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, *et al.* Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell*. 2015;162(5):974–86. doi: 10.1016/j.cell.2015.07.011. PubMed PMID: 26317466; PubMed Central PMCID: PMC4556003. ⏎

Chien F, Michaud M, Bakhtiari M, Ross JL, Schroff C, Snuderl M, *et al.* MDB-106. Medulloblastoma tumor microenvironments show intra- and inter-tumoral heterogeneity on spatial transcriptomics. *Neuro-Oncol*. 2024;26(Suppl_4):0. doi: 10.1093/neuonc/noae064.554. ⏎

Cho DH, Choi GB. Fungus packs a punch in the gut. *Immunity*. 2022;55(4):586–8. doi: 10.1016/j.immuni.2022.03.012. PubMed PMID: 35417672. ⏎

Choi E, Murray B, Choi S. Biofilm and cancer: interactions and future directions for cancer therapy. *Int J Mol Sci*. 2023;24(16). Epub 20230816. doi: 10.3390/ijms241612836. PubMed PMID: 37629016; PubMed Central PMCID: PMC10454087. ⏎

Chung HC, Ros W, Delord JP, Perets R, Italiano A, Shapira-Frommer R, *et al.* Efficacy and safety of pembrolizumab in previously treated advanced cervical cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol*. 2019;37(17):1470–8. Epub 20190403. doi: 10.1200/JCO.18.01265. PubMed PMID: 30943124. ⏎

Cisar C, Keener N, Ruffalo M, Paten B. A unified pipeline for FISH spatial transcriptomics. *Cell Genom*. 2023;3(9):100384. Epub 20230821. doi: 10.1016/j.xgen.2023.100384. PubMed PMID: 37719153; PubMed Central PMCID: PMC10504669. ⏎

Cooper E, Posner DA, Lee CYC, Hu L, Bonner S, Taylor JT, *et al.* Childhood brain tumors instruct cranial hematopoiesis and immunotolerance. *Nat Genet*. 2026. Epub 20260203. doi: 10.1038/s41588-025-02499-2. PubMed PMID: 41634415. ⏎

Cortellini A, Ricciuti B, Facchinetti F, Alessi JVM, Venkatraman D, Dall'Olio FG, *et al.* Antibiotic-exposed patients with non-small-cell lung cancer preserve efficacy outcomes following first-line chemo-immunotherapy. *Ann Oncol*. 2021;32(11):1391–9. Epub 20210813. doi: 10.1016/j.annonc.2021.08.1744. PubMed PMID: 34400292. ⏎

Coticchio G, Borini A, Zaca C, Makrakis E, Sfontouris I. Fertilization signatures as biomarkers of embryo quality. *Hum Reprod*. 2022;37(8):1704–11. doi: 10.1093/humrep/deac123. PubMed PMID: 35640036. ⏎

Cui J, Li X, Zhang Q, Du B, Ding Z, Yan C, *et al.* Existence and distribution of the microbiome in tumour tissues of children with hepatoblastoma. *Heliyon*. 2024;10(21):e39547. Epub 20241018. doi: 10.1016/j.heliyon.2024.e39547. PubMed PMID: 39553581; PubMed Central PMCID: PMC11564952. ⏎

Cui X, Liu S, Song H, Xu J, Sun Y. Single-cell and spatial transcriptomic analyses revealing tumor microenvironment remodeling after neoadjuvant chemoimmunotherapy in non-small cell lung cancer. *Mol Cancer*. 2025;24(1):111. Epub 20250409. doi: 10.1186/s12943-025-02287-w. PubMed PMID: 40205583; PubMed Central PMCID: PMC11980172. ⏎

D'Antonio DL, Zenoniani A, Umme S, Piattelli A, Curia MC. Intratumoral *Fusobacterium nucleatum* in pancreatic cancer: current and future perspectives. *Pathogens*. 2024;14(1). Epub 20241226. doi: 10.3390/pathogens14010002. PubMed PMID: 39860963; PubMed Central PMCID: PMC11768203. ⏎

Dambuza IM, Brown GD. Fungi accelerate pancreatic cancer. *Nature*. 2019;574(7777):184–5. doi: 10.1038/d41586-019-02892-y. PubMed PMID: 31591542. ⏎

Datorre JG, Dos Reis MB, Sorroche BP, Teixeira GR, Hatano SS, de Carvalho AC, *et al.* Intratumoral *Fusobacterium nucleatum* is associated with better cancer-specific survival in head and neck cancer patients. *J Oral Microbiol*. 2025;17(1):2487644. Epub 20250401. doi: 10.1080/20002297.2025.2487644. PubMed PMID: 40182114; PubMed Central PMCID: PMC11966973. ⏎

Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr*. 2003;133(7 Suppl):2485S–93S. doi: 10.1093/jn/133.7.2485S. PubMed PMID: 12840228. ⏎

de Koning W, Feenstra FF, Calkoen FGJ, van der Lugt J, Kester LA, Mustafa DAM. Characterizing the tumor immune microenvironment of ependymomas using targeted gene expression profiles and RNA sequencing. *Cancer Immunol Immunother*. 2023;72(8):2659–70. Epub 20230419. doi: 10.1007/s00262-023-03450-2. PubMed PMID: 37072536; PubMed Central PMCID: PMC10361846. ⏎

Deng R, Zong GF, Wang X, Yue BJ, Cheng P, Tao RZ, *et al.* Promises of natural products as clinical applications for cancer. *Biochim Biophys Acta Rev Cancer*. 2025a;1880(1):189241. Epub 20241212. doi: 10.1016/j.bbcan.2024.189241. PubMed PMID: 39674416.

Deng S, Kim W, Cheng K, Yang Q, Singh Y, Bae G, *et al.* Identification and impact of microbiota-derived metabolites in ascites of ovarian and gastrointestinal cancer. *Cancer Metab*. 2025b;13(1):21. Epub 20250513. doi: 10.1186/s40170-025-00391-5. PubMed PMID: 40361187; PubMed Central PMCID: PMC12076955. ⏎

Di Paola M, Sani C, Clemente AM, Iossa A, Perissi E, Castronovo G, *et al.* Characterization of cervico-vaginal microbiota in women developing persistent high-risk human papillomavirus infection. *Sci Rep*. 2017;7(1):10200. Epub 20170831. doi: 10.1038/s41598-017-09842-6. PubMed PMID: 28860468; PubMed Central PMCID: PMC5579045. ⏎

Ding R, Lian SB, Tam YC, Oh CC. The cutaneous microbiome in skin cancer—A systematic review. *JDDG: J Deutsch Dermatol Ges*. 2024;22(2):177–84. doi: https://doi.org/10.1111/ddg.15294. ⏎

Ding T, Liu C, Li Z. The mycobiome in human cancer: analytical challenges, molecular mechanisms, and therapeutic implications. *Mol Cancer*. 2025;24(1):18. Epub 20250115. doi: 10.1186/s12943-025-02227-8. PubMed PMID: 39815314; PubMed Central PMCID: PMC11734361. ⏎

Dinney CP, McConkey DJ, Millikan RE, Wu X, Bar-Eli M, Adam L, *et al.* Focus on bladder cancer. *Cancer Cell*. 2004;6(2):111–6. doi: 10.1016/j.ccr.2004.08.002. PubMed PMID: 15324694. ⏎

Dohlman AB, Klug J, Mesko M, Gao IH, Lipkin SM, Shen X, *et al.* A pan-cancer mycobiome analysis reveals fungal involvement in gastrointestinal and lung tumors. *Cell*. 2022;185(20):3807–22.e12. doi: 10.1016/j.cell.2022.09.015. PubMed PMID: 36179671; PubMed Central PMCID: PMC9564002. ⏎

Dong B, Huang Y, Cai H, Chen Y, Li Y, Zou H, *et al.* Prevotella as the hub of the cervicovaginal microbiota affects the occurrence of persistent human papillomavirus infection and cervical lesions in women of childbearing age via host NF-kappaB/C-myc. *J Med Virol*. 2022;94(11):5519–34. Epub 20220730. doi: 10.1002/jmv.28001. PubMed PMID: 35835717. ⏎

Du X, Wu J, Xia S, Zheng H, Zhang B. Unraveling the microbial landscape in prostate cancer: pathogenesis to therapy. *npj Biofilms Microbiomes*. 2026;12(1):34. Epub 20260106. doi: 10.1038/s41522-025-00900-w. PubMed PMID: 41495053. ⏎

Duman N, Oraloğlu G, Ece D, Caner A. *Staphylococcus aureus* as a signature species of skin microbiome in actinic keratosis and squamous cell carcinoma: a narrative review.

Dermatol Sin. 2025;43(2):100–6. doi: 10.4103/ds.DS-D-24-00248. PubMed PMID: 00701581-202504000-00004. ⏎

Dumont-Leblond N, Veillette M, Racine C, Joubert P, Duchaine C. Non-small cell lung cancer microbiota characterization: prevalence of enteric and potentially pathogenic bacteria in cancer tissues. *PLoS One*. 2021;16(4):e0249832. Epub 20210423. doi: 10.1371/journal.pone.0249832. PubMed PMID: 33891617; PubMed Central PMCID: PMC8064568. ⏎

Edwards P, Kang BW, Chau I. Targeting the stroma in the management of pancreatic cancer. *Front Oncol*. 2021;11:691185. Epub 20210714. doi: 10.3389/fonc.2021.691185. PubMed PMID: 34336679; PubMed Central PMCID: PMC8316993. ⏎

Elkrief A, Mendez-Salazar EO, Maillou J, Vanderbilt CM, Gogia P, Desilets A, *et al.* Antibiotics are associated with worse outcomes in lung cancer patients treated with chemotherapy and immunotherapy. *npj Precis Oncol*. 2024;8(1):143. Epub 20240716. doi: 10.1038/s41698-024-00630-w. PubMed PMID: 39014160; PubMed Central PMCID: PMC11252311. ⏎

Ervin SM, Li H, Lim L, Roberts LR, Liang X, Mani S, *et al.* Gut microbial beta-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J Biol Chem*. 2019;294(49):18586–99. Epub 20191021. doi: 10.1074/jbc.RA119.010950. PubMed PMID: 31636122; PubMed Central PMCID: PMC6901331. ⏎

Fang Y, Liu X, Ren J, Wang X, Zhou F, Huang S, *et al.* Integrated analysis of microbiome and metabolome reveals signatures in PDAC tumorigenesis and prognosis. *Microbiol Spect*. 2024;12(11):e00962–24. doi: doi:10.1128/spectrum.00962-24. ⏎

Fierer N, Leung PM, Lappan R, Eisenhofer R, Ricci F, Holland SI, *et al.* Guidelines for preventing and reporting contamination in low-biomass microbiome studies. *Nat Microbiol*. 2025;10(7):1570–80. Epub 20250620. doi: 10.1038/s41564-025-02035-2. PubMed PMID: 40542287. ⏎

Filomena A, Giovanni S, Ginevra S, Santiago N, Di Fasano Miriam S, Peppino M, *et al.* Identification of a circular RNA isoform of WASHC2A as a prognostic factor for high-risk paediatric B-ALL patients. *Biomed Pharmacother*. 2024;177:116903. Epub 20240624. doi: 10.1016/j.biopha.2024.116903. PubMed PMID: 38917755. ⏎

Fu A, Yao B, Dong T, Cai S. Intracellular microbes empower cancer metastasis. *Life Med*. 2022a;1(2):61–3. Epub 20220628. doi: 10.1093/lifemedi/lnac009. PubMed PMID: 39871936; PubMed Central PMCID: PMC11749340.

Fu A, Yao B, Dong T, Chen Y, Yao J, Liu Y, *et al.* Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. *Cell*. 2022b;185(8):1356–72 e26. Epub 20220407. doi: 10.1016/j.cell.2022.02.027. PubMed PMID: 35395179. ⏎

Fu F, Yu Y, Wang B, Zhao X, Wang N, Yin J, *et al.* Prostate and urinary microbiomes in prostate cancer development: focus on *Cutibacterium acnes*. *Front Cell Infect Microbiol*. 2025a;15:1562729. Epub 20250521. doi: 10.3389/fcimb.2025.1562729. PubMed PMID: 40470262; PubMed Central PMCID: PMC12133773. ⏎

Fu Y, Tao J, Liu T, Liu Y, Qiu J, Su D, *et al.* Unbiasedly decoding the tumor microenvironment with single-cell multiomics analysis in pancreatic cancer. *Mol Cancer*. 2024;23(1):140. doi: 10.1186/s12943-024-02050-7. ⏎

Fu Y, Yi Y, Shao Y, Jiang J, Deng Q. Single-cell and spatial transcriptomic insights into glioma cellular heterogeneity and metabolic adaptations. *Front Immunol*. 2025b;16:1561388. Epub 20250404. doi: 10.3389/fimmu.2025.1561388. PubMed PMID: 40255400; PubMed Central PMCID: PMC12006195.

Gajer P, Brotman RM, Bai G, Sakamoto J, Schutte UM, Zhong X, *et al.* Temporal dynamics of the human vaginal microbiota. *Sci Transl Med*. 2012;4(132):132ra52. doi: 10.1126/scitranslmed.3003605. PubMed PMID: 22553250; PubMed Central PMCID: PMC3722878. ⏎

Galasso L, Termite F, Mignini I, Esposto G, Borriello R, Vitale F, *et al.* Unraveling the role of *Fusobacterium nucleatum* in colorectal cancer: molecular mechanisms and pathogenic insights. *Cancers (Basel)*. 2025;17(3). Epub 20250123. doi: 10.3390/cancers17030368. PubMed PMID: 39941737; PubMed Central PMCID: PMC11816155. ⏎

Gao F, Yu B, Rao B, Sun Y, Yu J, Wang D, *et al.* The effect of the intratumoral microbiome on tumor occurrence, progression, prognosis and treatment. *Front Immunol*. 2022;13:1051987. Epub 20221118. doi: 10.3389/fimmu.2022.1051987. PubMed PMID: 36466871; PubMed Central PMCID: PMC9718533. ⏎

Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, *et al.* Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science*. 2017;357(6356):1156–60. doi: 10.1126/science.aah5043. PubMed PMID: 28912244; PubMed Central PMCID: PMC5727343. ⏎

Gherasim R-D, Chibelean C, Porav-Hodade D, Todea-Moga C, Tătaru S-O, Reman T-L, *et al.* Microbiome shifts in bladder cancer: a narrative review of urobiome composition. Progression, and therapeutic impact. *Medicina*. 2025;61(8):1401. PubMed PMID: doi:10.3390/medicina61081401. ⏎

Gigi E, Gavert N, Raijman-Nagar L, Zwang Y, Zemach H, Livyatan I, *et al.* Characterization of the tumor microbiome of brain metastases and glioblastoma reveals tumor-type-specific and location-specific microbial signatures. *Nat Cancer*. 2025a;6(11):1761–76. Epub 20251114. doi: 10.1038/s43018-025-01073-3. PubMed PMID: 41238775. ⏎

Ginwala R, Bukavina L, Sindhani M, Nachman E, Peri S, Franklin J, *et al.* Bladder cancer microbiome and its association with chemoresponse. *Front Oncol*. 2025;15:1506319. Epub 20250721. doi: 10.3389/fonc.2025.1506319. PubMed PMID: 40761254; PubMed Central PMCID: PMC12318727. ⏎

Ginwala R, Franklin J, White JR, Satyal U, Srivastava A, Abbosh PH. Abstract 2912: changes in mouse bladder cancer microbiome associated with exposure to bladder carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine. *Cancer Res*. 2021;81(13_Suppl):2912. doi: 10.1158/1538-7445.AM2021-2912. ⏎

Gok Yavuz B, Khanmammadova N, Majeed Z, Ali MIH, Hasanov M, Bilen MA, *et al*. Mapping heterogeneity in the tumor microenvironment of renal cell carcinoma through single-cell omics. *Trends Cancer*. 2025. Epub 20251202. doi: 10.1016/j.trecan.2025.11.001. PubMed PMID: 41339185. ⏎

Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, *et al.* Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. *Science*. 2018;359(6371):97–103. doi: doi:10.1126/science.aan4236. ⏎

Gozlan EC, Huda TI, Quach JU, Varkhedi M, Desantis JE, Blanck G. High level immunoglobulin gene expression and reduced intra-tumoral bacteria associated with the transition from initial to progressive diffuse intrinsic pontine glioma. *Anticancer Res*. 2024;44(6):2325–33. doi: 10.21873/anticanres.17039. PubMed PMID: 38821589. ⏎

Greathouse KL, Stone JK, Harris CC. Cancer-type-specific bacteria: freeloaders or partners? *Cancer Cell*. 2020;38(2):158–60. Epub 20200709. doi: 10.1016/j.ccell.2020.06.017. PubMed PMID: 32649886. ⏎

Griesinger AM, Riemondy K, Eswaran N, Donson AM, Willard N, Prince EW, *et al.* Multi-omic approach identifies hypoxic tumor-associated myeloid cells that drive immunobiology of high-risk pediatric ependymoma. *iScience*. 2023;26(9):107585. Epub 20230809. doi: 10.1016/j.isci.2023.107585. PubMed PMID: 37694144; PubMed Central PMCID: PMC10484966. ⏎

Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140(6):883–99. doi: 10.1016/j.cell.2010.01.025. PubMed PMID: 20303878; PubMed Central PMCID: PMC2866629. ⏎

Gu J, Lv X, Li W, Li G, He X, Zhang Y, *et al.* Deciphering the mechanism of Peptostreptococcus anaerobius-induced chemoresistance in colorectal cancer: the important roles of MDSC recruitment and EMT activation. *Front Immunol*. 2023;14:1230681. Epub 20230913. doi: 10.3389/fimmu.2023.1230681. PubMed PMID: 37781363; PubMed Central PMCID: PMC10533913. ⏎

Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, *et al.* Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity*. 2015;42(2):344–55. Epub 20150210. doi: 10.1016/j.immuni.2015.01.010. PubMed PMID: 25680274; PubMed Central PMCID: PMC4361732. ⏎

Hakimjavadi H, George SH, Taub M, Dodds LV, Sanchez-Covarrubias AP, Huang M, *et al.* The vaginal microbiome is associated with endometrial cancer grade and histology. *Cancer Res Commun*. 2022;2(6):447–55. Epub 20220616. doi: 10.1158/2767-9764.CRC-22-0075. PubMed PMID: 35928983; PubMed Central PMCID: PMC9345414. ⏎

Hamada M, Inaba H, Nishiyama K, Yoshida S, Yura Y, Matsumoto-Nakano M, *et al.* Potential role of the intratumoral microbiota in prognosis of head and neck cancer. *Int J Mol Sci*. 2023;24(20). Epub 20231022. doi: 10.3390/ijms242015456. PubMed PMID: 37895136; PubMed Central PMCID: PMC10607002. ⏎

Hamada M, Nishiyama K, Nomura R, Akitomo T, Mitsuhata C, Yura Y, *et al.* Clinical relationships between the intratumoral microbiome and risk factors for head and neck cancer. *Heliyon*. 2024;10(20):e39284. Epub 20241013. doi: 10.1016/j.heliyon.2024.e39284. PubMed PMID: 39497974; PubMed Central PMCID: PMC11533578. ⏎

Hardy L, Jespers V, Van den Bulck M, Buyze J, Mwambarangwe L, Musengamana V, *et al.* The presence of the putative *Gardnerella vaginalis* sialidase A gene in vaginal specimens is associated with bacterial vaginosis biofilm. *PLoS One*. 2017;12(2):e0172522. Epub 20170227. doi: 10.1371/journal.pone.0172522. PubMed PMID: 28241058; PubMed Central PMCID: PMC5328246. ⏎

He Z, Yu J, Gong J, Wu J, Zong X, Luo Z, *et al.* Campylobacter jejuni-derived cytolethal distending toxin promotes colorectal cancer metastasis. *Cell Host Microbe*. 2024;32(12):2080–91 e6. Epub 20241202. doi: 10.1016/j.chom.2024.11.006. PubMed PMID: 39626677. ⏎

Hill JH, Round JL. Intestinal fungal–host interactions in promoting and maintaining health. *Cell Host Microbe*. 2024;32(10):1668–80. doi: 10.1016/j.chom.2024.09.010. ⏎

Horsley H, Malone-Lee J, Holland D, Tuz M, Hibbert A, Kelsey M, *et al. Enterococcus faecalis* subverts and invades the host urothelium in patients with chronic urinary tract infection. *PLoS One*. 2013;8(12):e83637. Epub 20131210. doi: 10.1371/journal.pone.0083637. PubMed PMID: 24363814; PubMed Central PMCID: PMC3868479. ⏎

Hourigan SK, Zhu WSW, Wong W, Clemency NC, Provenzano M, Vilboux T, *et al.* Studying the urine microbiome in superficial bladder cancer: samples obtained by midstream voiding versus cystoscopy. *BMC Urology*. 2020;20(1):5. doi: 10.1186/s12894-020-0576-z. ⏎

Hsu BB, Gibson TE, Yeliseyev V, Liu Q, Lyon L, Bry L, *et al.* Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host Microbe*. 2019;25(6):803–14.e5. Epub 20190604. doi: 10.1016/j.chom.2019.05.001. PubMed PMID: 31175044; PubMed Central PMCID: PMC6579560. ⏎

Hu Y, Li R, Feng J, Sun N, Zheng W, Jin X, *et al. Pseudomonas aeruginosa* induces tumor pyroptosis and immune activation to enhance checkpoint blockade in colorectal cancer. *Cancer Immunol Immunother*. 2026;75(1):32. doi: 10.1007/s00262-025-04266-y. ⏎

Huang Z, Chen H, Wang S, Wei H, Wang X, Shen R, *et al.* NLRP3 overexpression associated with poor prognosis and presented as an effective therapeutic target in osteosarcoma. *Front Pharmacol*. 2021;12:724923. Epub 20210728. doi: 10.3389/fphar.2021.724923. PubMed PMID: 34393801; PubMed Central PMCID: PMC8355743. ⏎

Huycke MM, Abrams V, Moore DR. *Enterococcus faecalis* produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. *Carcinogenesis*. 2002;23(3):529–36. doi: 10.1093/carcin/23.3.529. PubMed PMID: 11895869. ⏎

Iliev ID, Leonardi I. Fungal dysbiosis: immunity and interactions at mucosal barriers. *Nat Rev Immunol*. 2017;17(10):635–46. doi: 10.1038/nri.2017.55. ⏎

Irfan M, Delgado RZR, Frias-Lopez J. The oral microbiome and cancer. *Front Immunol*. 2020;11:591088. Epub 20201023. doi: 10.3389/fimmu.2020.591088. PubMed PMID: 33193429; PubMed Central PMCID: PMC7645040. ⏎

Islam R, Heyer J, Figura M, Wang X, Nie X, Nathaniel B, *et al.* T cells in testicular germ cell tumors: new evidence of fundamental contributions by rare subsets. *Br J Cancer*. 2024;130(12):1893–903. Epub 20240422. doi: 10.1038/s41416-024-02669-9. PubMed PMID: 38649788; PubMed Central PMCID: PMC11183042. ⏎

Jani S, Church CD, Nghiem P. Insights into anti-tumor immunity via the polyomavirus shared across human Merkel cell carcinomas. *Front Immunol*. 2023;14:1172913.

Epub 20230523. doi: 10.3389/fimmu.2023.1172913. PubMed PMID: 37287968; PubMed Central PMCID: PMC10242112. ⏎

Jawahar J, Byrd AL. Building a blueprint for intra-tumoral microbial studies. *Nat Med*. 2025;31(11):3612–3. doi: 10.1038/s41591-025-03980-5. PubMed PMID: 41238914. ⏎

Jiang L, Li M, Zhao Z. Intra-tumoral microbial heterogeneity of breast cancer: roles in tumorigenesis, therapeutic responses, and future directions. *J Appl Microbiol*. 2025a;136(9). doi: 10.1093/jambio/lxaf230. PubMed PMID: 41001760.

Jiang S, Redelman-Sidi G. BCG in bladder cancer immunotherapy. *Cancers*. 2022;14(13):3073. PubMed PMID: doi:10.3390/cancers14133073. ⏎

Jiang SS, Chen YX, Fang JY. *Fusobacterium nucleatum*: ecology, pathogenesis and clinical implications. *Nat Rev Microbiol*. 2025b. Epub 20250922. doi: 10.1038/s41579-025-01237-z. PubMed PMID: 40983729. ⏎

Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E, *et al.* Identification of bacteria-derived HLA-bound peptides in melanoma. *Nature*. 2021;592(7852):138–43. doi: 10.1038/s41586-021-03368-8. ⏎

Kang DH, Kim Y, Lee JH, Kang HS, Chung C. Spatial transcriptomics in lung cancer and pulmonary diseases: a comprehensive review. *Cancers (Basel)*. 025;17(12):1912. Epub 20250609. doi: 10.3390/cancers17121912. PubMed PMID: 40563563; PubMed Central PMCID: PMC12191356. ⏎

Karta J, Meyers M, Rodriguez F, Koncina E, Gilson C, Klein E, *et al. Fusobacterium nucleatum* interacts with cancer-associated fibroblasts to promote colorectal cancer. *EMBO J*. 2025;44(19):5375–93. Epub 20250822. doi: 10.1038/s44318-025-00542-w. PubMed PMID: 40846900; PubMed Central PMCID: PMC12488894. ⏎

Kashyap P, Dutt N, Ahirwar DK, Yadav P. lung microbiome in lung cancer: a new horizon in cancer study. *Cancer Prev Res (Phila)*. 2024;17(9):401–14. doi: 10.1158/1940-6207.CAPR-24-0147. PubMed PMID: 38787628. ⏎

Keith JS, Khalil MI, Kamel MH, Davis R, Eltahawy E. Effects and complications of intravesical instillation of Bacillus Calmette–Guerin therapy. *Cur Bladder Dysfunct Rep*. 2019;14(2):58–66. doi: 10.1007/s11884-019-00506-x. ⏎

Khaliq AM, Rajamohan M, Saeed O, Mansouri K, Adil A, Zhang C, *et al.* Spatial transcriptomic analysis of primary and metastatic pancreatic cancers highlights tumor microenvironmental heterogeneity. *Nat Genet*. 2024;56(11):2455–65. doi: 10.1038/s41588-024-01914-4. ⏎

Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer*. 2015;15(1):25–41. doi: 10.1038/nrc3817. PubMed PMID: 25533674. ⏎

Kodikara S, Mao J, San Valentin EMD, Do KA, Reyes-Gibby CC, Le Cao KA. Oral microbial signatures of head and neck cancer patients with diverse longitudinal oral mucositis severity patterns. *bioRxiv*. 2025. Epub 20250718. doi: 10.1101/2025.07.15.665024. PubMed PMID: 40791415; PubMed Central PMCID: PMC12338723. ⏎

Komiyama S, Yamada T, Takemura N, Kokudo N, Hase K, Kawamura YI. Profiling of tumour-associated microbiota in human hepatocellular carcinoma. *Sci Rep*. 2021;11(1):10589. Epub 20210519. doi: 10.1038/s41598-021-89963-1. PubMed PMID: 34012007; PubMed Central PMCID: PMC8134445. ⏎

Kostic Aleksandar D, Chun E, Robertson L, Glickman Jonathan N, Gallini Carey A, Michaud M, *et al. Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. 2013;14(2):207–15. doi: 10.1016/j.chom.2013.07.007. ⏎

Kovaleva O, Podlesnaya P, Gratchev A. Resident microbiome of kidney tumors. *Oncol Rev*. 2024;18:1393664. Epub 20240521. doi: 10.3389/or.2024.1393664. PubMed PMID: 38835643; PubMed Central PMCID: PMC11148557. ⏎

Krueger A, Zaugg J, Chisholm S, Linedale R, Lachner N, Teoh SM, *et al.* Secreted toxins from *Staphylococcus aureus* strains isolated from keratinocyte skin cancers mediate pro-tumorigenic inflammatory responses in the skin. *Front Microbiol*. 2021;12:789042. Epub 20220125. doi: 10.3389/fmicb.2021.789042. PubMed PMID: 35145494; PubMed Central PMCID: PMC8822148. ⏎

Krueger A, Zaugg J, Lachner N, Bialasiewicz S, Lin LL, Gabizon S, *et al.* Changes in the skin microbiome associated with squamous cell carcinoma in transplant recipients. *ISME Commun*. 2022;2(1):13. Epub 20220201. doi: 10.1038/s43705-022-00095-7. PubMed PMID: 37938715; PubMed Central PMCID: PMC9723734. ⏎

Kushwaha M, Nukala V, Singh AK, Makharia GK, Mohan A, Kumar A, *et al.* Emerging implications of bacterial biofilm in cancer biology: recent updates and major perspectives. *Gut Microbes Rep*. 2024;1(1):2339270. doi: 10.1080/29933935.2024.2339270. ⏎

L'Orphelin JM, Dompmartin A, Dréno B. The skin microbiome: a new key player in melanoma, from onset to metastatic stage. *Pigment Cell Melanoma Res*. 2025;38(2):e13224. doi: 10.1111/pcmr.13224. PubMed PMID: 40016867; PubMed Central PMCID: PMC11868406. ⏎

Lambert I, Pervent M, Le Quere A, Clement G, Tauzin M, Severac D, *et al.* Responses of mature symbiotic nodules to the whole-plant systemic nitrogen signaling. *J Exp Bot*. 2020;71(16):5039–52. doi: 10.1093/jxb/eraa221. PubMed PMID: 32386062; PubMed Central PMCID: PMC7410188. ⏎

Lammers J, Calkoen F, Kranendonk M, Federico A, Kool M, Kester L, *et al.* P02.07 Characterization of the tumor immune microenvironment of pediatric posterior fossa A ependymomas. *J Immunother Cancer*. 2021;9(Suppl 1). doi: 10.1136/jitc-2021-ITOC8.19. ⏎

Lawlor MS, O'Connor C, Miller VL. Yersiniabactin is a virulence factor for *Klebsiella pneumoniae* during pulmonary infection. *Infect Immun*. 2007;75(3):1463–72. Epub 20070112. doi: 10.1128/IAI.00372-06. PubMed PMID: 17220312; PubMed Central PMCID: PMC1828572. ⏎

Leake I. Exploring cancer mycobiomes. *Nat Cancer*. 2022;3(12):1442. doi: 10.1038/s43018-022-00465-z. PubMed PMID: 36539485. ⏎

Leclercq SY, Sullivan MJ, Ipe DS, Smith JP, Cripps AW, Ulett GC. Pathogenesis of *Streptococcus* urinary tract infection depends on bacterial strain and beta-hemolysin/cytolysin that mediates cytotoxicity, cytokine synthesis, inflammation and virulence. *Sci Rep*. 2016;6:29000. Epub 20160707. doi: 10.1038/srep29000. PubMed PMID: 27383371; PubMed Central PMCID: PMC4935997. ⏎

Lee S, Meslier V, Bidkhori G, Garcia-Guevara F, Etienne-Mesmin L, Clasen F, *et al.* Transient colonizing microbes promote gut dysbiosis and functional impairment. *npj Biofilms Microbiomes*. 2024;10(1):80. Epub 20240908. doi: 10.1038/s41522-024-00561-1. PubMed PMID: 39245657; PubMed Central PMCID: PMC11381545. ⏎

Leonov G, Starodubova A, Makhnach O, Goldshtein D, Salikhova D. Intratumoral microbiome: impact on cancer progression and cellular immunotherapy. *Cancers (Basel)*. 2025;18(1). Epub 20251229. doi: 10.3390/cancers18010100. PubMed PMID: 41514613; PubMed Central PMCID: PMC12784895. ⏎

Li J, Zhai X, Chen C, Zhang R, Huang X, Liu Y. The intrahepatic bacterial metataxonomic signature of patients with hepatocellular carcinoma. *Sci Rep*. 2024;14(1):29077. Epub 20241123. doi: 10.1038/s41598-024-80246-z. PubMed PMID: 39580523; PubMed Central PMCID: PMC11585554. ⏎

Li Q, Luo W, Xiao L, Xu X, Peng X, Cheng L, *et al.* Microbial manipulators: *Fusobacterium nucleatum* modulates the tumor immune microenvironment in colorectal cancer. *J Oral Microbiol*. 2025;17(1):2544169. Epub 20250805. doi: 10.1080/20002297.2025.2544169. PubMed PMID: 40771747; PubMed Central PMCID: PMC12326384. ⏎

Li X, Saxena D. The tumor mycobiome: a paradigm shift in cancer pathogenesis. *Cell*. 2022;185(20):3648–51. doi: 10.1016/j.cell.2022.09.013. PubMed PMID: 36179665; PubMed Central PMCID: PMC10189653. ⏎

Liang R, Chen X, Chen L, Wan F, Chen K, Sun Y, *et al.* STAT3 signaling in ovarian cancer: a potential therapeutic target. *J Cancer*. 2020;11(4):837–48. Epub 20200101.

doi: 10.7150/jca.35011. PubMed PMID: 31949487; PubMed Central PMCID: PMC6959025. ⏎

Licht P, Dominelli N, Kleemann J, Pastore S, Muller ES, Haist M, *et al.* The skin microbiome stratifies patients with cutaneous T cell lymphoma and determines event-free survival. *npj Biofilms Microbiomes*. 2024;10(1):74. Epub 20240829. doi: 10.1038/s41522-024-00542-4. PubMed PMID: 39198450; PubMed Central PMCID: PMC11358159. ⏎

Littman Dan R, Pamer Eric G. Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe*. 2011;10(4):311–23. doi: 10.1016/j.chom.2011.10.004. ⏎

Liu D, Qiu X, Xiong X, Chen X, Pan F. Current updates on the role of reactive oxygen species in bladder cancer pathogenesis and therapeutics. *Clin Transl Oncol*. 2020;22(10):1687–97. doi: 10.1007/s12094-020-02330-w. ⏎

Liu H, Luo Z, Luo F, Wang X, Wei H, Yu C-Y. Intratumoral microbiota: a new perspective in cancer initiation, development, and therapy. *RSC Pharmaceutics*. 2025a;2(5):865–81. doi: 10.1039/D5PM00045A.

Liu L, Lin J, He H. Identification of potential crucial genes associated with the pathogenesis and prognosis of endometrial cancer. *Front Genet*. 2019;10:373. Epub 20190426. doi: 10.3389/fgene.2019.00373. PubMed PMID: 31105744; PubMed Central PMCID: PMC6499025. ⏎

Liu M, Wang Y, Zhang Y, Hu D, Tang L, Zhou B, *et al.* Landscape of small nucleic acid therapeutics: moving from the bench to the clinic as next-generation medicines. *Signal Transduct Target Ther*. 2025b;10(1):73. doi: 10.1038/s41392-024-02112-8. ⏎

Liu NN, Ma Q, Ge Y, Yi CX, Wei LQ, Tan JC, *et al.* Microbiome dysbiosis in lung cancer: from composition to therapy. *npj Precis Oncol*. 2020;4(1):33. Epub 20201210. doi: 10.1038/s41698-020-00138-z. PubMed PMID: 33303906; PubMed Central PMCID: PMC7730185. ⏎

Liu S, Xiong X, Hao T, Shi X, Zhao Y. The intratumor microbiota and thyroid cancer: a review. *Front Endocrinol (Lausanne)*. 2025c;16:1536155. Epub 20250630. doi: 10.3389/fendo.2025.1536155. PubMed PMID: 40661745; PubMed Central PMCID: PMC12256260. ⏎

Liu W, Li Y, Wu P, Guo X, Xu Y, Jin L, *et al.* The intratumoral microbiota: a new horizon in cancer immunology. *Front Cell Infect Microbiol*. 2024;14:1409464. Epub 20240729. doi: 10.3389/fcimb.2024.1409464. PubMed PMID: 39135638; PubMed Central PMCID: PMC11317474. ⏎

Longmore GD. Bacteria in tumors “hit the road” together. *Cell*. 2022;185(8):1292–1294. doi: 10.1016/j.cell.2022.03.013. PubMed PMID: 35427497; PubMed Central

PMCID: PMC9289786. ⏎

Lou K, Chi J, Wu J, Ma J, Liu S, Cui Y. Research progress on the microbiota in bladder cancer tumors. *Front Cell Infect Microbiol*. 2024;14:1374944. Epub 20240408. doi: 10.3389/fcimb.2024.1374944. PubMed PMID: 38650736; PubMed Central PMCID: PMC11033431. ⏎

Lu, Wu J, Yu X, Qian J, Song Z. Unveiling the interplay between microbiota and PD1/PD-L1 axis in tumor immunity and immunotherapy. *Front Immunol*. 2025a;16:1690374. Epub 20251127. doi: 10.3389/fimmu.2025.1690374. PubMed PMID: 41394818; PubMed Central PMCID: PMC12695803. ⏎

Lu J, Wei W, Zheng D. *Fusobacterium nucleatum* in colorectal cancer: ally mechanism and targeted therapy strategies. *Research (Wash DC)*. 2025b;8:0640. Epub 20250409. doi: 10.34133/research.0640. PubMed PMID: 40207017; PubMed Central PMCID: PMC11979337.

Lu W, He F, Lin Z, Liu S, Tang L, Huang Y, *et al.* Dysbiosis of the endometrial microbiota and its association with inflammatory cytokines in endometrial cancer. *Int J Cancer*. 2021;148(7):1708–16. Epub 20201231. doi: 10.1002/ijc.33428. PubMed PMID: 33285000. ⏎

Lu Y, Xu L, Chen W, Liu W, Zhang Y, Zhou Q, *et al.* Intrahepatic microbial heterogeneity in multifocal hepatocellular carcinoma and its association with host genomic and transcriptomic alterations. *Cancer Discov*. 2025c;15(8):1630–48. doi: 10.1158/2159-8290.CD-24-1259. PubMed PMID: 40287964; PubMed Central PMCID: PMC12319405. ⏎

Lu YQ, Qiao H, Tan XR, Liu N. Broadening oncological boundaries: the intratumoral microbiota. *Trends Microbiol*. 2024;32(8):807–22. Epub 20240203. doi: 10.1016/j.tim.2024.01.007. PubMed PMID: 38310023. ⏎

Luo C, Min X, Zhang D. New insights into the mechanisms of the immune microenvironment and immunotherapy in osteosarcoma. *Front Immunol*. 2024;15:1539696. Epub 20250117. doi: 10.3389/fimmu.2024.1539696. PubMed PMID: 39896817; PubMed Central PMCID: PMC11782189. ⏎

Luo YC, Huang XT, Wang R, Lin YJ, Sun JX, Li KF, *et al.* Advancements in understanding tumor-resident bacteria and their application in cancer therapy. *Mil Med Res*. 2025;12(1):38. Epub 20250725. doi: 10.1186/s40779-025-00623-1. PubMed PMID: 40713785; PubMed Central PMCID: PMC12291306. ⏎

MacGibeny MA, Adjei S, Pyle H, Bunick CG, Ghannoum M, Grada A, *et al.* The human skin microbiome in health. *J Am Acad Dermatol*. 2025;93(2):329–36. Epub 20240819. doi: 10.1016/j.jaad.2024.07.1498. PubMed PMID: 39168311; PubMed Central PMCID: PMC11912297. ⏎

Makarewicz J, Kazmierczak-Siedlecka K, Sobocki BK, Dobrucki IT, Kalinowski L, Stachowska E. Anti-cancer management of head and neck cancers and oral microbiome—what can we clinically obtain? *Front Cell Infect Microbiol*. 2024;14:1329057. Epub 20240228. doi: 10.3389/fcimb.2024.1329057. PubMed PMID: 38481661; PubMed Central PMCID: PMC10933093. ⏎

Mansour O, Morciano L, Zion K, Elgrabli R, Zenvirth D, Simchen G, *et al.* Timing of appearance of new mutations during yeast meiosis and their association with recombination. *Curr Genet*. 2020;66(3):577–92. Epub 20200113. doi: 10.1007/s00294-019-01051-0. PubMed PMID: 31932974. ⏎

Marci R, Gentili V, Bortolotti D, Lo Monte G, Caselli E, Bolzani S, *et al.* Presence of HHV-6A in endometrial epithelial cells from women with primary unexplained infertility. *PLoS One*. 2016;11(7):e0158304. Epub 20160701. doi: 10.1371/journal.pone.0158304. PubMed PMID: 27367597; PubMed Central PMCID: PMC4930213. ⏎

Mekadim C, Skalnikova HK, Cizkova J, Cizkova V, Palanova A, Horak V, *et al.* Dysbiosis of skin microbiome and gut microbiome in melanoma progression. *BMC Microbiol*. 2022;22(1):63. Epub 20220225. doi: 10.1186/s12866-022-02458-5. PubMed PMID: 35216552; PubMed Central PMCID: PMC8881828. ⏎

Meng Y, Deng J, Deng W, Sun Z. Intra-tumoral bacteria in head and neck cancer: holistic integrative insight. *Cancer Biol Med*. 2025;22(2):113–36. doi: 10.20892/j.issn.2095-3941.2024.0311. PubMed PMID: 39969204; PubMed Central PMCID: PMC11899592. ⏎

Messiaen J, Jacobs SA, De Smet F. The tumor micro-environment in pediatric glioma: friend or foe? *Front Immunol*. 2023;14:1227126. Epub 20231013. doi: 10.3389/fimmu.2023.1227126. PubMed PMID: 37901250; PubMed Central PMCID: PMC10611473. ⏎

Michaud M, Chien F, Bakhtia M, Snuderl M, Schroff C, Schneiderjan M, *et al.* TMIC-04. Spatial sequencing of medulloblastoma reveals biological niches correlated with high-risk features and relapse. *Neuro-Oncol*. 2024;26(Suppl_8):viii297–8. doi: 10.1093/neuonc/noae165.1182. ⏎

Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, *et al. Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut*. 2016;65(12):1973–80. Epub 20150826. doi: 10.1136/gutjnl-2015-310101. PubMed PMID: 26311717; PubMed Central PMCID: PMC4769120. ⏎

Mitra A, MacIntyre DA, Lee YS, Smith A, Marchesi JR, Lehne B, *et al.* Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Sci Rep*. 2015;5:16865. Epub 20151117. doi:

10.1038/srep16865. PubMed PMID: 26574055; PubMed Central PMCID: PMC4648063. ⏎

Miyake M, Matsuyama H, Teramukai S, Kinoshita F, Yokota I, Matsumoto H, *et al.* A new risk stratification model for intravesical recurrence, disease progression, and cancer-specific death in patients with non-muscle invasive bladder cancer: the J-NICE risk tables. *Int J Clin Oncol*. 2020;25(7):1364–76. doi: 10.1007/s10147-020-01654-5. ⏎

Molina MA, Coenen BA, Leenders WPJ, Andralojc KM, Huynen MA, Melchers WJG. Assessing the cervicovaginal microbiota in the context of hrHPV infections: temporal dynamics and therapeutic strategies. *mBio*. 2022;13(5):e01619-22. doi: doi:10.1128/mbio.01619-22. ⏎

Montanari E, Bernardo G, Le Noci V, Anselmi M, Pupa SM, Tagliabue E, *et al.* Biofilm formation by the host microbiota: a protective shield against immunity and its implication in cancer. *Mol Cancer*. 2025;24(1):148. Epub 20250521. doi: 10.1186/s12943-025-02348-0. PubMed PMID: 40399923; PubMed Central PMCID: PMC12093748. ⏎

Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer*. 2010;10(12):878–89. Epub 20101124. doi: 10.1038/nrc2961. PubMed PMID: 21102637; PubMed Central PMCID: PMC3718018. ⏎

Morad G, Damania AV, Melendez B, Singh BB, Veguilla FJ, Soto RA, *et al.* Microbial signals in primary and metastatic brain tumors. *Nat Med*. 2025;31(11):3675–88. Epub 20251114. doi: 10.1038/s41591-025-03957-4. PubMed PMID: 41238915; PubMed Central PMCID: PMC12618227. ⏎

Morup N, Main AM, Jorgensen N, Daugaard G, Juul A, Almstrup K. The seminal plasma microbiome of men with testicular germ cell tumours described by small RNA sequencing. *Andrology*. 2023;11(4):756–69. Epub 20221020. doi: 10.1111/andr.13305. PubMed PMID: 36168917. ⏎

Mou W, Deng Z, Zhu L, Jiang A, Lin A, Xu L, *et al.* Intratumoral mycobiome heterogeneity influences the tumor microenvironment and immunotherapy outcomes in renal cell carcinoma. *Sci Adv*. 2025;11(15):eadu1727. Epub 20250409. doi: 10.1126/sciadv.adu1727. PubMed PMID: 40203108; PubMed Central PMCID: PMC11980860. ⏎

Muzny CA, Schwebke JR. Pathogenesis of bacterial vaginosis: discussion of current hypotheses. *J Infect Dis*. 2016;214(Suppl 1):S1–5. doi: 10.1093/infdis/jiw121. PubMed PMID: 27449868; PubMed Central PMCID: PMC4957507. ⏎

Mysorekar IU, Hultgren SJ. Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract. *Proc Natl Acad Sci USA*. 2006;103(38):14170–5. Epub 20060912. doi: 10.1073/pnas.0602136103. PubMed PMID: 16968784; PubMed Central PMCID: PMC1564066. ⏎

Najafi S, Abedini F, Azimzadeh Jamalkandi S, Shariati P, Ahmadi A, Gholami Fesharaki M. The composition of lung microbiome in lung cancer: a systematic review and meta-analysis. *BMC Microbiol*. 2021;21(1):315. Epub 20211111. doi: 10.1186/s12866-021-02375-z. PubMed PMID: 34763672; PubMed Central PMCID: PMC8582175. ⏎

Nakatsuji T, Chen TH, Butcher AM, Trzoss LL, Nam S-J, Shirakawa KT, *et al.* A commensal strain of *Staphylococcus epidermidis* protects against skin neoplasia. *Sci Adv*. 2018;4(2):eaao4502. doi: doi:10.1126/sciadv.aao4502. ⏎

Narunsky-Haziza L, Sepich-Poore GD, Livyatan I, Asraf O, Martino C, Nejman D, *et al.* Pan-cancer analyses reveal cancer-type-specific fungal ecologies and bacteriome interactions. *Cell*. 2022;185(20):3789–806 e17. doi: 10.1016/j.cell.2022.09.005. PubMed PMID: 36179670; PubMed Central PMCID: PMC9567272. ⏎

Nazir A, Hussain FHN, Raza A. Advancing microbiota therapeutics: the role of synthetic biology in engineering microbial communities for precision medicine. *Front Bioeng Biotechnol* . 2024;12. ⏎

Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, *et al.* The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science*. 2020;368(6494):973–80. doi: 10.1126/science.aay9189. PubMed PMID: 32467386; PubMed Central PMCID: PMC7757858. ⏎

Noorbakhsh N, Hayatmoghadam B, Jamali M, Golmohammadi M, Kavianpour M. The Hippo signaling pathway in leukemia: function, interaction, and carcinogenesis. *Cancer Cell Int*. 2021;21(1):705. Epub 20211225. doi: 10.1186/s12935-021-02408-7. PubMed PMID: 34953494; PubMed Central PMCID: PMC8710012. ⏎

Okobi TJ, Uhomoibhi TO, Akahara DE, Odoma VA, Sanusi IA, Okobi OE, *et al.* Immune checkpoint inhibitors as a treatment option for bladder cancer: current evidence. *Cureus*. 2023;15(6):e40031. Epub 20230606. doi: 10.7759/cureus.40031. PubMed PMID: 37425564; PubMed Central PMCID: PMC10323982. ⏎

Okuno K, Tokunaga M, Von Hoff D, Kinugasa Y, Goel A, Group PBW. Intratumoral *Malassezia globosa* levels predict survival and therapeutic response to adjuvant chemotherapy in patients with pancreatic ductal adenocarcinoma. *Gastroenterology*. 2023;165(2):502–4.e2. Epub 20230428. doi: 10.1053/j.gastro.2023.04.017. PubMed PMID: 37119937; PubMed Central PMCID: PMC10405668. ⏎

Palermo M, Massimi L, Tamburrini G, Olivi A, Doglietto F, Albanese A, *et al.* Algorithm-based assessment of T-cell dysfunction and exclusion to forecast ICB sensitivity in pediatric brain ependymoma. *J Neurooncol*. 2025;176(2):128. Epub 20251219. doi: 10.1007/s11060-025-05384-4. PubMed PMID: 41417285; PubMed Central PMCID: PMC12717181. ⏎

Papavassiliou KA, Gargalionis AN, Papavassiliou AG. Direct YAP/TAZ-TEAD inhibitor paves the way toward realizing cancer mechanomedicine. *Pharmacol Res*. 2024;206:107287. Epub 20240627. doi: 10.1016/j.phrs.2024.107287. PubMed PMID: 38944219. ⏎

Peng G, Lin Y, Zou Q, Peng H, Lei A, Zou X, *et al. Malassezia restricta* as an unexpected cause of infectious osteomyelitis diagnosed by metagenomic sequencing: a case report and literature review. *BMC Infect Dis*. 2024;24(1):643. doi: 10.1186/s12879-024-09512-9. ⏎

Plachta I, Kleibert M, Czarnecka AM, Spalek M, Szumera-Cieckiewicz A, Rutkowski P. Current diagnosis and treatment options for cutaneous adnexal neoplasms with apocrine and eccrine differentiation. *Int J Mol Sci*. 2021;22(10). Epub 20210511. doi: 10.3390/ijms22105077. PubMed PMID: 34064849; PubMed Central PMCID: PMC8151110. ⏎

Podlipnik S, Martin BJ, Morgan-Linnell SK, Bailey CN, Siegel JJ, Petkov VI, *et al.* The 31-gene expression profile test outperforms AJCC in Stratifying risk of recurrence in patients with stage I cutaneous melanoma. *Cancers (Basel)*. 2024;16(2). Epub 20240109. doi: 10.3390/cancers16020287. PubMed PMID: 38254778; PubMed Central PMCID: PMC10814308. ⏎

Poore GD, Kopylova E, Zhu Q, Carpenter C, Fraraccio S, Wandro S, *et al.* RETRACTED ARTICLE: Microbiome analyses of blood and tissues suggest cancerdiagnostic approach. *Nature*. 2020;579(7800):567–74. doi: 10.1038/s41586-020-2095-1. ⏎

Porto JG, Arbelaez MCS, Pena B, Khandekar A, Malpani A, Nahar B, *et al.* The Influence of the microbiome on urological malignancies: a systematic review. *Cancers (Basel)*. 2023;15(20). Epub 20231014. doi: 10.3390/cancers15204984. PubMed PMID: 37894351; PubMed Central PMCID: PMC10605095. ⏎

Purcell RV, Pearson J, Aitchison A, Dixon L, Frizelle FA, Keenan JI. Colonization with enterotoxigenic *Bacteroides fragilis* is associated with early-stage colorectal neoplasia. *PLoS One*. 2017;12(2):e0171602. Epub 20170202. doi: 10.1371/journal.pone.0171602. PubMed PMID: 28151975; PubMed Central PMCID: PMC5289627. ⏎

Qin M, Pirouz A, Kim M-H, Krutzik SR, Garbán HJ, Kim J. *Propionibacterium acnes* Induces IL-1β Secretion via the NLRP3 inflammasome in human monocytes. *Journal of Investigative Dermatology*. 2014;134(2):381–8. doi: doi.org/10.1038/jid.2013.309. ⏎

Qiu K, Xie Q, Zeng D, Huang Y, Lin T. Intratumor microbiota *Delftia lacustiris* correlated with METTL3 to control development of papillary thyroid carcinoma. *Cancer Cell Int*. 2026. Epub 20260129. doi: 10.1186/s12935-026-04183-9. PubMed PMID: 41612397. ⏎

Rad SK, Yeo KKL, Wu F, Li R, Nourmohammadi S, Tomita Y, *et al.* A systematic review and meta-analysis of 16S rRNA and cancer microbiome atlas datasets to characterize microbiota signatures in normal breast, mastitis, and breast cancer. *Microorganisms*. 2025;13(2):467. Epub 20250219. doi: 10.3390/microorganisms13020467. PubMed PMID: 40005832; PubMed Central PMCID: PMC11858161. ⏎

Rademacher A, Huseynov A, Bortolomeazzi M, Wille SJ, Schumacher S, Sant P, *et al.* Comparison of spatial transcriptomics technologies using tumor cryosections. *Genome Biol*. 2025;26(1):176. Epub 20250620. doi: 10.1186/s13059-025-03624-4. PubMed PMID: 40542418; PubMed Central PMCID: PMC12180266. ⏎

Ravel J, Brotman RM. Translating the vaginal microbiome: gaps and challenges. *Genome Med*. 2016;8(1):35. Epub 20160401. doi: 10.1186/s13073-016-0291-2. PubMed PMID: 27036316; PubMed Central PMCID: PMC4818402. ⏎

Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res*. 2006;47(2):241–59. Epub 20051118. doi: 10.1194/jlr.R500013-JLR200. PubMed PMID: 16299351. ⏎

Riquelme E, Zhang Y, Zhang L, Montiel M, Zoltan M, Dong W, *et al.* Tumor Microbiome diversity and composition influence pancreatic cancer outcomes. *Cell*. 2019;178(4):795–806.e12. doi: 10.1016/j.cell.2019.07.008. PubMed PMID: 31398337; PubMed Central PMCID: PMC7288240. ⏎

Roy V, Jung W, Linde C, Coates E, Ledgerwood J, Costner P, *et al.* Differences in HPV-specific antibody Fc-effector functions following Gardasil(R) and Cervarix(R) vaccination. *npj Vaccines*. 2023;8(1):39. Epub 20230315. doi: 10.1038/s41541-023-00628-8. PubMed PMID: 36922512; PubMed Central PMCID: PMC10017795. ⏎

Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe*. 2013;14(2):195–206. doi: 10.1016/j.chom.2013.07.012. PubMed PMID: 23954158; PubMed Central PMCID: PMC3770529. ⏎

Ruiz-Moreno C, Salas SM, Samuelsson E, Minaeva M, Ibarra I, Grillo M, *et al.* Charting the single-cell and spatial landscape of IDH-wild-type glioblastoma with GBmap. *Neuro-Oncol*. 2025;27(9):2281–95. doi: 10.1093/neuonc/noaf113. PubMed PMID: 40312969; PubMed Central PMCID: PMC12526130. ⏎

Ryu H, Bi TM, Pulliam TH, Sarkar K, Church CD, Kumar N, *et al.* Merkel cell polyomavirus-specific and CD39(+)CLA(+) CD8 T cells as blood-based predictive biomarkers for PD-1 blockade in Merkel cell carcinoma. *Cell Rep Med*. 2024;5(2):101390. Epub 20240209. doi: 10.1016/j.xcrm.2023.101390. PubMed PMID: 38340724; PubMed Central PMCID: PMC10897544. ⏎

Sabeel Z, Yang Z. Microbiome-targeted nanoplatforms and engineering approaches in breast cancer therapy. *Mol Cancer*. 2025;24(1):276. Epub 20251030. doi: 10.1186/s12943-025-02456-x. PubMed PMID: 41168838; PubMed Central PMCID: PMC12574306. ⏎

Sahin TK, Sonmezer MC. The role of the microbiome in head and neck squamous cell cancers. *Eur Arch Otorhinolaryngol*. 2025;282(2):623–37. Epub 20240921. doi: 10.1007/s00405-024-08966-6. PubMed PMID: 39306588. ⏎

Sandoval-Clavijo A, Marti-Marti I, Ferrandiz-Pulido C, Verdaguer-Faja J, Jaka A, Toll A. Human papillomavirus-related cutaneous squamous cell carcinoma. *Cancers (Basel)*. 2025;17(5). Epub 20250305. doi: 10.3390/cancers17050897. PubMed PMID: 40075744; PubMed Central PMCID: PMC11898954. ⏎

Schorr L, Mathies M, Elinav E, Puschhof J. Intracellular bacteria in cancer-prospects and debates. *npj Biofilms Microbiomes*. 2023;9(1):76. Epub 20231009. doi: 10.1038/s41522-023-00446-9. PubMed PMID: 37813921; PubMed Central PMCID: PMC10562400. ⏎

Schratz KE, Flasch DA, Atik CC, Cosner ZL, Blackford AL, Yang W, *et al.* T cell immune deficiency rather than chromosome instability predisposes patients with short telomere syndromes to squamous cancers. *Cancer Cell*. 2023;41(4):807–17 e6. Epub 20230402. doi: 10.1016/j.ccell.2023.03.005. PubMed PMID: 37037617; PubMed Central PMCID: PMC10188244. ⏎

Schulz C, Vilchez-Vargas R, Öcal E, Koch N, Puhr-Westerheide D, Burnell LF, *et al.* Profiling of the tumor-associated microbiome in patients with hepatocellular carcinoma. *Gut Pathog*. 2025;17(1):53. doi: 10.1186/s13099-025-00727-y. ⏎

Semertzidou A, Whelan E, Smith A, Ng S, Roberts L, Brosens JJ, *et al.* Microbial signatures and continuum in endometrial cancer and benign patients. *Microbiome*. 2024;12(1):118. Epub 20240701. doi: 10.1186/s40168-024-01821-0. PubMed PMID: 38951935; PubMed Central PMCID: PMC11218081. ⏎

Sepich-Poore GD, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R. The microbiome and human cancer. *Science*. 2021;371(6536). doi: 10.1126/science.abc4552. PubMed PMID: 33766858; PubMed Central PMCID: PMC8767999. ⏎

Sgamato C, Rocco A, Compare D, Priadko K, Romano M, Nardone G. Exploring the link between *Helicobacter pylori*, gastric microbiota and gastric cancer. *Antibiotics (Basel)*. 2024;13(6). Epub 20240524. doi: 10.3390/antibiotics13060484. PubMed PMID: 38927151; PubMed Central PMCID: PMC11201017. ⏎

Shen J, Sun H, Chu J, Gong X, Liu X. Cervicovaginal microbiota: a promising direction for prevention and treatment in cervical cancer. *Infect Agent Cancer*. 2024;19(1):13. Epub 20240419. doi: 10.1186/s13027-024-00573-8. PubMed PMID: 38641803; PubMed Central PMCID: PMC11027553. ⏎

Sheng D, Yue K, Li H, Zhao L, Zhao G, Jin C, *et al.* The interaction between intratumoral microbiome and immunity is related to the prognosis of ovarian cancer. *Microbiol Spectr*. 2023;11(2):e0354922. Epub 20230328. doi: 10.1128/spectrum.03549-22. PubMed PMID: 36975828; PubMed Central PMCID: PMC10100779. ⏎

Sheraz M, Shi H, Banerjee S. Human microbiome and bacteriophages: impacts on health and disease. *Curr Clin Microbiol Rep*. 2025;12(1):7. doi: 10.1007/s40588-025-00243-2. ⏎

Shi S, Chu Y, Liu H, Yu L, Sun D, Yang J, *et al.* Predictable regulation of survival by intratumoral microbe-immune crosstalk in patients with lung adenocarcinoma. *Microb Cell*. 2024;11:29–40. Epub 20240219. doi: 10.15698/mic2024.02.813. PubMed PMID: 38375207; PubMed Central PMCID: PMC10876218. ⏎

Silver N, Dai J, Kerr TD, Alban T, Gross N, Sikora AG, *et al.* 1144 Total intratumoral bacteria abundance drives immunosuppression and immunotherapy resistance. *J Immunother Cancer*. 2025;13(Suppl 2). doi: 10.1136/jitc-2025-SITC2025.1144. ⏎

Sipos L, Banczerowski P, Juhasz J, Fedorcsak I, Berenyi G, Makra N, *et al.* Brain tumors and beyond: Multi-compartment microbiome and mycobiome analysis. *Int J Mol Sci*. 2025;26(3):991. Epub 20250124. doi: 10.3390/ijms26030991. PubMed PMID: 39940760; PubMed Central PMCID: PMC11817552. ⏎

Skipper PL, Tannenbaum SR, Ross RK, Yu MC. Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2003;12(6):503–7. PubMed PMID: 12814994. ⏎

So KA, Yang EJ, Kim NR, Hong SR, Lee JH, Hwang CS, *et al.* Changes of vaginal microbiota during cervical carcinogenesis in women with human papillomavirus infection. *PLoS One*. 2020;15(9):e0238705. Epub 20200917. doi:

10.1371/journal.pone.0238705. PubMed PMID: 32941440; PubMed Central PMCID: PMC7498004. ⏎

Sohail S, Burns MB. Integrating current analyses of the breast cancer microbiome. *PLoS One*. 2023;18(9):e0291320. Epub 20230912. doi: 10.1371/journal.pone.0291320. PubMed PMID: 37699008; PubMed Central PMCID: PMC10497162. ⏎

Somboonna N, Wan R, Ojcius DM, Pettengill MA, Joseph SJ, Chang A, *et al.* Hypervirulent *Chlamydia trachomatis* clinical strain is a recombinant between lymphogranuloma venereum (L(2)) and D lineages. *mBio*. 2011;2(3):e00045-11. Epub 20110503. doi: 10.1128/mBio.00045-11. PubMed PMID: 21540364; PubMed Central PMCID: PMC3088116. ⏎

Song J, Abraham SN. Innate and adaptive immune responses in the urinary tract. *Eur J Clin Invest*. 2008;38(Suppl 2):21–8. doi: 10.1111/j.1365-2362.2008.02005.x. PubMed PMID: 18826478. ⏎

Song M, Giovannucci E. Preventable incidence and mortality of carcinoma associated with lifestyle factors among white adults in the United States. *JAMA Oncol*. 2016;2(9):1154–61. doi: 10.1001/jamaoncol.2016.0843. PubMed PMID: 27196525; PubMed Central PMCID: PMC5016199. ⏎

Speth C, Bellotti R, Schäfer G, Rambach G, Texler B, Thurner GC, *et al.* Complement and fungal dysbiosis as prognostic markers and potential targets in PDAC treatment. *Curr Oncol*. 2022;29(12):9833–54. PubMed PMID: doi:10.3390/curroncol29120773. ⏎

Stanfill SB, Hecht SS, Joerger AC, González PJ, Maia LB, Rivas MG, *et al.* From cultivation to cancer: formation of N-nitrosamines and other carcinogens in smokeless tobacco and their mutagenic implications. *Crit Rev Toxicol*. 2023;53(10):658–701. doi: 10.1080/10408444.2023.2264327. ⏎

Strack R. Imaging-based spatial transcriptomics goes electric. *Nat Methods*. 2022;19(11):1338. doi: 10.1038/s41592-022-01678-x. PubMed PMID: 36329278. ⏎

Strathdee SA, Hatfull GF, Mutalik VK, Schooley RT. Phage therapy: from biological mechanisms to future directions. *Cell*. 2023;186(1):17–31. doi: 10.1016/j.cell.2022.11.017. PubMed PMID: 36608652; PubMed Central PMCID: PMC9827498. ⏎

Sun L, Ke X, Guan A, Jin B, Qu J, Wang Y, *et al.* Intratumoural microbiome can predict the prognosis of hepatocellular carcinoma after surgery. *Clin Transl Med*. 2023;13(7):e1331. doi: doi.org/10.1002/ctm2.1331. ⏎

Tanaka C, Ofuchi T, Ninomiya K, Inoue D, Sugimoto K, Murofushi K, *et al.* Surgery for older cancer patients: cross-organ review and good practice statement by the Japanese Geriatric Oncology Guideline Committee. *Ann Gastroenterol Surg*. 2025;9(6):1128–

36. Epub 20250531. doi: 10.1002/ags3.70048. PubMed PMID: 41199971; PubMed Central PMCID: PMC12586954. ⏎

Tetens AR, Findlay TR, Craig-Schwartz J, Liapodimitri A, Camacho O, Skalitzky KO, *et al.* Disordered DNA methylation leads to targetable transcriptional plasticity in ATRT. *Acta Neuropathol Commun*. 2025;14(1):22. doi: 10.1186/s40478-025-02173-y. ⏎

Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017;474(11):1823–36. doi: 10.1042/bcj20160510. ⏎

Tingting Y, Xiaoling Z, Yu Z, Yan L, Yang L, Xueqin S, *et al.* Interaction of intratumoral microbiota with the tumor immune microenvironment and its impact on cancer progression. *Front Oncol*. 2025;15:1609889. Epub 20251210. doi: 10.3389/fonc.2025.1609889. PubMed PMID: 41450920; PubMed Central PMCID: PMC12727591. ⏎

Trabert B, Graubard BI, Erickson RL, McGlynn KA. Childhood infections, orchitis and testicular germ cell tumours: a report from the STEED study and a meta-analysis of existing data. *Br J Cancer*. 2012;106(7):1331–4. Epub 20120216. doi: 10.1038/bjc.2012.45. PubMed PMID: 22343618; PubMed Central PMCID: PMC3314781. ⏎

Tzeng A, Sangwan N, Jia M, Liu CC, Keslar KS, Downs-Kelly E, *et al.* Human breast microbiome correlates with prognostic features and immunological signatures in breast cancer. *Genome Med*. 2021;13(1):60. Epub 20210416. doi: 10.1186/s13073-021-00874-2. PubMed PMID: 33863341; PubMed Central PMCID: PMC8052771. ⏎

Umemoto T, Inomoto T, Ueda K, Hamaguchi M, Kioka N. v-Src-mediated transformation suppresses the expression of focal adhesion protein vinexin. *Cancer Lett*. 2009;279(1):22–9. Epub 20090212. doi: 10.1016/j.canlet.2009.01.017. PubMed PMID: 19217206. ⏎

Valour F, Senechal A, Dupieux C, Karsenty J, Lustig S, Breton P, *et al.* Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. *Infect Drug Resist*. 2014;7:183–97. Epub 20140705. doi: 10.2147/IDR.S39601. PubMed PMID: 25045274; PubMed Central PMCID: PMC4094581. ⏎

Van den Ende B, Riva M, De Smet F, Jacobs S, Hulleman E, Coosemans A. Exploring the tumor microenvironment in diffuse intrinsic pontine glioma: immunological insights and therapeutic challenges. *J Immunother Cancer*. 2025;13(6). Epub 20250612. doi: 10.1136/jitc-2025-012009. PubMed PMID: 40514066; PubMed Central PMCID: PMC12164617. ⏎

Vella G, Rescigno M. Cancer microbiota: a focus on tumor-resident bacteria. *EMBO Rep*. 2025;26(12):2977–93. Epub 20250528. doi: 10.1038/s44319-025-00482-w.

PubMed PMID: 40437288; PubMed Central PMCID: PMC12187936. ⏎

Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannani D, *et al.* The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science*. 2013;342(6161):971–6. doi: 10.1126/science.1240537. PubMed PMID: 24264990; PubMed Central PMCID: PMC4048947. ⏎

Voigt AY, Emiola A, Johnson JS, Fleming ES, Nguyen H, Zhou W, *et al.* Skin microbiome variation with cancer progression in human cutaneous squamous cell carcinoma. *J Invest Dermatol*. 2022;142(10):2773–82 e16. Epub 20220404. doi: 10.1016/j.jid.2022.03.017. PubMed PMID: 35390349; PubMed Central PMCID: PMC9509417. ⏎

Vuong L, Kotecha RR, Voss MH, Hakimi AA. Tumor Microenvironment dynamics in clear-cell renal cell carcinoma. *Cancer Dis*. 2019;9(10):1349–57. doi: 10.1158/2159-8290.Cd-19-0499. ⏎

Vuotto C, Longo F, Balice MP, Donelli G, Varaldo PE. Antibiotic resistance related to biofilm formation in *Klebsiella pneumoniae*. *Pathogens*. 2014;3(3):743–58. Epub 20140905. doi: 10.3390/pathogens3030743. PubMed PMID: 25438022; PubMed Central PMCID: PMC4243439. ⏎

Walker SP, Tangney M, Claesson MJ. Sequence-based characterization of intratumoral bacteria-A guide to best practice. *Frontiers in Oncology*. 2020;10:179. Epub 20200221. doi: 10.3389/fonc.2020.00179. PubMed PMID: 32154174; PubMed Central PMCID: PMC7046755. ⏎

Walther-Antonio MR, Chen J, Multinu F, Hokenstad A, Distad TJ, Cheek EH, *et al.* Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med*. 2016;8(1):122. Epub 20161125. doi: 10.1186/s13073-016-0368-y. PubMed PMID: 27884207; PubMed Central PMCID: PMC5123330. ⏎

Wang H, Zhao M, Shi F, Zheng S, Xiong L, Zheng L. A review of signal pathway induced by virulent protein CagA of *Helicobacter pylori*. *Front Cell Infect Microbiol*. 2023;13. doi: 10.3389/fcimb.2023.1062803. ⏎

Wang J, Li X, Wu X, Wang Z, Zhang C, Cao G, *et al.* Uncovering the microbiota in renal cell carcinoma tissue using 16S rRNA gene sequencing. *J Cancer Res Clin Oncol*. 2021;147(2):481–91. doi: 10.1007/s00432-020-03462-w. ⏎

Wang JW, Ge X, Liu JH, Zhang X. Oncolytic virus therapy for osteosarcoma: mechanisms, opportunities, and challenges. *Front Immunol*. 2025a;16:1724768. Epub 20251210. doi: 10.3389/fimmu.2025.1724768. PubMed PMID: 41451200; PubMed Central PMCID: PMC12728009.

Wang N, Wu S, Huang L, Hu Y, He X, He J, *et al.* Intratumoral microbiome: implications for immune modulation and innovative therapeutic strategies in cancer. *J*

Biomed Sci. 2025b;32(1):23. Epub 20250219. doi: 10.1186/s12929-025-01117-x. PubMed PMID: 39966840; PubMed Central PMCID: PMC11837407. ⏎

Wang Q, Wang K, Tan X, Li Z, Wang H. Immunomodulatory role of metalloproteases in cancers: current progress and future trends. *Front Immunol*. 2022a;13:1064033. Epub 20221216. doi: 10.3389/fimmu.2022.1064033. PubMed PMID: 36591235; PubMed Central PMCID: PMC9800621. ⏎

Wang Y, Wang Y, Wang J. A comprehensive analysis of intratumor microbiome in head and neck squamous cell carcinoma. *Eur Arch Otorhinolaryngol*. 2022b;279(8):4127–36. Epub 20220205. doi: 10.1007/s00405-022-07284-z. PubMed PMID: 35122129. ⏎

Wang Z, Zhang T, Liu Y. Emerging technologies and current challenges in intratumoral microbiota research. *Front Cell Infect Microbiol*. 2025c;15:1685862. Epub 20260102. doi: 10.3389/fcimb.2025.1685862. PMID: 41552720; PubMed Central PMCID: PMC12808383.

Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, *et al.* CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet*. 2006;38(7):787–93. doi: 10.1038/ng1834. ⏎

Wen H, Zheng S, Zhu X, Wang L, Chen D. Characteristics of the tumor microenvironment and potential immunotherapy strategies in renal cell carcinoma. *Front Immunol*. 2025;16:1643533. Epub 20250902. doi: 10.3389/fimmu.2025.1643533. PubMed PMID: 40963620; PubMed Central PMCID: PMC12436424. ⏎

Weng Z, Yang N, Shi S, Xu Z, Chen Z, Liang C, *et al.* Outer membrane vesicles from *Acinetobacter baumannii*: biogenesis, functions, and vaccine application. *Vaccines*. 2024;12(1):49. PubMed PMID: doi:10.3390/vaccines12010049. ⏎

Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. 2015;15(8):486–99. doi: 10.1038/nri3862. PubMed PMID: 26205583; PubMed Central PMCID: PMC4889009. ⏎

Willing C, Peich M, Danescu A, Kehlen A, Fowler PA, Hombach-Klonisch S. Estrogen-independent actions of environmentally relevant AhR-agonists in human endometrial epithelial cells. *Mol Hum Reprod*. 2011;17(2):115–26. Epub 20100927. doi: 10.1093/molehr/gaq081. PubMed PMID: 20876610. ⏎

Wong-Rolle A, Dong Q, Zhu Y, Divakar P, Hor JL, Kedei N, *et al.* Spatial meta-transcriptomics reveal associations of intratumor bacteria burden with lung cancer cells showing a distinct oncogenic signature. *J Immunother Cancer*. 2022;10(7). doi: 10.1136/jitc-2022-004698. PubMed PMID: 35793869; PubMed Central PMCID: PMC9260850. ⏎

Wong-Rolle A, Wei HK, Zhao C, Jin C. Unexpected guests in the tumor microenvironment: microbiome in cancer. *Protein Cell*. 2021;12(5):426–35. Epub 20201209. doi: 10.1007/s13238-020-00813-8. PubMed PMID: 33296049; PubMed Central PMCID: PMC8106554. ⏎

Woo YR, Cho SH, Lee JD, Kim HS. The human microbiota and skin cancer. *Int J Mol Sci*. 2022;23(3). Epub 20220205. doi: 10.3390/ijms23031813. PubMed PMID: 35163734; PubMed Central PMCID: PMC8837078. ⏎

Wu J, Dai T, Li Z, Pan M, Zhang W, Chen H, *et al.* Integrating multi-modal transcriptomics identifies cellular subtypes with distinct roles in PDAC progression. *Cell Oncol*. 2025;48(5):1571–92. doi: 10.1007/s13402-025-01100-6. ⏎

Wu J, Zhang P, Mei W, Zeng C. Intratumoral microbiota: implications for cancer onset, progression, and therapy. *Front Immunol*. 2023;14:1301506. Epub 20240116. doi: 10.3389/fimmu.2023.1301506. PubMed PMID: 38292482; PubMed Central PMCID: PMC10824977. ⏎

Wu K, Li Y, Ma K, Zhao W, Yao Z, Zheng Z, *et al.* The microbiota and renal cell carcinoma. *Cell Oncol (Dordr)*. 2024;47(2):397–413. Epub 20231025. doi: 10.1007/s13402-023-00876-9. PubMed PMID: 37878209. ⏎

Xia P, Zhou J, Shen R, Wang D. Deciphering the cellular and molecular landscape of cervical cancer progression through single-cell and spatial transcriptomics. *npj Precis Oncol*. 2025;9(1):158. doi: 10.1038/s41698-025-00948-z. ⏎

Xie C, Pocha C. Crosstalk between gut microbiota and hepatocellular carcinoma. *Gastrointest Disord*. 2023;5(2):127–43. PubMed PMID: doi:10.3390/gidisord5020013. ⏎

Xie C, Yan Q, Zhang P, Zhang L, Yan G. Intratumoral microbiota omics analysis in head and neck squamous cell carcinoma. *Front Microbiol*. 2025a;16:1711139. Epub 20251127. doi: 10.3389/fmicb.2025.1711139. PubMed PMID: 41395468; PubMed Central PMCID: PMC12695817. ⏎

Xie Z, Wu Z, Liu Y, Gu Y, Niu J, Lv K. Intratumoral microbiota: implications for cancer progression and treatment. *Front Microbiol*. 2025b;16:1551515. Epub 20250728. doi: 10.3389/fmicb.2025.1551515. PubMed PMID: 40792257; PubMed Central PMCID: PMC12336131.

Xu H, Leng J, Liu F, Chen T, Qu J, Yang Y, *et al.* Tumor microbiota of renal cell carcinoma affects clinical prognosis by influencing the tumor immune microenvironment. *Heliyon*. 2024;10(19). doi: 10.1016/j.heliyon.2024.e38310. ⏎

Xu S, Ducroux A, Ponnurangam A, Vieyres G, Franz S, Musken M, *et al.* cGAS-mediated innate immunity spreads intercellularly through HIV-1 Env-induced

membrane fusion sites. *Cell Host Microbe*. 2016;20(4):443–57. doi: 10.1016/j.chom.2016.09.003. PubMed PMID: 27736643. ⏎

Xu Y, Wang MC. The intratumoral microbiota in breast cancer: from basic research to clinical translation. *Gut Microbes*. 2025;17(1):2560695. Epub 20250915. doi: 10.1080/19490976.2025.2560695. PubMed PMID: 40948461; PubMed Central PMCID: PMC12439558. ⏎

Xue C, Chu Q, Zheng Q, Yuan X, Su Y, Bao Z, *et al.* Current understanding of the intratumoral microbiome in various tumors. *Cell Rep Med*. 2023;4(1):100884. doi: 10.1016/j.xcrm.2022.100884. PubMed PMID: 36652905; PubMed Central PMCID: PMC9873978. ⏎

Xue C, Gu X, Shi Q, Ma X, Jia J, Su Y, *et al.* The interaction between intratumoral bacteria and metabolic distortion in hepatocellular carcinoma. *J Transl Med*. 2024;22(1):237. doi: 10.1186/s12967-024-05036-7. ⏎

Xue C, Jia J, Gu X, Zhou L, Lu J, Zheng Q, *et al.* Intratumoral bacteria interact with metabolites and genetic alterations in hepatocellular carcinoma. *Signal Transduct Target Ther*. 2022;7(1):335. Epub 20220928. doi: 10.1038/s41392-022-01159-9. PubMed PMID: 36167691; PubMed Central PMCID: PMC9515207. ⏎

Yang L, Li A, Wang Y, Zhang Y. Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy. *Signal Transduct Target Ther*. 2023;8(1):35. Epub 20230116. doi: 10.1038/s41392-022-01304-4. PubMed PMID: 36646684; PubMed Central PMCID: PMC9842669. ⏎

Yang X, Ning T, Zhang W, Hou S, Su J, Li W, *et al. Fusobacterium nucleatum* drives gastric cancer metastasis via Gbp-CypA-NF-kappaB-mediated CXCL8 crosstalk between tumor cells and mast cells. *Cell Commun Signal*. 2025a;24(1):6. Epub 20251128. doi: 10.1186/s12964-025-02561-w. PubMed PMID: 41316398; PubMed Central PMCID: PMC12777487. ⏎

Yang X, Yin L, Tian Z, Zhou Q. Intratumoral microbiota in lung cancer: emerging roles in TME modulation and immunotherapy response. *Int J Mol Sci*. 2025b;27(1). Epub 20251225. doi: 10.3390/ijms27010255. PubMed PMID: 41516132; PubMed Central PMCID: PMC12786020.

Yao B, Delaidelli A, Vogel H, Sorensen PH. Pediatric brain tumours: lessons from the immune microenvironment. *Curr Oncol*. 2023;30(5):5024–46. Epub 20230515. doi: 10.3390/curroncol30050379. PubMed PMID: 37232837; PubMed Central PMCID: PMC10217418. ⏎

Ye P, Bergerson JRE, Brownell I, Starrett GJ, Abraham RS, Anderson MV, *et al.* Resolution of squamous-cell carcinoma by restoring T-cell receptor signaling. *N Engl*

J Med. 2025;393(5):469–78. doi: 10.1056/NEJMoa2502114. PubMed PMID: 40742260; PubMed Central PMCID: PMC12370287. ⏎

Yeo ELL, Hong BH, Tay SH, Neo J, Ong EHW, Chow WM, *et al.* Tumor immune microenvironment delineates progression trajectories of distinct nasopharyngeal carcinoma phenotypes. *Cell Rep Med*. 2025;6(6):102143. Epub 20250523. doi: 10.1016/j.xcrm.2025.102143. PubMed PMID: 40412382; PubMed Central PMCID: PMC12208333. ⏎

Yeo K, Li R, Wu F, Bouras G, Mai LTH, Smith E, *et al.* Identification of consensus head and neck cancer-associated microbiota signatures: a systematic review and meta-analysis of 16S rRNA and The Cancer Microbiome Atlas datasets. *J Med Microbiol*. 2024;73(2). doi: 10.1099/jmm.0.001799. PubMed PMID: 38299619. ⏎

Yin H, Pu N, Chen Q, Zhang J, Zhao G, Xu X, *et al.* Gut-derived lipopolysaccharide remodels tumoral microenvironment and synergizes with PD-L1 checkpoint blockade via TLR4/MyD88/AKT/NF-kappaB pathway in pancreatic cancer. *Cell Death Dis*. 2021;12(11):1033. Epub 20211030. doi: 10.1038/s41419-021-04293-4. PubMed PMID: 34718325; PubMed Central PMCID: PMC8557215. ⏎

Yu AI, Zhao L, Eaton KA, Ho S, Chen J, Poe S, *et al.* Gut microbiota modulate CD8 T cell responses to influence colitis-associated tumorigenesis. *Cell Rep*. 2020;31(1):107471. doi: 10.1016/j.celrep.2020.03.035. PubMed PMID: 32268087; PubMed Central PMCID: PMC7934571. ⏎

Yu J, Zhuang A, Gu X, Hua Y, Yang L, Ge S, *et al.* Nuclear PD-L1 promotes EGR1-mediated angiogenesis and accelerates tumorigenesis. *Cell Discov*. 2023;9(1):33. Epub 20230328. doi: 10.1038/s41421-023-00521-7. PubMed PMID: 36977660; PubMed Central PMCID: PMC10050073. ⏎

Yu Y, Zhao W, Yang M, Wu B, Yuan X. Tumor-promoting gut microbes in colorectal cancer: mechanisms and translational perspectives. *Int J Med Sci*. 2026;23(1):63–75. Epub 20260101. doi: 10.7150/ijms.123494. PubMed PMID: 41399387; PubMed Central PMCID: PMC12701968. ⏎

Yuan L, Yang P, Wei G, Hu X, Chen S, Lu J, *et al.* Tumor microbiome diversity influences papillary thyroid cancer invasion. *Commun Biol*. 2022;5(1):864. Epub 20220824. doi: 10.1038/s42003-022-03814-x. PubMed PMID: 36002642; PubMed Central PMCID: PMC9402670. ⏎

Zapatka M, Borozan I, Brewer DS, Iskar M, Grundhoff A, Alawi M, *et al.* The landscape of viral associations in human cancers. *Nat Genet*. 2020;52(3):320–30. Epub 20200205. doi: 10.1038/s41588-019-0558-9. PubMed PMID: 32025001; PubMed Central PMCID: PMC8076016. ⏎

Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, *et al*. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 2013;39(2):372–85. doi: 10.1016/j.immuni.2013.08.003. PubMed PMID: 23973224. ⏎

Zeng R, Gou H, Lau HCH, Yu J. Stomach microbiota in gastric cancer development and clinical implications. *Gut*. 2024;73(12):2062–73. Epub 20241111. doi: 10.1136/gutjnl-2024-332815. PubMed PMID: 38886045; PubMed Central PMCID: PMC11672014. ⏎

Zhang H, Li S, Wang D, Liu S, Xiao T, Gu W, *et al.* Metabolic reprogramming and immune evasion: the interplay in the tumor microenvironment. *Biomark Res*. 2024a;12(1):96. Epub 20240903. doi: 10.1186/s40364-024-00646-1. PubMed PMID: 39227970; PubMed Central PMCID: PMC11373140.

Zhang H, Mu C, Gu Y, Meng F, Qin X, Cao H. Selection strategy of second-line biologic therapies in adult patients with ulcerative colitis following prior biologic treatment failure: systematic review and meta-analysis. *Pharmacol Res*. 2024b;202:107108. Epub 20240224. doi: 10.1016/j.phrs.2024.107108. PubMed PMID: 38403257.

Zhang J, You Z, Li X, Hu J, Li J, Jing Z. Harnessing intratumoral microbiota: new horizons in immune microenvironment and immunotherapy. *J Transl Med*. 2025a;23(1):897. Epub 20250812. doi: 10.1186/s12967-025-06916-2. PubMed PMID: 40797326; PubMed Central PMCID: PMC12341252. ⏎

Zhang Z, Liang L, Jiang X, Shan J, Li S, Liu J, *et al.* Skin microbiome influences the progression of cutaneous squamous cell carcinoma through the immune system. *World J Surg Oncol*. 2025b;23(1):129. Epub 20250409. doi: 10.1186/s12957-025-03791-5. PubMed PMID: 40205611; PubMed Central PMCID: PMC11980248. ⏎

Zhang Z, Ma Q, Zhang L, Ma L, Wang D, Yang Y, *et al.* Human papillomavirus and cervical cancer in the microbial world: exploring the vaginal microecology. *Front Cell Infect Microbiol*. 2024c;14:1325500. Epub 20240125. doi: 10.3389/fcimb.2024.1325500. PubMed PMID: 38333037; PubMed Central PMCID: PMC10850380. ⏎

Zhao K, Hu Y. Microbiome harbored within tumors: a new chance to revisit our understanding of cancer pathogenesis and treatment. *Signal Transduct Target Ther*. 2020;5(1):136. Epub 20200729. doi: 10.1038/s41392-020-00244-1. PubMed PMID: 32728023; PubMed Central PMCID: PMC7391753. ⏎

Zhao Y, Yang Z, Wu D, Zhao H. Dissecting the intratumoral microbiome landscape in lung cancer. *Front Immunol*. 2025;16:1614731. Epub 20250724. doi:

10.3389/fimmu.2025.1614731. PubMed PMID: 40777004; PubMed Central PMCID: PMC12328415. ⏎

Zhou M, Wang Q, Yang R, Zong Z, Huang B, Chen J, *et al.* Microbial diagnostic features identified across populations possess potential antitumor properties in breast cancer. *mSystems*. 2025;10(7):e0027125. Epub 20250623. doi: 10.1128/msystems.00271-25. PubMed PMID: 40548719; PubMed Central PMCID: PMC12282184. ⏎

Zhou X, An R, Li X. Cervical cancer immune microenvironment: mechanisms of HPV-mediated immune evasion and advances in immunotherapy (Review). *Oncol Lett*. 2026;31(1):22. Epub 20251105. doi: 10.3892/ol.2025.15375. PubMed PMID: 41246557; PubMed Central PMCID: PMC12612796. ⏎

Zhu W, Wang JZ, Liu Z, Wei JF. The bacteria inside human cancer cells: mainly as cancer promoters. *Front Oncol*. 2022;12:897330. Epub 20220812. doi: 10.3389/fonc.2022.897330. PubMed PMID: 36033476; PubMed Central PMCID: PMC9411745. ⏎

Zhu Y, Liu W, Wang M, Wang X, Wang S. Causal roles of skin microbiota in skin cancers suggested by genetic study. *Front Microbiol*. 2024;15:1426807. Epub 20240805. doi: 10.3389/fmicb.2024.1426807. PubMed PMID: 39161599; PubMed Central PMCID: PMC11330880. ⏎

Zong Z, Zhou F, Zhang L. The fungal mycobiome: a new hallmark of cancer revealed by pan-cancer analyses. *Signal Transduct Target Ther*. 2023;8(1):50. Epub 20230201. doi: 10.1038/s41392-023-01334-6. PubMed PMID: 36725834; PubMed Central PMCID: PMC9892023. ⏎

Qiao, H., Tan, X. R., Li, H., Li, J. Y., Chen, X. Z., Li, Y. Q., Li, W. F., Tang, L. L., Zhou, G. Q., Zhang, Y., Liang, Y. L., He, Q. M., Zhao, Y., Huang, S. Y., Gong, S., Li, Q., Ye, M. L., Chen, K. L., Sun, Y., Ma, J., … Liu, N. (2022). Association of Intratumoral Microbiota With Prognosis in Patients With Nasopharyngeal Carcinoma From 2 Hospitals in China. *JAMA oncology*, *8*(9), 1301–1309. https://doi.org/10.1001/jamaoncol.2022.2810.

Tan, X. R., Qiao, H., Li, Y. Q., Jiang, W., Huang, S. Y., Gong, S., Li, W. F., Tang, L. L., Zhou, G. Q., Liang, Y. L., Li, H., He, Q. M., Bai, J. W., Ye, M. L., Wang, J. Y., Huang, S. W., Li, J. Y., Gan, C. Q., Li, Y. Q., Zhao, Y., … Liu, N. (2025). Tissue-resident microbiota signature in nasopharyngeal carcinoma. *Microbiome*, *13*(1), 125. https://doi.org/10.1186/s40168-025-02114-w.

15

Microbiome Profiling in Clinical Practices: Bridging the Gap in Human Health

Jyoti Kant Choudhari, Shashank Purwar, Akanksha Singh, Anna Antony, and Abhi Tyagi

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15.1 Introduction

The human microbiome comprises trillions of diverse microorganisms—including bacteria, archaea, fungi, protozoa, and viruses—that colonize different regions of the body. This elaborate network of microbes is integral to maintaining the body's health and balance by supporting key physiological processes, including digestion, nutrient uptake, immune regulation, and protection against harmful pathogens (Hooper et al., 2012; Turnbaugh et al., 2007). Advancements in high-throughput sequencing and metagenomic approaches have revolutionized our understanding of the human microbiome's composition and functional capabilities. Through 16S ribosomal RNA (rRNA) gene profiling and metagenomic sequencing, researchers have identified a broad spectrum of microbial life, its adaptability, and its associations with various diseases. The Human Microbiome Project 2 (HMP2) has demonstrated considerable variability in the gut microbiota of individuals diagnosed with inflammatory bowel disease (IBD), highlighting the potential of microbial communities as diagnostic or prognostic biomarkers (Lloyd-Price et al., 2019). Metagenomics enables the study of uncultivated microbes by analyzing genetic material directly from environmental samples. As highlighted by Choudhari et al. (2022), it reveals microbial diversity and function using high-throughput sequencing and computational tools. While it has advanced our understanding of microbial roles in health, industry, and the environment, challenges remain in data integration and functional annotation (Choudhari et al., 2022). The gut microbiome–metabolome interaction plays a key role in glucose regulation, with one-third

of altered blood metabolites in type 2 diabetes linked to microbial changes. Lifestyle interventions, such as diet and exercise, can modulate these metabolites, making the microbiome a potential target for diabetes management (Wu et al., 2025). The gut microbiome also plays a central role in mediating the effects of diet on human health by co-metabolizing nutrients and influencing immune, metabolic, and neural pathways. Dietary patterns, food quality, and nutrient composition all influence gut microbial diversity and function, thereby affecting outcomes such as inflammation, barrier integrity, and the production of metabolites. Microbially derived metabolites—such as short-chain fatty acids (SCFAs), polyphenol derivatives, and vitamin by-products—modulate intestinal immunity and systemic physiology. This bidirectional interaction highlights the microbiome's significance in personalized and precision nutrition, where dietary interventions can be tailored to support beneficial microbiota and enhance health outcomes (Sanz et al., 2025). Furthermore, a diverse early-life gut microbiome, particularly one with high *Bacteroides* abundance, is associated with a better response to and increased shedding of the RV3-BB rotavirus vaccine when administered at birth, highlighting the microbiome's role in shaping the efficacy of oral vaccines (Wagner et al., 2025).

15.1.1 Gut Microbiota

The gut microbiota, primarily found in the colon, is the most diverse microbial community in the body and performs numerous interconnected physiological processes crucial for human health (Gebrayel et al., 2022). The fermentation of dietary fibers by the microbiota produces SCFAs, specifically butyrate, acetate, and propionate, which serve as vital energy sources for colonocytes, providing approximately 10% of daily calories and supporting mitochondrial function in intestinal epithelial cells (O'Riordan et al., 2022). At the same time, the microbiota synthesizes important B-complex vitamins (B1, B2, B6, B12, and folate) and vitamin K, which are absorbed through specific colonic transport mechanisms, greatly boosting the host's micronutrient stores, especially in groups with limited dietary vitamin intake (Wan et al., 2022). The microbiota maintains intestinal barrier integrity through SCFA-mediated mechanisms that strengthen tight junctions, inhibit histone deacetylases, and activate protective G-protein-coupled receptors, thereby preventing the translocation of harmful bacteria and lipopolysaccharides (LPS) into the systemic circulation (Di Vincenzo et al., 2024). Moreover, the microbiota guides the immune system in distinguishing between pathogenic threats and harmless antigens, primarily through SCFA-producing bacteria that support the development of regulatory T cells (Tregs) and control inflammatory responses via histone acetylation and pattern recognition receptor (PRR) signaling (Ma et al., 2022).

The disruption of this mutualistic relationship, known as dysbiosis, serves as a significant pathogenic mechanism linked to various modern diseases, including metabolic disorders (such as type 2 diabetes, non-alcoholic fatty liver disease, obesity, and

atherosclerosis), autoimmune diseases, and IBD (Zhang et al., 2025). Dysbiosis is characterized by a reduction in microbial diversity, a decline in beneficial bacteria such as *Bacteroidetes* and *Firmicutes*, and an increase in potentially harmful organisms, resulting in a pro-inflammatory state. This state is caused by a decrease in the production of SCFAs, lower vitamin production, and increased translocation of bacterial LPS, which impairs immune tolerance (Zhang et al., 2025). Recognizing these essential functions has led to the development of microbiome profiling as a new clinical tool, which utilizes next-generation sequencing (NGS) technologies to analyze microbial composition and function. Its applications include disease diagnosis, risk evaluation, phenotype classification, and monitoring microbiota-targeted therapies, such as specific dietary changes, prebiotics, probiotics, and fecal microbiota transplantation (FMT). This systems-level approach highlights the gut microbiota as a key therapeutic target in modern medicine, combining laboratory research with chronic disease management through personalized, microbiome-based treatments.

15.1.2 Oral Microbiota

The oral microbiota is a diverse and intricate ecosystem that comprises approximately 700 bacterial groups, as well as viruses, fungi, archaea, and protozoa (Zhang et al., 2018). These microorganisms inhabit various regions of the mouth, such as the tongue, teeth, cheeks, periodontal pockets, palate, and gingival surfaces. This microbial ecology maintains a balance between commensal organisms that enhance oral health and pathogenic species that may induce disease during dysbiosis. The mouth cavity exhibits the second-highest microbial diversity in the human body, surpassed only by the gastrointestinal system (Zhang et al., 2018). It is distinguished by the presence of multiple bacterial phyla, including *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria*. Gram-positive cocci, especially *Streptococcus* species, play a crucial role in maintaining oral health (Rajasekaran et al., 2024). Helpful commensal microbes, such as *Streptococcus sanguinis* and *S. salivarius*, produce antimicrobial agents that directly prevent pathogen growth and protect enamel (Reichardt et al., 2024). However, opportunistic bacteria like *Streptococcus mutans* can take advantage of favorable conditions to become harmful. The makeup of the oral microbiota is significantly influenced by diet, age, smoking, alcohol consumption, antibiotics, medications, genetics, and hormonal changes during pregnancy—particularly increased dietary sugar intake, which substantially increases the presence of bacteria that cause cavities. Bacteriophages, along with bacteria, represent an ecologically important but understudied group (Li et al., 2022; Santacroce et al., 2023). Their presence exhibits an inverse relationship with host bacterial populations, which is shaped by the lytic and lysogenic cycles (Naureen et al., 2020). Recent findings show that bacteriophage diversity varies significantly between individuals with healthy gums and those with periodontal disease. This variation is linked to the development of periodontal conditions

and indicates potential for diagnostic and therapeutic uses through targeted bacteriophage treatments (Edlund et al., 2015).

It is essential for dental health by suppressing the growth of pathogenic species linked to dental caries and periodontal disease, primarily through competitive exclusion and the production of antimicrobial substances. *Streptococcus mutans*, the primary agent of dental caries, possesses three key pathogenic traits (Lemos et al., 2019; Zhang et al., 2018): the capacity to generate significant quantities of extracellular polysaccharides, especially glucans, from dietary sucrose via glucosyltransferase activity, which promotes persistent adhesion to dental surfaces and biofilm development (Koo et al., 2013, 2010); the ability to transport and metabolize various carbohydrates into organic acids, enhancing acidogenicity that results in tooth enamel demineralization (Bowen and Koo, 2011); and the capacity to survive in acidic environments through the expression of acid tolerance genes (Lemos and Burne, 2008; Murray et al., 1991). The biofilm generated by *Streptococcus mutans* and associated acidogenic bacteria creates a sustained low-pH microenvironment on tooth surfaces, promoting demineralization (Gao et al., 2024). Disease progression occurs when pathogenic, acid-producing bacteria outnumber the harmless, non-cariogenic microbes. Additionally, consuming sucrose in the diet worsens this by providing the primary substrate for glucan formation and aiding biofilm growth. Periodontal disease results from a microbial imbalance, marked by an increase in harmful species and a decrease in beneficial ones. This imbalance creates a pro-inflammatory environment where dysbiotic communities secrete virulence factors, such as proteases, LPS, and toxins, directly damaging periodontal tissues and disrupting critical host-microbe interactions necessary for immune defense. These pathogenic bacteria can evade immune detection and modify the host immune response, promoting inflammation that leads to tissue damage. This inflammatory response enhances tissue destruction through feedback loops and hampers tissue repair, speeding up the development of periodontal disease. Microbiome profiling can predict treatment success. Probiotics may prevent and treat infections by producing bacteriocins and enzymes, reducing the growth of pathogenic bacteria, and modulating the immune system. Future methods include microbiota diagnostics and personalized treatments using NGS and biomarkers to enhance oral health.

15.1.3 Skin Microbiota

The skin microbiota is a complex system comprising approximately 1,000 bacterial species from 19 bacterial phyla, significantly influenced by spatial variations related to the unique microenvironments of skin regions (Yang et al., 2024). Dry skin areas show low diversity and are mainly inhabited by *Cutibacterium* species, while damp regions display high diversity, dominated by *Staphylococcus* and *Corynebacterium* (Wang et al., 2025). The microbial composition is more influenced by local microenvironmental factors than by host-specific features, particularly the vasculature (Bay et al., 2024). Additionally, the skin

microbiome undergoes significant changes with age due to variations in hazardous lipids, antimicrobial peptides (AMPs), and immune function, which influence disease susceptibility (Kenshi and Richard, 2008). The skin microbiota defends against pathogenic infections via three mechanisms: competitive exclusion, antimicrobial production, and immune modulation (Flowers and Grice, 2020). Beneficial microorganisms occupy ecological niches, diminishing nutrient availability for pathogens and detrimental viruses. Coagulase-negative Staphylococci generate compounds that disrupt *Staphylococcus aureus* quorum sensing, while *Cutibacterium acnes* synthesizes cutimycin, which inhibits *S. aureus* colonization (Flowers and Grice, 2020; Suri et al., 2025). The skin also maintains an acidic mantle, with an optimal pH of around 4.7, which promotes the growth of beneficial bacteria and inhibits the growth of pathogens (Brooks et al., 2025). This acidic environment enhances host enzyme activities related to lipid processing and skin integrity (Baker et al., 2023). Alkaline personal care products disrupt the skin's pH, impairing bacterial stability and health. Thus, pH plays a vital role in skin health (Kim et al., 2009). The microbiota also influences immune responses by interacting with keratinocytes and Langerhans cells (Kreouzi et al., 2025). These epidermal cells help distinguish between harmful and harmless stimuli, supporting immune protection. Keratinocytes, which make up about 95% of the epidermis, act as sentinel cells, using PRRs like toll-like receptors (TLRs) and NOD-like receptors (NLRs) to detect bacterial signals (Yousef et al., 2025). Dead keratinocytes release defensins within this layer, which are part of the first line of defense against pathogens (Karim et al., 2019). These signals trigger innate immune responses that strengthen the skin barrier. Upon detecting microbes, keratinocytes release cytokines and chemokines, such as tumor necrosis factor (TNF), interleukin-1beta (IL-1β), and interleukin-6 (IL-6), which stimulate the production of AMPs, including cathelicidin, and recruit immune cells to respond effectively (Aristizábal and González, 2013). Langerhans cells work closely with keratinocytes to sample and present antigens, facilitating T-cell activation in lymph nodes and promoting protective adaptive immunity while reducing overreactions to benign microbes through the induction of Tregs (Zhou et al., 2022). Modern skin therapies increasingly favor microbiota-focused strategies. Probiotics and treatments aimed at restoring microbial diversity are becoming increasingly preferred over traditional antimicrobials, which can harm protective commensal bacteria (De Almeida et al., 2023). Evidence indicates that the initial composition of the microbiome can predict treatment outcomes for skin and gum diseases (Mahmud et al., 2022). Strategies such as using moisturizers to restore skin pH or applying targeted anti-*S. aureus* treatments can improve results by supporting beneficial bacteria. Future dermatology practices may incorporate microbiota profiling and customized interventions to manage dysbiosis, emphasizing the importance of standardized microbiota sampling and individualized protocols (Chen, 2025).

15.1.4 Respiratory and Urogenital Tract Microbiota

Site-specific microbiotas play a critical role in mucosal immunity and host protection across multiple body compartments. The respiratory tract microbiota, predominantly residing in the upper respiratory tract (nasal cavity and throat), supports immune surveillance and confers resistance against pathogens such as *Streptococcus pneumoniae* and influenza viruses. Dysbiosis is associated with asthma, chronic obstructive pulmonary disease (COPD), and recurrent respiratory infections. Similarly, the urogenital microbiota, especially in females, is dominated by *Lactobacillus* species that produce lactic acid to maintain a low vaginal pH (~4.5), thereby inhibiting pathogenic growth and preventing urinary tract infections (UTIs) and bacterial vaginosis. Disruptions to this microbial balance can lead to fertility issues and increased susceptibility to sexually transmitted infections. Beyond these localized effects, dysbiosis—defined as an imbalance in microbial composition—has been implicated in a wide range of systemic conditions, including IBD, obesity, diabetes, cardiovascular diseases, neurodegenerative disorders, and autoimmune diseases. The gut–brain axis further underscores the microbiome's influence on mental health, with microbial imbalances linked to depression, anxiety, and Alzheimer's disease (Cryan and Dinan, 2012; Mayer et al., 2014).

15.2 The Approach of Microbiome Profiling

Microbiome profiling refers to the comprehensive analysis and identification of microbial communities present in a specific biological or environmental sample. Unlike traditional culture-based techniques, which are limited in scope because over 99% of microbial species cannot be cultivated under standard laboratory conditions, modern profiling relies on molecular biology and computational approaches to overcome these limitations. Advancements in high-throughput sequencing technologies, combined with powerful bioinformatics tools, have revolutionized microbiome research. These methods not only provide detailed taxonomic profiles—indicating which microorganisms are present—but also offer insights into their functional roles and ecological interactions within the community. Below is an overview of the major techniques employed in microbiome profiling.

15.2.1 16S rRNA Gene Sequencing

This method focuses on the 16S rRNA gene, which contains both conserved and variable regions. This structure enables universal amplification and genus-level identification of bacteria. It is cost-effective and commonly employed for initial microbiome assessments. While excellent for bacterial classification, this sequencing approach has limited phylogenetic resolution at the species level and offers poor discrimination among certain

genera. To achieve precise taxonomic resolution, DNA-relatedness studies are required (Choudhari et al., 2022; Janda and Abbott, 2007).

15.2.2 Shotgun Metagenomics

Shotgun metagenomics is a sequencing approach used to profile the gut microbiota and its functional potential. This approach sequences all DNA in a sample, capturing the full microbial community, including bacteria, archaea, viruses, fungi, and other eukaryotes. It enables strain-level resolution and functional annotation of genes and metabolic pathways (Quince et al., 2017). By aligning to various genomic locations throughout the genome, it can provide better resolution, higher sensitivity, and a more comprehensive characterization of microbial community structures than 16S rRNA gene amplicon sequencing (Dai et al., 2018).

15.2.3 Metatranscriptomics

Metatranscriptomics is a high-throughput, culture-independent technique that measures gene expression in complex microbial communities. It goes beyond DNA-based methods by assessing the community's actual activity and potential. Using signals from both phylogenetic and metabolic markers, it can simultaneously identify the composition and activity of the microbes (Zhi et al., 2014). The characterization of the RNA content in samples differs from DNA analysis, which uses the metagenomic approaches described earlier. Like metagenomics, metatranscriptomics can identify microbial members of an ecosystem and offer functional insights into the community. Since it utilizes RNA transcripts instead of DNA, it reveals the community's dynamic nature, allowing for conclusions about how environmental changes trigger broad shifts in gene expression (Kaakoush, 2020).

15.2.4 Metaproteomics and Metabolomics

Metaproteomics is the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time (Chen et al., 2017). Examining the human microbiome using metaproteomics, metabolomics, and integrative multi-omics approaches may provide critical insights into indicators of health and illness, facilitating the development of microbiome-targeted therapies for conditions associated with dysbiosis. Metaproteomics facilitates the extraction of functional activity data from microbiome samples, while metabolomics offers insights into the comprehensive metabolic conditions that influence and characterize host-microbiome interactions. This approach also offers significant benefits compared to other omics methods for functional analysis. Metagenomic data primarily reflect the microbial potential of a system without providing insights into

actual microbial activity. Conversely, metatranscriptomics advances the identification of active metabolic pathways; however, it does not account for translational regulation, as a confirmed lack of connection exists between messenger RNA (mRNA) levels and protein levels. Metaproteomics provides substantial insights into microbial activity, alongside metabolomics, which examines the intermediate and final products of biological activities (De Angelis and Calasso, 2014). Combining these functional-omic platforms with microbiome composition profiling enables a comprehensive understanding of the microbiome's functional and metabolic state, as well as its impact on human health. Metabolite analysis, in contrast to metagenome sequencing, elucidates the metabolically active population of microbes rather than their genetic potential. With the use of genome sequence information, the genetic background of the microbe is considered while evaluating transcriptional activity (Kaakoush, 2020).

15.3 Resources of Microbiome Data

Microbiome data resources encompass comprehensive primary sequence databases, specialized portals for microbiomes, and databases focused on specific diseases or anatomical areas (Table 15.1), all of which facilitate data deposition, reutilization, and meta-analyses. Major databases such as the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) and other International Nucleotide Sequence Database Collaboration (INSDC) collaborators (the European Nucleotide Archive (ENA) and DRA) house extensive amounts of raw 16S amplicon and shotgun metagenomic sequences derived from various microbiome investigations. Additionally, specific to the microbiome field, tools such as Metagenomics Rapid Annotation using Subsystem Technology (MG-RAST), MGnify, and the Human Microbiome Project Data Coordination Center (HMPDCC) provide curated data, standardized procedures, and multi-omic datasets to facilitate exploratory research and inter-study comparisons. Recent systems, such as Gut Microbiome data repository (GMrepo) and other curated databases focused on gut or body sites, systematically categorize human microbiome data according to illnesses, host characteristics, and sample locations, therefore facilitating the rapid identification of microbial markers and supporting comprehensive microbiome research (Mitchell et al., 2020; Sengupta et al., 2023).

TABLE 15.1

Microbiome Database and Web Server Resource for Data Storage and Analysis

Database Name	Purpose	Link	Reference

Database Name	Purpose	Link	Reference
NCBI SRA (metagenome collections)	Primary archive for raw sequencing data, including 16S and shotgun metagenomes from diverse environments and hosts.	www.ncbi.nlm.nih.gov/sra	Katz et al. (2022)
MG-RAST	Public server for storage, quality control, annotation, and comparative analysis of metagenomic data; supports shotgun and amplicon datasets.	www.mg-rast.org	Katz et al. (2022)
MGnify (EBI)	EBI metagenomics resource providing analysis and taxonomic/functional annotation of amplicon and shotgun datasets; hosts public microbiome projects.	www.ebi.ac.uk/metagenomics	Mitchell et al. (2020)
IMG/M (Integrated Microbial Genomes and Microbiomes)	DOE-JGI system integrating genomes and microbiomes; supports functional and comparative analysis of metagenomic datasets.	https://img.jgi.doe.gov	Chen et al. (2021)

Database Name	Purpose	Link	Reference
Qiita	Platform for sharing, curation and analysis of microbiome studies, particularly 16S/ITS/shotgun data with rich sample metadata	https://qiita.ucsd.edu	Shi et al. (2019)
Human Microbiome Project DACC	Portal to all public HMP phase I/II data (16S, metagenomes, metatranscriptomes, metadata) across multiple body sites	www.hmpdacc.org	Creasy et al. (2021)
GMrepo	Gut microbiome database stratified by human phenotypes and diseases; integrates taxonomic profiles and phenotype information.	https://gmrepo.humangut.info	Wu et al. (2020)
mBodyMap	Curated database of microbes across human body sites with health/disease associations and abundance distributions.	http://mbodymap.microbiome.cloud	Jin et al. (2022)
HMDAD	Human Microbe–Disease Association Database; text-mined and curated microbe–disease relationships across body sites.	www.cuilab.cn/hmdad	Ma et al. (2017)

Database Name	Purpose	Link	Reference
Disbiome	Database of reported microbe–disease associations with strength estimates based on source credibility.	https://disbiome.ugent.be	Janssens et al. (2018)
MicroPhenoDB	Resource linking microbes to human diseases and phenotypes, integrating multiple data sources.	https://ngdc.cncb.ac.cn/databasecommons/database/id/7628	Yao et al. (2020)
Human Gut Microbiome Atlas	Atlas summarizing metagenome-wide association studies for 23 diseases, highlighting disease-susceptible species.	www.microbiomeatlas.org	Maghini et al. (2025)
MiMeDB (Microbial Metabolome Database)	Database of small-molecule metabolites from the human microbiome, linking metabolites, microbes, host, bioactivity, and exposure data.	https://mimedb.org	Wishart et al. (2023)
MicrobiomeAnalyst	Web platform for statistical and functional analysis of microbiome and metabolomics data, including integrative workflows.	www.microbiomeanalyst.ca	Lu et al. (2023)

Database Name	Purpose	Link	Reference
Meta2DB	Curated shotgun metagenomic and metadata database providing standardized taxonomic feature tables across many studies and diseases.	https://github.com (search "Meta2DB"; check exact project URL)	Lu et al. (2023)
MDB: Microbiome Database (CNGB)	Microbiome database organizing sequencing projects, samples and analysis resources for microbiome research.cngb	https://db.cngb.org/microbiome/	
Ocean Gene Atlas	Web service to explore gene biogeography in marine plankton metagenomes and metatranscriptomes.	https://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/	Vernette et al. (2022)
FoodMicroDB	Freely accessible database focused on food microbiome composition and temporal dynamics, organized by project and food/organism.	www.bic.ac.cn/FoodMicro/	Li et al. (2024)
Metapeptide databases (Sixgill)	Metaproteomic-oriented metapeptide databases generated from shotgun reads using Sixgill; provides peptide databases and example metagenomic datasets.	https://noble.gs.washington.edu/proj/metapeptide/	May et al. (2016)

Database Name	Purpose	Link	Reference
mBodyMap-linked disease databases (GMrepo, MVP)	MVP catalogues microbe–phage associations; often used with GMrepo and mBodyMap for integrated analyses.	(See MVP database homepage)	Jin et al. (2021)
SILVA	Comprehensive ribosomal RNA gene reference database widely used for 16S/18S taxonomic assignment in microbiome studies.	www.arb-silva.de	Quast et al. (2013)

15.4 Tools for Microbiome Data Analysis

15.4.1 Preprocessing and Quality Control

Microbiome analysis begins with raw sequencing reads or taxa tables organized by taxonomic levels, requiring thorough preprocessing and quality control to ensure accurate downstream interpretation. Depending on the sequencing method, 16S rRNA or shotgun metagenomics tools are used to perform key steps such as quality filtering, length trimming, and the removal of chimeras and contaminants, as shown in Table 15.2. Quality is typically assessed using Phred scores, while low-abundance or low-prevalence features are filtered to reduce noise (Ibrahimi et al., 2023).

TABLE 15.2
List of Tools for Metagenome Data Processing and Analysis ⏎

Tools	Purpose	Web Link	Reference
QIIME2	Comprehensive platform for preprocessing, diversity analysis, taxonomy assignment, and visualization.	https://qiime2.org/	Bolyen et al. (2019)

Tools	Purpose	Web Link	Reference
DADA2	High-resolution denoising and error-correction of amplicon data; often integrated into QIIME2 or R pipelines.	https://github.com/benjjneb/dada2	Callahan et al. (2016)
USEARCH/VSEARCH	Quality filtering, OTU clustering, chimera detection (VSEARCH is open source).	https://github.com/rcedgar/usearch_old_binaries/	Zhou et al. (2024)
Cutadapt	Adapter trimming and quality filtering.	https://github.com/marcelm/cutadapt/	Martin (2011)
Trimmomatic	General-purpose read trimming and quality control.	https://github.com/usadellab/Trimmomatic	Bolger et al. (2014)
FASTQC	Generates quality reports on raw reads.	www.bioinformatics.babraham.ac.uk/projects/fastqc/	Patel and Jain (2012)
MultiQC	Aggregates quality control reports across samples.	http://multiqc.info	Patel and Jain (2012)
BBTools (BBMap, BBDuk, BBMerge)	Filtering, adapter trimming, error correction, and merging paired end reads.	https://hpc.nih.gov/apps/bbtools.html (?)	Niazai et al. (2023)

15.4.2 Taxonomic Classification and Functional Annotation

Taxonomic classification of microbiota involves identifying microorganisms in a sample using sequencing approaches, such as 16S rRNA for bacteria, internal transcribed spacer (ITS) for fungi, or shotgun metagenomics for all microbes. These sequences are compared to reference databases such as SILVA, UNITE, or NCBI using tools like QIIME2, Kraken2, or MetaPhlAn (Table 15.3). This process reveals the composition and diversity of microbial communities, providing insight into their ecological or clinical roles.

TABLE 15.3

List of Tools for Taxonomic Classification and Function Annotation of Metagenome Data ⏎

Tools	Purpose	Web Link	Reference
Greengenes/SILVA/RDP	Reference databases for taxonomic classification.	http://ftp.microbio.me/greengenes_release/	McDonald et al. (2024)
Kraken2	Ultrafast taxonomic classification of metagenomic sequences.	https://benlangmead.github.io/aws-indexes/k2	Wood et al. (2019)
Centrifuge	Classification of metagenomic reads against large genome databases.	https://github.com/mourisl/centrifuger.	Song and Langmead (2024)
MetaPhlAn3	Taxonomic profiling using clade-specific marker genes (shotgun).	https://huttenhower.sph.harvard.edu/metaphlan3/	Blanco-Míguez et al. (2023)
Kaiju	Protein-level classification for metagenomic reads.	https://github.com/bioinformatics-centre/kaiju	Menzel et al. (2016)
PICRUSt2	Predicts metagenome functional content from 16S data.	https://github.com/picrust/picrust2	Aßhauer et al. (2015)
Tax4Fun	Functional profiling from 16S using KEGG orthologs.	http://tax4fun.gobics.de/	Pereira-Marques et al. (2024)
HUMAnN3	Functional and pathway profiling from shotgun metagenomics.	https://huttenhower.sph.harvard.edu/humann/	Beghini et al. (2021)

15.4.3 Diversity Analysis and Statistical Testing

Diversity analysis in microbiota studies assesses the variety and distribution of microbial species within (alpha diversity) and between (beta diversity) samples using metrics like Shannon index, Simpson index, and Bray-Curtis dissimilarity. These analyses help identify differences in microbial communities across conditions or treatments, which are often visualized with ordination methods such as principal coordinate analysis (PCoA) or non-metric multidimensional scaling (NMDS). Statistical testing, including permutational

multivariate analysis of variance (PERMANOVA), analysis of similarities (ANOSIM), and Kruskal–Wallis tests, is then applied to determine whether observed differences are statistically significant and biologically meaningful (Table 15.4).

TABLE 15.4
List of Tools for Statistical Analysis

Tools	Purpose	Web link	Reference
Phyloseq (R), Vegan (R), Microeco (R)	R package for ecological analysis of microbiome data. Ecological diversity metrics, PERMANOVA, ordinations (NMDS, PCoA). High-level functions for microbiome statistics.	https://joey711.github.io/phyloseq/ https://github.com/vegandevs/vegan	McMurdie and Holmes (2012)
LEfSe	Identifies differentially abundant taxa using LDA effect size.	https://huttenhower.sph.harvard.edu/lefse/	Segata et al. (2011)
ALDEx2	Compositional data analysis for differential abundance.	www.bioconductor.org/packages/release/bioc/ALDEx2.html	Gloor et al. (2024)
DESeq2/edgeR	Originally for RNA-seq, adapted for microbiome count data.	www.bioconductor.org/packages/release/bioc/DESeq2.html	Love et al. (2014)

15.4.4 Visualization

Visualization of microbiota data facilitates the interpretation of complex microbial community structures through charts and plots that represent taxonomic composition, diversity, and functional profiles. Common visualizations include bar plots and pie charts for relative abundance, heatmaps for taxonomic or functional clustering, and ordination plots like PCoA or NMDS for beta diversity analysis. Tools such as QIIME2, R (specifically the phyloseq and ggplot2 packages), and Python (specifically the matplotlib

and seaborn libraries) are widely used to create these informative and interactive visual outputs (Table 15.5).

TABLE 15.5
List of Tools for Data Visualization

Tools	Purpose	Web Link	Reference
QIIME2 View	Interactive result visualization via web	https://qiime2.org/ [2]	Bolyen et al. 2019
EMPeror	Ordination plot visualizations (e.g., PCoA).	http://qiime.org/emperor/installation_index.html [22]	Vázquez-Baeza et al. (2013)
ggplot2 (R)	General plotting for customized data visualization.	https://ggplot2.tidyverse.org/	Wickham (2011)
Krona	Hierarchical pie chart visualizations of taxa.	http://krona.sourceforge.net	Ondov et al. (2011)

15.4.5 Multi-Omics Integration Tools

Multi-omics studies integrate data from various biological layers, including genomics, transcriptomics, proteomics, metabolomics, and microbiomics, to provide a comprehensive view of biological systems. Tools and platforms such as Illumina and Oxford Nanopore generate genomic and transcriptomic data, while mass spectrometry (MS) and LC-MS/MS support proteomic and metabolomic profiling. Meanwhile metagenomic sequencing tools like QIIME2 and MetaPhlAn capture microbiome profiles. Integration platforms such as mixOmics, MOFA+, and MicrobiomeAnalyst facilitate combined analysis, enabling deeper insights into health, disease, or environmental interactions (Table 15.6).

TABLE 15.6
List of Tools for Omics Data Integration

Tools	Purpose	Web Link	Reference
mixOmics	Integrative analysis of multi-omics data.	www.mixOmics.org	(Rohart et al. 2017)
MOFA+	Multi-omics factor analysis for identifying shared patterns.	https://github.com/bioFAM/MOFA2	(Walter et al. 2023)

Tools	Purpose	Web Link	Reference
MicrobiomeAnalyst	Also supports multi-omics module.	www.microbiomeanalyst.ca/	(Lu et al. 2023)

15.5 Challenges in Microbiome Profiling

Microbiome analysis presents several experimental and computational challenges that can significantly impact the reliability and reproducibility of results. One of the foundational issues lies in study design and setup. Inadequate or inconsistent sample sizes can lead to misleading interpretations due to variations in microbial load, even among biologically similar replicates. This is especially problematic in clinical research, where confounding variables such as age, gender, diet, genotype, and housing conditions influence microbial composition and can mask biological signals if not properly controlled (Fritz et al., 2013; Martin et al., 2018). While cross-sectional studies are simpler to execute, they often fail to capture temporal microbial shifts, whereas longitudinal studies—though more informative—often lack robust downstream analysis methods (Fritz et al., 2013).

Sample collection and handling are critical yet vulnerable stages in microbiome studies. Variability in microbial biomass, such as lower levels in the skin compared to the gut, complicates DNA extraction and can skew sequencing outcomes (Salter et al., 2014). Contamination from environmental exposure or cross-sample mixing is a common concern, as are transportation and storage issues, which can alter microbial community structures if not immediately stabilized through freezing or chemical preservation (Dominianni et al., 2014; Lu et al., 2015).

The DNA extraction and library preparation steps also introduce challenges. Different extraction protocols yield variable DNA quality and quantity, which directly affects polymerase chain reaction (PCR) amplification and sequencing. Additionally, biases can be introduced through different library preparation kits and PCR-based workflows, influencing genome representation and functional prediction (Jones et al., 2015; Koren and Phillippy, 2015).

Sequencing technologies present another layer of complexity. Short-read platforms, such as Illumina, are high-throughput and cost-effective but limited in their ability to resolve complex or polyploid genomes due to their short read lengths (Nakano et al., 2017). Long-read technologies, such as PacBio and Oxford Nanopore, offer better resolution for complex or repetitive sequences but have traditionally been hindered by high error rates, more expensive instrumentation, and more stringent sample preparation requirements (Levy and Myers, 2016).

Computationally, microbiome data are vast and heterogeneous, making standardization of analysis a major hurdle. A lack of common data formats and inadequate metadata limits

reproducibility and complicates cross-study comparisons (Treangen and Salzberg, 2012). Software tools vary in usability and performance, and differing outputs from different tools can lead to conflicting interpretations, underscoring the need for consistent, open-source pipelines. In amplicon sequencing, distinguishing sequencing errors from true variants remains a challenge. Operational taxonomic unit (OTU)-based tools cluster reads by similarity but may reduce taxonomic resolution, while amplicon sequence variant (ASV)-based methods provide finer resolution but are more computationally demanding and prone to over-splitting (McIntyre et al., 2017).

Metagenomic sequencing, although broader in scope, presents difficulties in selecting the optimal tools for assembly, binning, and annotation due to the sheer number of available options. Assemblers often struggle with uneven coverage and repeat sequences, especially since many were not originally developed for metagenomic data (Westcott and Schloss, 2015). Binning and annotation are further complicated by shared sequences and variable genome coverage. Tools like MetaBAT and MaxBin employ different clustering methods, but even the best pipelines have limitations in assessing genome completeness and contamination (Zakrzewski et al., 2017).

15.6 Future Scope of Microbiome Profiling

The future landscape of microbiome profiling in clinical practice represents a paradigm shift toward precision medicine, where microbial communities serve as both diagnostic indicators and therapeutic targets. Recent advances in sequencing technologies, bioinformatics platforms, and our understanding of host–microbe interactions are converging to create unprecedented opportunities for clinical applications. The integration of microbiome data into routine healthcare promises to revolutionize disease prevention, diagnosis, and treatment across a wide range of medical specialties. As we move toward an era of personalized medicine, microbiome profiling stands at the forefront of innovative approaches that could fundamentally transform our understanding and management of human health. With the growing availability of high-performance metagenomic, metatranscriptomic, and metaproteomic technologies, microbiome research is evolving into a clinically actionable domain. For instance, recent studies have demonstrated that metaproteomic profiling of gut microbiota can identify protein biomarkers predictive of cardiovascular risk and treatment response in patients with chronic heart failure (Yang et al., 2025). Furthermore, multi-view statistical models now enable the integration of microbiome, metabolome, and transcriptome data to dissect the causal links between dysbiosis and diseases, such as IBD (Fang et al., 2024). These integrative approaches provide a more nuanced, systems-level understanding of host–microbiome interactions, facilitating diagnosis, prognosis, and therapeutic design.

15.6.1 Advanced Diagnostic and Biomarker Applications

Microbial biomarkers are emerging as valuable tools for disease detection and risk stratification. The gut microbiome, in particular, has shown promise due to its intimate connection with the host's immune and metabolic systems. Altered abundances of specific taxa have been linked to a range of pathologies—such as a decline in butyrate-producing bacteria in type 2 diabetes and an enrichment of *Fusobacterium nucleatum* and *Porphyromonas* species in colorectal cancer (CRC) (Poore et al., 2020; Wang et al., 2023). A 2024 study further classified subtypes of *F. nucleatum* with distinct virulence properties and associations with CRC progression and metastasis, suggesting their potential use in subtype-specific diagnostics and targeted therapies (Mehta et al., 2017; Sun et al., 2019). Beyond single-pathogen associations, recent research emphasizes the utility of complex microbial signatures, including co-metabolic relationships and functional modules, for multi-disease screening. Duvallet et al. (2017) demonstrated that combined microbial functional profiles could be used to detect conditions such as IBD, liver cirrhosis, and hepatocellular carcinoma with high accuracy. Such biomarkers are being increasingly integrated into predictive models powered by deep learning and transformer-based architectures (Yan et al., 2025), thereby greatly enhancing their diagnostic performance.

15.6.2 Multi-Disease Screening and Precision Diagnostics

Sophisticated machine learning models and statistical frameworks are now enabling the identification of microbial patterns across diseases, facilitating simultaneous screening for multiple diseases. Shi et al. (2025) developed a semi-parametric model capable of uncovering robust co-metabolism patterns in microbiota that correlate with disease states, further enhancing screening precision. The ability to assess multiple conditions using a single, non-invasive sample (e.g., stool or saliva) could drastically reduce diagnostic burden and healthcare costs while improving patient comfort and accessibility. In oncology, metagenome-informed cancer screening is gaining traction. Poore et al. (2020) demonstrated that tumor-associated microbial DNA from blood or tissue samples can be used to differentiate among multiple cancer types. Their approach is now being expanded in clinical studies with real-time validation, pushing microbiome-based liquid biopsies toward mainstream diagnostics.

15.6.3 Technological Advancements and Culture-Free Profiling Platforms

The integration of NGS has enabled comprehensive and high-resolution microbial profiling directly from clinical samples. Novel culture-free platforms, validated in recent studies (Benoit et al., 2024; Wang et al., 2023), combine automated sequencing pipelines with user-friendly, web-based interfaces for real-time clinical interpretation. These systems can detect both viable and non-culturable pathogens, improving sensitivity for fastidious organisms

and supporting more targeted antibiotic therapy. Moreover, automated interpretation and visualization of data through machine learning are making microbiome insights more accessible to clinicians. By simplifying complex bioinformatics analyses, these tools accelerate clinical translation and enable faster decision-making.

15.6.4 Multi-Omics Integration and Functional Insight

Microbiome-based diagnostics are moving beyond taxonomic identification to include functional and transcriptional insights through metatranscriptomics and metaproteomics. This approach enables researchers to analyze gene expression and protein activity in situ, providing dynamic snapshots of microbial behavior in both healthy and diseased states (Franzosa et al., 2019). More recently, Muller et al. (2024) emphasized the power of multi-omics to predict disease onset and therapeutic response. Functional characterization of the microbiome is essential for identifying drug-microbiome interactions. Recent evidence demonstrates that the gut microbiota metabolizes more than 150 U.S. Food and Drug Administration (FDA)-approved pharmaceuticals, with direct implications for drug efficacy and toxicity profiles. Consequently, microbiome profiling enables precision medicine approaches by informing drug selection, dosage optimization, and personalized treatment strategies (Zimmermann et al., 2019).

15.6.5 Therapeutic Applications and Clinical Implementation

Beyond diagnosis, microbiome profiling is increasingly being applied in therapeutic contexts. Engineered microbial consortia have demonstrated clinical promise in treating recurrent *Clostridioides difficile* infection through synthetic bacteriotherapy (Liu et al., 2023; Vazquez-Castellanos et al., 2019). Precision probiotics and postbiotics tailored to individual microbial signatures are under active development, with several now undergoing clinical trials (Singh et al., 2023). In the realm of personalized medicine, microbiome-informed interventions are being trialed in metabolic syndrome, IBD, and neuropsychiatric conditions. Future therapies may include CRISPR-engineered microbes, microbiome-encoded drug delivery systems, or microbiome-guided dietary prescriptions.

15.6.6 Challenges and Future Directions

Despite these advances, challenges remain. Technical limitations—such as a lack of strain-level resolution, inconsistencies in sample processing, and the absence of global clinical standards—impede widespread clinical adoption. Furthermore, distinguishing correlation from causation in microbiome–disease relationships requires longitudinal studies and mechanistic experiments.

Future research should focus on:

- Longitudinal cohort tracking to understand temporal dynamics;
- Functional validation of microbial biomarkers;
- Standardized pipelines for data integration and clinical interpretation; and
- Ethical frameworks for personalized microbiome data usage.

15.7 Conclusion

The integration of advanced bioinformatics pipelines and computational tools has enabled researchers to carry out comprehensive investigations of the classification and function of microbial communities. Microbiome profiling has revolutionized biological research and therapeutic treatment. As this chapter has demonstrated, microbial communities inhabiting a range of anatomical niches, including the gut, oral cavity, skin, respiratory tract, and urogenital system, play a critical role in maintaining homeostasis, regulating immunological responses, facilitating metabolic processes, and even influencing neurocognitive processes. Since traditional culture-based microbiology gave way to high-throughput sequencing methods such as 16S rRNA sequencing, shotgun metagenomics, metatranscriptomics, and metabolomics, our understanding of these complex ecosystems has significantly expanded. The integration of advanced bioinformatics pipelines and computational tools has enabled researchers to conduct comprehensive investigations into the classification and function of microbial communities. Publicly available repositories, tools, and web servers, such as the Human Microbiome Project (HMP), MG-RAST, and Qiita, as well as computational tools like QIIME2, MetaPhlAn, and HUMAnN, have contributed to data collection and the development of reproducible methods. Furthermore, the recent development of multi-omics integration technologies, such as mixOmics and MOFA+, has significantly enhanced our ability to identify predictive biomarkers and gain mechanistic insights by facilitating the connection between microbial signals and host transcriptomic and metabolomic changes.

References

Aristizábal, B., González, Á., 2013. Innate immune system, in: *Autoimmunity: From Bench to Bedside* [Internet]. El Rosario University Press. ⏎

Aßhauer, K.P., Wemheuer, B., Daniel, R., Meinicke, P., 2015. Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31, 2882–2884. https://doi.org/10.1093/bioinformatics/btv287 ⏎

Baker, P., Huang, C., Radi, R., Moll, S.B., Jules, E., Arbiser, J.L., 2023. Skin barrier function: The interplay of physical, chemical, and immunologic properties. *Cells* 12, 2745. https://doi.org/10.3390/cells12232745 ⏎

Bay, L., Jemec, G.B., Ring, H.C., 2024. Microenvironmental host–microbe interactions in chronic inflammatory skin diseases. *APMIS* 132, 974–984. https://doi.org/10.1111/apm.13464 ⏎

Beghini, F., McIver, L.J., Blanco-Míguez, A., Dubois, L., Asnicar, F., Maharjan, S., Mailyan, A., Manghi, P., Scholz, M., Thomas, A.M., Valles-Colomer, M., Weingart, G., Zhang, Y., Zolfo, M., Huttenhower, C., Franzosa, E.A., Segata, N., 2021. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife* 10, e65088. https://doi.org/10.7554/eLife.65088 ⏎

Benoit, P., Brazer, N., de Lorenzi-Tognon, M., Kelly, E., Servellita, V., Oseguera, M., Nguyen, J., Tang, J., Omura, C., Streithorst, J., Hillberg, M., Ingebrigtsen, D., Zorn, K., Wilson, M.R., Blicharz, T., Wong, A.P., O'Donovan, B., Murray, B., Miller, S., Chiu, C.Y., 2024. Seven-year performance of a clinical metagenomic next-generation sequencing test for diagnosis of central nervous system infections. *Nature Medicine* 30, 3522–3533. https://doi.org/10.1038/s41591-024-03275-1 ⏎

Blanco-Míguez, A., Beghini, F., Cumbo, F., McIver, L.J., Thompson, K.N., Zolfo, M., Manghi, P., Dubois, L., Huang, K.D., Thomas, A.M., Nickols, W.A., Piccinno, G., Piperni, E., Punčochář, M., Valles-Colomer, M., Tett, A., Giordano, F., Davies, R., Wolf, J., Berry, S.E., Spector, T.D., Franzosa, E.A., Pasolli, E., Asnicar, F., Huttenhower, C., Segata, N., 2023. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology* 41, 1633–1644. https://doi.org/10.1038/s41587-023-01688-w ⏎

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170 ⏎

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft,

J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9 ⏎

Bowen, W.H., Koo, H., 2011. Biology of *Streptococcus mutans*-derived glucosyltransferases: Role in extracellular matrix formation of cariogenic biofilms. *Caries Research* 45, 69–86. https://doi.org/10.1159/000324598 ⏎

Brooks, S.G., Mahmoud, R.H., Lin, R.R., Fluhr, J.W., Yosipovitch, G., 2025. The Skin acid mantle: An update on skin pH. *Journal of Investigative Dermatology* 145, 509–521. https://doi.org/10.1016/j.jid.2024.07.009 ⏎

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13, 581–583. https://doi.org/10.1038/nmeth.3869 ⏎

Chen, G., Chen, C., Lei, Z., 2017. Meta-omics insights in the microbial community profiling and functional characterization of fermented foods. *Trends in Food Science & Technology* 65, 23–31. https://doi.org/10.1016/j.tifs.2017.05.002 ⏎

Chen, I.-M.A., Chu, K., Palaniappan, K., Ratner, A., Huang, J., Huntemann, M., Hajek, P., Ritter, S., Varghese, N., Seshadri, R., Roux, S., Woyke, T., Eloe-Fadrosh, E.A., Ivanova, N.N., Kyrpides, N.C., 2021. The IMG/M data management and analysis system v.6.0: New tools and advanced capabilities. *Nucleic Acids Research* 49, D751–D763. https://doi.org/10.1093/nar/gkaa939 ⏎

Chen, X., 2025. The dual role of skin microbiome modulation in precision care for atopic dermatitis: A review. *Health Sciences Review* 17, 100245. https://doi.org/10.1016/j.hsr.2025.100245 ⏎

Choudhari, J.K., Choubey, J., Verma, M.K., Chatterjee, T., Sahariah, B.P., 2022. Metagenomics: The boon for microbial world knowledge and current challenges, in: *Bioinformatics: Methods and Applications*. Academic Press/Elsevier, pp. 159–175. https://doi.org/10.1016/B978-0-323-89775-4.00022-5 ⏎

Creasy, H.H., Felix, V., Aluvathingal, J., Crabtree, J., Ifeonu, O., Matsumura, J., McCracken, C., Nickel, L., Orvis, J., Schor, M., Giglio, M., Mahurkar, A., White, O., 2021. HMPDACC: A human microbiome project multi-omic data resource. *Nucleic Acids Research* 49, D734–D742. https://doi.org/10.1093/nar/gkaa996 ⏎

Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Review Neuroscience* 13, 701–712. https://doi.org/10.1038/nrn3346 ⏎

Dai, D., Rhoads, W.J., Edwards, M.A., Pruden, A., 2018. Shotgun metagenomics reveals taxonomic and functional shifts in hot water microbiome due to temperature setting and stagnation. *Frontiers in Microbiology* 9. https://doi.org/10.3389/fmicb.2018.02695 ⏎

De Almeida, C.V., Antiga, E., Lulli, M., 2023. Oral and topical probiotics and postbiotics in skincare and dermatological therapy: A concise review. *Microorganisms* 11, 1420. https://doi.org/10.3390/microorganisms11061420 ⏎

De Angelis, M., Calasso, M., 2014. MOLECULAR BIOLOGY | Proteomics, in: Batt, C.A., Tortorello, M.L. (Eds.), *Encyclopedia of Food Microbiology* (Second Edition). Academic Press, Oxford, pp. 793–802. https://doi.org/10.1016/B978-0-12-384730-0.00376-1 ⏎

Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L.R., Scaldaferri, F., 2024. Gut microbiota, intestinal permeability, and systemic inflammation: A narrative review. *Internal and Emergency Medicine* 19, 275–293. https://doi.org/10.1007/s11739-023-03374-w ⏎

Dominianni, C., Wu, J., Hayes, R.B., Ahn, J., 2014. Comparison of methods for fecal microbiome biospecimen collection. *BMC Microbiology* 14, 103. https://doi.org/10.1186/1471-2180-14-103 ⏎

Duvallet, C., Gibbons, S.M., Gurry, T., Irizarry, R.A., Alm, E.J., 2017. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nat Commun* 8, 1784. https://doi.org/10.1038/s41467-017-01973-8 ⏎

Edlund, A., Santiago-Rodriguez, T.M., Boehm, T.K., Pride, D.T., 2015. Bacteriophage and their potential roles in the human oral cavity. *Journal of Oral Microbiology* 7, 27423. https://doi.org/10.3402/jom.v7.27423 ⏎

Fang, L., Wang, Y., Ye, C., 2024. Integration of multiview microbiome data for deciphering microbiome–metabolome–disease pathways. *arXiv:2402.08222*. https://doi.org/10.48550/arXiv.2402.08222 ⏎

Flowers, L., Grice, E.A., 2020. The skin microbiota: Balancing risk and reward. *Cell Host Microbe* 28, 190–200. https://doi.org/10.1016/j.chom.2020.06.017 ⏎

Franzosa, E.A., Sirota-Madi, A., Avila-Pacheco, J., Fornelos, N., Haiser, H.J., Reinker, S., Vatanen, T., Hall, A.B., Mallick, H., McIver, L.J., Sauk, J.S., Wilson, R.G., Stevens, B.W., Scott, J.M., Pierce, K., Deik, A.A., Bullock, K., Imhann, F., Porter, J., Zhernakova, A., Fu, J., Weersma, R.K., Wijmenga, C., Clish, C.B., Vlamakis, H., Huttenhower, C., Xavier, R.J., 2019. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nature Microbiology* 4, 293–305. https://doi.org/10.1038/s41564-018-0306-4 ⏎

Fritz, J.V., Desai, M.S., Shah, P., Schneider, J.G., Wilmes, P., 2013. From meta-omics to causality: Experimental models for human microbiome research. *Microbiome* 1, 14. https://doi.org/10.1186/2049-2618-1-14 ⏎

Gao, Z., Chen, X., Wang, C., Song, J., Xu, J., Liu, X., Qian, Y., Suo, H., 2024. New strategies and mechanisms for targeting *Streptococcus mutans* biofilm formation to prevent dental caries: A review. *Microbiological Research* 278, 127526. https://doi.org/10.1016/j.micres.2023.127526 ⏎

Gebrayel, P., Nicco, C., Al Khodor, S., Bilinski, J., Caselli, E., Comelli, E.M., Egert, M., Giaroni, C., Karpinski, T.M., Loniewski, I., Mulak, A., Reygner, J., Samczuk, P., Serino, M., Sikora, M., Terranegra, A., Ufnal, M., Villeger, R., Pichon, C., Konturek, P., Edeas, M., 2022. Microbiota medicine: Towards clinical revolution. *Journal of Translational Medicine* 20, 111. https://doi.org/10.1186/s12967-022-03296-9 ⏎

Gloor, G.B., Nixon, M.P., Silverman, J.D., 2024. Explicit scale simulation for analysis of RNA-sequencing with ALDEx2. *bioRxiv*. https://doi.org/10.1101/2023.10.21.563431 ⏎

Hooper, L.V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. *Science* 336, 1268–1273. https://doi.org/10.1126/science.1223490 ⏎

Ibrahimi, E., Lopes, M.B., Dhamo, X., Simeon, A., Shigdel, R., Hron, K., Stres, B., D'Elia, D., Berland, M., Marcos-Zambrano, L.J., 2023. Overview of data preprocessing for machine learning applications in human microbiome research. *Frontiers in Microbiology* 14. https://doi.org/10.3389/fmicb.2023.1250909 ⏎

Janda, J.M., Abbott, S.L., 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology* 45, 2761–2764. https://doi.org/10.1128/JCM.01228-07 ⏎

Janssens, Y., Nielandt, J., Bronselaer, A., Debunne, N., Verbeke, F., Wynendaele, E., Van Immerseel, F., Vandewynckel, Y.-P., De Tré, G., De Spiegeleer, B., 2018. Disbiome database: Linking the microbiome to disease. *BMC Microbiology* 18, 50. https://doi.org/10.1186/s12866-018-1197-5 ⏎

Jin, H., Hu, G., Sun, C., Duan, Y., Zhang, Z., Liu, Z., Zhao, X.-M., Chen, W.-H., 2022. mBodyMap: A curated database for microbes across human body and their associations with health and diseases. *Nucleic Acids Research* 50, D808–D816. https://doi.org/10.1093/nar/gkab973 ⏎

Jones, M.B., Highlander, S.K., Anderson, E.L., Li, W., Dayrit, M., Klitgord, N., Fabani, M.M., Seguritan, V., Green, J., Pride, D.T., Yooseph, S., Biggs, W., Nelson, K.E., Venter, J.C., 2015. Library preparation methodology can influence genomic and functional predictions in human microbiome research. *Proceedings of the National Academy of Sciences United States of America* 112, 14024–14029. https://doi.org/10.1073/pnas.1519288112 ⏎

Kaakoush, N.O., 2020. The microbiome in oesophageal disease—A year in review. *Microbiota in Health and Disease* 2, e310. ⏎

Karim, N., Phinney, B.S., Salemi, M., Wu, P.-W., Naeem, M., Rice, R.H., 2019. Human stratum corneum proteomics reveals cross-linking of a broad spectrum of proteins in cornified envelopes. *Experimental Dermatology* 28, 618–622. https://doi.org/10.1111/exd.13925 ⏎

Katz, K., Shutov, O., Lapoint, R., Kimelman, M., Brister, J.R., O'Sullivan, C., 2022. The Sequence Read Archive: A decade more of explosive growth. *Nucleic Acids Research*

50, D387–D390. https://doi.org/10.1093/nar/gkab1053 ⏎

Kenshi, Y., Richard, L.G., 2008. Antimicrobial peptides in human skin disease. *European Journal of Dermatology* 18, 11–21. https://doi.org/10.1684/ejd.2008.0304 ⏎

Kim, E., Kim, S., Nam, G.W., Lee, H., Moon, S., Chang, I., 2009. The alkaline pH-adapted skin barrier is disrupted severely by SLS-induced irritation. *International Journal of Cosmetic Science* 31, 263–269. https://doi.org/10.1111/j.1468-2494.2009.00491.x ⏎

Koo, H., Falsetta, M.L., Klein, M.I., 2013. The exopolysaccharide matrix: A virulence determinant of cariogenic biofilm. *Journal of Dental Research* 92, 1065–1073. https://doi.org/10.1177/0022034513504218 ⏎

Koo, H., Xiao, J., Klein, M.I., Jeon, J.G., 2010. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *Journal of Bacteriology* 192, 3024–3032. https://doi.org/10.1128/JB.01649-09 ⏎

Koren, S., Phillippy, A.M., 2015. One chromosome, one contig: Complete microbial genomes from long-read sequencing and assembly. *Current Opinion in Microbiology, Host–Microbe Interactions: Bacteria • Genomics* 23, 110–120. https://doi.org/10.1016/j.mib.2014.11.014 ⏎

Kreouzi, M., Theodorakis, N., Nikolaou, M., Feretzakis, G., Anastasiou, A., Kalodanis, K., Sakagianni, A., 2025. Skin microbiota: Mediator of interactions between metabolic disorders and cutaneous health and disease. *Microorganisms* 13, 161. https://doi.org/10.3390/microorganisms13010161 ⏎

Lemos, J.A., Burne, R.A., 2008. A model of efficiency: Stress tolerance by *Streptococcus mutans*. *Microbiology (Reading)* 154, 3247–3255. https://doi.org/10.1099/mic.0.2008/023770-0 ⏎

Lemos, J.A., Palmer, S.R., Zeng, L., Wen, Z.T., Kajfasz, J.K., Freires, I.A., Abranches, J., Brady, L.J., 2019. The biology of *Streptococcus mutans*. *Microbiology Spectrum* 7. https://doi.org/10.1128/microbiolspec.gpp3-0051-2018 ⏎

Levy, S.E., Myers, R.M., 2016. Advancements in next-generation sequencing. *Annual Review of Genomics and Human Genetics* 17, 95–115. https://doi.org/10.1146/annurev-genom-083115-022413 ⏎

Li, X., Liu, Y., Yang, X., Li, C., Song, Z., 2022. The oral microbiota: Community composition, influencing factors, pathogenesis, and interventions. *Frontiers in Microbiology* 13. https://doi.org/10.3389/fmicb.2022.895537 ⏎

Li, Y., Lyu, H., Yang, H., Ju, Z., Ma, C., Hou, H., Wang, Y., Zhou, Y., Gao, Y., Yang, J., Xu, S., Bai, D., Luo, H., Yousuf, S., Zhang, T., Xun, J., Zeng, M., Qi, H., Chen, T., Liu, Y.-X., 2024. FoodMicroDB: A microbiome database for composition and time-series research in food. *iMetaOmics* 1, e40. https://doi.org/10.1002/imo2.40 ⏎

Liu, J., Zhu, W., Lessing, D.J., Chu, W., 2023. Synthetic microbial consortia for the treatment of *Clostridioides difficile* infection in mice model. *Microbial Biotechnology*

16, 1985–2006. https://doi.org/10.1111/1751-7915.14333 ⏎

Lloyd-Price, J., Arze, C., Ananthakrishnan, A.N., Schirmer, M., Avila-Pacheco, J., Poon, T.W., Andrews, E., Ajami, N.J., Bonham, K.S., Brislawn, C.J., Casero, D., Courtney, H., Gonzalez, A., Graeber, T.G., Hall, A.B., Lake, K., Landers, C.J., Mallick, H., Plichta, D.R., Prasad, M., Rahnavard, G., Sauk, J., Shungin, D., Vázquez-Baeza, Y., White, R.A., IBDMDB Investigators, Braun, J., Denson, L.A., Jansson, J.K., Knight, R., Kugathasan, S., McGovern, D.P.B., Petrosino, J.F., Stappenbeck, T.S., Winter, H.S., Clish, C.B., Franzosa, E.A., Vlamakis, H., Xavier, R.J., Huttenhower, C., 2019. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 569, 655–662. https://doi.org/10.1038/s41586-019-1237-9 ⏎

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15, 550. https://doi.org/10.1186/s13059-014-0550-8 ⏎

Lu, Y., Hugenholtz, P., Batstone, D.J., 2015. Evaluating DNA extraction methods for community profiling of pig hindgut microbial community. *PLoS One* 10, e0142720. https://doi.org/10.1371/journal.pone.0142720 ⏎

Lu, Y., Zhou, G., Ewald, J., Pang, Z., Shiri, T., Xia, J., 2023. MicrobiomeAnalyst 2.0: Comprehensive statistical, functional and integrative analysis of microbiome data. *Nucleic Acids Research* 51, W310–W318. https://doi.org/10.1093/nar/gkad407 ⏎

Ma, J., Piao, X., Mahfuz, S., Long, S., Wang, J., 2022. The interaction among gut microbes, the intestinal barrier and short chain fatty acids. *Animal Nutrition* 9, 159–174. https://doi.org/10.1016/j.aninu.2021.09.012 ⏎

Ma, W., Zhang, L., Zeng, P., Huang, C., Li, J., Geng, B., Yang, J., Kong, W., Zhou, X., Cui, Q., 2017. An analysis of human microbe–disease associations. *Brief Bioinform* 18, 85–97. https://doi.org/10.1093/bib/bbw005 ⏎

Maghini, D.G., Oduaran, O.H., Olubayo, L.A.I., Cook, J.A., Smyth, N., Mathema, T., Belger, C.W., Agongo, G., Boua, P.R., Choma, S.S.R., Gómez-Olivé, F.X., Kisiangani, I., Mashaba, G.R., Micklesfield, L., Mohamed, S.F., Nonterah, E.A., Norris, S., Sorgho, H., Tollman, S., Wafawanaka, F., Tluway, F., Ramsay, M., Wirbel, J., Bhatt, A.S., Hazelhurst, S., 2025. Expanding the human gut microbiome atlas of Africa. *Nature* 638, 718–728. https://doi.org/10.1038/s41586-024-08485-8 ⏎

Mahmud, Md.R., Akter, Sharmin, Tamanna, S.K., Mazumder, L., Esti, I.Z., Banerjee, S., Akter, Sumona, Hasan, Md.R., Acharjee, M., Hossain, Md.S., Pirttilä, A.M., 2022. Impact of gut microbiome on skin health: Gut–skin axis observed through the lenses of therapeutics and skin diseases. *Gut Microbes* 14, 2096995. https://doi.org/10.1080/19490976.2022.2096995 ⏎

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17, 10–12. https://doi.org/10.14806/ej.17.1.200 ⏎

Martin, T.C., Visconti, A., Spector, T.D., Falchi, M., 2018. Conducting metagenomic studies in microbiology and clinical research. *Applied Microbiology Biotechnology* 102, 8629–8646. https://doi.org/10.1007/s00253-018-9209-9 ⏎

May DH, Timmins-Schiffman E, Mikan MP, Harvey HR, Borenstein E, Nunn BL, Noble WS. An Alignment-Free "Metapeptide" Strategy for Metaproteomic Characterization of Microbiome Samples Using Shotgun Metagenomic Sequencing. *J Proteome Res*. 2016 Aug 5;15(8):2697–705. doi: 10.1021/acs.jproteome.6b00239.

Mayer, E.A., Knight, R., Mazmanian, S.K., Cryan, J.F., Tillisch, K., 2014. Gut microbes and the brain: Paradigm shift in neuroscience. *Journal of Neuroscience* 34, 15490–15496. https://doi.org/10.1523/JNEUROSCI.3299-14.2014 ⏎

McDonald, D., Jiang, Y., Balaban, M., Cantrell, K., Zhu, Q., Gonzalez, A., Morton, J.T., Nicolaou, G., Parks, D.H., Karst, S.M., Albertsen, M., Hugenholtz, P., DeSantis, T., Song, S.J., Bartko, A., Havulinna, A.S., Jousilahti, P., Cheng, S., Inouye, M., Niiranen, T., Jain, M., Salomaa, V., Lahti, L., Mirarab, S., Knight, R., 2024. Greengenes2 unifies microbial data in a single reference tree. *Nature Biotechnology* 42, 715–718. https://doi.org/10.1038/s41587-023-01845-1 ⏎

McIntyre, A.B.R., Ounit, R., Afshinnekoo, E., Prill, R.J., Hénaff, E., Alexander, N., Minot, S.S., Danko, D., Foox, J., Ahsanuddin, S., Tighe, S., Hasan, N.A., Subramanian, P., Moffat, K., Levy, S., Lonardi, S., Greenfield, N., Colwell, R.R., Rosen, G.L., Mason, C.E., 2017. Comprehensive benchmarking and ensemble approaches for metagenomic classifiers. *Genome Biology* 18, 182. https://doi.org/10.1186/s13059-017-1299-7 ⏎

McMurdie, P.J., Holmes, S., 2012. Phyloseq: A bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. *Pacific Symposium on Biocomputing* 235–246. ⏎

Mehta, R.S., Nishihara, R., Cao, Y., Song, M., Mima, K., Qian, Z.R., Nowak, J.A., Kosumi, K., Hamada, T., Masugi, Y., 2017. Association of dietary patterns with risk of colorectal cancer subtypes classified by *Fusobacterium nucleatum* in tumor tissue. *JAMA Oncology* 3, 921–927. ⏎

Menzel, P., Ng, K.L., Krogh, A., 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nature Communications* 7, 11257. https://doi.org/10.1038/ncomms11257 ⏎

Mitchell, A.L., Almeida, A., Beracochea, M., Boland, M., Burgin, J., Cochrane, G., Crusoe, M.R., Kale, V., Potter, S.C., Richardson, L.J., Sakharova, E., Scheremetjew, M., Korobeynikov, A., Shlemov, A., Kunyavskaya, O., Lapidus, A., Finn, R.D., 2020. MGnify: The microbiome analysis resource in 2020. *Nucleic Acids Research* 48, D570–D578. https://doi.org/10.1093/nar/gkz1035 ⏎

Muller, E., Shiryan, I., Borenstein, E., 2024. Multi-omic integration of microbiome data for identifying disease-associated modules. *Nature Communications* 15, 2621. ⏎

Murray, P.A., Winkler, J.R., Peros, W.J., French, C.K., Lippke, J.A., 1991. DNA probe detection of periodontal pathogens in HIV-associated periodontal lesions. *Oral Microbiology and Immunology* 6, 34–40. https://doi.org/10.1111/j.1399-302x.1991.tb00449.x ⏎

Nakano, K., Shiroma, A., Shimoji, M., Tamotsu, H., Ashimine, N., Ohki, S., Shinzato, M., Minami, M., Nakanishi, T., Teruya, K., Satou, K., Hirano, T., 2017. Advantages of genome sequencing by long-read sequencer using SMRT technology in medical area. *Human Cell* 30, 149–161. https://doi.org/10.1007/s13577-017-0168-8 ⏎

Naureen, Z., Dautaj, A., Anpilogov, K., Camilleri, G., Dhuli, K., Tanzi, B., Maltese, P.E., Cristofoli, F., De Antoni, L., Beccari, T., Dundar, M., Bertelli, M., 2020. Bacteriophages presence in nature and their role in the natural selection of bacterial populations. *Acta Biomedica* 91, e2020024. https://doi.org/10.23750/abm.v91i13-S.10819 ⏎

Niazai, S., Rahimzai, A.A., Atifnigar, H., 2023. Applications of MATLAB in natural sciences: A comprehensive review. *European Journal of Theoretical and Applied Sciences* 1, 1006–1015. https://doi.org/10.59324/ejtas.2023.1(5).87 ⏎

Ondov, B.D., Bergman, N.H., Phillippy, A.M., 2011. Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics* 12, 385. https://doi.org/10.1186/1471-2105-12-385 ⏎

O'Riordan, K.J., Collins, M.K., Moloney, G.M., Knox, E.G., Aburto, M.R., Fülling, C., Morley, S.J., Clarke, G., Schellekens, H., Cryan, J.F., 2022. Short chain fatty acids: Microbial metabolites for gut–brain axis signalling. *Molecular and Cellular Endocrinology* 546, 111572. https://doi.org/10.1016/j.mce.2022.111572 ⏎

Patel, R.K., Jain, M., 2012. NGS QC Toolkit: A toolkit for quality control of next generation sequencing data. *PLoS One* 7, e30619. https://doi.org/10.1371/journal.pone.0030619 ⏎

Pereira-Marques, J., Ferreira, R.M., Figueiredo, C., 2024. A metatranscriptomics strategy for efficient characterization of the microbiome in human tissues with low microbial biomass. *Gut Microbes* 16, 2323235. https://doi.org/10.1080/19490976.2024.2323235 ⏎

Poore, G.D., Kopylova, E., Zhu, Q., Carpenter, C., Fraraccio, S., Wandro, S., Kosciolek, T., Janssen, S., Metcalf, J., Song, S.J., 2020. RETRACTED ARTICLE: Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature* 579, 567–574. ⏎

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* 41, D590–D596. https://doi.org/10.1093/nar/gks1219 ⏎

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology* 35, 833–844. https://doi.org/10.1038/nbt.3935 ⏎

Rajasekaran, J.J., Krishnamurthy, H.K., Bosco, J., Jayaraman, V., Krishna, K., Wang, T., Bei, K., 2024. Oral microbiome: A review of its impact on oral and systemic health. *Microorganisms* 12, 1797. https://doi.org/10.3390/microorganisms12091797 ⏎

Reichardt, E., Shyp, V., Alig, L., Verna, C., Kulik, E.M., Bornstein, M.M., 2024. Antimicrobial effect of probiotic bacteriocins on *Streptococcus* mutans biofilm in a dynamic oral flow chamber model—An in vitro study. *Journal of Oral Microbiology* 16, 2304971. https://doi.org/10.1080/20002297.2024.2304971 ⏎

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology* 12, 87. https://doi.org/10.1186/s12915-014-0087-z ⏎

Santacroce, L., Passarelli, P.C., Azzolino, D., Bottalico, L., Charitos, I.A., Cazzolla, A.P., Colella, M., Topi, S., Godoy, F.G., D'Addona, A., 2023. Oral microbiota in human health and disease: A perspective. *Experimental Biology and Medicine (Maywood)* 248, 1288–1301. https://doi.org/10.1177/15353702231187645 ⏎

Sanz, Y., Cryan, J.F., Deschasaux-Tanguy, M., Elinav, E., Lambrecht, R., Veiga, P., 2025. The gut microbiome connects nutrition and human health. *Nature Reviews Gastroenterology and Hepatology* 1–22. https://doi.org/10.1038/s41575-025-01077-5 ⏎

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biology* 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60 ⏎

Sengupta, P., Sivabalan, S.K.M., Mahesh, A., Palanikumar, I., Kuppa Baskaran, D.K., Raman, K., 2023. Big Data for a small world: A review on databases and resources for studying microbiomes. *Journal of the Indian Institute of Science* 103, 891–907. https://doi.org/10.1007/s41745-023-00370-z ⏎

Shi, H., Wang, Y., Cheng, D., 2025. Microbial correlation: A semi-parametric model for investigating microbial co-metabolism. *arXiv:2504.05450*. https://doi.org/10.48550/arXiv.2504.05450 ⏎

Shi, W., Qi, H., Sun, Q., Fan, G., Liu, S., Wang, J., Zhu, B., Liu, H., Zhao, F., Wang, X., Hu, X., Li, W., Liu, J., Tian, Y., Wu, L., Ma, J., 2019. gcMeta: A Global Catalogue of Metagenomics platform to support the archiving, standardization and analysis of microbiome data. *Nucleic Acids Research* 47, D637–D648. https://doi.org/10.1093/nar/gky1008 ⏎

Singh, A., Alexander, S.G., Martin, S., 2023. Gut microbiome homeostasis and the future of probiotics in cancer immunotherapy. *Frontiers in Immunology* 14, 1114499. ⏎

Song, L., Langmead, B., 2024. Centrifuger: Lossless compression of microbial genomes for efficient and accurate metagenomic sequence classification. *Genome Biology* 25, 106. https://doi.org/10.1186/s13059-024-03244-4 ⏎

Sun, C., Li, B., Wang, B., Zhao, J., Zhang, X., Li, T., Li, W., Tang, D., Qiu, M., Wang, X., Zhu, C., Qian, Z., 2019. The role of *Fusobacterium nucleatum* in colorectal cancer: From carcinogenesis to clinical management. *Chronic Diseases and Translational Medicine* 5, 178–187. https://doi.org/10.1016/j.cdtm.2019.09.001 ⏎

Suri, Hitakshika, Suri, Harshika, Nagda, N., Misra, T., Vuppu, S., 2025. Current perspectives on the human skin microbiome: Functional insights and strategies for therapeutic modulation. *Biomedicine & Pharmacotherapy* 193, 118655. https://doi.org/10.1016/j.biopha.2025.118655 ⏎

Treangen, T.J., Salzberg, S.L., 2012. Repetitive DNA and next-generation sequencing: Computational challenges and solutions. *Nature Reviews Genetics* 13, 36–46. https://doi.org/10.1038/nrg3117 ⏎

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I., 2007. The human microbiome project. *Nature* 449, 804–810. https://doi.org/10.1038/nature06244 ⏎

Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPeror: A tool for visualizing high-throughput microbial community data. *Gigascience* 2, 2047-217X-2-16. https://doi.org/10.1186/2047-217X-2-16 ⏎

Vazquez-Castellanos, J.F., Biclot, A., Vrancken, G., Huys, G.R., Raes, J., 2019. Design of synthetic microbial consortia for gut microbiota modulation. *Current Opinion in Pharmacology* 49, 52–59. ⏎

Villar, E., Vannier, T., Vernette, C., Lescot, M., Cuenca, M., Alexandre, A., Bachelerie, P., Rosnet, T., Pelletier, E., Sunagawa, S., Hingamp, P., 2018. The Ocean Gene Atlas: Exploring the biogeography of plankton genes online. *Nucleic Acids Research* 46, W289–W295. https://doi.org/10.1093/nar/gky376

Wagner, J., Handley, A., Donato, C.M., Lyons, E.A., Pavlic, D., Ong, D.S., Bonnici, R., Bogdanovic-Sakran, N., Parker, E.P.K., Bronowski, C., Thobari, J.A., Satria, C.D., Nirwati, H., Witte, D., Jere, K.C., Mpakiza, A., Watts, E., Turner, A., Boniface, K., Mandolo, J., Justice, F., Bar-Zeev, N., Iturriza-Gomara, M., Buttery, J.P., Cunliffe, N.A., Soenarto, Y., Bines, J.E., 2025. Early-life gut microbiome associates with positive vaccine take and shedding in neonatal schedule of the human neonatal rotavirus vaccine RV3-BB. *Nature Communications* 16, 3432. https://doi.org/10.1038/s41467-025-58632-6 ⏎

Wan, Z., Zheng, J., Zhu, Z., Sang, L., Zhu, J., Luo, S., Zhao, Y., Wang, R., Zhang, Y., Hao, K., Chen, L., Du, J., Kan, J., He, H., 2022. Intermediate role of gut microbiota in vitamin B nutrition and its influences on human health. *Frontiers in Nutrition* 9, 1031502. https://doi.org/10.3389/fnut.2022.1031502 ⏎

Wang, J., Li, B., Zhang, Yuanman, Ma, W., Jia, T., Yang, J., Zhang, Yexiang, Qi, F., Jia, Y., Song, L., 2025. Analysis of microbial composition in different dry skin areas of Beijing

women. *Frontiers in Microbiology* 16, 1504054. https://doi.org/10.3389/fmicb.2025.1504054 ⏎

Wang, Y., Chen, T., Zhang, S., Zhang, L., Li, Q., Lv, Q., Kong, D., Jiang, H., Ren, Y., Jiang, Y., Li, Y., Huang, W., Liu, P., 2023. Clinical evaluation of metagenomic next-generation sequencing in unbiased pathogen diagnosis of urinary tract infection. *Journal of Translational Medicine* 21, 762. https://doi.org/10.1186/s12967-023-04562-0 ⏎

Westcott, S.L., Schloss, P.D., 2015. De novo clustering methods outperform reference-based methods for assigning 16S rRNA gene sequences to operational taxonomic units. *PeerJ* 3, e1487. https://doi.org/10.7717/peerj.1487 ⏎

Wickham, H., 2011. ggplot2. *WIREs Computational Statistics* 3, 180–185. https://doi.org/10.1002/wics.147 ⏎

Wishart, D.S., Oler, E., Peters, H., Guo, A., Girod, S., Han, S., Saha, S., Lui, V.W., LeVatte, M., Gautam, V., Kaddurah-Daouk, R., Karu, N., 2023. MiMeDB: The Human Microbial Metabolome Database. *Nucleic Acids Research* 51, D611–D620. https://doi.org/10.1093/nar/gkac868 ⏎

Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. *Genome Biology* 20, 257. https://doi.org/10.1186/s13059-019-1891-0 ⏎

Wu, H., Lv, B., Zhi, L., Shao, Y., Liu, X., Mitteregger, M., Chakaroun, R., Tremaroli, V., Hazen, S.L., Wang, R., Bergström, G., Bäckhed, F., 2025. Microbiome–metabolome dynamics associated with impaired glucose control and responses to lifestyle changes. *Nature Medicine* 31, 2222–2231. https://doi.org/10.1038/s41591-025-03642-6 ⏎

Wu, S., Sun, C., Li, Y., Wang, T., Jia, L., Lai, S., Yang, Y., Luo, P., Dai, D., Yang, Y.-Q., Luo, Q., Gao, N.L., Ning, K., He, L., Zhao, X.-M., Chen, W.-H., 2020. GMrepo: A database of curated and consistently annotated human gut metagenomes. *Nucleic Acids Research* 48, D545–D553. https://doi.org/10.1093/nar/gkz764 ⏎

Yan, B., Nam, Y., Li, L., Deek, R.A., Li, H., Ma, S., 2025. Recent advances in deep learning and language models for studying the microbiome. *Frontiers in Genetics* 15, 1494474. ⏎

Yang, C., Camargo Tavares, L., Lee, H.-C., Steele, J.R., Ribeiro, R.V., Beale, A.L., Yiallourou, S., Carrington, M.J., Kaye, D.M., Head, G.A., Schittenhelm, R.B., Marques, F.Z., 2025. Faecal metaproteomics analysis reveals a high cardiovascular risk profile across healthy individuals and heart failure patients. *Gut Microbes* 17, 2441356. https://doi.org/10.1080/19490976.2024.2441356 ⏎

Yang, Y., Huang, J., Zeng, A., Long, X., Yu, N., Wang, X., 2024. The role of the skin microbiome in wound healing. *Burns Trauma* 12, tkad059. https://doi.org/10.1093/burnst/tkad059 ⏎

Yao, G., Zhang, W., Yang, M., Yang, H., Wang, J., Zhang, H., Wei, L., Xie, Z., Li, W., 2020. MicroPhenoDB associates metagenomic data with pathogenic microbes. *Microbial*

Core Genes, and Human Disease Phenotypes. Genomics Proteomics Bioinformatics 18, 760–772. https://doi.org/10.1016/j.gpb.2020.11.001 ⏎

Yousef, H., Alhajj, M., Fakoya, A.O., Sharma, S., 2025. Anatomy, skin (integument), epidermis, in: *StatPearls*. StatPearls Publishing, Treasure Island (FL). ⏎

Zakrzewski, M., Proietti, C., Ellis, J.J., Hasan, S., Brion, M.-J., Berger, B., Krause, L., 2017. Calypso: A user-friendly web-server for mining and visualizing microbiome–environment interactions. *Bioinformatics* 33, 782–783. https://doi.org/10.1093/bioinformatics/btw725 ⏎

Zhang, H., Yang Lee, B.J., Wang, T., Xiang, X., Tan, Y., Han, Y., Bi, Y., Zhi, F., Wang, X., He, F., Salminen, S.J., Zhu, B., Yang, R., 2025. Microbiota, chronic inflammation, and health: The promise of inflammatome and inflammatomics for precision medicine and health care. *hLife* 3, 307–326. https://doi.org/10.1016/j.hlife.2025.04.004 ⏎

Zhang, Y., Wang, X., Li, H., Ni, C., Du, Z., Yan, F., 2018. Human oral microbiota and its modulation for oral health. *Biomedicine & Pharmacotherapy* 99, 883–893. https://doi.org/10.1016/j.biopha.2018.01.146 ⏎

Zhi, W., Ge, Z., He, Z., Zhang, H., 2014. Methods for understanding microbial community structures and functions in microbial fuel cells: A review. *Bioresource Technology* 171, 461–468. https://doi.org/10.1016/j.biortech.2014.08.096 ⏎

Zhou, L., Jiang, A., Veenstra, J., Ozog, D.M., Mi, Q.-S., 2022. The roles of skin Langerhans cells in immune tolerance and cancer immunity. *Vaccines (Basel)* 10, 1380. https://doi.org/10.3390/vaccines10091380 ⏎

Zhou, Y., Liu, Y.-X., Li, X., 2024. USEARCH 12: Open-source software for sequencing analysis in bioinformatics and microbiome. *iMeta* 3, e236. https://doi.org/10.1002/imt2.236 ⏎

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., Goodman, A.L., 2019. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature* 570, 462–467. ⏎

16

The Cervicovaginal Microbiome: Structure, Classification, and Implications for Women's Health

Mykhaylo Usyk and Robert D. Burk

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16.1 Introduction

The human cervicovaginal tract harbors a complex microbial ecosystem comprising bacteria, fungi, protozoa, and viruses that collectively influence genital tract homeostasis. For much of the 20th century, knowledge of this microbiota was limited by visual-microscopic and culture-dependent methods. Modern sequencing technologies have revealed that healthy reproductive-aged women typically harbor communities dominated by one or a few species of *Lactobacillus* (Ravel et al., 2011; Smith et al., 2016) (Figure 16.1).

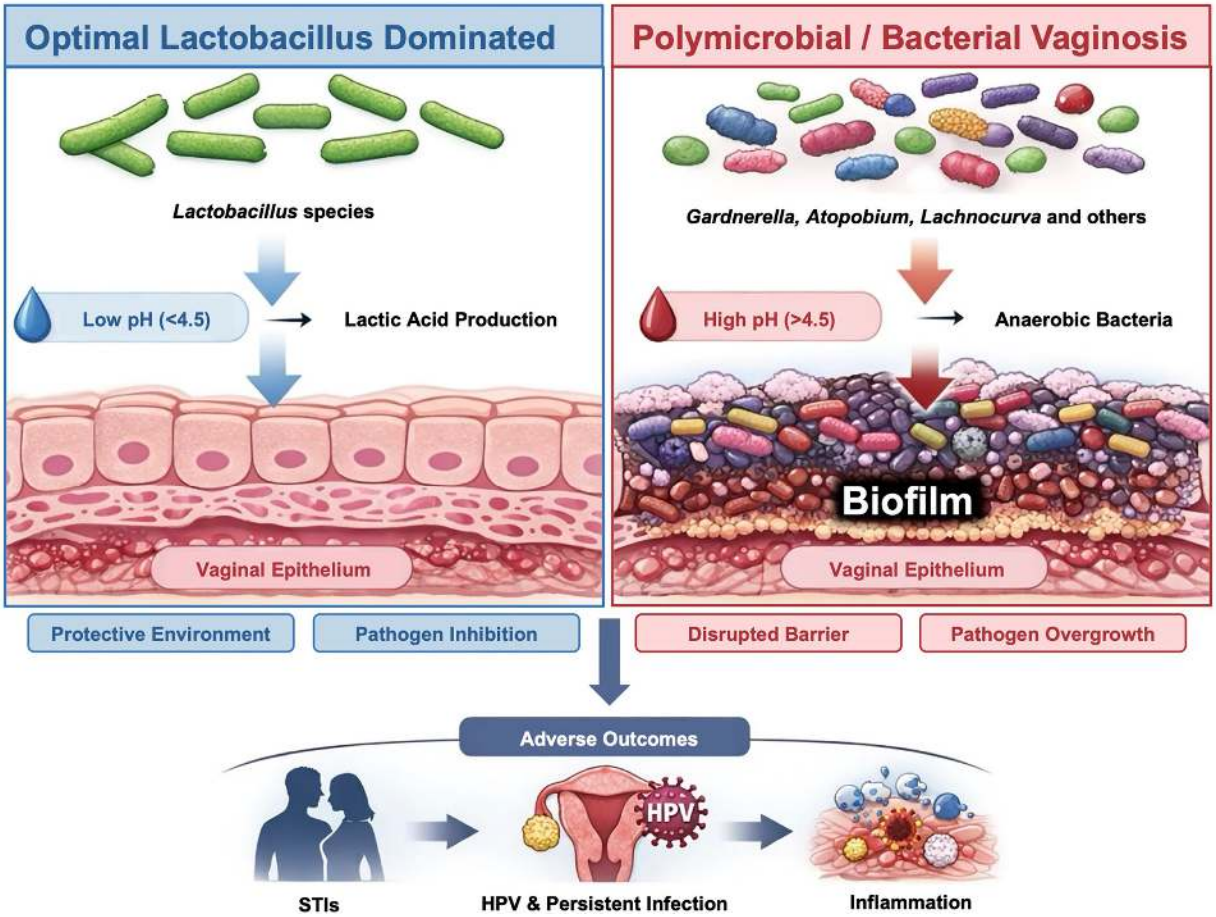

FIGURE 16.1 Conceptual overview of the cervicovaginal microbiome. Healthy *Lactobacillus*-dominated communities maintain epithelial integrity and pathogen resistance, whereas dysbiotic BV-associated communities promote inflammation, infection susceptibility, and adverse reproductive outcomes. ⏎

These microbes produce lactic acid, lowering vaginal pH to <4.5, and generate bacteriocins and hydrogen peroxide that help exclude pathogens. Conversely, dysbiosis involving the depletion of *Lactobacillus* and the proliferation of facultative and obligate anaerobes is termed bacterial vaginosis (BV). Clinically, BV is characterized by abnormal discharge, elevated pH, and a fishy odor, and it is diagnosed using the Amsel criteria or Nugent scoring (Amsel et al., 1983; Chaijareenont, Sirimai, Boriboonhirunsarn, & Kiriwat, 2004). BV is the most common vaginal disorder among reproductive-age women, with a prevalence approaching

30% in the United States, and rates reaching 50% or higher in high-risk populations (McKinnon et al., 2019; Usyk et al., 2022).

Beyond simple binary states of optimal or healthy cervicovaginal microbiome (CVM) versus BV, sequencing studies have identified discrete community configurations, called community state types (CSTs), that are associated with distinct physiologic and pathologic states (France et al., 2020; Ravel et al., 2011). These insights, coupled with the development of molecular diagnostic tools, have transformed our understanding of the CVM and its role in reproductive health. In this chapter, we examine the key bacterial components of the CVM, the epidemiologic and clinical significance of CVM variation, and the methods used to characterize CVM communities. Particular attention is paid to categorical CSTs, continuous measures as informed by the molecular BV (*molBV*) algorithm, and their relevance to cervical pre-cancer and sexually transmitted infections (STIs).

16.2 Structure of the Cervicovaginal Microbiome

16.2.1 Dominant *Lactobacillus* Species

Lactic acid-producing *Lactobacillus* spp. are central to a healthy CVM and represent what is considered to be the "optimal" protective state in reproductive-age women (Bing, Forney, & Ravel, 2012). Four species—*Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus gasseri*, and *Lactobacillus jensenii*—account for the majority of *Lactobacillus*-dominated communities (Bing et al., 2012). Among these, *L. crispatus* and *Lactobacillus iners* most commonly dominate the CVM, whereas *L. gasseri* and *L. jensenii* are observed less frequently and are absent or rare in some populations (Figure 16.2).

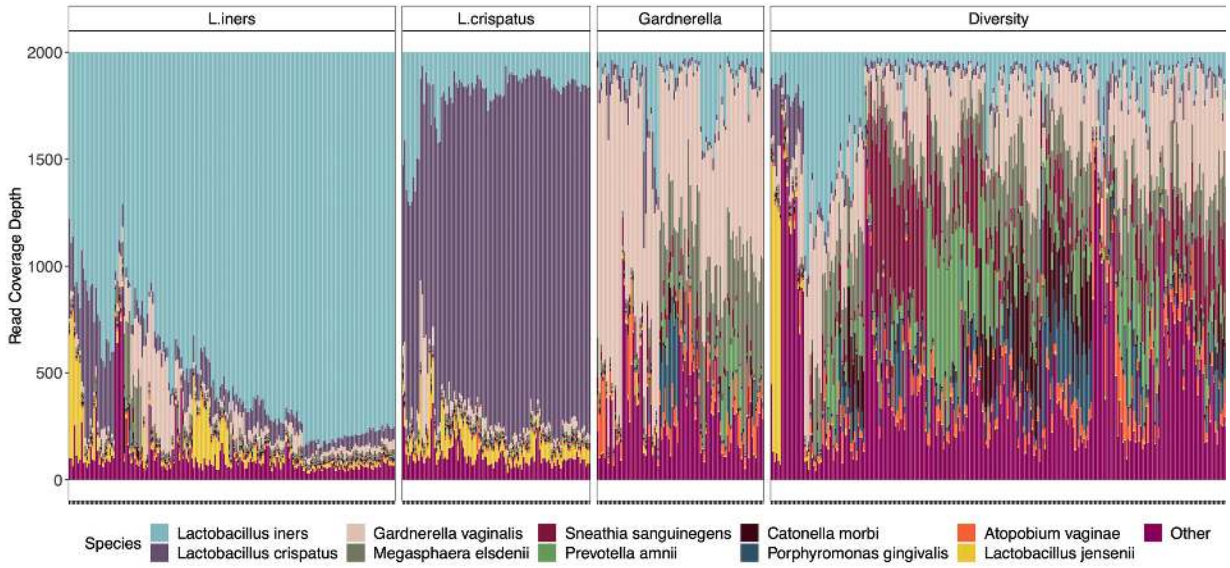

FIGURE 16.2 Empirical cervicovaginal microbiome profiles (each column represents the top bacteria found in a given participant's CVM with color differentiating species) illustrating dominant *Lactobacilli* and dysbiotic community states. Representative sequencing-based abundance profiles from a cohort demonstrating communities dominated by *Lactobacillus iners* or *Lactobacillus crispatus*, contrasted with *Gardnerella*-dominated and high-diversity, *Lactobacillus*-depleted states. These profiles illustrate the predominance of *L. crispatus* and *L. iners* among *Lactobacillus*-dominated communities, whereas *L. gasseri* and *L. jensenii* are absent. ⏎

These organisms metabolize host-derived glycogen breakdown products into lactic acid, thereby maintaining an acidic vaginal milieu that suppresses pathogen growth, shapes microbial community structure, and modulates epithelial immune responses. Beyond acidification, vaginal lactobacilli contribute to colonization resistance through the production of bacteriocins, hydrogen peroxide, and competitive exclusion at the epithelial surface.

Among these species, *L. crispatus* is most consistently associated with vaginal health and community stability. It possesses the largest genome of the vaginal lactobacilli and encodes extensive carbohydrate utilization pathways, stress response systems, and antimicrobial functions that support persistence in the cervicovaginal niche (France, Mendes-Soares, & Forney, 2016). Communities dominated by *L. crispatus* are typically characterized

by low vaginal pH, low microbial diversity, and reduced inflammatory tone. Host genetic studies indicate that *L. crispatus* dominance is heritable among women of European ancestry, whereas women of African ancestry more frequently harbor *L. iners* dominated or diverse communities (Wright et al., 2021). Epidemiologically, *L. crispatus*-dominated CVMs are associated with a lower risk of preterm birth, BV, and STIs, supporting its role as a keystone protective species (France et al., 2020; Wright et al., 2021).

In contrast, *L. iners* occupies a more ambiguous ecological position. Its relatively small genome reflects extensive gene loss and metabolic dependency on the host and surrounding microbiota, yet it retains stress tolerance mechanisms and adhesins that facilitate epithelial colonization (Mancabelli et al., 2021). *L. iners* is notable for its ability to persist across both *Lactobacillus*-dominated and dysbiotic states, often appearing during transitions between health and BV. Unlike *L. crispatus*, *L. iners* does not consistently maintain a strongly acidic environment and may coexist with, or precede, the expansion of anaerobic taxa. This dual presence has led to the view that *L. iners* functions as a transitional or permissive species rather than a strictly protective one (Mancabelli et al., 2021).

L. gasseri and *L. jensenii* are less frequently dominant but contribute to vaginal acidity and antimicrobial defense through lactic acid and bacteriocin production (Mancabelli et al., 2021). *L. jensenii*-dominated communities define CST V and are generally associated with favorable clinical profiles, though they appear less stable than *L. crispatus*-dominated states. Collectively, these species illustrate that *Lactobacillus* dominance is not a single entity; rather, distinct *Lactobacillus* taxa confer differing degrees of ecological stability and protection, with important implications for susceptibility to dysbiosis and the resulting disease risk.

16.2.2 BV-Associated and Other Key Taxa

BV occurs when protective lactobacilli are depleted and replaced by polymicrobial communities of facultative and obligate anaerobic bacteria that fundamentally alter the cervicovaginal niche (McKinnon et al., 2019;

Usyk et al., 2022). Rather than a simple overgrowth of a single organism, BV represents a structured ecological state characterized by increased microbial diversity (Figure 16.2), elevated vaginal pH, biofilm formation, and disruption of epithelial and immune homeostasis.

The best-characterized BV-associated bacterium is *Gardnerella vaginalis*, a facultative anaerobe that plays a central role in initiating dysbiosis. *G. vaginalis* adheres to the vaginal epithelium and forms dense biofilms that act as a physical and metabolic scaffold for secondary colonizers (Shvartsman, Hill, Sandstrom, & MacDonald, 2023). This epithelial adherence underlies the formation of "clue cells," (Figure 16.3), which are defined as vaginal epithelial cells coated with adherent bacteria and considered a hallmark of BV in Gram-stained smears and a core component of the Amsel diagnostic criteria.

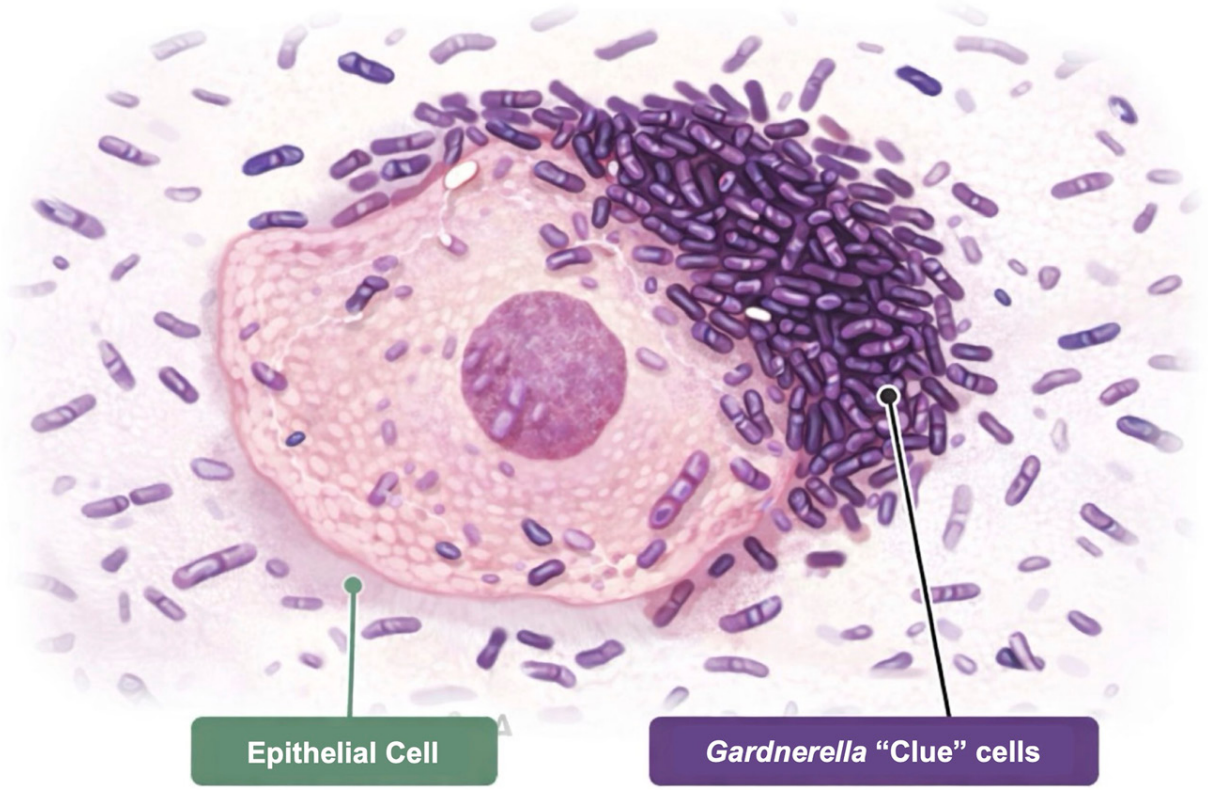

FIGURE 16.3 Illustrative depiction of BV-associated "clue cells". Schematic illustration of a vaginal epithelial cell densely coated with adherent *Gardnerella* cells (colored purple to indicate gram staining), forming a "clue cell," a hallmark feature of BV observed in Gram-stained vaginal smears and incorporated into the Amsel diagnostic criteria. ⏎

Through the secretion of vaginolysin and the production of sialidases and proteases, *G. vaginalis* degrades mucin layers and compromises epithelial barrier integrity, facilitating colonization by additional anaerobic taxa (Shvartsman et al., 2023). Expansion of *G. vaginalis*-dominated communities has been linked to infertility, adverse pregnancy outcomes, pelvic inflammatory disease, and increased susceptibility to HIV and other STIs, underscoring its role as a key driver of BV-associated pathology (Shvartsman et al., 2023).

Another recently characterized BV-associated pathobiont is *Candidatus Lachnocurva vaginae* (formerly *BVAB1*; Holm et al., 2020), which is frequently enriched in highly dysbiotic communities. Genomic analyses

indicate that this curved, rod-shaped organism utilizes d-lactate, a metabolic feature that may provide a competitive advantage in environments where lactobacilli are depleted (Osei Sekyere, Oyenihi, Trama, & Adelson, 2023). *Candidatus L. vaginae* produces trimethylamine, contributing to the characteristic malodor of BV, and encodes flagellar machinery suggestive of motility and active niche exploration (Osei Sekyere et al., 2023). A high abundance of *Candidatus L. vaginae* is strongly associated with specific *molBV* subtypes and has been linked to an increased risk of incident *Chlamydia trachomatis* infection (Usyk et al., 2025), highlighting its relevance beyond symptomatic BV.

In addition to *Gardnerella* and *Candidatus L. vaginae*, BV-associated communities commonly include anaerobic genera such as *Prevotella*, *Megasphaera*, *Atopobium*, and *Clostridium* (France et al., 2020). These organisms engage in metabolic cross-feeding within polymicrobial biofilms, producing short-chain fatty acids (SCFAs), amines, and other metabolites that further elevate vaginal pH and promote inflammation. Among these, *Atopobium vaginae* has emerged as a clinically relevant taxon; its enrichment defines the CST IV-B subtype and is associated with heightened odds of concurrent cervical infection and adverse reproductive outcomes (France et al., 2020). Similarly to the *Lactobacillus*-dominated communities, these species-specific features of BV-associated bacteria illustrate that BV is not a uniform condition but a spectrum of dysbiotic states driven by distinct microbial consortia with differing pathogenic potential.

16.3 Epidemiology of the Cervicovaginal Microbiome and Interactions with Health

The balance between *Lactobacillus* dominance and anaerobic proliferation is dynamic. When lactobacilli produce high concentrations of L-lactic acid and bacteriocins, they acidify the niche, inhibit anaerobe growth, and modulate epithelial immune responses. Conversely, factors such as

antibiotic use, hormonal fluctuations, or sexual activity can tip the balance toward dysbiosis. Dysbiotic communities produce SCFAs, amines, and other metabolites that neutralize the pH and disrupt mucosal integrity. These shifts have clinical ramifications: BV is linked to infertility, preterm birth, miscarriage, and an elevated risk of acquiring *C. trachomatis*, *Mycoplasma genitalium*, HIV, and high-risk HPV (hrHPV) (Mohanty, Doke, & Khuroo, 2023; Peters, Usyk, & Burk, 2026; Usyk et al., 2022). Epidemiologic studies have also associated BV with obesity and metabolic syndrome (Srinivasan et al., 2015), underscoring its systemic impact.

Large cross-sectional studies demonstrate that CVM composition varies with age, ethnicity, and behavioral factors. Ravel et al. (2011) classified vaginal communities into five major CSTs: CST I (*L. crispatus*), CST II (*L. gasseri*), CST III (*L. iners*), CST V (*L. jensenii*), and CST IV (*Lactobacillus*-depleted, diverse communities rich in anaerobes). They observed that CST IV was more prevalent among Black and Hispanic women (38–40%) than among non-Hispanic white or Asian women (11–21%) (France et al., 2020; Ravel et al., 2011). Vaginal pH was higher in women with CST IV, and these communities often contained *Gardnerella*, *Atopobium*, and other anaerobes. Importantly, non-*Lactobacillus* communities may be stable and asymptomatic for some women, challenging the notion that *lactobacillus* dominance is universally required for health (McKinnon et al., 2019; Usyk et al., 2025).

BV is highly prevalent. Data from the US National Health and Nutrition Examination Survey (NHANES) indicate a prevalence of approximately 29% among reproductive-age women (Qi, Dai, Song, & Zhang, 2024), while global estimates range from 23% to 37%. BV prevalence varies by race/ethnicity and socioeconomic status; Black and Hispanic women have higher rates and more frequent recurrences, reflecting a complex interplay between host genetics, behaviors, and access to care. Diagnosed BV also carries a significant economic burden, estimated at > $5 billion annually (Peebles, Velloza, Balkus, McClelland, & Barnabas, 2019). Importantly, both BV and CVM composition fluctuate across the menstrual cycle,

pregnancy, and menopause, highlighting the influence of hormones on microbial ecology.

16.4 High-Throughput Approaches to Characterize the CVM

Amplicon sequencing of variable regions of the bacterial 16S rRNA gene has become the workhorse for profiling the CVM (Figure 16.4).

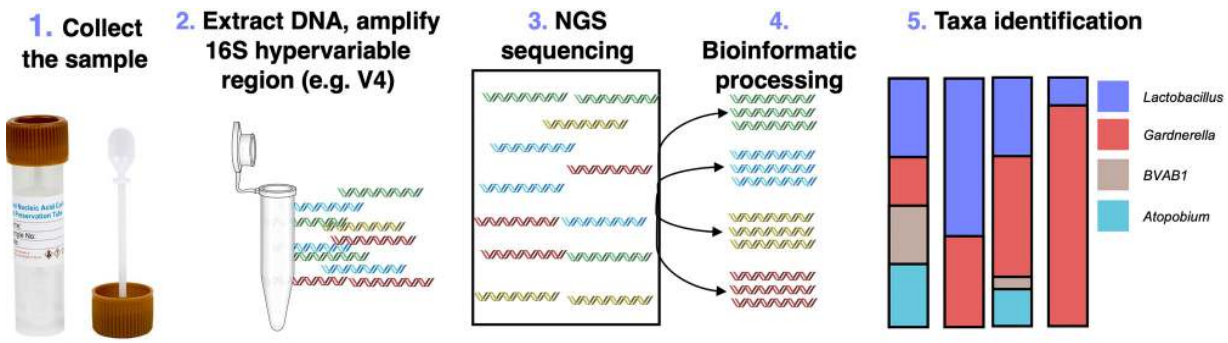

FIGURE 16.4 High-throughput sequencing workflow for characterizing the cervicovaginal microbiome. Schematic overview of a typical 16S rRNA gene amplicon sequencing workflow used to profile the cervicovaginal microbiome, including sample collection, DNA extraction and amplification of hypervariable regions, next-generation sequencing, bioinformatic processing, and taxonomic assignment. This approach enables culture-independent characterization of bacterial taxa and downstream analyses such as those involving risk analyses. ⏎

It enables culture-independent identification of bacteria at the genus or species level with relatively low cost and high throughput. The choice of 16S rRNA gene variable region (e.g., V3–V4 or V4) influences taxonomic resolution, but across regions, the overall community structures are comparable, with certain notable exceptions. For example, the V1 hypervariable region demonstrates a poor ability to detect *Gardnerella* compared to the V4 region (Graspeuntner, Loeper, Künzel, Baines, & Rupp, 2018). A key limitation of 16S sequencing is its inability to discern strains or detect non-bacterial organisms, such as fungi or viruses (Bradford &

Ravel, 2017). Whole-metagenome shotgun sequencing sequences all DNA within a sample and can resolve community members to the species and strain level (Usyk, Peters, et al., 2023), detect virulence genes, and infer metabolic pathways. However, the high cost and the dominance of human DNA in cervicovaginal samples limit its routine use. Furthermore, relative abundance data can obscure absolute changes in biomass (Morton et al., 2019).

After sequencing, reads are processed using pipelines like DADA2 (Callahan et al., 2016) (in the case of amplicon sequencing) or mothur (Schloss, 2020) to denoise sequences and assign taxonomy. Taxonomic classifiers rely on curated databases, but misclassifications can occur, especially for closely related taxa. CST assignment is often performed using distance-based clustering or *k*-nearest neighbor approaches (France et al., 2020). VALENCIA implements a nearest-centroid classifier trained on a large, annotated dataset to assign CSTs, and it has been widely adopted (France et al., 2020). High-resolution profiling enables the identification of CST subgroups (e.g., CST IV-A vs. IV-B) that correlate with clinical outcomes (Usyk et al., 2025). The concept of CSTs simplifies the diversity of CVM profiles into discrete categories, providing a framework for epidemiologic and clinical studies. In the original classification, CST I is dominated by *L. crispatus*, CST II by *L. gasseri*, CST III by *L. iners*, CST V by *L. jensenii*, and CST IV by a diverse mix of anaerobic bacteria (Ravel et al., 2011). Advances in sequencing have revealed that CST IV encompasses multiple subtypes. CST IV-A is characterized by a high abundance of *Candidatus L. vaginae* and/or *G. vaginalis*, while CST IV-B is enriched for *Atopobium vaginae* and *Megasphaera* species. These subtypes have distinct metabolic profiles and associations with disease (France et al., 2020; Usyk et al., 2025). Another refinement is the identification of transitional CSTs (e.g., CST I-A vs. CST I-B) distinguished by the presence of other *Lactobacillus* species or mixtures of *L. crispatus* and *L. iners*. High-resolution techniques also detect driver versus passenger species, supporting the ecological model that a few

"driver" taxa, such as *L. crispatus*, dictate community structure while other species follow.

16.5 *molBV* and Clinical diagnostics

Traditional BV diagnostics rely on clinical signs (Amsel criteria) or Gram-stain-based Nugent scoring, both of which depend on subjective interpretation and require trained personnel (Chaijareenont et al., 2004). While these approaches have served as clinical standards for decades, they provide only coarse categorization of a biologically heterogeneous condition and are limited in their ability to capture underlying microbial and immunologic variation. To address these limitations, the *molBV* algorithm was developed to generate a quantitative "molecular Nugent" score directly from 16S rRNA sequencing data (Usyk et al., 2022). More specifically, the *molBV* algorithm computes a weighted abundance score based on key BV-associated taxa, enabling objective and reproducible classification of samples across different 16S hypervariable regions and study populations. In multiple confirmatory cohorts, *molBV* closely reproduced gold-standard Nugent scoring while generalizing across diverse ethnic groups and sequencing protocols (Usyk et al., 2022) (Figure 16.5).

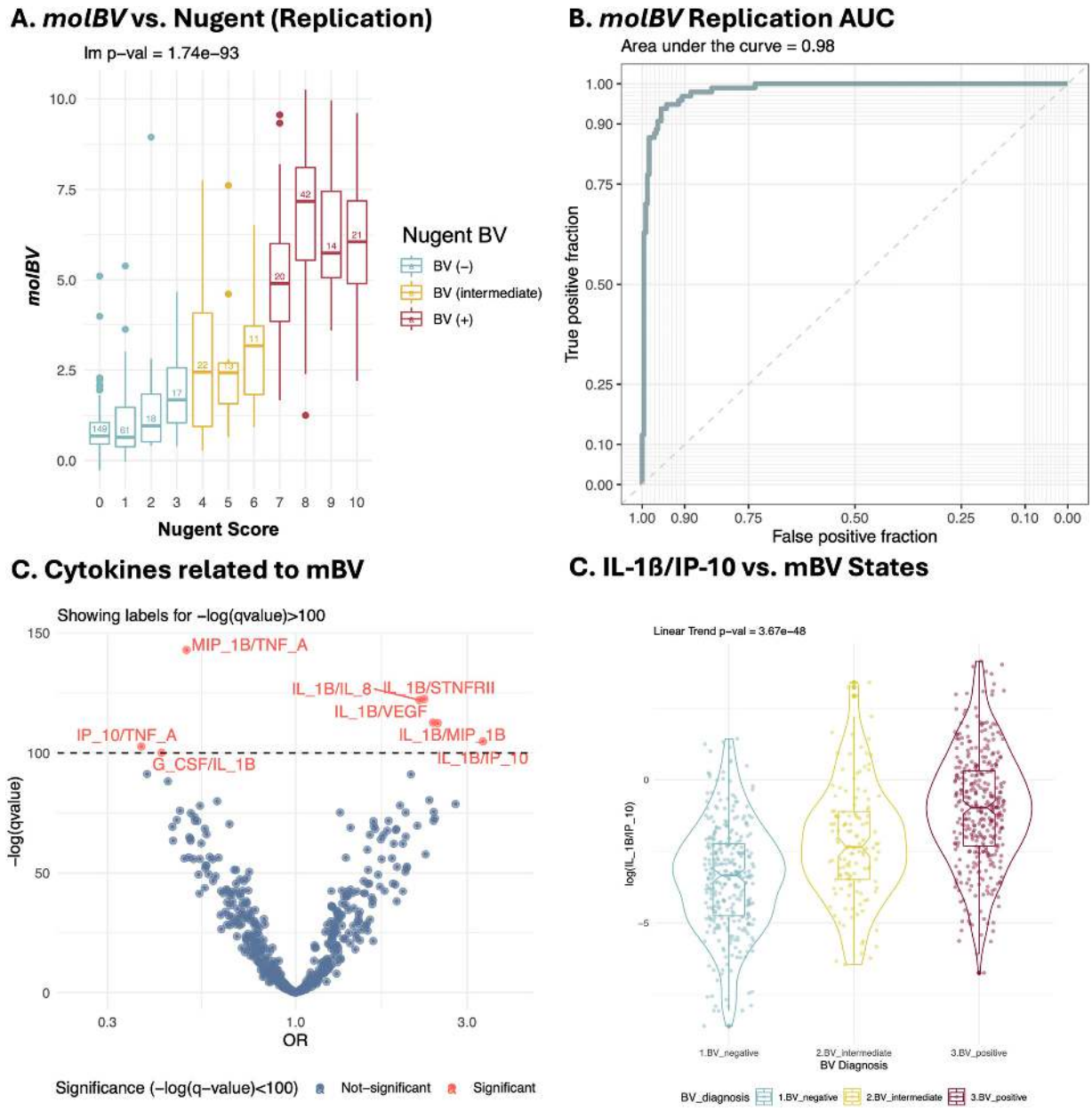

FIGURE 16.5 Validation of *molBV* as a quantitative and biologically informative diagnostic. Empirical validation of the *molBV* framework across multiple analytical dimensions. (A) Correlation between *molBV* scores and traditional Nugent scores demonstrates strong concordance between molecular and microscopy-based BV assessment. (B) Receiver operating characteristic analysis shows high accuracy of *molBV* for classification of Nugent-defined BV, indicating robust diagnostic performance. (C) Differential cytokine (small proteins that indicate activity of host immune cells) associations across mBV states reveal distinct

inflammatory profiles linked to *molBV* status. (D) Variation in the IL-1β/IP-10 ratio across mBV-negative, mBV-intermediate and mBV-positive states illustrates progressive immunologic shifts associated with molecularly defined BV, a cytokine signature independently linked to disease-associated dysbiotic states. ⏎

Importantly, *molBV* classifies samples along a continuous spectrum that can be discretized into three clinically interpretable categories: mBV-negative, mBV-intermediate, and mBV-positive. This classification mirrors traditional Nugent categories while preserving underlying quantitative information.

Beyond diagnostic concordance, *molBV* captures biologically meaningful heterogeneity that is not discernible by microscopy alone. The mBV-positive status is associated with distinct inflammatory cytokine profiles, indicating that molecularly defined BV states differ in their host immune context. Elevated IL-1β/IP-10 ratios in BV were associated with the clearance of high-risk HPV infection, whereas elevated TNF-α/MIP-1β ratios were prospectively associated with progression to cervical intraepithelial neoplasia (Usyk et al., 2020, 2022). Thus, *molBV* integrates microbial composition with host response, providing insight into divergent disease trajectories.

The *molBV* framework has been further extended to identify clinically relevant subtypes. In a cohort of Black and Hispanic adolescent women, a subtype designated mBV-A conferred a 2.38-fold increased risk of incident *C. trachomatis* infection compared with mBV-negative status (Usyk et al., 2025). The mBV-A subtype was distinguished by a marked enrichment of *Candidatus L. vaginae* relative to an alternative subtype, mBV-B, highlighting that specific microbial configurations, rather than BV status in general, drive susceptibility to infection. Together, these findings position *molBV* as a scalable molecular diagnostic that moves beyond binary BV classification toward biologically informed risk stratification with direct clinical relevance.

16.6 Public Health Relevance of Cervicovaginal Microbiome Variation

16.6.1 Cervical Precancer and Human Papillomavirus

Persistent infection with high-risk human papillomavirus (hrHPV) is the necessary cause of cervical cancer. However, only a subset of hrHPV infections progress to high-grade lesions; host and microbial factors modulate this trajectory. The depletion of lactobacilli, elevated vaginal pH, and colonization by anaerobic bacteria have been linked to the persistence of hrHPV and progression to cervical intraepithelial neoplasia (Usyk et al., 2020). Women with CST IV communities harbor higher viral loads and are more likely to develop high-grade lesions than those with *L. crispatus*-dominated CST I. High-resolution sequencing further showed that *Gardnerella*- and *L. iners*-dominated subtypes differ in their association with HPV persistence (Di Paola et al., 2017; Usyk et al., 2020).

The inflammatory host response associated with dysbiosis provides a plausible mechanistic link between CVM composition and HPV outcomes (Usyk et al., 2022). BV-associated communities are characterized by altered cytokine profiles that may impair effective antiviral immune responses while promoting chronic inflammation. In the *molBV* study, BV-associated increases in the IL-1β/IP-10 cytokine ratio were associated with the decreased clearance of incident hrHPV infection, whereas a TNF-α/MIP-1β–driven inflammatory signature was prospectively associated with progression to high-grade cervical lesions (Figure 16.6).

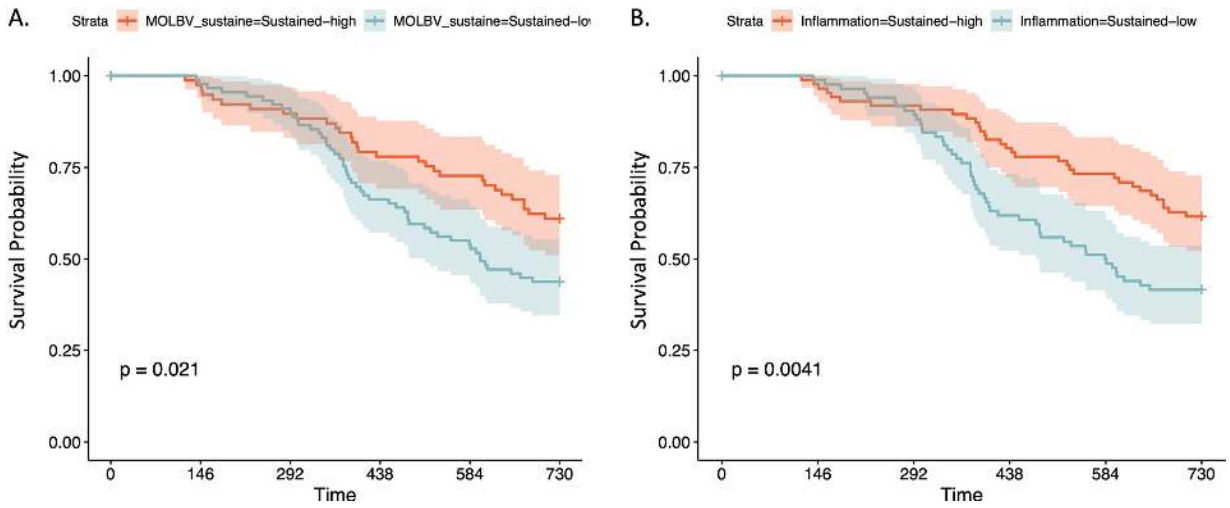

FIGURE 16.6 molBV and inflammatory signatures associated with clearance of hrHPV infection. Kaplan–Meier analyses illustrating associations between molBV, inflammatory cytokine profiles, and clearance of incident hrHPV infection. (A) Women with sustained high molBV scores exhibited altered probabilities of hrHPV clearance compared with those with sustained low molBV scores, indicating that molecularly defined BV states are associated with divergent viral outcomes. (B) Sustained elevation of the IL-1β/IP-10 cytokine ratio, an inflammatory signature linked to BV-associated microbial states, was associated with differential hrHPV clearance over time. ⏎

These findings suggest that molecularly defined BV states reflect not only microbial composition but also distinct immunologic environments that differentially shape HPV natural history. These observations support a model in which the CVM modulates cervical mucosal immunity, influencing whether hrHPV infection is cleared, persists, or progresses to precancer. Incorporating CVM structure and molBV subtypes into studies of HPV natural history should provide critical context for understanding inter-individual variability in disease risk and identify potentially modifiable microbial targets for the prevention of cervical cancer.

16.6.1.1 C. trachomatis and Other STIs

C. trachomatis is the most common bacterial STI worldwide, with over 130 million cases annually (Fu et al., 2022). Adolescents and young adults account for the majority of infections, and there are marked racial and ethnic disparities, with Black and Hispanic women experiencing five-fold higher rates than non-Hispanic white women (Huai et al., 2020). Besides sexual behavior and age, BV is a recognized risk factor for *C. trachomatis* acquisition (Fu et al., 2022). The longitudinal study of Usyk and colleagues integrated *molbv* and CST classification to dissect CVM-*chlamydia* interaction (i.e., by splitting mBV into high- and low-risk subtypes) (Usyk et al., 2025). They found that mBV-A, corresponding to features of CST IV-A, significantly increased the odds of incident *C. trachomatis* infection (OR = 2.38). A polymicrobial risk score incorporating ten genera, including *Candidatus L. vaginae*, *Prevotella*, *Megasphaera*, and *Clostridium* conferred a 2.5-fold increase in *chlamydia* risk per unit increase. Post-treatment, the same dysbiotic communities re-emerged in a subset of cases, contributing to reinfection and highlighting the resilience of BV-associated communities. The mBV-positive and mBV-intermediate states at the time of infection were also significantly associated with *chlamydia* detection (OR = 3.66 and 2.08, respectively). These findings underscore that specific mBV subtypes, rather than BV per se, drive susceptibility to *C. trachomatis*.

Beyond chlamydia, mBV and CST IV communities are linked to the acquisition of other STIs, including *Mycoplasma genitalium*, HIV, and *Trichomonas vaginalis* (Bautista et al., 2016; Reiter & Kellogg Spadt, 2019; Usyk, Schlecht, et al., 2023). For example, the prevalence of BV is higher among women with HIV, and BV treatment reduces HIV viral shedding (Peters et al., 2026); however, recurrence is common. The interplay between the CVM and STIs is bidirectional: infections such as herpes simplex virus can disrupt the microbiome, while dysbiotic communities can impair mucosal immunity and facilitate viral entry.

16.6.2 Reproductive and Pregnancy Outcomes

Dysbiosis has broader reproductive implications. BV has been associated with infertility, pelvic inflammatory disease, and adverse pregnancy outcomes, such as miscarriage, preterm birth, and low birth weight (McKinnon et al., 2019; Ravel, Moreno, & Simon, 2021). *L. crispatus*-dominated communities are protective; women with *Lactobacillus-dominated CVMs* have a lower risk of preterm birth, while those with BV are at increased risk (Mohanty et al., 2023). Mechanistically, anaerobic bacteria produce pro-inflammatory mediators and proteases that may weaken the cervical mucus plug or trigger labor. Dysbiosis has also been implicated in infertility and inflammatory changes in the endometrium. Therefore, restoring and maintaining a healthy CVM may improve fertility and pregnancy outcomes.

16.7 Conclusions and Future Directions

The cervicovaginal microbiome profoundly influences women's reproductive and sexual health. Communities dominated by *L. crispatus* or *L. jensenii* maintain a protective acidic environment, whereas the depletion of lactobacilli and the overgrowth of anaerobes (BV) disrupt homeostasis and predispose individuals to infections and adverse outcomes. Discrete CSTs and molBV subtypes provide a framework for understanding CVM variation and its clinical implications. The *molbv* algorithm offers an objective, scalable diagnostic tool that is ideal for analyses of BV when only DNA extracted from cervicovaginal samples is available. This measure of molBV captures microbial and immunologic heterogeneity and predicts the risks of HPV persistence, cervical pre-cancer, and chlamydia acquisition. Future research should focus on longitudinal studies across diverse populations, the mechanistic exploration of host–microbe–immune interactions, and clinical trials of targeted interventions, such as probiotics, microbial transplantation, or personalized antimicrobials. Integration of CVM profiling into public-health strategies may enable precision prevention of STIs, cervical cancer, and adverse pregnancy outcomes.

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References

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *American Journal of Medicine*, *74*(1), 14–22. doi:10.1016/0002-9343(83)91112-9 ⏎

Bautista, C. T., Wurapa, E., Sateren, W. B., Morris, S., Hollingsworth, B., & Sanchez, J. L. (2016). Bacterial vaginosis: A synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. *Military Medical Research*, *3*(1), 4. doi:10.1186/s40779-016-0074-5 ⏎

Bing, M., Forney, L., & Ravel, J. (2012). The vaginal microbiome: Rethinking health and diseases. *Annual Review of Microbiology*, *66*, 371–389. ⏎

Bradford, L. L., & Ravel, J. (2017). The vaginal mycobiome: A contemporary perspective on fungi in women's health and diseases. *Virulence*, *8*(3), 342–351. ⏎

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. doi:10.1038/nmeth.3869 ⏎

Chaijareenont, K., Sirimai, K., Boriboonhirunsarn, D., & Kiriwat, O. (2004). Accuracy of Nugent's score and each Amsel's criteria in the diagnosis of bacterial vaginosis. *Journal of the Medical Association of Thailand*, *87*(11), 1270–1274. ⏎

Di Paola, M., Sani, C., Clemente, A. M., Iossa, A., Perissi, E., Castronovo, G., … Cavalieri, D. (2017). Characterization of cervico-vaginal microbiota in women developing persistent high-risk human papillomavirus infection. *Scientific Reports*, *7*(1), 10200. ⏎

France, M. T., Ma, B., Gajer, P., Brown, S., Humphrys, M. S., Holm, J. B., … Ravel, J. (2020). VALENCIA: A nearest centroid classification method for vaginal microbial communities based on composition. *Microbiome*, *8*(1), 166. doi:10.1186/s40168-020-00934-6 ⏎

France, M. T., Mendes-Soares, H., & Forney, L. J. (2016). Genomic comparisons of *Lactobacillus crispatus* and *Lactobacillus iners* reveal potential ecological drivers of community composition in the vagina. *Appl Environ Microbiol*, *82*(24), 7063–7073. doi:10.1128/AEM.02385-16 ⏎

Fu, L., Sun, Y., Han, M., Wang, B., Xiao, F., Zhou, Y., … Li, P. (2022). Incidence trends of five common sexually transmitted infections excluding HIV from 1990 to 2019 at the global, regional, and national levels: Results from the Global Burden of Disease Study 2019. *Frontiers in Medicine*, 9.

Graspeuntner, S., Loeper, N., Künzel, S., Baines, J. F., & Rupp, J. (2018). Selection of validated hypervariable regions is crucial in 16S-based microbiota studies of the female genital tract. *Scientific Reports*, *8*(1), 9678. ⏎

Holm, J. B., France, M. T., Ma, B., McComb, E., Robinson, C. K., Mehta, A., … Ravel, J. (2020). Comparative metagenome-assembled genome analysis of "*Candidatus Lachnocurva vaginae*", formerly known as Bacterial Vaginosis-Associated Bacterium− 1 (BVAB1). *Frontiers in Cellular and Infection Microbiology*, *10*, 117. ⏎

Huai, P., Li, F., Chu, T., Liu, D., Liu, J., & Zhang, F. (2020). Prevalence of genital *Chlamydia trachomatis* infection in the general population: A meta-analysis. *BMC Infectious Diseases*, *20*(1), 1–8. ⏎

Mancabelli, L., Mancino, W., Lugli, G. A., Milani, C., Viappiani, A., Anzalone, R., … Turroni, F. (2021). Comparative genome analyses of

Lactobacillus crispatus isolates from different ecological niches reveal an adaptation of this species to the human vaginal environment. *Applied and Environmental Microbiology*, *87*(8), e02899–02820. ⏎

McKinnon, L. R., Achilles, S. L., Bradshaw, C. S., Burgener, A., Crucitti, T., Fredricks, D. N., … Klatt, N. (2019). The evolving facets of bacterial vaginosis: Implications for HIV transmission. *AIDS Research and Human Retroviruses*, *35*(3), 219–228. ⏎

Mohanty, T., Doke, P. P., & Khuroo, S. R. (2023). Effect of bacterial vaginosis on preterm birth: A meta-analysis. *Archives of Gynecology and Obstetrics*, *308*(4), 1247–1255. ⏎

Morton, J. T., Marotz, C., Washburne, A., Silverman, J., Zaramela, L. S., Edlund, A., … Knight, R. (2019). Establishing microbial composition measurement standards with reference frames. *Nature Communications*, *10*(1), 2719. doi:10.1038/s41467-019-10656-5 ⏎

Osei Sekyere, J., Oyenihi, A. B., Trama, J., & Adelson, M. E. (2023). Species-specific analysis of bacterial vaginosis-associated bacteria. *Microbiology Spectrum*, *11*(4), e04676–04622. ⏎

Peebles, K., Velloza, J., Balkus, J. E., McClelland, R. S., & Barnabas, R. V. (2019). High global burden and costs of bacterial vaginosis: A systematic review and meta-analysis. *Sexually Transmitted Diseases*, *46*(5), 304–311. ⏎

Peters, B. A., Usyk, M., & Burk, R. D. (2026). The female microbiome in HIV prevention, pathogenesis, and treatment. *Current Opinion in HIV and AIDS*, *21*(1), 46–51. ⏎

Qi, J., Dai, C., Song, L., & Zhang, J. (2024). Association between bacterial vaginosis with human papillomavirus in the United States (NHANES 2003–2004). *BMC Women's Health*, *24*(1), 138. ⏎

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., McCulle, S. L., … Forney, L. J. (2011). Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences U S A*, 108 (Suppl 1), 4680–4687. doi:10.1073/pnas.1002611107 ⏎

Ravel, J., Moreno, I., & Simon, C. (2021). Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. *Am J Obstet Gynecol*, *224*(3), 251–257. doi:10.1016/j.ajog.2020.10.019 ⏎

Reiter, S., & Kellogg Spadt, S. (2019). Bacterial vaginosis: A primer for clinicians. *Postgraduate Medicine*, *131*(1), 8–18. ⏎

Schloss, P. D. (2020). Reintroducing mothur: 10 years later. *Applied and Environmental Microbiology*, *86*(2), e02343-02319.

Shvartsman, E., Hill, J. E., Sandstrom, P., & MacDonald, K. S. (2023). Gardnerella revisited: Species heterogeneity, virulence factors, mucosal immune responses, and contributions to bacterial vaginosis. *Infection and Immunity*, *91*(5), e00390-00322. ⏎

Smith, B. C., Zolnik, C. P., Usyk, M., Chen, Z., Kaiser, K., Nucci-Sack, A., … Burk, R. D. (2016). Distinct ecological niche of anal, oral, and cervical mucosal microbiomes in adolescent women. *Yale Journal of Biology and Medicine*, *89*, 277–284. ⏎

Srinivasan, S., Morgan, M. T., Fiedler, T. L., Djukovic, D., Hoffman, N. G., Raftery, D., … Fredricks, D. N. (2015). Metabolic signatures of bacterial vaginosis. *MBio*, *6*(2), e00204-15. doi:10.1128/mbio.00204–00215 ⏎

Usyk, M., Carlson, L., Schlecht, N. F., Sollecito, C. C., Grassi, E., Wiek, F., … Diaz, A. (2025). Cervicovaginal microbiome and natural history of *Chlamydia trachomatis* in adolescents and young women. *Cell*, *188*(4):1051–1061.e12. doi: 10.1016/j.cell.2024.12.011 ⏎

Usyk, M., Peters, B. A., Karthikeyan, S., McDonald, D., Sollecito, C. C., Vazquez-Baeza, Y., … Burk, R. D. (2023). Comprehensive evaluation of shotgun metagenomics, amplicon sequencing, and harmonization of these platforms for epidemiological studies. *Cell Reports Methods*, *3*, 100391. doi:10.1016/j.crmeth.2022.100391 ⏎

Usyk, M., Schlecht, N. F., Pickering, S., Williams, L., Sollecito, C. C., Gradissimo, A., … Herrero, R. (2022). molBV reveals immune landscape of bacterial vaginosis and predicts human papillomavirus infection natural history. *Nature Communications*, *13*(1), 1–12. ⏎

Usyk, M., Schlecht, N. F., Viswanathan, S., Gradissimo, A., Valizadegan, N., Sollecito, C. C., … Burk, R. D. (2023). TRiCit: A high-throughput approach to detect *Trichomonas vaginalis* from ITS1 amplicon sequencing. *International Journal of Molecular Sciences*, *24*. doi:10.3390/ijms241411839 ⏎

Usyk, M., Zolnik, C. P., Castle, P. E., Porras, C., Herrero, R., Gradissimo, A., … Burk, R. D. (2020). Cervicovaginal microbiome and natural history of HPV in a longitudinal study. *PLoS Pathogens*, *16*, e1008376. doi:10.1371/journal.ppat.1008376 ⏎

Wright, M. L., Fettweis, J. M., Eaves, L. J., Silberg, J. L., Neale, M. C., Serrano, M. G., … Borzelleca Jr, J. F. (2021). Vaginal microbiome *Lactobacillus crispatus* is heritable among European American women. *Communications Biology*, *4*(1), 872. ⏎

Section IV

System-Level Understanding of Microbiomes

17

Microglia and the Microbiome: Distant yet Connected

Cameo Volk and Marie Ève Tremblay

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17.1 Introduction

Microglia were discovered in 1919 by the Spanish neuroscientist Pio del Rio-Hortega. A pioneer in staining technology, del Rio-Hortega quickly distinguished these cells from astrocytes and neurons primarily on the basis of their small somas and extensive networks of long, outstretched branches, now known to play a significant role in assessing their local environment for threats (Boullerne & Feinstein, 2020). Early histological accounts noted the cells' extensive morphological plasticity and helped to develop much of the terminology used today. The concept of "resting" microglia, which has been replaced by the "surveillant" nomenclature, refers to the cells in their normal homeostatic state, displaying a small cell body housing a complex web of outstretched processes that can reach an area 10-fold the size of their body (Paolicelli et al., 2022; Kettenmann et al., 2013). In contrast, upon encountering a disturbance, the cells traditionally became classified as "activated," retracting their branches and adopting an ameboid configuration with short, stubby outcroppings (Cao et al., 2025). The term "activated" microglia is still prevalent in research and often used as a shorthand to refer to the highly mobile, phagocytic state. However, there is a

push to discontinue the term, as it insinuates ramified or "resting" microglia are not actively contributing to their environment. Microglia are always active, in health and disease; ramified microglia are not inherently less participatory, but simply better suited to regulate homeostatic conditions (Paolicelli et al., 2022). The former phenotype reflects microglia's role as tissue-resident macrophages and is accompanied by increased phagocytic activity, aligning closer with their macrophage counterparts. Early investigations into microglia quickly found the rounded form prevalent in diseased and postmortem tissues, and spurred theories that the cells conveyed neurotoxic effects (Hellwig et al., 2013). Unfortunately, investigations into microglial functions were halted for the better part of the past century, only garnering renewed attention in the past decade.

Microglia have emerged as an extremely important cell population, gaining interest for their contributions to neurodevelopment, maintaining optimal brain health, and their roles in aging and neurodegenerative disorders (Lannes et al., 2017). They are the sentinels of the central nervous system's (CNS's) innate immune system and make up ~5–10% of total brain cells. Increased research has highlighted their functions outside of immunity, notably their contributions to refining synaptic circuits early in development, alongside aiding the differentiation of neurons and other glial cells. Under steady-state conditions, microglia regulate neurotransmission and maintain synaptic integrity while serving as key immune sentinels that respond to pathological insults and mediate neuroinflammatory responses (Frost & Schafer, 2016). Even in homeostatic conditions, microglia are continually responding to neurotransmitters released into the synaptic cleft. Glutamate, gamma-aminobutyric acid (GABA), and adenosine triphosphate (ATP) are efficiently recognized by microglia and help characterize nearby neuronal activity. Microglia are rich in receptors integral to positive and negative feedback loops, establishing a strong bidirectional connection between themselves and nearby neurons (Liu et al., 2016; Nakamura & Shichita, 2025). The traditional view that microglia are "docile spectators" is vastly outdated, with early theories denoting them as quiescent cells repeatedly debunked. Microglia continuously engage with their environment, and they are now considered one of the most dynamic and active cellular populations (Bilbo & Stevens, 2017).

Microglia's extensive resume of functions is largely attributed to their plastic nature. Microglia are considered some of the most morphologically advanced

cells, capable of rapidly shifting their phenotype to respond to specific environmental cues. Broadly speaking, microglia can be classified into four overarching categories: ramified, hyper-ramified, ameboid, and rod (Table 17.1). Of note, these categories exist across a continuum with various intermediates; however, this polychotomy is used to help easily speculate on function and response type. Under homeostatic conditions, microglia exhibit long, thin processes that stretch outwards from a small cell body. Live-imaging techniques have documented the process by which these thin tendrils, tens of microns in length, repeatedly extend and retract to probe their local surroundings and intervene whenever needed (Andoh & Koyama, 2021). Beyond surveillance, ramified microglia are capable of phagocytosis, often achieved by a bulbous structure at the end of their processes. Microglial processes are rich in a vast array of receptors, primed to respond to neurotransmitters, cytokines, or immune stimuli. Interactions with these receptors can "activate" microglia, a process often marked by de-ramification. The transition into the ameboid state elicits a large-scale retraction of processes into short stubs surrounding a larger and rounder soma (Vidal-Itriago et al., 2022). In this form, microglia are more migratory, demonstrate higher phagocytic activity, and even have a faster turnover rate than their surveillant counterparts (Parakalan et al., 2012). Alongside their higher prevalence in disease states as part of the neuroinflammatory response, ameboid microglia are also common in aging tissue (Ritzel et al., 2015). Documenting microglial morphology across brain regions, sexes, ages, and health conditions has become a burgeoning research field.

Beyond the predominant ramified and amoeboid morphologies are two less commonly reported subsets: rod and hyper-ramified. Rod microglia have a thin soma surrounded by planar processes and are commonly found alongside damaged neurons (Taylor et al., 2014). Their function is largely unexplored but believed to be a part of the CNS immune response during trauma, neurodegeneration, or aging. Other studies point toward an organizational role during the repair of neuronal circuitry after injury (Au & Ma, 2017). The final phenotype, hype-ramified, lacks substantial literature. Sometimes considered an extreme form of surveillant microglia, hyper-ramification is primarily documented to occur in chronic stress states and is accompanied by increased ionized calcium-binding adaptor molecule 1 (Iba1) expression (Fujikawa & Jinno, 2022; Vidal-Itriago et al., 2022). Iba1 is the predominant marker used in

immunohistochemical analysis of microglia and is generally considered to reliably stain all phenotypes (with the exception of dark microglia, an elusive and under-researched phenotype wherein Iba1 can be downregulated) (Bisht et al., 2016; Lier et al., 2021). Staining of this calcium-binding protein is a quick and efficient way to discern microglia from other neural counterparts, namely astrocytes, oligodendrocytes, and neurons. However, it does run the risk of staining infiltrating macrophages, as sometimes observed in pathological CNS conditions (Gheorghe et al., 2020; Kenkhuis et al., 2022). Overall, microglia's ability to rapidly and effectively respond to their environment, coupled with their morphological complexity, makes them uniquely qualified to form a strong connection with fringe inhabitants.

TABLE 17.1

Simplified Characterization of Microglial Subgroups, Incorporating Nomenclature from a Variety of Sources ⏎

Morphology	Amoeboid	Ramified	Hyper Ramified	Rod	References
Description	Rounded cell body with short, retracted processes and compacted shape	Long processes creating a branching network	Extensive network of long, thin processes; high arborization beyond ramified counterparts	Elongated cell bodies with polarized processes	Green and Rowe (2024), Paolicelli et al. (2022), Vidal-Itriago et al. (2022)

Morphology	Amoeboid	Ramified	Hyper Ramified	Rod	References
Significance	High phagocytic activity; migratory; cleanup cellular debris, respond to immune challenge; disappearance of long, thin processes associated with "activation	Termed the "resting" state; surveil the brain milieu and associated with homeostatic functions	May display an upregulation in ramification-promoting proteins like β1 integrin; presumed to aid in the modulation of synaptic transmission during stress	Join to form multicellular "strings" that could serve a neuroprotective role when aligned against uninjured neurons in compromised settings; considered another "active" phenotype	
Environment	Seen in the inflammatory response; neural degeneration	Homeostasis; healthy tissue	Associated with acute and chronic stress; ecotoxicity	Aging tissues; injury states	

Morphology	Amoeboid	Ramified	Hyper Ramified	Rod	References
Synonyms	Fully active, phagocytic, M1/M2 (used sparingly; outdated/rejected from widespread use), de-ramified, disease associated microglia (DAM), reactive, "activated"	Resting, homeostatic, non-reactive, surveillant, M0	Bushy, primed	N/A	
Example					Images exported from BioRender

Early studies of microglia assumed they were shielded from the circulatory system by the blood–brain barrier (BBB). All inputs were thought to originate from the CNS, limiting communication to local stimuli and encounters (Paolicelli et al., 2022). However, this theory has since been disproven with the discovery of the gut–brain axis (GBA) (Figure 17.1). A bidirectional communication network between the microbiota and the emotional and cognitive centers of the CNS, the GBA facilitates complex signaling using neural, endocrine, immune, and humoral links (Rutsch et al., 2020). Microglia–microbiota crosstalk has become its own emerging niche, with the microbiota seen as a master regulator of microglial activity. A landmark study from 2015 demonstrated that germ-free conditions in mice drastically hindered microglial development, leading to a variety of downstream consequences, such as changes in neuronal density and function. This ultimately sparked a deep interest in discerning microglia–microbiota relations, particularly during neurodevelopmental and

neurodegenerative diseases (Erny et al., 2015). Dysbiosis is present in many neurological conditions known to be concurrent with abnormal microglial function (Bano et al., 2024). Patients with Alzheimer's disease (AD) exhibit reduced *Firmicutes* and *Bifidobacterium*, alongside higher levels of proinflammatory *Bacteroidetes* species (Liang et al., 2024). Other studies, such as one investigating the impact of fecal microbiota transplant (FMT) using microbes from human patients with Parkinson's disease (PD), found impaired motor function (following transference into mice) compared to healthy controls (Sampson et al., 2016). These reports lend credence to the importance of the GBA. Though not a new concept, as altered microbiome composition is implicated in a range of diseases outside the gastrointestinal (GI) tract, researchers see targeting the microbiome as a novel therapeutic strategy for neurodevelopmental, neurodegenerative, and psychiatric disorders. In this chapter, we will review microglia's basic properties (i.e., their maturation, function, and participation in neural pathologies) and investigate the influences of the microbiome, as mediated through the GBA.

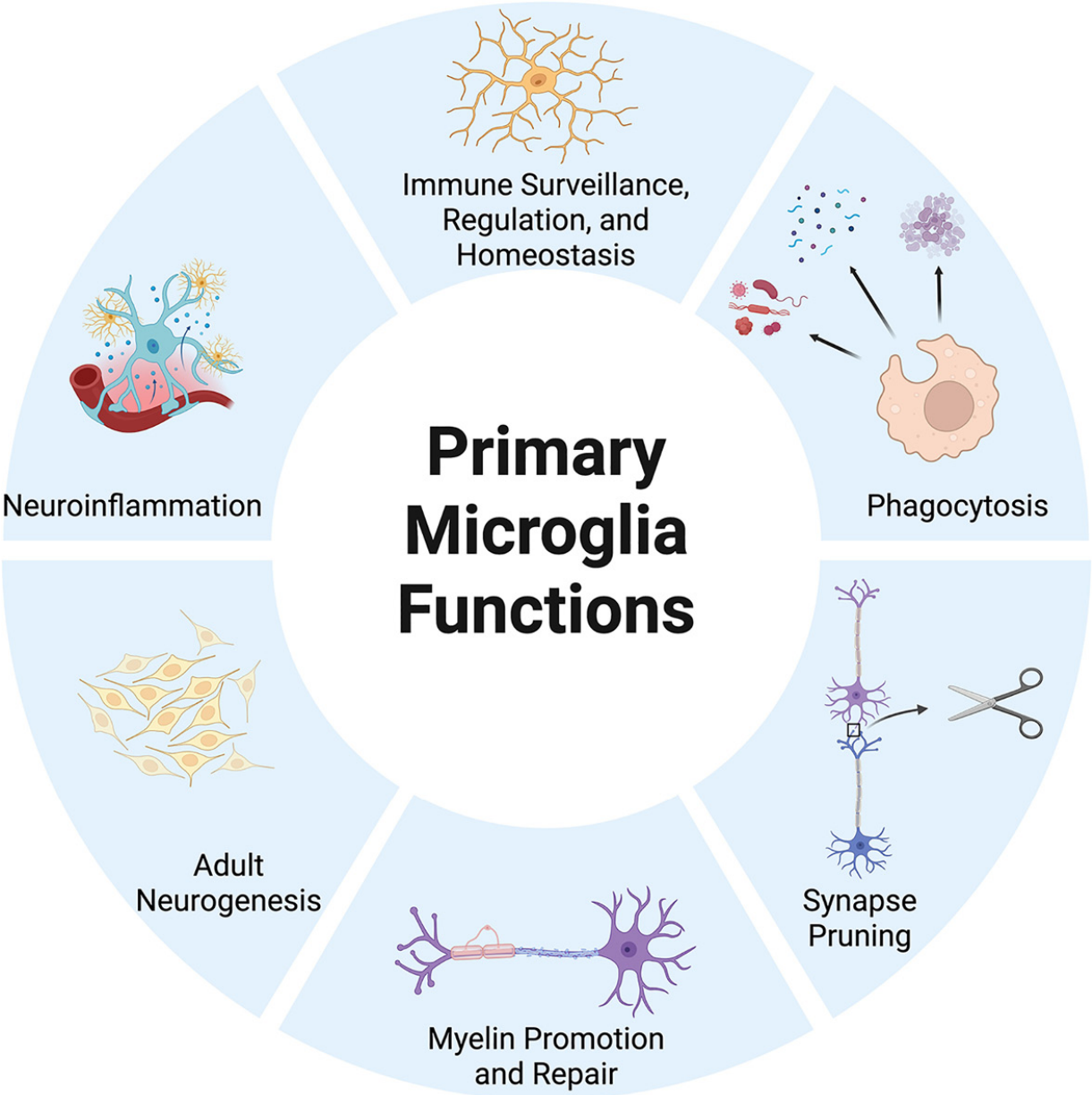


FIGURE 17.1 Simplified schematic of the GBA. This bidirectional communication system allows for intimate crosstalk between the gut's inhabitants and the central nervous system. The network utilizes various signaling pathways to transmit information necessary for homeostatic function as well as disease responses. Figure generated using BioRender. ⏎

17.2 Microglia

17.2.1 Microglial Maturation

Until recently, the origin of microglia was unknown. They were largely speculated to arise from the same lineage as peripheral macrophages, due to their analogous structures and functions. However, this has since been disproven, primarily based on microglia's prenatal origin, capacity for self-renewal, longevity, and their migratory nature (Yin et al., 2017). Lineage-tracing studies from 2010 deduced that microglia are products of unique precursors in the yolk sac, a temporary extranatal membranous structure that serves as a site of primitive hematopoiesis around embryonic day (E)7.0 in mice (Ginhoux et al., 2010, 2013). Mice are the principal model for understanding microglial morphology, but they do exemplify altered epigenomic landscapes and stage-dependent processes compared to humans (Han et al., 2023).

Microglial development is a highly refined process that requires ongoing input from genetic and environmental cues. The first defined step in microglial formation begins at E7.5, when microglia, alongside CNS-resident macrophages, arise from erythromyeloid progenitor cells (EPCs) and begin developing in the yolk sac. These early macrophages express a series of transcription factors (TFs), including runt-related transcription factor 1 (RUNX1), JUN, PU.1, and interferon regulatory factor 8 (IRF8). Proteins such as RUNX1 promote the transition into ramified morphologies, whereas IRF8 and other TFs help with chromatin accessibility to facilitate initiation of the microglia-specific transcriptome (Saeki et al., 2024; Zusso et al., 2012). By day E8.5 post-conception, the cells are equipped to colonize the fetal liver and differentiate into erythrocytes or cells from myeloid lineages. A subset of these migratory cells will develop microglia-specific markers such as CX3CR1, CD11b, and F4/80 (Ginhoux et al., 2010). As microglia progenitors, they move to the brain over E9.5–10.5, becoming some of the earliest colonizers of the CNS. During this time, microglia also begin to assume their characteristic "ramified" structure, with 40–80% of cells losing their ameboid characteristics by the end of their journey. The ameboid phenotype facilitates large migration patterns (Okajima & Tsuruta, 2018). Ramification is assumed to reflect the cells reaching their destination, as they take up residence in the CNS. Microglial migration to the neural tube and eventual population of the developing brain and spinal cord coincides with the formation of the BBB, a fundamental structure in shielding microglia and other CNS cells from

potentially damaging substances from the circulation and the periphery throughout their lifetime (Mayer & Fischer, 2024). The mass movement of microglia preceding their proliferation and spatial distribution in the CNS is dependent on the neuronal expression and secretion of interleukin (IL)-34, which in turn requires sensing from colony-stimulating factor-1 receptor (CSF1R) on erythromyeloid progenitors (EMPs) and developing microglia (Devlin et al., 2025).

Once established in the brain, microglial populations rise proportionally to brain growth. Their rapid proliferation results from intraparenchymal proliferation (clonal expansion), which is largely governed by CSF1 and IL-34 (two ligands for CSF1R), as well as transforming growth factor beta 1 (TGF-β1) (Barry-Carroll et al., 2023; Spittau et al., 2020). Eventually, upon achieving a stable mosaic distribution during late postnatal development, expansion slows, and microglial density levels out around postnatal day (P)14 (Barry-Carroll et al., 2023). Their numbers remain relatively constant into adulthood, with a stable rate of proliferation that is independent of progenitors and/or monocytes. This feature of self-renewal in the CNS ultimately helps define microglia from their macrophage counterparts (Rossi & Lewis, 2018).

17.2.2 Early Identity and Function

Advances in transcriptomic analysis have helped elucidate a concise maturation process for microglia following their prenatal colonization and postnatal proliferation. Researchers have simplified the gene-expression profile and epigenetic signatures responsible for microglia's stepwise program into three defining steps, occurring in tandem with brain development (Matcovitch-Natan et al., 2016). The first of these temporary stages, early-microglia, is dominated by genes associated with cellular development, growth, and proliferation. They are generally ameboid to facilitate phagocytosis of excess neuronal structures and highly migratory as they guide axonal growth. From E14 to several weeks postnatal in mice, they begin transitioning into ramified morphologies and refocus their efforts on synaptic pruning and promoting axonal myelination. Beyond their traditional immunological roles, microglia have emerged as leaders in the refinement of neural circuits. Eliminating weak or inefficient synapses is crucial for proper neuronal signaling and, in its absence, can lead to impaired

cognitive function (Paolicelli et al., 2011). Microglia are the sole glial cell type at this stage, with astrocytes and oligodendrocytes entering the brain only around E18 in mice (Akdemir et al., 2020). Researchers are now trying to discern the way in which a subpopulation of microglia contributes to white matter development (Santos & Fields, n.d.). Eventually, they conclude in their "housekeeper" form, ramified and expressing gene signatures rich in immune signaling pathways. The adult transcriptome is distinct from tissue-resident macrophages but does converge at neuroinflammatory pathways, coinciding with both cell populations' role as immune mediators (Grassivaro et al., 2020).

17.2.3 Adult Microglia Differentiation and Function

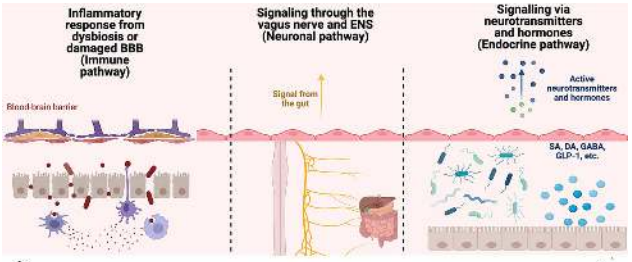
Though there is no definitive marker of maturation, researchers deduce that the adult microglia population establishes itself around 2 weeks postnatal. This has been substantiated in studies wherein, following complete inhibition using CSF1R antagonists, repopulation occurs within ~14 days, or sometimes as early as 7 days (Najafi et al., 2018). At this point, the cells outgrow their highly phagocytic ameboid form, and their proliferation is significantly reduced. In mice, population decline begins at 3 weeks post-birth, continuing until 6 weeks, at which point only 50% of the cells remain. Numbers remain relatively steady until at least 9 months of age, and over the course of a lifetime, microglia renew at a median rate of 28% a year. On average, microglia will replace themselves every 4.2 years, but they have been known to last more than two decades (Réu et al., 2017). This positions them uniquely among other immune cells, which rarely outlast a year before regenerating (De Boer & Perelson, 2013; Macallan et al., 2005).

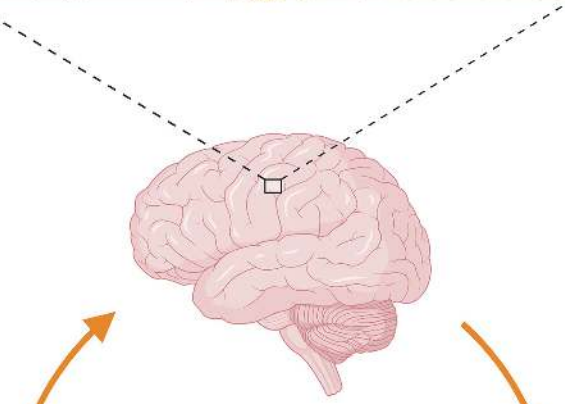
Adult microglia exhibit the extensive morphological spectrum detailed earlier in the chapter. This tool complements their varied functions and allows them to quickly and effectively respond to changes in the environment (Vidal-Itriago et al., 2022). In the absence of disease, microglia detect disturbances in their environment while supporting other cells in the brain parenchyma. They continually contribute to adult neurogenesis, maintaining a balance between new neurons and the elimination of weak or old cells, while also refining existing synapses. In some circumstances, microglia will interact directly with neuronal

cell bodies to form purinergic junctions and regulate signaling activities (Zhao et al., 2024).

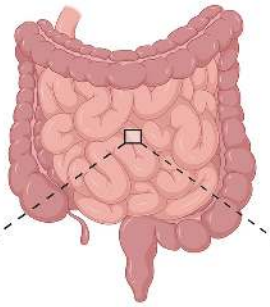
Outside of neurons, microglia maintain the brain's neurovasculature. A complex network of arteries, veins, and capillaries, neurovascular units are essential for the proper distribution of oxygen and glucose, alongside the elimination of waste products like carbon dioxide (Schaeffer & Iadecola, 2021). Microglia have been shown to promote angiogenesis and even regulate capillary vascular tone (Kisler et al., 2021; Zhao et al., 2018). Other maintenance functions of microglia include, but are not limited to, supporting the growth and differentiation of astrocytes and precursor oligodendrocytes. In the case of oligodendrocytes, microglia contribute to a healthy myelin sheath and the remyelinating process during disturbances (Baaklini et al., 2023; Borst et al., 2021).

Microglia's predominant function, hinted at previously, is to serve as the immune cells of the CNS (Figure 17.2). They are equipped with a diverse range of receptors, notably pattern recognition receptors (PRRs) at the membrane, suitable for sensing erroneous endogenous material (e.g., DNA, RNA) or foreign debris (e.g., bacteria or viral fragments) (Saijo et al., 2013). Depending on the insult, microglia will launch a pro- or anti-inflammatory signaling cascade. Pro-inflammatory responses are often met with the emission of pro-inflammatory cytokines (interleukin-1 beta (IL-1β), IL-6, tumor necrosis factor- alpha (TNF-α)), alongside reactive species (reactive oxygen species (ROS)) (Rodríguez-Gómez et al., 2020). Further, microglia will upregulate proteins such as pentraxin 3, a time-dependent protein that facilitates microglia's phagocytic form (Oggioni et al., 2021). The primary goals are to neutralize the threat, sometimes at the cost of mounting a damaging immune response. For anti-inflammatory reactions, microglia can migrate to the site of injury. Once there, they release a repertoire of anti-inflammatory cytokines (IL-10) and neurotrophic factors. In contrast to pro-inflammatory responses, this approach aims to dampen chronic inflammation and spur tissue repair (Cherry et al., 2014).





The Gut-Brain Axis



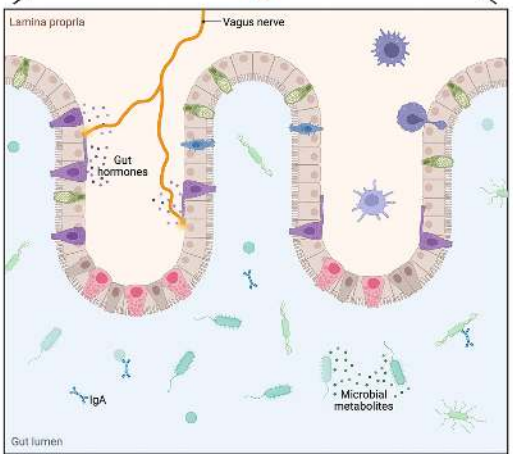


FIGURE 17.2 Primary functions of adult microglia. Mature microglia exhibit a vast repertoire of functions not limited to the CNS's immune system. They are fundamental for maintaining the homeostatic environment by constantly testing their surroundings for disturbances and responding when necessary. The brain relies heavily on microglial interactions with neuronal elements to ensure synapse and myelin integrity, with newer findings showing microglia aid in adult neurogenesis throughout life. Taken together, microglia prove to be a significant mediator of CNS health and baseline function. Figure generated using BioRender. ⏎

Overall, microglia's diverse functions demonstrate how vital they are for CNS health and function. Disruptions in their behaviors, or failure to meet maturation, can have far-reaching consequences, sometimes brought on by faulty GBA communication.

17.3 GBA Influences on Microglia Development

It has long been suspected that the gut and brain share an intimate relationship. Accounts as far back as ancient China and Greece report on links between digestion and personal disposition, with some cultures using primitive forms of FMT in traditional medicine (Lewandowska-Pietruszka et al., 2022). While microbes were yet to be discovered, the importance of optimal GI health was becoming clearer. In contrast to current interpretations of the GBA, scientists believed gut health was subject to one's mental state.

One of the earliest experiments establishing a direct link between emotion and digestion stemmed from the work of Dr. William Beaumont. An army surgeon, Beaumont experimentally demonstrated that a patient's emotional state (such as anger or fear) has a direct effect on the rate of digestion. Though his methods were less than ideal—as he primarily used the gastric secretions and an optimally placed fistula from a wounded soldier—his work provided hard evidence for a gut-brain communication network (Beaumont & Daniels, 1833; Gale, 2021). Later contributions to the field were less crude, such as Ivan Pavlov's conditioning experiments from 1904, wherein he found that sensory signals could stimulate gastric and pancreatic secretions during the cephalic phase of digestion (Chunduri et al., 2022). These findings complemented the growing

sentiment of "nervous sympathy," first coined in 1765 and popularized in the 19th century. Foundational to this idea was the large number of nerve endings in the intestines, believed to be part of an "abdominal brain" (Chunduri et al., 2022). Microscopic proof of a stable microbiota in the rodent stomach by Dubos *et al*., seemed to be the missing piece, marrying the aforementioned concepts together and giving birth to new research into the GBA (Rasmussen, 2022).

Neuroscientists' increased interest in the GBA mirrors the advancement of techniques and bioinformatic tools used to study the microbiome. The advancement of technologies has allowed researchers to gain critical insight into the variety of ways in which the gut microbiota is paramount to CNS physiology. Beyond the CNS, the microbiome has established itself as a necessary player in development. Early microbes are foundational to educating the immune system, fortifying the BBB, and aiding in nutrient availability, among other functions (Muramatsu & Winter, 2024; Parker et al., 2019; Zheng et al., 2020). Even before birth, the maternal microbiota is postulated to be a key determinant of fetal health, playing into nutrient absorption and immune tolerance (Dominguez-Bello et al., 2019).

Focusing on the CNS, microbes use a combination of signals ingrained in immune, endocrine, enteric, and neuronal pathways to deliver information along the GBA. When these physical and chemical connections are severed or exploited, it can have dire influences on the host. Germ-free (GF) mice experience both anatomical and cognitive deficits, some of which can be repaired with the correct combination of pre- or probiotics (Bidgoli et al., 2025). Investigations into GF mice have lent credence to the importance of a robust microbiome to ensure optimal brain health. Among many other factors, GF mice exhibit increased BBB permeability, differential expression of synapse-related genes, increased stress hormone signaling, and insufficient control of neurogenesis (Luczynski et al., 2016). However, more topical to the present chapter, GF mice give rise to immature microglia with impaired immune responses and other global deficits (Prabakaran, 2015). With these findings in mind, work is being done to tease out the impact of the gut microbiota on microglia in early development, as well as later in life.

17.3.1 The Maternal Microbiota Is Key to Prenatal Microglia Maturation

The womb, like the brain, is canonically a sterile environment (Perez-Muñoz et al., 2017). However, while that holds, it does not mean it is detached from microbial influences. Evidence exists highlighting the importance of the maternal microbiome for proper development and maturation (EBioMedicine, 2021). Given the multifaceted role of microglia in development, researchers have logically started to deduce the impact of the maternal microbiome on neonatal microglial development.

The fetal microglia from GF dams experience significant differences in transcriptomic and chromatin accessibility levels, suggesting the maternal microbiota has a role for these cells in utero (Kimmel et al., 2024). Furthermore, the overall effect appears to be sex- and stage-dependent. At embryonic day 14.5 (E14.5), there are minimal changes in gene expression between mice born from GF or specific-pathogen-free (SPF) parents. However, closer to birth at E18.5, there is a vast increase in differentially expressed genes (DEGs) that persists into adulthood. Beyond GF and SPF parameters, gene expression is also dependent on sex, with male GF embryos having differential expression of genes linked to translation and metabolism, versus inflammatory responses and transcriptional regulators in their GF female counterparts (Thion et al., 2018).

The notion of the microbiome helping inform the developing immune system may begin at the maternal microbiota (Zheng et al., 2020). Genes such as *Ly86* and *Aoah*, both essential for the innate immune response to lipopolysaccharide (LPS), were downregulated in offspring from GF parents (Su et al., 2014; Yang et al., 2020). Taken together, the parental microbiome may be a key player in priming and fine-tuning the offspring's immune system (Thion et al., 2018).

17.3.2 Gut Microbiota Shapes Adult Microglial Identity

It is well characterized that GF mice display global deficits and changes, and this is a pivotal argument for the importance of gut microbes in development (Luczynski et al., 2016). This rings especially true for microglia, as exemplified by research into microglia taken from GF mice. Genome-wide mRNA expression profiles in microglia from GF mice reveal notable decreases in the expression of genes necessary for cell "activation" and immunity. Lowered expression of PRR-associated proteins and other immunogenic mediators could suggest insufficient protection from downstream challenges. Experiments testing this suggestion

found a reduced cytokine expression profile and overall innate immune response in GF mice challenged with LPS. In addition, there was a significant attenuation of pro-inflammatory genes (Erny et al., 2015). In contrast, GF mice upregulate genes associated with survival factors and cell growth (Erny et al., 2015). This aligns nicely with the observation that GF mice display higher microglia density than SPF mice (Castillo-Ruiz et al., 2018). Even within their regions, GF microglia fail to appropriately alter their morphology, sometimes remaining arrested in a "bushy" state reminiscent of early development. When they do reach ramified morphologies, they make excessive contacts with neighboring cells and do not adopt accepted cellular boundaries (Erny et al., 2015).

When compared to other cells of the CNS, microglia are preferentially affected by deficits in the microbiome. One study from 2023 explored how different cell types (notably neurons, astrocytes, and oligodendrocytes) were affected in a GF mouse model (Huang et al., 2023). Looking at SPF and GF mice, glial cells displayed the most DEGs, with GF microglia highly enriched for genes associated with pro-inflammatory and complement signaling pathways. Microbial colonization seemingly reversed these transcriptional changes, suggesting that microbiota-depletion effects can be rescued. In addition, single-cell resolution demonstrated that the microbiome's absence confers region-specific changes. Individual microglia from the prefrontal cortex (PFC) and hippocampus did not exhibit the same transcriptome differences (Huang et al., 2023). Altogether, this adds another layer of complexity to the microbiome's influence on microglial signaling.

Researchers have also investigated the impact of antibiotic (ABX) cocktails on microglia, finding similar, though not identical, effects as GF models. Microglia from ABX-treated mice do not experience the same enrichment of neuroinflammatory genes, potentially due to the incomplete eradication of gut microbes (Cordella et al., 2021). However, some reports find heightened levels of circulating chemokines and cytokines after sustained ABX treatment (Minter et al., 2016). Further, they were not as susceptible to morphological changes often seen in GF mice. Similar to GF models, microglia from ABX-treated samples exist at higher densities and are reported to have increased basal motility, oftentimes favoring homeostatic functions in lieu of deficient immune functions (Cordella et al., 2021)

Many of the studies looking at microglial deficits from dysbiosis or GF models all function to better interpret the role of the microbiome in disease, particularly neurodegenerative disorders known to be concurrent with imbalances in the gut microbiota (GM).

17.4 Microglia in Neurodegenerative Diseases

As the brain's immune cells, microglia are the supposed protectors against disturbances to cognitive function and impede further injury. However, research into microglia's role in neurodegenerative disease presents them more as a double-edged sword: capable of diminishing threats, but easily overactive and contributing to pathological environments (Wyss-Coray & Mucke, 2002). A highly cited example of this occurs in AD, a neurodegenerative disease characterized by the deposition of beta-amyloid (Aβ) plaques and tangles of hyperphosphorylated tau proteins, alongside synaptic loss and pervasive CNS inflammation. This manifests as severe cognitive deficits and is the leading cause of dementia (Aarsland et al., 2025). In response to Aβ plaques, microglia will migrate to the source and attempt to phagocytose the misfolded proteins. Though clearance can initially be beneficial, it can quickly promote an inflammatory environment, leading to profound synapse loss and further neuronal damage (Fruhwürth et al., 2024). This pattern continues for a host of other neurological conditions and has made microglia a highly sought-after target for developing novel treatments.

Alongside interest in microglia's pathological contributions comes mounting evidence as to the role of dysbiosis in promoting or affecting neurological health. Specific bacterial species and metabolites have been linked to neurodegenerative disorders, as well as several mental illnesses (Clapp et al., 2017; Intili et al., 2023). Given the immense crosstalk between the brain and gut, it is natural to suggest that variations in the microbiome factor into disease progression. Connecting the two concepts suggests the microbiome's effect on microglia could be a risk factor for poor brain health.

Erroneous communication between the microbiome and microglia has been implicated in advancing various neurodegenerative disorders. Dysbiosis, and its resulting impact on microglia, is perhaps the best documented. However, reliable evidence suggests dysbiosis in PD—namely, fewer SCFA-producing bacteria—is

also receiving increasing attention (Tizabi et al., 2025). The GBA, though necessary for health, development, and homeostatic functions, can be co-opted as a driver of disease in pathological states (Ullah et al., 2023).

17.4.1 Alzheimer's Disease

As previously stated, microglial dysregulation can induce pro-inflammatory environments in disease, namely through the release of chemokines, cytokines, and reactive species. The cycle is perpetuated by increased neuronal damage, reinvigorating the immune cells and contributing to disease pathology. A leading theory explaining microglia's shift to damaging phenotypes has not been established, but many are now proposing it could be induced by alterations to the gut microbiome (Intili et al., 2023). Broadly speaking, patients with AD have a consistent decrease in *Firmicutes* and *Bifidobacteria*, and an increase in *Bacteroidetes* and *Proteobacteria* species. Changes in the composition of gut bacteria at the onset of disease are so well documented that some propose using it as an early biomarker. Dysbiotic environments can promote the production of pro-inflammatory mediators and suppress beneficial bacterial species (Manfredi et al., 2025). Certain bacterial metabolites, such as trimethylamine *N*-oxide (TMAO), are known to promote tau and Aβ aggregation (Gao et al., 2019).

There are several mechanisms by which the microbiome may promote inappropriate microglial activation in AD. One explanation draws on the decreased abundance of beneficial bacterial species. Butyrate is an SCFA with promising neuroprotective properties, and several studies indicate it helps enhance memory and cognition (Tu et al., 2025). However, butyrate-producing gut microbes are at lower abundances in aging adults and patients with AD, offering a potential source of cognitive decline. Microglia treated with butyrate exhibit improved anti-inflammatory properties. Thus, the interaction (or lack thereof) of microglia and bacterial metabolites important for anti-inflammatory phenotypes may favor pro-inflammatory behaviors. Further, supplementing patients with AD with these metabolites or their producers may be enough to rescue certain disease behaviors (Naomi et al., 2021; Zhang et al., 2025).

A more direct avenue by which the microbiome contributes to aberrant microglial phenotypes is by microbiota-driven neuroinflammation. Dysbiosis can cause increased gut permeability, facilitating the emergence of harmful

substances into the bloodstream (Lei et al., 2025). The leakage of pro-inflammatory mediators can cross the BBB, stimulating widespread inflammation in the brain and overburdening microglia. The once favorable phagocytic cells aiding in A**β**-plaque clearance decline early in disease and become a double-edged sword. Being able to control and limit gut permeability, and by extension maintain the integrity of the BBB, is essential for limiting systemic inflammation and preventing further AD progression. At present, researchers are working on probiotic "cocktails" to curtail the inflammatory GI environment with varying degrees of success (Prajapati et al., 2025).

17.5 The Microbiome as a Contributing Factor for Mental Health

Going back to the origins of the GBA, researchers deduced that one's emotional state can have a direct influence on digestion. Individuals suffering from anxiety, depression, and other mental illnesses experience higher degrees of gut dysbiosis (Bibbò et al., 2022). Alongside these disturbances, individuals struggling with their mental health may also present with altered levels of bacterial metabolites, such as higher levels of TMAO in depression. These substances may cross the BBB and induce inflammation, perpetuating psychiatric distress (Liu et al., 2023).

The concept of the microbiome affecting mental health was cemented following FMT experiments involving the colonization of GF mice with fecal samples from anxiety- and depression-like mouse models. Upon receiving the stressed fecal samples, GF mice took on both the behaviors and neuroinflammatory triggers of the mice subjected to chronic, unpredictable stress (Li et al., 2019). Additional studies using similar methods have corroborated these results, while also spurring discussion regarding FMT as a promising intervention for treating mental health conditions (Meng et al., 2024). Overall, there is an abundance of evidence pointing toward microbial contributions to both mental illness progression and remedies.

Akin to the dual role of microglia and the microbiome in neurodegenerative diseases, microglia also contribute to psychiatric disorders. Though an exact mechanism has not been established, microglia-induced inflammation has been accepted as a contributing factor in depression. Further, microglia appear to affect the transcriptional activity of dorsal raphe nucleus (DRN) neurons,

exacerbating symptoms associated with suicidal ideation (Brisch et al., 2022). Some studies point toward the microglial response to chronic stress, wherein they appear to excessively phagocytose neuronal synapses. This excessive loss can lead to dysfunction, contributing to cognitive impairment and acute injury (Chen et al., 2025; De Schepper et al., 2023).

Investigations into how crosstalk between microglia and the microbiome could influence psychiatric conditions showed that this pathway can confer both detrimental and beneficial effects. Microglia from FMT-treated mice from a depression-model donor found microglia were more primed to pro-inflammatory pathways. Specifically, in the dentate gyrus, recipients had hyper-ramified morphologies that were highly sensitive to LPS or chronic stress challenges. Non-immune functions were also hindered, with researchers observing impaired hippocampal neurogenesis and the GF mice adopting depressive-like behaviors (Hui He et al., 2024).

Chronic stress is a hallmark of many psychiatric conditions, such as anxiety and depression. There is a difference in the microbial composition of stress-resistant and stress-sensitive subjects. Simply put, stress-resistant mice have a higher alpha diversity. Studies looking into stress-resistant mice find lower expression levels of immune responses and a higher resistance to the reductions in synaptic plasticity observed in stress-sensitive mice. Microglia in mice with a stress-sensitive microbial composition are usually denser and favor ameboid morphologies alongside the increased emission of pro-inflammatory cytokines. Transplantation of stress-resistant fecal matter into stress-sensitive models can confer resistance to stress, showing how the gut microbiota can rescue cell-specific behaviors (Haili He et al., 2024).

17.6 Conclusion

Investigations into microglia, the microbiome, and how the two interact through the GBA constitute a quickly expanding field with widespread implications for human health. This chapter aimed to provide a surface-level understanding of the field and hopefully encourage readers to continue reading on the topic. In the interest of simplicity and space, many topics were overlooked—such as the specific pathways utilized in the GBA, microglia- and microbiome-based treatments in disease, and the contribution of specific bacterial species. However,

we hope this primer leaves you with a better understanding and appreciation for both your glial cells and the diverse inhabitants of the GI tract. Though spatially distant, the intimate connection between your brain and gut brings them closer than ever.

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References

Aarsland, D., Sunde, A. L., Tovar-Rios, D. A., Leuzy, A., Fladby, T., Zetterberg, H., Blennow, K., Tan, K., De Santis, G., Yakoub, Y., Arslan, B., Huber, H., Pola, I., Grötschel, L., Di Molfetta, G., Skjellegrind, H. K., Selbaek, G., & Ashton, N. J. (2025). Prevalence of Alzheimer's disease pathology in the community. *Nature*, 1–5. https://doi.org/10.1038/s41586-025-09841-y ⏎

Akdemir, E. S., Huang, A. Y.-S., & Deneen, B. (2020). Astrocytogenesis: Where, when, and how. *F1000Research*, *9*, F1000 Faculty Rev-233. https://doi.org/10.12688/f1000research.22405.1 ⏎

Andoh, M., & Koyama, R. (2021). Assessing microglial dynamics by live imaging. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.617564 ⏎

Au, N. P. B., & Ma, C. H. E. (2017). Recent advances in the study of bipolar/rod-shaped microglia and their roles in neurodegeneration. *Frontiers in Aging Neuroscience*, *9*, 128. https://doi.org/10.3389/fnagi.2017.00128 ⏎

Baaklini, C. S., Ho, M. F. S., Lange, T., Hammond, B. P., Panda, S. P., Zirngibl, M., Zia, S., Himmelsbach, K., Rana, H., Phillips, B., Antoszko, D., Ibanga, J., Lopez, M., Lee, K. V., Keough, M. B., Caprariello, A. V., Kerr, B. J., & Plemel, J. R. (2023). Microglia promote remyelination independent of their

role in clearing myelin debris. *Cell Reports*, *42*(12), 113574. https://doi.org/10.1016/j.celrep.2023.113574 ⏎

Bano, N., Khan, Sameera, Ahamad, S., Kanshana, J. S., Dar, N. J., Khan, Sumbul, Nazir, A., & Bhat, S. A. (2024). Microglia and gut microbiota: A double-edged sword in Alzheimer's disease. *Ageing Research Reviews*, *101*, 102515. https://doi.org/10.1016/j.arr.2024.102515 ⏎

Barry-Carroll, L., Greulich, P., Marshall, A. R., Riecken, K., Fehse, B., Askew, K. E., Li, K., Garaschuk, O., Menassa, D. A., & Gomez-Nicola, D. (2023). Microglia colonize the developing brain by clonal expansion of highly proliferative progenitors, following allometric scaling. *Cell Reports*, *42*(5), 112425. https://doi.org/10.1016/j.celrep.2023.112425 ⏎

Beaumont, W., & Daniels, W. B. (Worth B. (with U.S. National Library of Medicine). (1833). *Experiments and Observations on the Gastric Juice, and the Physiology of Digestion*. Plattsburgh, NY: F. P. Allen. https://archive.org/details/2543009R.nlm.nih.gov ⏎

Bibbò, S., Fusco, S., Ianiro, G., Settanni, C. R., Ferrarese, D., Grassi, C., Cammarota, G., & Gasbarrini, A. (2022). Gut microbiota in anxiety and depression: Pathogenesis and therapeutics. *Frontiers in Gastroenterology*, *1*. https://doi.org/10.3389/fgstr.2022.1019578 ⏎

Bidgoli, F. A., Talaei, S. A., Tameh, A. A., & Salami, M. (2025). Probiotics restore impaired spatial cognition and synaptic plasticity of prenatally-stressed male rats: Focus on hippocampal and intestinal tight junctions. *Neurobiology of Stress*, *37*, 100736. https://doi.org/10.1016/j.ynstr.2025.100736 ⏎

Bilbo, S., & Stevens, B. (2017). Microglia: The brain's first responders. *Cerebrum: The Dana Forum on Brain Science*, *2017*, cer-14-17. ⏎

Bisht, K., Sharma, K. P., Lecours, C., Gabriela Sánchez, M., El Hajj, H., Milior, G., Olmos-Alonso, A., Gómez-Nicola, D., Luheshi, G., Vallières, L., Branchi, I., Maggi, L., Limatola, C., Butovsky, O., & Tremblay, M. (2016). Dark microglia: A new phenotype predominantly associated with pathological states. *Glia*, *64*(5), 826–839. https://doi.org/10.1002/glia.22966 ⏎

Borst, K., Dumas, A. A., & Prinz, M. (2021). Microglia: Immune and non-immune functions. *Immunity*, *54*(10), 2194–2208. https://doi.org/10.1016/j.immuni.2021.09.014 ⏎

Boullerne, A. I., & Feinstein, D. L. (2020). History of neuroscience I. Pío del Río-Hortega (1882–1945): The discoverer of microglia and oligodendroglia. *ASN Neuro*, *12*, 1759091420953259. https://doi.org/10.1177/1759091420953259 ⏎

Brisch, R., Wojtylak, S., Saniotis, A., Steiner, J., Gos, T., Kumaratilake, J., Henneberg, M., & Wolf, R. (2022). The role of microglia in neuropsychiatric disorders and suicide. *European Archives of Psychiatry and Clinical Neuroscience*, *272*(6), 929–945. https://doi.org/10.1007/s00406-021-01334-z ⏎

Cao, M., Zhang, Y., Li, Y., Zhang, X., & Ma, M. (2025). Ameboid microglia as a scavenger role in phagocytosis of photoreceptor outer segment in an experimental retinal detachment model. *Investigative Ophthalmology & Visual Science*, *66*(3), 4. https://doi.org/10.1167/iovs.66.3.4 ⏎

Castillo-Ruiz, A., Mosley, M., George, A. J., Mussaji, L. F., Fullerton, E. F., Ruszkowski, E. M., Jacobs, A. J., Gewirtz, A. T., Chassaing, B., & Forger, N. G. (2018). The microbiota influences cell death and microglial colonization in the perinatal mouse brain. *Brain, Behavior, and Immunity*, *67*, 218–229. https://doi.org/10.1016/j.bbi.2017.08.027 ⏎

Chen, X., Zhang, K., Li, Y., Wang, C., Lou, H., & Yu, S. (2025). Stress induces behavioral disorders by promoting microglial phagocytosis via decreasing neuronal Dkk3. *Molecular Psychiatry*, *30*(12), 5731–5748. https://doi.org/10.1038/s41380-025-03314-4 ⏎

Cherry, J. D., Olschowka, J. A., & O'Banion, M. K. (2014). Neuroinflammation and M2 microglia: The good, the bad, and the inflamed. *Journal of Neuroinflammation*, *11*(1), 98. https://doi.org/10.1186/1742-2094-11-98 ⏎

Chunduri, A., Reddy, S. D. M., Jahanavi, M., & Reddy, C. N. (2022). Gut–brain axis, neurodegeneration and mental health: A personalized medicine perspective. *Indian Journal of Microbiology*, *62*(4), 505–515. https://doi.org/10.1007/s12088-022-01033-w ⏎

Clapp, M., Aurora, N., Herrera, L., Bhatia, M., Wilen, E., & Wakefield, S. (2017). Gut microbiota's effect on mental health: The gut–brain axis. *Clinics and Practice*, *7*(4), 987. https://doi.org/10.4081/cp.2017.987 ⏎

Cordella, F., Sanchini, C., Rosito, M., Ferrucci, L., Pediconi, N., Cortese, B., Guerrieri, F., Pascucci, G. R., Antonangeli, F., Peruzzi, G., Giubettini, M.,

Basilico, B., Pagani, F., Grimaldi, A., D'Alessandro, G., Limatola, C., Ragozzino, D., & Di Angelantonio, S. (2021). Antibiotics treatment modulates microglia–synapses interaction. *Cells*, *10*(10), 2648. https://doi.org/10.3390/cells10102648 ⏎

De Boer, R. J., & Perelson, A. S. (2013). Quantifying T lymphocyte turnover. *Journal of Theoretical Biology*, *327*, 45–87. https://doi.org/10.1016/j.jtbi.2012.12.025 ⏎

De Schepper, S., Ge, J. Z., Crowley, G., Ferreira, L. S. S., Garceau, D., Toomey, C. E., Sokolova, D., Rueda-Carrasco, J., Shin, S.-H., Kim, J.-S., Childs, T., Lashley, T., Burden, J. J., Sasner, M., Sala Frigerio, C., Jung, S., & Hong, S. (2023). Perivascular cells induce microglial phagocytic states and synaptic engulfment via SPP1 in mouse models of Alzheimer's disease. *Nature Neuroscience*, *26*(3), 406–415. https://doi.org/10.1038/s41593-023-01257-z ⏎

Devlin, B. A., Nguyen, D. M., Ribeiro, D., Grullon, G., Clark, M. J., Finn, A., Ceasrine, A. M., Oxendine, S., Deja, M., Shah, A., Ati, S., Schaefer, A., & Bilbo, S. D. (2025). Excitatory-neuron-derived interleukin-34 supports cortical developmental microglia function. *Immunity*, *58*(8), 1948–1965.e6. https://doi.org/10.1016/j.immuni.2025.06.002 ⏎

Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., & Blaser, M. J. (2019). Role of the microbiome in human development. *Gut*, *68*(6), 1108–1114. https://doi.org/10.1136/gutjnl-2018-317503 ⏎

EBioMedicine. (2021). The maternal microbiome: Another bridge linking mothers and infants. *eBioMedicine*, *71*. https://doi.org/10.1016/j.ebiom.2021.103602 ⏎

Erny, D., de Angelis, A. L. H., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W. S., McCoy, K. D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., & Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, *18*(7), 965–977. https://doi.org/10.1038/nn.4030 ⏎

Frost, J. L., & Schafer, D. P. (2016). Microglia: Architects of the developing nervous system. *Trends in Cell Biology*, *26*(8), 587–597. https://doi.org/10.1016/j.tcb.2016.02.006 ⏎

Fruhwürth, S., Zetterberg, H., & Paludan, S. R. (2024). Microglia and amyloid plaque formation in Alzheimer's disease—Evidence, possible mechanisms, and future challenges. *Journal of Neuroimmunology*, *390*, 578342. https://doi.org/10.1016/j.jneuroim.2024.578342 ⏎

Fujikawa, R., & Jinno, S. (2022). Identification of hyper-ramified microglia in the CA1 region of the mouse hippocampus potentially associated with stress resilience. *European Journal of Neuroscience*, *56*(8), 5137–5153. https://doi.org/10.1111/ejn.15812 ⏎

Gale, A. (2021). Dr. William Beaumont: Founding father of gastroenterology. *Missouri Medicine*, *118*(6), 518–519. ⏎

Gao, Q., Wang, Y., Wang, X., Fu, S., Zhang, X., Wang, R.-T., & Zhang, X. (2019). Decreased levels of circulating trimethylamine N-oxide alleviate cognitive and pathological deterioration in transgenic mice: A potential therapeutic approach for Alzheimer's disease. *Aging (Albany NY)*, *11*(19), 8642–8663. https://doi.org/10.18632/aging.102352 ⏎

Gheorghe, R.-O., Deftu, A., Filippi, A., Grosu, A., Bica-Popi, M., Chiritoiu, M., Chiritoiu, G., Munteanu, C., Silvestro, L., & Ristoiu, V. (2020). Silencing the cytoskeleton protein Iba1 (ionized calcium binding adapter protein 1) interferes with BV2 microglia functioning. *Cellular and Molecular Neurobiology*, *40*(6), 1011–1027. https://doi.org/10.1007/s10571-020-00790-w ⏎

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., & Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, NY)*, *330*(6005), 841–845. https://doi.org/10.1126/science.1194637 ⏎

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., & Huber, T. (2013). Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience*, *7*. https://doi.org/10.3389/fncel.2013.00045 ⏎

Grassivaro, F., Menon, R., Acquaviva, M., Ottoboni, L., Ruffini, F., Bergamaschi, A., Muzio, L., Farina, C., & Martino, G. (2020). Convergence between microglia and peripheral macrophages phenotype during development and neuroinflammation. *The Journal of Neuroscience*, *40*(4), 784–795. https://doi.org/10.1523/JNEUROSCI.1523-19.2019 ⏎

Green, T. R. F., & Rowe, R. K. (2024). Quantifying microglial morphology: An insight into function. *Clinical and Experimental Immunology*, *216*(3), 221–229. https://doi.org/10.1093/cei/uxae023 ⏎

Han, C. Z., Li, R. Z., Hansen, E., Trescott, S., Fixsen, B. R., Nguyen, C. T., Mora, C. M., Spann, N. J., Bennett, H. R., Poirion, O., Buchanan, J., Warden, A. S., Xia, B., Schlachetzki, J. C. M., Pasillas, M. P., Preissl, S., Wang, A., O'Connor, C., Shriram, S., … Coufal, N. G. (2023). Human microglia maturation is underpinned by specific gene regulatory networks. *Immunity*, *56*(9), 2152–2171.e13. https://doi.org/10.1016/j.immuni.2023.07.016 ⏎

He, Haili, He, Hui, Mo, L., Yuan, Q., Xiao, C., Ma, Q., Yi, S., Zhou, T., You, Z., & Zhang, J. (2024). Gut microbiota regulate stress resistance by influencing microglia–neuron interactions in the hippocampus. *Brain, Behavior, & Immunity—Health*, *36*, 100729. https://doi.org/10.1016/j.bbih.2024.100729 ⏎

He, Hui, He, Haili, Mo, L., You, Z., & Zhang, J. (2024). Priming of microglia with dysfunctional gut microbiota impairs hippocampal neurogenesis and fosters stress vulnerability of mice. *Brain, Behavior, and Immunity*, *115*, 280–294. https://doi.org/10.1016/j.bbi.2023.10.031 ⏎

Hellwig, S., Heinrich, A., & Biber, K. (2013). The brain's best friend: Microglial neurotoxicity revisited. *Frontiers in Cellular Neuroscience*, *7*, 71. https://doi.org/10.3389/fncel.2013.00071 ⏎

Huang, Y., Wu, J., Zhang, H., Li, Y., Wen, L., Tan, X., Cheng, K., Liu, Y., Pu, J., Liu, L., Wang, H., Li, W., Perry, S. W., Wong, M.-L., Licinio, J., Zheng, P., & Xie, P. (2023). The gut microbiome modulates the transformation of microglial subtypes. *Molecular Psychiatry*, *28*(4), 1611–1621. https://doi.org/10.1038/s41380-023-02017-y ⏎

Intili, G., Paladino, L., Rappa, F., Alberti, G., Plicato, A., Calabrò, F., Fucarino, A., Cappello, F., Bucchieri, F., Tomasello, G., Carini, F., & Pitruzzella, A. (2023). From dysbiosis to neurodegenerative diseases through different communication pathways: An overview. *Biology*, *12*(2), 195. https://doi.org/10.3390/biology12020195 ⏎

Kenkhuis, B., Somarakis, A., Kleindouwel, L. R. T., van Roon-Mom, W. M. C., Höllt, T., & van der Weerd, L. (2022). Co-expression patterns of microglia markers Iba1, TMEM119 and P2RY12 in Alzheimer's disease. *Neurobiology of Disease*, *167*, 105684. https://doi.org/10.1016/j.nbd.2022.105684 ⏎

Kettenmann, H., Kirchhoff, F., & Verkhratsky, A. (2013). Microglia: New roles for the synaptic stripper. *Neuron*, *77*(1), 10–18. https://doi.org/10.1016/j.neuron.2012.12.023 ⏎

Kimmel, M. C., Verosky, B., Chen, H. J., Davis, O., & Gur, T. L. (2024). The maternal microbiome as a map to understanding the impact of prenatal stress on offspring psychiatric health. *Biological Psychiatry*, *95*(4), 300–309. https://doi.org/10.1016/j.biopsych.2023.11.014 ⏎

Kisler, K., Nikolakopoulou, A. M., & Zlokovic, B. V. (2021). Microglia have a grip on brain microvasculature. *Nature Communications*, *12*(1), 5290. https://doi.org/10.1038/s41467-021-25595-3 ⏎

Lannes, N., Eppler, E., Etemad, S., Yotovski, P., & Filgueira, L. (2017). Microglia at center stage: A comprehensive review about the versatile and unique residential macrophages of the central nervous system. *Oncotarget*, *8*(69), 114393–114413. https://doi.org/10.18632/oncotarget.23106 ⏎

Lei, W., Cheng, Y., Liu, X., Gao, J., Zhu, Z., Ding, W., Xu, X., Li, Y., Ling, Z., Jiang, R., & Chen, X. (2025). Gut microbiota-driven neuroinflammation in Alzheimer's disease: From mechanisms to therapeutic opportunities. *Frontiers in Immunology*, *16*, 1582119. https://doi.org/10.3389/fimmu.2025.1582119 ⏎

Lewandowska-Pietruszka, Z., Figlerowicz, M., & Mazur-Melewska, K. (2022). The history of the intestinal microbiota and the gut–brain axis. *Pathogens*, *11*(12), 1540. https://doi.org/10.3390/pathogens11121540 ⏎

Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., & Li, X. (2019). Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice via the gut microbiota–inflammation–brain axis. *Stress*, *22*(5), 592–602. https://doi.org/10.1080/10253890.2019.1617267 ⏎

Liang, Y., Liu, C., Cheng, M., Geng, L., Li, J., Du, W., Song, M., Chen, N., Yeleen, T. A. N., Song, L., Wang, X., Han, Y., & Sheng, C. (2024). The link between gut microbiome and Alzheimer's disease: From the perspective of new revised criteria for diagnosis and staging of Alzheimer's disease. *Alzheimer's & Dementia*, *20*(8), 5771–5788. https://doi.org/10.1002/alz.14057 ⏎

Lier, J., Streit, W. J., & Bechmann, I. (2021). Beyond activation: Characterizing microglial functional phenotypes. *Cells*, *10*(9), 2236.

https://doi.org/10.3390/cells10092236 ⏎

Liu, H., Leak, R. K., & Hu, X. (2016). Neurotransmitter receptors on microglia. *Stroke and Vascular Neurology*, *1*(2), 52–58. https://doi.org/10.1136/svn-2016-000012 ⏎

Liu, L., Wang, H., Chen, X., Zhang, Y., Zhang, H., & Xie, P. (2023). Gut microbiota and its metabolites in depression: From pathogenesis to treatment. *eBioMedicine*, *90*. https://doi.org/10.1016/j.ebiom.2023.104527 ⏎

Luczynski, P., McVey Neufeld, K.-A., Oriach, C. S., Clarke, G., Dinan, T. G., & Cryan, J. F. (2016). Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology* 19(8):pyw020. https://doi.org/10.1093/ijnp/pyw020 ⏎

Macallan, D. C., Wallace, D. L., Zhang, Y., Ghattas, H., Asquith, B., de Lara, C., Worth, A., Panayiotakopoulos, G., Griffin, G. E., Tough, D. F., & Beverley, P. C. L. (2005). B-cell kinetics in humans: Rapid turnover of peripheral blood memory cells. *Blood*, *105*(9), 3633–3640. https://doi.org/10.1182/blood-2004-09-3740 ⏎

Manfredi, J. N., Gupta, S. K., Vyavahare, S., Deak, F., Lu, X., Buddha, L., Wankhade, U., Lohakare, J., Isales, C., & Fulzele, S. (2025). Gut microbiota dysbiosis in Alzheimer's disease (AD): Insights from human clinical studies and the mouse AD models. *Physiology & Behavior*, *290*, 114778. https://doi.org/10.1016/j.physbeh.2024.114778 ⏎

Matcovitch-Natan, O., Winter, D. R., Giladi, A., Vargas Aguilar, S., Spinrad, A., Sarrazin, S., Ben-Yehuda, H., David, E., Zelada González, F., Perrin, P., Keren-Shaul, H., Gury, M., Lara-Astaiso, D., Thaiss, C. A., Cohen, M., Bahar Halpern, K., Baruch, K., Deczkowska, A., Lorenzo-Vivas, E., … Amit, I. (2016). Microglia development follows a stepwise program to regulate brain homeostasis. *Science*, *353*(6301), aad8670. https://doi.org/10.1126/science.aad8670 ⏎

Mayer, M. G., & Fischer, T. (2024). Microglia at the blood brain barrier in health and disease. *Frontiers in Cellular Neuroscience*, *18*, 1360195. https://doi.org/10.3389/fncel.2024.1360195 ⏎

Meng, Y., Sun, J., & Zhang, G. (2024). Pick fecal microbiota transplantation to enhance therapy for major depressive disorder. *Progress in Neuro-*

Psychopharmacology and Biological Psychiatry, *128*, 110860. https://doi.org/10.1016/j.pnpbp.2023.110860 ⏎

Minter, M. R., Zhang, C., Leone, V., Ringus, D. L., Zhang, X., Oyler-Castrillo, P., Musch, M. W., Liao, F., Ward, J. F., Holtzman, D. M., Chang, E. B., Tanzi, R. E., & Sisodia, S. S. (2016). Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Scientific Reports*, *6*(1), 30028. https://doi.org/10.1038/srep30028 ⏎

Muramatsu, M. K., & Winter, S. E. (2024). Nutrient acquisition strategies by gut microbes. *Cell Host & Microbe*, *32*(6), 863–874. https://doi.org/10.1016/j.chom.2024.05.011 ⏎

Najafi, A. R., Crapser, J., Jiang, S., Ng, W., Mortazavi, A., West, B. L., & Green, K. N. (2018). A limited capacity for microglial repopulation in the adult brain. *Glia*, *66*(11), 2385–2396. https://doi.org/10.1002/glia.23477 ⏎

Nakamura, A., & Shichita, T. (2025). Interaction between neurons and microglia in healthy and disease states. *International Immunology*, dxaf057. https://doi.org/10.1093/intimm/dxaf057 ⏎

Naomi, R., Embong, H., Othman, F., Ghazi, H. F., Maruthey, N., & Bahari, H. (2021). Probiotics for Alzheimer's disease: A systematic review. *Nutrients*, *14*(1), 20. https://doi.org/10.3390/nu14010020 ⏎

Oggioni, M., Mercurio, D., Minuta, D., Fumagalli, S., Popiolek-Barczyk, K., Sironi, M., Ciechanowska, A., Ippati, S., De Blasio, D., Perego, C., Mika, J., Garlanda, C., & De Simoni, M.-G. (2021). Long pentraxin PTX3 is upregulated systemically and centrally after experimental neurotrauma, but its depletion leaves unaltered sensorimotor deficits or histopathology. *Scientific Reports*, *11*(1), 9616. https://doi.org/10.1038/s41598-021-89032-7 ⏎

Okajima, T., & Tsuruta, F. (2018). Microglial dynamics during brain development. *Neural Regeneration Research*, *13*(2), 222–223. https://doi.org/10.4103/1673-5374.226386 ⏎

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., & Gross, C. T. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science*, *333*(6048), 1456–1458. https://doi.org/10.1126/science.1202529 ⏎

Paolicelli, R. C., Sierra, A., Stevens, B., Tremblay, M.-E., Aguzzi, A., Ajami, B., Amit, I., Audinat, E., Bechmann, I., Bennett, M., Bennett, F., Bessis, A., Biber, K., Bilbo, S., Blurton-Jones, M., Boddeke, E., Brites, D., Brône, B., Brown, G. C., … Wyss-Coray, T. (2022). Microglia states and nomenclature: A field at its crossroads. *Neuron*, *110*(21), 3458–3483. https://doi.org/10.1016/j.neuron.2022.10.020 ⏎

Parakalan, R., Jiang, B., Nimmi, B., Janani, M., Jayapal, M., Lu, J., Tay, S. S., Ling, E.-A., & Dheen, S. Thameem. (2012). Transcriptome analysis of amoeboid and ramified microglia isolated from the corpus callosum of rat brain. *BMC Neuroscience*, *13*, 64. https://doi.org/10.1186/1471-2202-13-64 ⏎

Parker, A., Fonseca, S., & Carding, S. R. (2019). Gut microbes and metabolites as modulators of blood–brain barrier integrity and brain health. *Gut Microbes*, *11*(2), 135–157. https://doi.org/10.1080/19490976.2019.1638722 ⏎

Perez-Muñoz, M. E., Arrieta, M.-C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, *5*, 48. https://doi.org/10.1186/s40168-017-0268-4 ⏎

Prabakaran, S. (2015). Microglia get a gut feeling. *Science Signaling*, *8*(385), ec194–ec194. https://doi.org/10.1126/scisignal.aac9922 ⏎

Prajapati, S. K., Wang, S., Mishra, S. P., Jain, S., & Yadav, H. (2025). Protection of Alzheimer's disease progression by a human-origin probiotics cocktail. *Scientific Reports*, *15*(1), 1589. https://doi.org/10.1038/s41598-024-84780-8 ⏎

Rasmussen, N. (2022). René Dubos, the autochthonous flora, and the discovery of the microbiome. *Journal of the History of Biology*, *55*(3), 537–558. https://doi.org/10.1007/s10739-022-09692-7 ⏎

Réu, P., Khosravi, A., Bernard, S., Mold, J. E., Salehpour, M., Alkass, K., Perl, S., Tisdale, J., Possnert, G., Druid, H., & Frisén, J. (2017). The lifespan and turnover of microglia in the human brain. *Cell Reports*, *20*(4), 779–784. https://doi.org/10.1016/j.celrep.2017.07.004 ⏎

Ritzel, R. M., Patel, A. R., Pan, S., Crapser, J., Hammond, M., Jellison, E., & McCullough, L. D. (2015). Age- and location-related changes in microglial function. *Neurobiology of Aging*, *36*(6), 2153–2163. https://doi.org/10.1016/j.neurobiolaging.2015.02.016 ⏎

Rodríguez-Gómez, J. A., Kavanagh, E., Engskog-Vlachos, P., Engskog, M. K. R., Herrera, A. J., Espinosa-Oliva, A. M., Joseph, B., Hajji, N., Venero, J. L., & Burguillos, M. A. (2020). Microglia: Agents of the CNS pro-inflammatory response. *Cells*, *9*(7), 1717. https://doi.org/10.3390/cells9071717 ⏎

Rossi, F., & Lewis, C. (2018). Microglia's heretical self-renewal. *Nature Neuroscience*, *21*(4), 455–456. https://doi.org/10.1038/s41593-018-0123-3 ⏎

Rutsch, A., Kantsjö, J. B., & Ronchi, F. (2020). The gut–brain axis: How microbiota and host inflammasome influence brain physiology and pathology. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.604179 ⏎

Saeki, K., Pan, R., Lee, E., Kurotaki, D., & Ozato, K. (2024). IRF8 defines the epigenetic landscape in postnatal microglia, thereby directing their transcriptome programs. *Nature Immunology*, *25*(10), 1928–1942. https://doi.org/10.1038/s41590-024-01962-2 ⏎

Saijo, K., Crotti, A., & Glass, C. K. (2013). Regulation of microglia activation and deactivation by nuclear receptors. *Glia*, *61*(1), 104–111. https://doi.org/10.1002/glia.22423 ⏎

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., Challis, C., Schretter, C. E., Rocha, S., Gradinaru, V., Chesselet, M.-F., Keshavarzian, A., Shannon, K. M., Krajmalnik-Brown, R., Wittung-Stafshede, P., Knight, R., & Mazmanian, S. K. (2016). Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*, *167*(6), 1469–1480.e12. https://doi.org/10.1016/j.cell.2016.11.018 ⏎

Santos, E. N., & Fields, R. D. (n.d.). Regulation of myelination by microglia. *Science Advances*, *7*(50), eabk1131. https://doi.org/10.1126/sciadv.abk1131 ⏎

Schaeffer, S., & Iadecola, C. (2021). Revisiting the neurovascular unit. *Nature Neuroscience*, *24*(9), 1198–1209. https://doi.org/10.1038/s41593-021-00904-7 ⏎

Spittau, B., Dokalis, N., & Prinz, M. (2020). The role of TGFβ signaling in microglia maturation and activation. *Trends in Immunology, Special Issue: Microglia and Astrocytes*, *41*(9), 836–848. https://doi.org/10.1016/j.it.2020.07.003 ⏎

Su, S., Zhu, H., Xu, X., Wang, Xin, Dong, Y., Kapuku, G., Treiber, F., Gutin, B., Harshfield, G., Snieder, H., & Wang, Xiaoling. (2014). DNA methylation of the LY86 gene is associated with obesity, insulin resistance, and inflammation.

Twin Research and Human Genetics: The Official Journal of the International Society for Twin Studies, *17*(3), 183–191. https://doi.org/10.1017/thg.2014.22 ⏎

Taylor, S. E., Morganti-Kossmann, C., Lifshitz, J., & Ziebell, J. M. (2014). Rod microglia: A morphological definition. *PLoS One*, *9*(5), e97096. https://doi.org/10.1371/journal.pone.0097096 ⏎

Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P., Schulte-Schrepping, J., Blecher, R., Ulas, T., Squarzoni, P., Hoeffel, G., Coulpier, F., Siopi, E., David, F. S., Scholz, C., Shihui, F., Lum, J., Amoyo, A. A., Larbi, A., Poidinger, M., … Garel, S. (2018). Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell*, *172*(3), 500–516.e16. https://doi.org/10.1016/j.cell.2017.11.042 ⏎

Tizabi, Y., Getachew, B., Mendieta, L., Palafox-Sánchez, V., Tsytsarev, V., Manaye, K. F., Tinkov, A. A., da Silva, V. D. A., & Aschner, M. (2025). A narrative review on the role of microbiota and microglia in premotor symptoms of Parkinson's disease. *Neurotoxicity Research*, *43*(6), 45. https://doi.org/10.1007/s12640-025-00768-w ⏎

Tu, J., Zhang, J., & Chen, G. (2025). Higher dietary butyrate intake is associated with better cognitive function in older adults: Evidence from a cross-sectional study. *Frontiers in Aging Neuroscience*, *17*. https://doi.org/10.3389/fnagi.2025.1522498 ⏎

Ullah, H., Arbab, S., Tian, Y., Liu, C., Chen, Y., Qijie, L., Khan, M. I. U., Hassan, I. U., & Li, K. (2023). The gut microbiota–brain axis in neurological disorder. *Frontiers in Neuroscience*, *17*. https://doi.org/10.3389/fnins.2023.1225875 ⏎

Vidal-Itriago, A., Radford, R. A. W., Aramideh, J. A., Maurel, C., Scherer, N. M., Don, E. K., Lee, A., Chung, R. S., Graeber, M. B., & Morsch, M. (2022). Microglia morphophysiological diversity and its implications for the CNS. *Frontiers in Immunology*, *13*, 997786. https://doi.org/10.3389/fimmu.2022.997786 ⏎

Wyss-Coray, T., & Mucke, L. (2002). Inflammation in neurodegenerative disease—A double-edged sword. *Neuron*, *35*(3), 419–432. https://doi.org/10.1016/S0896-6273(02)00794-8 ⏎

Yang, W., Yaggie, R. E., Schaeffer, A. J., & Klumpp, D. J. (2020). AOAH remodels arachidonic acid-containing phospholipid pools in a model of interstitial cystitis pain: A MAPP Network study. *PLoS One*, *15*(9), e0235384. https://doi.org/10.1371/journal.pone.0235384 ⏎

Yin, J., Valin, K. L., Dixon, M. L., & Leavenworth, J. W. (2017). The role of microglia and macrophages in CNS homeostasis, autoimmunity, and cancer. *Journal of Immunology Research*, *2017*, 5150678. https://doi.org/10.1155/2017/5150678 ⏎

Zhang, B., Sun, Z., Song, W., Huang, H., & Wu, Y. (2025). Engineered butyrate-producing yeasts mitigate Alzheimer-associated phenotypes. *Signal Transduction and Targeted Therapy*, *10*(1), 369. https://doi.org/10.1038/s41392-025-02474-7 ⏎

Zhao, S., Umpierre, A. D., & Wu, L.-J. (2024). Tuning neural circuits and behaviors by microglia in the adult brain. *Trends in Neurosciences*, *47*(3), 181–194. https://doi.org/10.1016/j.tins.2023.12.003 ⏎

Zhao, X., Eyo, U. B., Murguan, M., & Wu, L.-J. (2018). Microglial interaction with the neurovascular system in physiology and pathology. *Developmental Neurobiology*, *78*(6), 604–617. https://doi.org/10.1002/dneu.22576 ⏎

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, *30*(6), 492–506. https://doi.org/10.1038/s41422-020-0332-7 ⏎

Zusso, M., Methot, L., Lo, R., Greenhalgh, A. D., David, S., & Stifani, S. (2012). Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. *The Journal of Neuroscience*, *32*(33), 11285–11298. https://doi.org/10.1523/JNEUROSCI.6182-11.2012 ⏎

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From Neurodevelopmental Alterations to Neurodiversity: The Microbiome Perspective

Elena Niccolai, Nevena Todorovic, and Amedeo Amedei

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18.1 Introduction

As if biology, medicine, and sociology were not already complex enough and, in many ways, still insufficiently understood, delving into the microbiome and its disturbances in the neurodiversity scenario makes these fields even more intricate. Before addressing the potential influence of the microbiome, it is essential to rightly define the concepts of neurodiversity and neurodivergence and to outline the group of conditions referred to as neurodevelopmental disorders (NDDs), since these terms are frequently conflated in scientific literature.

Neurodiversity, a relatively recent social concept that is gaining increasing attention and conceptual refinement in both medicine and the social sciences, stands in contrast to the conventional medical framework that typically interprets neurological variation as a deficiency or pathology requiring correction (Sharma et al., 2025). The neurodiversity paradigm instead recognizes these variations as valuable forms of human strength and alternative ways of experiencing the world. The sociologist Judy Singer introduced, or at least formalized and popularized, this term in 1998, although some authors noted that related ideas had been discussed earlier by other activists within autism communities. Singer described neurodiversity as the recognition that differences in neurocognitive functioning are part of the natural range of human variations rather than intrinsically pathological. Her work encouraged society to embrace individuals described as *persons* with neurodiversity and to move away from the search for cures and strict diagnostic parameters (McLennan et al., 2025). This has led to "neurodiversity movement" and the development of specific terms, besides neurodiversity, such as neurodiverse, neurodivergent, and neurotypical (Shah et al., 2022; Botha et al., 2022). Initially, the term was most often associated with autism spectrum disorder (ASD), but its scope has expanded to include attention-deficit/hyperactivity disorder (ADHD), dyslexia, dyscalculia, dyspraxia, Tourette syndrome (TS), and others (Stenning et al., 2021). Today, the scientific literature widely recognizes this concept as encompassing the natural and expected spectrum of human neurological and neurocognitive diversity, rejecting the notion of a single, universally superior or "default" cognitive style. Instead, it is grounded in shared principles that emphasize the diversity value, the recognition of individual variability, and a socially informed framework for understanding these differences (Dwyer, 2022; Cherewick et al., 2023; Xia et al., 2024).

But how does neurodivergence fit within this framework? Are neurodiversity and neurodivergence synonymous, or do they represent distinct concepts? This question invites a more nuanced discussion (Cleveland Clinic, 2023). Although the terms are often interchangeably used, recent scholarship emphasizes that they are not equivalent. In detail, one analysis conceptualizes neurodiversity as a "term of inclusion," while framing neurodivergence as a "term of exclusion." Within this perspective, individuals described as "neurotypical" are those whose cognitive functioning conforms to socially constructed norms, norms shaped and reinforced by socio-political forces rather than by any objective standard of neurological variation. Conversely, subjects whose cognitive profiles diverge from these norms, and who therefore do not benefit from the same epistemic authority or cognitive privilege, are categorized as "neuroatypical," with "neurodivergent" now being the more commonly used term (Legault et al., 2021).

On the other hand, NDDs, as defined in the International Classification of Diseases (ICD-11), are described by the World Health Organization (WHO) as conditions that emerge during the developmental period and are characterized by difficulties in acquiring or performing intellectual, motor, linguistic, or social functions. This category includes ASD, ADHD, disorders of intellectual development, developmental speech and language disorders, developmental learning disorder, developmental motor coordination disorder, stereotyped movement disorder, other NDDs, unspecified NDDs, primary tics and tic disorders, and secondary NDDs (WHO, 2023). These conditions often show multifactorial onset, arising from interactions among genetic, biological, social, and environmental factors. They are not viewed solely as medical diagnoses but as states with broad functional and social implications. NDDs exist along a continuum, from mild developmental differences to severe and complex presentations. Within this continuum, there are individuals with isolated difficulties, as well as those with multiple, co-occurring challenges (Gillberg, 2010; WHO and UNICEF, 2023). NDDs place significant strain on health, social structures, and economic resources, largely because their clinical management is rarely straightforward. Many of these conditions lack sharply defined boundaries, with overlapping features and atypical patterns of manifestation (Cainelli, 2023).

However, neurodiversity has emerged as an increasingly influential concept within current medicine, marking a significant shift in the ways we assess, diagnose, and treat those who fall within this spectrum. Notably, individuals may identify as neurodiverse or neurodivergent based on their sensory profiles, social cognition, learning styles, or affective characteristics, regardless of whether they meet diagnostic criteria for a developmental disorder (Thapar et al., 2017; Gillberg, 2010; Botha et al., 2022). Finally, neurocognitive differences represent the strongest link between the concepts of neurodiversity and NDDs, and that is the right focus of our exploration.

Building upon this conceptual framework, the present chapter examines NDDs through the lens of the human microbiome, exploring how alterations in microbial composition and function, collectively termed dysbiosis, may influence neurological development, behavior, and cognition. A growing body of evidence supports the existence of a bidirectional communication system between the gut and the brain, the microbiota–gut–brain axis, which integrates neural, immune, endocrine, and metabolic signaling pathways. Perturbations in this network have increasingly been linked to behavioral, cognitive, and affective characteristics seen across the neurodevelopmental spectrum.

Given the diversity and complexity of neurodivergent conditions, this chapter focuses on a set of representative and data-rich disorders in which microbiome research has advanced most robustly and yielded mechanistically informative insights. These include ASD, ADHD, Rett syndrome (RTT), TS, Down syndrome (DS), and Prader–Willi syndrome (PWS). Although clinically distinct and heterogeneous in their etiologies, these conditions share several relevant features: early-life onset, persistent impact on cognition and behavior, frequent gastrointestinal (GI) comorbidities, metabolic and immune dysregulation, and—critically—mounting evidence of microbiome alterations that may modulate symptom expression. These

shared characteristics make them mostly suitable models for examining the potential role of the microbiota–gut–brain axis in neurodevelopmental health.

ASD, the most extensively studied condition in this context, is characterized by differences in social communication, sensory processing, and behavioral patterns. Beyond its core features, ASD is strongly associated with GI dysfunction, immune anomalies, metabolic irregularities, and dietary restrictions. Numerous studies have documented differences in microbial diversity, short-chain fatty acids (SCFAs) production, mucosal permeability, and even gut viral communities, positioning ASD as a central condition in microbiome-based neurodevelopmental research. ADHD involves developmentally inappropriate levels of inattention, impulsivity, and hyperactivity. Recent findings suggest that metabolic, inflammatory, and gut-derived factors may contribute to these symptoms. Individuals with ADHD often present with altered dietary patterns, micronutrient insufficiencies, disrupted sleep, and elevated inflammatory markers. Microbiome studies have identified changes in taxa involved in neurotransmitter precursor metabolism, pointing toward gut microbial pathways relevant to dopaminergic and noradrenergic signaling.

RTT, a rare X-linked condition caused by pathogenic MECP2 variants, features developmental regression, motor impairment, autonomic dysfunction, epilepsy, and severe communicative challenges. Chronic GI disturbances—such as reflux, constipation, and dysmotility—are highly prevalent, and several studies reveal marked reductions in microbial diversity and enrichment of pro-inflammatory taxa, suggesting that dysbiosis may exacerbate systemic inflammation and autonomic symptoms.

TS, defined by persistent motor and vocal tics, frequently co-occurs with ADHD, anxiety, and obsessive–compulsive traits. Emerging microbiome analyses have identified characteristic alterations associated with tic severity and neuroimmune activation. Although still preliminary, these findings support potential interactions between gut microbial pathways, dopaminergic regulation, and immune signaling.

DS, or trisomy 21, presents a characteristic constellation of cognitive, physiological, and metabolic features. Individuals with DS display early immune dysregulation, hypotonia, altered oral and intestinal physiology, and high rates of GI and periodontal disorders. Both gut and oral microbiomes in DS show distinctive signatures linked to inflammatory status, metabolic function, and cognitive outcomes, making DS a valuable comparison between idiopathic NDDs and genetically defined neurodevelopmental syndromes.

Finally, PWS is a complex genetic disorder caused by the loss of paternally expressed genes on chromosome 15q11–q13, characterized by neonatal hypotonia, hyperphagia, obesity risk, endocrine dysfunction, and behavioral rigidity. PWS exhibits one of the most distinctive microbiome profiles among NDDs, with profound reductions in microbial diversity, enrichment of potentially inflammatory taxa, and depletion of SCFA producers. Given the centrality of appetite regulation, metabolism, and gut–brain signaling in PWS, it serves as a compelling model for understanding microbiome–host interactions.

In sum, together, these conditions span a broad neurodevelopmental continuum, from highly prevalent idiopathic disorders (ASD, ADHD) to rare genetic syndromes (RTT, DS, PWS), and collectively show the multifaceted ways in which microbiome alterations may contribute to neurobehavioral phenotypes.

Our selection rationale is threefold: (1) they represent clinically impactful conditions with substantial relevance to public health and quality of life; (2) they encompass both idiopathic and genetically determined NDDs, allowing for comparison across distinct biological frameworks; and (3) they are the NDDs most thoroughly investigated in relation to microbiome and their metabolites, and microbiota-associated therapeutic strategies.

Finally, the aim of this chapter is not to biologize neurodiversity or reduce neurocognitive variation to microbial signatures, but rather to remark how microbial ecology may intersect with neurodevelopmental processes, contributing to vulnerability, adaptation, and resilience within the neurodivergent spectrum.

18.2 Microbiome–Gut–Brain Interactions and Neurodiversity

18.2.1 Early-Life Development of the Microbiome and the Brain

The gut microbiome (GM) is largely shaped during early childhood and is strongly influenced by factors such as mode of birth (vaginal or cesarean), gestational age, early feeding practices (breast milk or formula, introduction of solid foods), antibiotic exposure, maternal age, lifestyle, and diet. Its development during the first 1000 days of life is a gradual process that unfolds through several developmental stages, during which the microbiome undergoes substantial changes (Robertson et al., 2019). This timeframe, often described as the "critical window," marks a period when the microbiome is mostly sensitive to external influences (Cox et al., 2014; Bäckhed et al., 2015). Any disturbances in this period may have adverse effects, contributing to the emergence of various disorders (Ratsika et al., 2021). This early-life ecosystem plays a vital role in shaping metabolic, immune, and neurological functions, essential for healthy growth and development (Martin et al., 2016).

During the first weeks of life, vaginally delivered infants acquire a GM derived largely from the mother's gut and vaginal microbiome, with *Bifidobacterium*, *Bacteroides*, *Lactobacillus, Clostridium*, and *Escherichia* commonly observed. In contrast, infants born by cesarean section more often harbor higher levels of *Klebsiella, Clostridium, Enterobacter, Staphylococcus*, and other opportunistic species, likely originating from the mother's skin and hospital environment (Ferretti et al., 2018; Shao et al., 2019).

Throughout the first six months of life, the GM composition begins to shift. This period is strongly influenced by the mode of feeding. In exclusively breastfed infants, the GM is typically dominated by *Bifidobacterium* and *Bacteroides*, whereas formula-fed infants generally show lower levels of these genera, greater microbial diversity, and more frequent colonization by *Collinsella, Enterococcus, Escherichia*, and *Clostridium* (Azad et al., 2015; Ma et al., 2020).

During mid-infancy, the introduction of solid foods leads to a rise in microbiome diversity. Increases are observed in genera such as *Bacteroides, Faecalibacterium, Blautia, Roseburia, Anaerostipes*, and other anaerobes (Wernroth et al., 2022; Bäckhed et al., 2015; Nunez et al., 2025). From late infancy into early toddlerhood, the GM continues to expand the diversity. By approximately 3 years of age, the microbiome generally reaches a more stable, adult-like configuration, characterized by the predominance of *Bacteroides, Faecalibacterium*, and various members of the Lachnospiraceae family. However, this ecosystem remains dynamic, with its structure and composition shifting throughout life (Stewart et al., 2018; Roswall et al., 2021).

Moreover, neonatal exposure to antibiotics can delay GM maturation and even cause a regression from its expected developmental trajectory, mirrored in a strong decrease of *Bifidobacterium* and an increase in *Enterobacteriaceae*. Additionally, they tend to show reduced strain-level diversity, which may affect the GM function and sometimes, these disruptions can persist long term (Gasparrini et al., 2019; Bokulich et al., 2016). On the other hand, physical activity influences GM composition by increasing beneficial species, enhancing microbial diversity, and promoting SCFAs producers. Encouraging physical activity in early childhood, together with a balanced diet, is relevant for healthy microbiome development. Some factors, such as the presence of siblings and pets, hygiene practices, and home environment, also contribute to this process and disruptions in these areas can significantly affect it. Maternal dysbiotic features induced by overnutrition may be transmitted to the infant during gestation, birth, or lactation, compromising early gut colonization and immune tolerance and increasing the risk of later-life diseases (Monda et al., 2017; Azad et al., 2013; Chu et al., 2016).

GM changes during early life correspond temporally with critical brain development events, including the migration and multiplication of neurons, proliferation, migration, and apoptosis of glia, elimination of

excess synapses, and myelination, accompanied by brain tissue volume changes and rapid emergence of cognitive functions. Insights from germ-free and antibiotic-treated mouse models reinforce the biological plausibility of this connection, documenting that the complete microbial missing or substantial microbial depletion leads to altered neurochemical profiles, disrupted gene expression, and behavioral abnormalities compared to conventionally colonized controls (Diaz Heijtz et al., 2011; Leclercq et al., 2017). These findings have motivated human studies to explore how early-life microbiome features may be detectable in neurodevelopmental outcomes. Carlson et al. (2018) conducted one of the first human studies to investigate links between infant GM and cognitive development, integrating stool microbiome profiling, cognitive assessments, and structural brain MRI data. The authors identified three distinct microbiome clusters, which differed significantly in cognitive performance at age 2. Contrary to expectations, greater microbial diversity at one year of age was associated with lower cognitive scores, especially in visual reception and expressive language domains. Exploratory neuroimaging analyses indicated minimal and inconsistent associations between GM and regional brain volumes (Carlson et al., 2018). Rather than pointing to major structural differences, these findings suggested that microbiome-related influences may act through more subtle or functional neurodevelopmental pathways. With the acknowledgment that the visual cortex shows sensitivity to GM changes, Bonham et al. (2025) conducted a longitudinal study following infants over the first 18 months of life. Using visual-evoked potentials (VEPs) to assess visual cortex development alongside metagenomic analysis of GM, they described that microbial genes involved in metabolizing neuroactive molecules, including GABA, glutamate, tryptophan, and SCFAs, were associated with visual cortical maturation. Notably, early microbiome composition showed a stronger association with future VEP measurements than concurrent ones, which could indicate that GM may influence early brain development during “critical window” (Bonham et al., 2025).

18.2.2 GM–Brain Axis

One of the most fascinating connections in a human body is the gut–brain axis, a bidirectional communication network. Its core components include the central nervous system (CNS), enteric nervous system (ENS), autonomic pathways, neuroendocrine signaling, immune system, intestinal epithelial barrier, and GM with its vast repertoire of metabolites. Recognition of the microbiome as a critical influencer of gut–brain communication led to the establishment of the GM-brain axis concept (Mehra et al., 2023).

The first indications of this interaction appeared in 2011, when germ-free mice were shown to exhibit altered motor activity and decreased anxiety-like behavior, alongside findings that *Lacticaseibacillus rhamnosus JB-1* possesses notable anxiolytic and antidepressant properties (Bravo et al., 2011). GM-brain communication appears to begin even prenatally, as microbial components can reach the developing fetus through the amniotic environment (Mehra et al., 2023). As previously reported, after birth, infants are rapidly colonized by diverse microbes and clinical and preclinical evidence documents that dysbiosis during perinatal period may affect neurodevelopment through GM-brain axis, contributing to outcomes such as ADHD, ASD, emotional dysregulation, anxiety, and aggression. In addition, this axis modulates brain processes, including myelination, microglial maturation, and neural plasticity, shaping complex behaviors (Borrego-Ruiz et al., 2024; Gulledge et al., 2023; Hoban et al., 2016). Current evidence suggests that GM–brain communication occurs through three major and interdependent pathways: immune, neuronal, and endocrine/systemic (Wang et al., 2023a, b).

GM members, together with their metabolites (including SCFAs, secondary bile acids, amino-acid derivatives) and other bioactive molecules, such as microbe-associated molecular patterns (MAMPs), shape intestinal immunity and influence CNS activity via systemic circulation, with microglia serving as key intermediaries. A healthy intestinal barrier maintains this balance, whereas dysbiosis can increase gut permeability and promote neuroinflammation (Cryan et al., 2019; Wang et al., 2023a, b; Brescia et al.,

2021). Additionally, GM "educates" immune cells that later act in the CNS by inhibiting or promoting neuroinflammation or by trapping pathogens (Sanmarco et al., 2021; Fitzpatrick et al., 2020; Schnell et al., 2021; Wang et al., 2023a, b). Moreover, microbial cell-wall components, such as peptidoglycan fragments, can cross the blood–brain barrier (BBB) and activate pattern-recognition receptors in regions including the prefrontal cortex, striatum, and cerebellum, affecting synaptogenesis and behaviors related to sociability, anxiety, and stress (Gonzalez-Santana et al., 2020). Cytokines and microbial metabolites form another key communication route. The GM produces numerous metabolites, neurotransmitters, and neuromodulators, such as gamma-aminobutyric acid (GABA), serotonin, dopamine, norepinephrine, acetylcholine, histamine, SCFAs, and secondary bile acids, that can influence neurodevelopment, either promoting or impairing it (Agirman et al., 2021). The microbiome also modulates neurodevelopment through the enteroendocrine system by generating signals sensed by various enteroendocrine cells (producers of glucagon-like peptide 1, peptide YY, cholecystokinin, substance P, and serotonin) (Wang et al., 2023a, b). Furthermore, microbial influences on oxytocin, vasopressin, and cortisol illustrate the broader neuroendocrine mechanisms, in addition to their capacity to synthesize and degrade hormones (Morais et al., 2020). Altogether, microbial metabolites, enteroendocrine signaling, and neuroendocrine pathways form a tightly interconnected system through which the GM shapes neurodevelopment. The neuronal component of the GM–brain axis relies mainly on the ENS and the vagus nerve (Marklund, 2022). Vagal sensory fibers detect GM-induced chemical signals (neurohormones), such as serotonin, released by enteroendocrine cells (Chambers et al., 2013). Alongside the vagus nerve, the ENS itself contributes significantly to this axis, mainly in conditions such as autism spectrum disorder (ASDR), where GI and neurological symptoms commonly co-occur (Rao et al., 2016). Finally, neuronal communication involves mechanical cues generated by gut distension, contraction, and luminal flow, adding yet another layer to this complex bidirectional system (Mercado-Perez et al., 2022).

Understanding how the microbiome shapes neurodevelopment provides a framework for the following chapter, which explores how microbial profiles differ among individuals across the neurodiversity spectrum.

18.2.3 Environmental Hazards and the Microbiome–Brain Axis in Early Development

In the centuries before industrialization, the development of the brain and the microbial ecosystem in early human life was shaped primarily by biological and natural environmental forces, including diet, maternal health, pathogens, and natural microbial richness of soil, animals, and outdoor environments. Today, however, the ecological context in which this co-development unfolds has changed radically toward an environment saturated not only with human-made chemicals but also with ultrafine airborne particles, industrial by-products, microplastics, and complex mixtures of pollutants whose biological effects are only beginning to be understood. These exposures are no longer marginal or rare, they are global, chronic, and largely unavoidable components of modern life.

Among the many environmental influences that may affect both the microbiome and neurodevelopment, several groups of hazards are constantly discussed: air pollution, heavy metals, endocrine-disrupting chemicals (EDCs), and micro- and nano-plastics (M/NPs).

Air pollution, especially fine particulate matter (PM2.5), together with nitrogen oxides and ozone, interacts with the respiratory and GI epithelium, inducing oxidative stress, systemic inflammation, and measurable GM shifts. Animal models have shown that prenatal and early-life exposures to PM2.5 alter microbial diversity, reduce the abundance of taxa such as *Lactobacillus* and *Bifidobacterium*, and increase pro-inflammatory bacterial groups (Nam et al., 2020; Mittal et al., 2014; Li et al., 2017; Wang et al., 2018). These microbial alterations are accompanied by changes in synaptic plasticity, microglial activation, and impairments in learning and memory. Recent experimental evidence from a real-world whole-body

exposure model further demonstrates that continuous exposure to ambient air pollution induces persistent neurobehavioral impairments in offspring mice, associated with pathological and inflammatory changes in key brain regions. These neurodevelopmental alterations are paralleled by dynamic, stage-specific disruptions in GM composition and function across development. Mediation analyses and antibiotic-based microbiome depletion indicate that GM alterations play a significant mechanistic role in linking air pollution exposure to neurodevelopmental damage, as antibiotic intervention markedly attenuated behavioral deficits, brain inflammation, and pathological differences between exposed and control animals (Yang et al., 2025). Observational human studies align with these findings, linking early urban air pollution to differences in attention, emotional regulation, and cognitive development (Zhou et al., 2024; Yuan et al., 2023; Sun et al., 2022).

Heavy metals, such as lead, mercury, cadmium, and arsenic, constitute another relevant group of hazards that can persist for decades, accumulating in food chains, air, water, and soil. Exposure to these metals is associated with disruption of intestinal epithelial barrier, increased intestinal permeability, reduced microbial diversity, altered microbial metabolic function, chronic low-grade inflammation, increased oxidative stress, and modulation of neurodevelopmental trajectories with measurable cognitive and behavioral consequences (Rezazadegan et al., 2025; Hoen et al., 2018; Laue et al., 2022; Ouyang et al., 2023).

EDCs, including bisphenols, phthalates, and related compounds, represent a third, increasingly recognized class of hazards. These substances are widely present in lubricants, flooring materials, personal care products (shampoos, soaps), food packaging, and polycarbonate plastics. Perinatal and early-life exposure to EDCs can reshape GM, increase intestinal permeability, and promote mucosal and systemic inflammation. In rodent models, developmental exposure to bisphenol A alters the GM of both parents and offspring and has been linked to immune, metabolic, and behavioral disturbances. Finally, human data, including maternal-infant cohorts, further indicate that higher prenatal or early-life levels of certain EDCs are associated with differences in infant GM, supporting the idea that hormone-like pollutants can modulate host-microbe interactions during the critical period when both the microbiome and brain are being established (Lei at al., 2019; Hu et al., 2017; Yang et al., 2019; Ryan et Vandenbergh, 2006; Roen et al., 2015; Evans et al., 2014; Braun et al., 2011; Perer et al., 2012; Xu et al., 2015).

Micro- and nano-plastics, once considered biologically inert, are now emerging as pervasive contaminants with potential relevance for the gut–brain axis. Humans are exposed to M/NPs through ingestion, inhalation, and dermal contact (Ramsperger, et al., 2023). Animal models indicates that exposure to micro- and nanoplastics (M/NPs) can disrupt gut homeostasis by inducing microbial dysbiosis, impairing epithelial integrity, and increasing intestinal permeability (Jin et al., 2019; Harusato et al., 2023; Lin et al., 2023; Kaluç et al., 2024). These alterations are consistently accompanied by local GI inflammation and, in some models, by signs of systemic immune activation, including elevated circulating inflammatory mediators (Lee et al., 2023). As a consequence of barrier dysfunction and inflammatory signaling, plastic particles and microbiome-derived pro-inflammatory molecules may gain access to the systemic circulation and accumulate in peripheral tissues, as suggested by evidence of particle translocation across biological barriers (Ragusa et al., 2021). Furthermore, experimental studies report coordinated metabolic disturbances affecting both gut and brain tissues following M//NPs exposure (Lee et al., 2024), supporting the concept that M/NPs may influence neural function primarily through microbiome-dependent and immune-mediated pathways, while direct neurotoxicity remains a plausible contributing mechanism (Yang et al., 2023a, b; Hoyo-Alvarez et al., 2022).

These environmental hazards rarely act in isolation. Rather, children and pregnant women are exposed to a complex mixture of particulates, metals, EDCs, microplastics, and other pollutants over an extended period. This has led to the broader concept of the exposome, defined as the totality of environmental

exposures across the life course, and to increasing interest in how the exposome intersects with the microbiome and brain development (Wild, 2005; Aburto et al., 2025). Within this framework, the microbiome can be viewed both as a target of environmental damage and as an active mediator that translates chemical and physical exposures into biological signals, including metabolites, cytokines, and neural inputs that reach the developing brain.

From the standpoint of neurodiversity and NDDs, these findings do not support a simplistic narrative in which pollution or microbiome alterations directly cause any specific diagnostic label. Instead, they suggest that environmental hazards may subtly shift the balance of risk by disturbing microbiome–brain communication during critical developmental window, in interaction with many other biological and social factors.

18.3 Microbiota Dysregulation across Neurodiversity: Evidence and Insights

18.3.1 Autism Spectrum Disorders

Over the past two decades, converging evidence has demonstrated that ASD individuals harbor a distinct gut microbial profile compared to neurotypical subjects (Parracho et al., 2005; Finegold et al., 2010; De Angelis et al., 2015; Strati et al., 2017; Liu et al., 2019; Chen et al., 2021; Zeng et al., 2025). Although findings vary across studies due to differences in age, diet, geography, sequencing technology, and analytical approaches, several recurring microbial patterns have emerged. Most investigations report an altered Bacillota/Bacteroidota ratio, often characterized by a relative enrichment of Bacillota and a depletion of Bacteroidota, together with a reduced overall microbial diversity, typically regarded as an indicator of intestinal dysbiosis (Finegold et al., 2012; Tomova et al., 2015; Kang et al., 2018). At the taxonomic level, ASD cohorts frequently show increased abundances of *Clostridium* spp. (notably *Clostridium bolteae* and *C*lostridium *perfringens*), Proteobacteria (including *Escherichia/Shigella*), *Sutterella*, and in some reports, *Lactobacillus* (Finegold et al., 2017; Gondalia et al., 2012; Luna et al., 2017). Conversely, beneficial commensals such as *Bifidobacterium*, *Faecalibacterium*, *Prevotella*, and *Akkermansia* (in some studies) are depleted in patients with ASD (De Angelis et al., 2013; Kang et al., 2013; Strati et al., 2017; Zou et al., 2020). Collectively, these microbial shifts point toward a pro-inflammatory and metabolically imbalanced intestinal ecosystem in ASD.

Beyond bacterial dysbiosis, recent studies have expanded the analysis to include the gut virome, providing new insights into the viral component of the intestinal ecosystem and its potential interaction with bacterial communities in ASD. Wan and colleagues performed metagenomic profiling of fecal samples from children with ASD and neurotypical controls, revealing a significant reduction in viral diversity and altered bacteriophage–bacterium interactions, particularly involving Caudovirales and Microviridae families (Wan et al., 2024). These alterations were associated with perturbations in bacterial metabolic pathways and SCFAs biosynthesis, suggesting that virome dysregulation may exacerbate bacterial imbalance and contribute to gut dysfunction in ASD. Complementarily, Yuan et al. (2025) compared gut viral communities between ASD and healthy children, identifying distinct viral signatures enriched in ASD, including phages targeting *Bacteroides* and *Clostridium* species, which may reinforce bacterial dysbiosis through top-down ecological control. Together, these findings underscore that ASD-associated gut alterations encompass both bacterial and viral components, with complex cross-kingdom interactions shaping intestinal ecology and probably influencing neurodevelopmental outcomes.

Beyond compositional differences, increasing evidence supports a mechanistic link between gut dysbiosis and ASD symptomatology through the GM–brain axis (Dan et al., 2020; Rose et al., 2018; Huang et al., 2021). GI disturbances, reported in up to 70% of ASD individuals, often parallel the degree of

microbial imbalance and correlate with behavioral symptom severity (Wang et al., 2023a, b; He et al., 2023). Several studies indicate that microbial metabolites may directly influence neurodevelopmental and behavioral outcomes. For example, altered SCFA levels, mainly propionic acid (PPA), have been implicated in ASD, as elevated PPA can induce autism-like behaviors in animal models (Liu et al., 2019; He et al., 2023; Chang et al., 2024). Other bacterial-derived metabolites such as p-cresol, which interfere with neurotransmitter metabolism, are also found at higher concentrations in some children with ASD (De Angelis et al., 2013; Rose et al., 2018). Furthermore, evidence of a "leaky gut" phenotype, characterized by increased intestinal permeability, suggests that bacterial components such as lipopolysaccharides (LPS) and toxic metabolites can translocate into the systemic circulation, promoting immune activation and neuroinflammation that may alter brain function and behavior (Tomova et al., 2015; Adams et al., 2018; Dan et al., 2020).

Altogether, these findings indicate that ASD-associated gut dysbiosis is not merely a secondary manifestation but may actively contribute to disease expression through immune, metabolic, and neuroactive signaling pathways. Understanding these interactions provides a biological basis for the development of microbiota-targeted interventions (including dietary modulation, pre/probiotics, and fecal microbiota transplantation (FMT) as potential therapeutic strategies for alleviating ASD-related symptoms.

A summary of the main studies investigating GM alterations in ASD is reported in Table 18.1.

TABLE 18.1
Summary of Original Studies Reporting Gut Microbiota Alterations in Autism Spectrum Disorder

No.	Reference	Country	Participants (ASD)/Controls (NT)	Type of Controls	Sequencing/Analysis Method	Main Findings
1	Parracho et al. (2005)	UK	18/20	Non-related NT	Culture-based	↑ *Clostridium*, ↓ *Bifidobacterium*.
2	Finegold et al. (2012)	USA	9/9	Non-related NT	Culture, biochemical	↑ *C. bolteae* in regressive autism.
3	Finegold et al. (2010)	USA	33/7	Non-related NT 1	16S rRNA (pyroseq.)	↑ *Desulfovibrio*, ↓ *Firmicutes*.
4	Strati et al. (2017)	Italy	40/40	Non-related NT	16S rRNA	↓ *Akkermansia*, *Faecalibacterium*, ↑ *Clostridium*.
5	Adams et al. (2018)	USA	67/50	Non-related NT	16S rRNA + clinical	Microbiota improved post-diet; ↑ *Bifidobacterium*.
6	Chen et al. (2021)	China	62/62	Non-related NT	16S rRNA	Dysbiosis correlated with ASD severity and IQ.
7	Chen et al. (2022)	Taiwan	57/40	Non-related NT	16S rRNA	Dysbiosis linked to behavior, not GI symptoms.
8	Dan et al. (2020)	China	62/64	Non-related NT	Metagenomics	↓ SCFA producers; metabolic dysregulation.
9	De Angelis et al. (2015)	Italy	10/10	Non-related NT	16S rRNA + metabolomics	↑ *Clostridium*, ↓ *Bifidobacterium*.
10	Ding et al. (2021)	China	40/40	Non-related NT	16S rRNA	↓ Diversity; ↑ *Bacteroides*, *Clostridium*.

No.	Reference	Country	Participants (ASD)/Controls (NT)	Type of Controls	Sequencing/Analysis Method	Main Findings
11	Huang et al. (2021)	China	80/80	Non-related NT	Metagenomics	Gut–brain correlations with behavior.
12	Zuffa et al. (2023)	Sweden	72 infants	High-risk vs. low-risk infants	16S rRNA	Early-life microbial shifts in ASD-prone infants.
13	Liu et al. (2019)	China	77/77	Non-related NT	16S rRNA + SCFA	↓ SCFA, ↓ *Prevotella*, ↑ *Clostridium*.
14	Niu et al. (2019)	China	62/62	Non-related NT	16S rRNA	Probiotics partially restored dysbiosis.
15	Pärtty et al. (2015)	Finland	75 (RCT)	Randomized infants	16S rRNA	Early *L. rhamnosus* GG ↓ neuropsychiatric risk.
16	Tomova et al. (2015)	Slovakia	20/20	Non-related NT	qPCR, 16S rRNA	↑ *Clostridium*, ↓ *Bifidobacterium*.
17	Wang et al. (2023a,b)	China	45/45	Non-related NT	16S rRNA	Distinct profile in ASD with GI symptoms.
18	Wu et al. (2020)	China	77/77	Non-related NT	16S rRNA	Microbiota discriminates ASD vs. control.
19	Zhai et al. (2019)	China	60/60	Non-related NT	16S rRNA	Dysbiosis linked to trace element imbalance.
20	Zou et al. (2020)	China	72/72	Non-related NT	16S rRNA	↓ Diversity; ↑ *Clostridium*, ↓ *Prevotella*.
21	Zhang et al. (2018)	China	62/62	Non-related NT	16S rRNA	↑ *Bacteroides*, *Clostridium*; ↓ *Prevotella*.
22	Kang et al. (2013)	USA	20/20	Non-related NT	16S rRNA	↓ *Prevotella* and fermenters.
23	Kang et al. (2018)	USA	21/21	Non-related NT	Metabolomics + 16S	Altered microbial metabolites in ASD.
24	Rose et al. (2018)	USA	90/90	Non-related NT	16S rRNA + immune	Dysbiosis correlated with immune activation.
25	Finegold et al. (2017)	USA	20/20	Non-related NT	PCR	*C. perfringens* toxin genes detected in ASD.
26	Hughes and Ashwood (2018)	USA	40/40	Non-related NT	Serological	↑ Anti-*Candida* IgG in ASD.
27	Kantarcioglu et al. (2016)	Turkey	20/20	Non-related NT	Culture	↑ Yeast species in ASD stool.
28	Kushak et al. (2017)	USA	14/10	Non-related NT	16S rRNA (duodenal)	Dysbiosis linked to carb maldigestion.
29	Luna et al. (2017)	USA	40/40	Non-related NT	16S rRNA + immune	Microbiome–neuroimmune signatures.

No.	Reference	Country	Participants (ASD)/Controls (NT)	Type of Controls	Sequencing/Analysis Method	Main Findings
30	Son et al. (2015)	USA	59 pairs	NT siblings	16S rRNA	Sibling differences in *Bacteroides* and *Clostridium*.
31	Gondalia et al. (2012)	Australia	23 families	NT siblings	16S rRNA	Dysbiosis more evident with GI symptoms.
32	De Angelis et al. (2013)	Italy	20/20	Non-related NT	16S rRNA + metabolomics	Altered metabolome in ASD.
33	Pulikkan et al. (2018)	India	30/30	Non-related NT	16S rRNA	↑ *Clostridium*, ↓ *Bacteroidetes*.
34	Ahmed et al. (2020)	Egypt	30/20	Non-related NT	16S rRNA	↑ *Firmicutes/Bacteroidetes* ratio.
35	Yap et al. (2021)	Australia	247/125	Non-related NT	16S rRNA + diet data	Diet mediates ASD–microbiome link.
36	He et al. (2023)	China	60/60	Non-related NT	16S rRNA + SCFA	Constipated ASD: ↓ SCFA, altered *Firmicutes*.
37	Chang et al. (2024)	China	80/80	Non-related NT	16S rRNA + metabolomics	Dysregulated amino acid metabolism.
38	Kurokawa et al. (2024)	Japan	200/200	Mixed (siblings and unrelated)	16S rRNA	↓ Diversity with restricted diet.
39	Toscano de Oliveira et al. (2025)	Brazil	40/40	Non-related NT	16S rRNA	Dysbiosis linked to selective feeding.
40	De Sales-Millán et al. (2024)	Mexico	60/60	Non-related NT	Metagenomics	Sex-based and metabolic differences.
41	Soltysova et al. (2025)	Slovakia	80/80	Non-related NT	16S rRNA	Shared dysbiosis in ASD, ADHD, anorexia.
42	Xiang et al. (2025)	China	90/90	Non-related NT	16S rRNA	Distinct phylogenetic clustering in ASD.
43	Kadiyska et al. (2025)	Bulgaria	72/72	Non-related NT	16S rRNA	Age-dependent dysbiosis; altered ratios.
44	Zeng et al. (2025)	China	100/100	Non-related NT	16S rRNA	Diversity inversely linked to symptom severity.
45	Bhusri et al. (2025)	Thailand	80/80	Non-related NT	16S rRNA	Gender- and age-specific microbiome shifts.

18.3.2 Attention-Deficit/Hyperactivity Disorder

Recent research has increasingly implicated GM alterations in the ADHD pathophysiology, suggesting that intestinal dysbiosis may influence neurodevelopmental and behavioral regulation via the GM–brain axis (Aarts et al., 2017; Jiang et al., 2018; Wang et al., 2020a, b; Cassidy-Bushrow et al., 2023; Novau-Ferré et al., 2025). Although ADHD is classically defined by neurobehavioral symptoms, namely inattention,

hyperactivity, and impulsivity, emerging evidence supports a biological contribution of microbial and metabolic imbalance, affecting neurotransmission, immune signaling, and energy homeostasis.

Across several studies, consistent microbial compositional differences have been documented between individuals with ADHD and neurotypical controls (including both unrelated controls and neurotypical siblings) (Prehn-Kristensen et al., 2018; Kurokawa et al., 2024). Common features include reduced microbial alpha diversity, a marker of gut ecosystem instability, and altered relative abundances of specific bacterial taxa. In detail, enrichment of *Bacteroides*, *Neisseria*, and *Prevotella* genera, alongside reductions in *Faecalibacterium* and *Bifidobacterium*, have been repeatedly reported (Jiang et al., 2018; Wang et al., 2020a, b; Wan et al., 2020). These taxa are involved in SCFAs metabolism and catecholamine precursor synthesis, suggesting a mechanistic link to altered dopaminergic and noradrenergic signaling—two neurotransmitter systems central to ADHD pathology (Aarts et al., 2017; Wan et al., 2020).

Mechanistically, gut-derived metabolites and immune mediators appear to contribute to the neurobiological alterations observed in ADHD. Reduced butyrate-producing bacteria (e.g., *Faecalibacterium prausnitzii*) may impair intestinal barrier integrity and modulate the release of inflammatory cytokines, leading to low-grade systemic inflammation and potential neuroimmune crosstalk (Wang et al., 2022). Altered microbial enzymatic pathways involved in phenylalanine, tyrosine, and tryptophan metabolism—precursors for dopamine and serotonin—have also been identified in ADHD microbiomes, potentially influencing synaptic signaling and reward anticipation (Aarts et al., 2017; Wan et al., 2020). Furthermore, dietary habits and restricted food diversity, common in children with ADHD, may exacerbate dysbiosis, as suggested by a comparative study showing lower microbial diversity and decreased dietary diversity in ADHD subjects versus both neurotypical siblings and unrelated controls (Kurokawa et al., 2024).

Table 18.2 offers a synthesis of the main studies investigating the GM in ADHD.

TABLE 18.2
Summary of Studies Examining Gut Microbiota Alterations in ADHD

Reference	Country/Population	Subjects (ADHD/Controls)	Control Type	Sequencing Method	Main Findings
Aarts et al., (2017) (*PLoS One*)	Netherlands	19 ADHD/77 controls	Unrelated neurotypical	16S rRNA (V4)	Altered abundance of *Bifidobacterium* linked to reward anticipation and dopamine precursor pathways.
Jiang et al. (2018) (*Behav. Brain Res.*)	China	51 ADHD (treatment-naïve)/32 controls	Unrelated neurotypical	16S rRNA (V3–V4)	Enrichment of *Neisseria* and *Bacteroides*; depletion of *Faecalibacterium*; altered SCFA-producing taxa.
Prehn-Kristensen et al. (2018) (*PLoS One*)	Germany	14 ADHD/17 controls	Unrelated neurotypical	16S rRNA (V1–V2)	Reduced alpha diversity; lower abundance of *Faecalibacterium* and *Lachnospiraceae*.
Wang et al. (2020a) (*Eur. Child Adolesc. Psychiatry*)	Taiwan	35 ADHD/35 controls	Unrelated neurotypical	16S rRNA (V3–V4)	Higher *Bacteroides* and *Prevotella*; lower *Faecalibacterium*; links between microbiota and dietary patterns.

Reference	Country/Population	Subjects (ADHD/Controls)	Control Type	Sequencing Method	Main Findings
Wan et al. (2020) (*Front. Neurosci.*)	China	30 ADHD/30 controls	Unrelated neurotypical	16S rRNA	Altered microbial pathways in dopamine, norepinephrine, and serotonin metabolism.
Wang et al. (2022) (*Transl. Psychiatry*)	Taiwan	60 ADHD/60 controls	Unrelated neurotypical	16S rRNA + cytokine profiling	Correlation between reduced butyrate-producing bacteria and elevated plasma cytokines (IL-6, TNF-α).
Cassidy-Bushrow et al. (2023) (*Pediatr. Res.*)	USA	297 preadolescents (prospective cohort)	Unrelated neurotypical	16S rRNA (infant and child stool)	Early-life microbial signatures (e.g., lower *Bifidobacterium*) predict ADHD diagnosis in later childhood.
Kurokawa et al. (2024) (*J Child Psychol Psychiatry*)	Japan	60 ASD/60 ADHD/60 Siblings/60 Controls	Both siblings and unrelated	16S rRNA (V3–V4)	ADHD group showed reduced microbial and dietary diversity vs. both sibling and unrelated controls.
Novau-Ferré et al. (2025) (*Res. Dev. Disabil.*)	Spain	80 (ADHD, ASD, controls; probiotic RCT)	Both	16S rRNA + probiotic intervention	Probiotic supplementation partially restored microbial balance and improved behavioral measures in ADHD subgroup.

18.3.3 Rett Syndrome

RTT is a severe, progressive NDD that predominantly affects females. Clinically, RTT is characterized by developmental regression, particularly involving loss of spoken language and purposeful hand use; together with the emergence of hand stereotypies and impaired ambulation, these features constitute the four core diagnostic criteria. In addition, affected individuals often exhibit a range of other neurological impairments and comorbidities, including seizures, breathing abnormalities, anxiety, and constipation. RTT is consistently associated with intestinal dysbiosis and altered microbial metabolism, which may contribute to the prominent GI comorbidities (notably constipation) and low-grade intestinal inflammation observed in affected individuals. In the first large molecular study, RTT subjects exhibited reduced bacterial richness and a distinct bacterial–fungal community dominated by taxa such as *Bifidobacterium*, several *Clostridia* (including *Anaerostipes* and *Clostridium clusters XIVa/XIVb*), *Escherichia/Shigella* and fungal *Candida*; these changes were associated with altered SCFA profiles and markers of subclinical intestinal inflammation (Strati et al., 2016). A follow-up, independent cohort likewise reported RTT-related differences in the fecal metabolome (including altered levels of GABA, tyrosine, and glutamate end-products) and showed that microbiome composition varied with clinical features (pubertal status, clinical severity and diet), suggesting phenotype-dependent microbiome signatures (Thapa et al., 2021).

Reviews and focused analyses emphasize that MeCP2 dysfunction, ENS impairment, chronic constipation, and altered host immunity in RTT create a permissive niche for a dysbiotic community that may reinforce GI dysfunction via altered metabolite production and immune activation (Borghi et al., 2017). For context, comparative studies in other NDD syndromes (e.g., DS) also report microbiome alterations linked to clinical and cognitive features, supporting the broader concept that chromosomal or monogenic NDD disorders can be accompanied by disease-specific microbiome and metabolome shifts

(Biagi et al., 2014; Ren et al., 2022). Taken together, these data support a model in which RTT-related host factors (genetic, neuroenteric, and immune) interact with the intestinal microbial ecosystem to shape metabolite output (SCFAs, amino-acid–derived compounds, etc.), which in turn may influence GI physiology and systemic/neuronal signaling. Table 18.3 summarizes main findings relating intestinal dysbiosis with RTT.

TABLE 18.3
Summary of Studies Examining Gut Microbiota Alterations in RS

No.	Reference (Year)	Country/Cohort Origin	Subjects (RTT or DS)/Controls	Control Type	Methods (Sequencing/Metabolomics/Assays)	Principal Findings
58	Strati et al. (2016) (Altered gut microbiota in Rett syndrome. *Microbiome*)	Italy	RTT 50/HC 28	Unrelated healthy controls	16S rRNA (V3–V5) sequencing + ITS fungal profiling; fecal SCFA measurement; inflammatory markers (fecal calprotectin, ESR)	RTT subjects showed reduced bacterial richness (lower α-diversity), altered bacterial and fungal community structure, enrichment of *Bifidobacterium*, several *Clostridia* (Anaerostipes, *Clostridium* XIVa/XIVb), *Escherichia/Shigella* and fungal *Candida*; altered SCFA profiles and mild intestinal inflammation.
59	Borghi et al. (2017) (Rett syndrome: a focus on gut microbiota. *Int. J. Mol. Sci.*)	Italy (review/synthesis of cohorts)	— (review)	—	Review integrating diet, microbiota, SCFA data	Reviewed existing evidence linking MeCP2 dysfunction, constipation, and microbiota shifts in RTT; highlighted altered SCFA production and proposed mechanistic links to GI comorbidity.

No.	Reference (Year)	Country/Cohort Origin	Subjects (RTT or DS)/Controls	Control Type	Methods (Sequencing/Metabolomics/Assays)	Principal Findings
60	Thapa et al. (2021) (Assessment of the gut bacterial microbiome and metabolome of girls and women with Rett syndrome. *PLoS One*)	USA (Baylor College of Medicine cohort)	RTT 44/Controls 21	Unrelated age-matched female controls (no GI disorders)	Illumina MiSeq 16S rRNA sequencing (V region) + LC-MS metabolomics (selected protein-metabolite end-products)	Microbiome composition within RTT varied with pubertal status, clinical severity, and diet; metabolome changes included lower fecal/derived GABA, tyrosine, and glutamate end-products in RTT vs. controls; authors highlighted phenotype-dependent microbiome–metabolome relationships.
61	Biagi et al. (2014) (Gut microbiome in Down syndrome. *PLoS One*)	Italy	DS adults 17/Healthy adults (matched)	Unrelated healthy adults	454 pyrosequencing (16S rRNA, V4)	Adult DS subjects display GM deviations relative to controls; study provided early evidence for disease-associated microbial changes in a chromosomal NDD.
62	Ren et al. (2022) (Altered gut microbiota correlates with cognitive impairment in Chinese children with Down's syndrome. *Eur. Child Adolesc. Psychiatry*)	China	DS children (reported n ≈ 15)/controls (≈15)	Unrelated age-matched healthy controls	16S rRNA sequencing; KEGG pathway analysis	Altered microbial composition in DS children (e.g., decreased *Acidaminococcaceae*); microbial functional modules (peptidases, pyrimidine metabolism) differed and correlated with cognitive scores—supporting microbiome–cognition associations in another neurodevelopmental condition.

18.3.4 Down Syndrome

DS, a trisomy of chromosome 21, is characterized by complex systemic manifestations—including immune dysregulation, metabolic alterations, and an increased risk of early-onset Alzheimer's disease—all of which may be influenced by host–microbiome interactions. Mounting evidence indicates that microbiome alterations are a consistent DS feature, extending beyond the gut to include the oral and lower airway ecosystems, thereby reflecting a systemic microbial imbalance (Biagi et al., 2014; Morishima et al., 2022; Mitsuhata et al., 2022; De Lausnay et al., 2021; Rosas et al., 2025).

Early 16S rRNA analyses revealed a distinct gut microbial signature in DS adults, with decreased microbial diversity and shifts in taxa involved in SCFAs production, mainly *Blautia*, *Coprococcus*, and *Faecalibacterium*—key butyrate producers associated with gut epithelial integrity and anti-inflammatory tone (Biagi et al., 2014). More recent studies in children confirmed altered GM profiles and demonstrated significant correlations with cognitive performance (Ren et al., 2022; Cai et al., 2023). Specifically, DS children showed depletion of SCFA-producing *Ruminococcaceae* and *Lachnospiraceae* alongside enrichment of *Enterobacteriaceae* and *Bacteroides*, accompanied by increased systemic inflammatory markers and altered microbial metabolic pathways linked to amino acid and lipid metabolism (Cai et al., 2023). These compositional and functional changes may contribute to gut barrier dysfunction, systemic inflammation, and neuroinflammatory priming that accelerate cognitive decline in DS—a hypothesis further supported by metagenomic and metabolomic studies proposing gut dysbiosis as a potential driver of early Alzheimer's-like pathology in this population (Rosas et al., 2025).

Beyond the gut, the oral microbiome of DS people displays a characteristic dysbiotic profile, likely mirroring both intrinsic immune alterations and frequent periodontal disease. Case-control studies revealed enrichment of pathogenic taxa such as *Porphyromonas gingivalis*, *Treponema denticola*, *Fusobacterium nucleatum*, and *Prevotella intermedia*, alongside a decrease in commensal *Streptococcus* species (Mitsuhata et al., 2022; Contaldo et al., 2021). These changes are associated with elevated inflammatory cytokines in saliva and a high prevalence of chronic periodontitis, which may contribute to systemic inflammation and microbial translocation into the bloodstream, thereby exacerbating immune activation and endothelial dysfunction. Interestingly, maternal GM in pregnancies resulting in DS newborns already showed compositional shifts, suggesting that microbial dysbiosis may precede and influence developmental trajectories (Hursitoglu et al., 2021).

Collectively, the available evidence supports a multisystemic microbiome imbalance in human DS, spanning gut, oral, and respiratory compartments, which may participate in the pathogenesis of both GI and neurocognitive complications through immune, metabolic, and neuroinflammatory pathways. This concept positions the microbiome as both a biomarker and a potential therapeutic target to mitigate inflammation and promote healthy aging in DS subjects.

18.3.5 Tourette Syndrome

Emerging evidence implicates the gut–brain axis in the TS pathophysiology, an NDD characterized by chronic motor and vocal tics often accompanied by obsessive–compulsive and attention-deficit symptoms. Although research in this area remains limited, available studies indicate significant GM alterations associated with symptom severity and treatment response.

Initial observations by Lee and Wong (2018) demonstrated that children with TS exhibit altered intestinal microbiota profiles, with differences in bacterial composition correlating with tic severity scores. These findings were supported and expanded by Xi et al. (2021), who reported a distinct microbial signature characterized by reduced diversity and compositional shifts in key taxa—including enrichment of *Ruminococcus*, *Bacteroides*, and *Prevotella* species, alongside depletion of *Faecalibacterium*—compared to neurotypical controls. The relative abundance of certain taxa correlated with tic intensity and behavioral

symptoms, suggesting a role for microbial metabolites or immune signaling in modulating dopaminergic and serotonergic pathways.

Moreover, interventional studies have provided preliminary functional insights into this association. In a pilot clinical trial, FMT significantly reduced tic severity and improved comorbid behavioral symptoms in children with TS, alongside partial normalization of GM composition (Zhao et al., 2020). These effects were accompanied by an increase in SCFA–producing bacteria and a decrease in potential pro-inflammatory taxa, pointing to a potential immuno-neurotransmitter regulatory mechanism. Similarly, a more recent investigation combining physiotherapy with dietary and behavioral interventions demonstrated both symptomatic improvement and modulation of GM composition, especially in butyrate-producing and bile acid–metabolizing genera (Bao et al., 2023).

Collectively, these findings suggest that gut dysbiosis contributes to the neuroimmune and neurochemical imbalance underlying TS, potentially through pathways involving microbial metabolites, vagal signaling, and immune activation. While causality remains to be firmly established, the GM emerges as a promising target for microbiota-based adjunctive therapies aimed at mitigating tics and improving neurobehavioral outcomes in TS.

18.3.6 Prader–Willi Syndrome

PWS is a complex genetic disorder characterized by hyperphagia, obesity, and distinctive neuroendocrine and behavioral features. Despite the presence of severe obesity, PWS patients often display unexpectedly high insulin sensitivity and distinct metabolic profiles, suggesting the involvement of unique biological modulators—including the GM. Recent studies provide converging evidence that gut microbial composition and functionality differ markedly in PWS compared to both obese and lean controls, and these alterations appear closely linked to metabolic homeostasis and dietary patterns.

In children with PWS, distinct GM signatures have been consistently reported, characterized by a higher relative abundance of *Akkermansia muciniphila*, *Christensenellaceae*, and *Ruminococcaceae*, along with lower levels of *Prevotella* and *Bacteroides* compared to matched controls (Peng et al., 2020; Zhong et al., 2024). These compositional features are thought to promote enhanced insulin sensitivity and reduced systemic inflammation, in contrast to the dysbiotic profiles typically observed in common obesity. Zhong et al. (2024) further demonstrated that the microbial community structure was associated with improved glycemic control and lipid metabolism, suggesting that microbiome-derived metabolites may contribute to the paradoxical metabolic phenotype of PWS.

Complementary metabolomic analyses have strengthened this connection; in detail, Hou et al. (2023) identified distinct plasma lipid signatures in PWS children, including higher levels of polyunsaturated fatty acids, which correlated with specific microbial taxa such as *Akkermansia* and *Blautia*, pointing toward gut–lipid metabolic crosstalk. In parallel, Garcia–Ribera's groups reported that dietary components, mainly fiber and fat intake, strongly influenced GM composition and obesity severity, emphasizing the nutritional modulation potential of the microbiome in PWS management (Garcia-Ribera et al., 2020).

In adults, Dahl and colleagues confirmed that the PWS GM remains distinct and less diverse than that of obese individuals without PWS, maintaining enrichment in taxa associated with leanness and metabolic resilience (Dahl et al., 2021). Similarly, Olsson showed that the GM of obese PWS subjects exhibited a healthier metabolic profile, characterized by reduced pro-inflammatory and endotoxin-producing bacteria, supporting the hypothesis that microbiota-mediated metabolic buffering contributes to the relatively preserved insulin sensitivity and lower incidence of metabolic complications in PWS (Olsson et al., 2020).

In sum, all these findings suggest that GM composition and function play a crucial mechanistic role in shaping the metabolic phenotype of PWS. Through modulation of gut barrier function, bile acid metabolism, and SCFA production, the microbiome may act as a key mediator between diet, metabolic

health, and neuroendocrine regulation, offering a promising therapeutic target for optimizing metabolic outcomes and weight management in human PWS.

18.4 Microbiome-Based Interventions to Improve Neurodevelopmental Health

Given the growing body of evidence linking microbial dysbiosis to the pathophysiology and symptom severity of neurodevelopmental and neurodivergent disorders, there is increasing interest in microbiota-directed interventions as potential adjunctive strategies to improve metabolic, GI, and neurobehavioral outcomes. Restoration of intestinal eubiosis may help modulate neuroimmune and metabolic signaling pathways, finally contributing to improved quality of life and symptom management in affected individuals. Therapeutic approaches to achieve this goal can be broadly categorized into three main strategies: (1) prebiotic, probiotic, and symbiotic supplementation, which aim to selectively promote beneficial taxa, restore microbial diversity, and enhance production of neuroactive metabolites such as SCFA and tryptophan derivatives; (2) dietary interventions, including both targeted nutritional modulation and comprehensive dietary pattern changes, which directly shape microbial composition and metabolic output; and (3) fecal microbiota transplantation, a more radical approach that introduces an entire healthy microbial consortium to reprogram gut ecosystem functionality.

In the following subsections, we summarize the clinical and experimental evidence evaluating these interventions across various NDDs, highlighting their potential mechanisms of action, efficacy, and current limitations.

18.4.1 Prebiotic, Probiotic, and Synbiotic Interventions

Over the past decade, growing recognition of the GM's pivotal role in neurodevelopment, immune regulation, and neurotransmission has fostered strong interest in microbiota-targeted strategies as adjunctive treatments for NDDs conditions. Prebiotics, probiotics, and synbiotics represent complementary approaches designed to restore intestinal eubiosis and modulate the microbiota–gut–brain axis through microbial, immune, and metabolic pathways.

Prebiotics—non-digestible dietary substrates such as galacto- or fructo-oligosaccharides—selectively stimulate the growth of beneficial commensals (notably *Bifidobacterium* and *Faecalibacterium*) and enhance the production of SCFAs, key metabolites, as previously reported, involved in epithelial integrity and neuroimmune regulation.

Probiotics, defined as live microorganisms conferring health benefits when administered in adequate amounts, act through multiple mechanisms, including competitive inhibition of pathogens, modulation of mucosal immunity, restoration of SCFAs and neurotransmitter homeostasis, and regulation of the hypothalamic–pituitary–adrenal (HPA) axis.

Finally, synbiotics—combinations of prebiotics and probiotics—aim to synergistically enhance microbial viability and metabolic output, thereby optimizing the host–microbiome interface.

Within this framework, the term "psychobiotics" has emerged to denote probiotic or prebiotic formulations that exert clinically relevant effects on brain function, mood, or behavior via microbiota-mediated mechanisms. Psychobiotics may influence neuroactive signaling molecules—including serotonin, GABA, dopamine, and tryptophan metabolites—while attenuating systemic inflammation and oxidative stress, both of which are frequently dysregulated in neurodevelopmental and neuropsychiatric conditions. This concept has been substantiated by mounting preclinical and clinical evidence showing that targeted microbial modulation can alleviate behavioral disturbances, cognitive dysfunction, and GI comorbidities in disorders such as ASD, ADHD, RTT, TS, and PWS.

18.4.1.1 Autism Spectrum Disorder

Among NDDs, ASD is the condition most extensively investigated in relation to microbiota-targeted interventions. The therapeutic rationale stems from the consistent evidence of GM dysbiosis and GI dysfunction in ASD, together with the demonstrated ability of specific probiotics and prebiotics to modulate microbial composition, immune responses, and gut–brain signaling. Over the past decade, different clinical trials have evaluated the effects of probiotics, prebiotics, or synbiotics—either alone or in combination with dietary modifications—on behavioral and GI outcomes in ASD populations.

Early open-label studies suggested that probiotic supplementation could alleviate GI symptoms and improve overall well-being (Shaaban et al., 2018; Sichel, 2013; Niu et al., 2019). Subsequent randomized controlled trials (RCTs) confirmed moderate improvements in GI disturbances, irritability, and adaptive behaviors, though effects on core ASD symptoms were less consistent. For instance, some authors reported significant declines in constipation and sensory abnormalities following *Lactobacillus* and *Bifidobacterium* supplementation, while others observed enhanced quality of life but minimal changes in autism severity (Santocchi et al., 2020; Arnold et al., 2019). Similarly, Guidetti *et al*. demonstrated that a multi-strain probiotic mixture improved both GI function and behavioral irritability, whereas Kong et al. (2021 b,c) found synergistic benefits when probiotics were combined with oxytocin therapy (Guidetti et al., 2022).

More recent trials have moved toward precision microbial interventions tailored to the ASD microbiome. A 2024 phase II RCT using *Bacteroides fragilis* BF839 showed improved social responsiveness and reduced hyperactivity, consistent with *B. fragilis*' known capacity to modulate gut permeability and GABA metabolism (Lin et al., 2024). In another precision approach, it was demonstrated that personalized synbiotic therapy increased microbial diversity and improved GI symptoms in children with ASD (Phan et al., 2024). Complementary work by Mazzone revealed that targeted microbial interventions enhanced social behavior and stress-related physiological responses, even in the absence of major changes in diagnostic severity scores (Mazzone et al., 2024).

In addition, prebiotic interventions have shown promise in restoring microbial balance and supporting neurobehavioral health. In a landmark crossover trial, Grimaldi et al. (2018) found that supplementation with galacto-oligosaccharides significantly increased *Bifidobacterium* abundance and improved social impairment and sleep quality. Likewise, it was reported that partially hydrolyzed guar gum relieved constipation and decreased irritability, while Raghavan et al. (2022) demonstrated improvements in behavioral regulation and reductions in α-synuclein levels after β-glucan supplementation (Inoue et al., 2019).

Combined dietary and probiotic interventions have further strengthened the microbiota–behavior link. In detail, Li et al. (2024) showed that a medium-carbohydrate diet plus probiotic therapy alleviated GI and behavioral symptoms while normalizing dysbiosis-associated metabolites. A recent metagenomic study also confirmed that anti-inflammatory dietary patterns and probiotics synergistically restored beneficial taxa such as *Faecalibacterium* and *Prevotella* (Valencia-Buitrago et al., 2025). Notably, a large 2025 RCT involving children with both ASD and ADHD demonstrated probiotic-induced modulation of GM composition and improvement in emotional regulation, further supporting the therapeutic potential of microbiome-based strategies (Novau-Ferré et al., 2025).

Although results across studies remain heterogeneous, the overall evidence supports the concept that probiotic, prebiotic, and synbiotic interventions can beneficially modulate the GM and improve GI, metabolic, and certain behavioral parameters in ASD, likely through SCFAs restoration, immune regulation, and modulation of serotonergic and dopaminergic pathways. Anyway, future multi-omic and precision-designed trials will be essential to determine strain specificity, dosage, and long-term efficacy in distinct ASD subgroups.

18.4.1.2 Attention-Deficit/Hyperactivity Disorder

Multiple RCTs now indicate that prebiotic, probiotic, and synbiotic supplementation can modulate microbial composition and improve behavioral, cognitive, and neurophysiological outcomes in both ADHD children and adults.

Early clinical work demonstrated that a synbiotic formulation containing *Lactobacillus* and *Bifidobacterium* strains improved attention, hyperactivity, and daily functioning, mainly in individuals with pronounced irritability, while modulating circulating cytokines and increasing fecal SCFAs concentrations (Skott et al., 2020). Follow-up trials by the same group extended these findings, showing that synbiotic intake significantly altered plasma immune activity markers—including reductions in IL-6 and TNF-α—and enhanced microbial pathways linked to butyrate production, thereby supporting an anti-inflammatory and neuroprotective effect (Yang et al., 2023a,b).

A large multicenter placebo-controlled "basket" trial provided robust evidence that synbiotic therapy reduced irritability and improved emotional regulation in adults with ADHD and/or borderline personality disorder, with mechanistic data indicating normalization of tryptophan–kynurenine metabolism and decreased peripheral inflammation (Arteaga-Henríquez et al., 2024). More recently, Trezzi et al. (2025) compared two synbiotic formulations enriched with pigmented corn extract or cornstarch in drug-naïve children with ADHD and reported improvements in attentional control, working memory, and EEG markers of cortical activation, along with microbiome enrichment in *Faecalibacterium* and *Roseburia*—key butyrate producers associated with cognitive flexibility.

Probiotic supplementation alone has also shown beneficial adjunctive effects in pediatric ADHD. In a 12-week double-blind RCT, it was observed that *Bifidobacterium bifidum* supplementation improved inattentive symptoms and parental ratings of emotional regulation, while enhancing microbial diversity and increasing SCFA levels (Wang et al., 2024a,b). Similarly, it was found that *Lactobacillus acidophilus* LB supplementation led to a significant reduction in total ADHD symptom scores and improved attention span compared to placebo (Elhossiny et al., 2023). A triple-blind trial reported that adding probiotics to standard methylphenidate therapy enhanced treatment response, principally in cognitive performance and hyperactivity reduction, supporting the role of microbiota modulation in optimizing pharmacologic efficacy (Sangsefidi et al., 2025).

Additional emerging evidence points toward neuroimmune and metabolic mechanisms mediating these effects. Synbiotic supplementation has been shown to alter plasma SCFAs and neurotransmitter precursors, modulate HPA-axis activity, and reduce systemic inflammation (Yang et al., 2023a,b; Trezzi et al., 2025). Finally, probiotic-induced restoration of beneficial taxa such as *Bifidobacterium* and *Faecalibacterium* may help rebalance catecholamine synthesis and improve dopaminergic signaling, processes directly implicated in ADHD pathophysiology.

Although study heterogeneity remains high in terms of microbial strains, duration, and participant characteristics, the collective data suggest that microbiota-targeted interventions hold therapeutic promise in ADHD—either as standalone nutritional strategies in drug-naïve patients or as adjunctive treatments enhancing cognitive and behavioral outcomes.

Surely, future precision-designed, multi-omic trials are warranted to define optimal strain combinations, treatment duration, and microbiome-mediated biomarkers of clinical response.

18.4.1.3 RTT, DS, and TS

Although clinical research on microbiota-based interventions in RTT and TS remains at an early stage, emerging pilot trials suggest that probiotics may offer neurological and behavioral benefits through modulation of gut–brain signaling, immune tone, and microbial metabolites.

In RTT, Wong et al. (2024) conducted the first randomized, double-blind, placebo-controlled pilot study assessing probiotic supplementation in 50 girls with classic RTT. Treatment with a *Lactobacillus*-dominant probiotic formulation for 12 weeks resulted in improved GI function, reduced frequency of abdominal discomfort, and modest gains in adaptive behavior and sleep regulation compared to placebo. These clinical effects were accompanied by increased fecal SCFA levels and a partial normalization of microbial diversity, supporting the hypothesis that microbiota modulation can influence enteric and neurophysiological symptoms in this population.

In TS and chronic tic disorders, probiotic therapy has also shown encouraging early results. In detail, the supplementation with *Lactobacillus plantarum* PS128 for two months led to significant reductions in tic severity and anxiety symptoms, along with improved sleep and quality of life in pediatric patients (Wu et al., 2021). More recently, Liang et al.)2025) evaluated *Limosilactobacillus reuteri* supplementation in children with chronic tic disorders and observed further declines in total tic scores over a 24-week period, coupled with improved attention and emotional regulation. The beneficial effects correlated with increased abundance of *L. reuteri* and *Bifidobacterium* species and enhanced SCFA production, thereby suggesting microbial metabolite–mediated neuromodulation.

In addition, preliminary microbiome-targeted interventions have also been explored in DS, primarily focusing on the oral microbiome and mucosal health. In a recent RCT, the sugar-free probiotic oral rinse significantly reduced *Candida albicans* colonization in DS children after oral rehabilitation, maintaining lower fungal loads over six months (Saha et al., 2025). Complementarily, it was demonstrated that a postbiotic-based gingival gel effectively decreased inflammation and improved gingival indices compared with placebo, highlighting the local immunomodulatory and antimicrobial effects of probiotic and postbiotic formulations in DS-associated oral dysbiosis (Scribante et al., 2025).

Collectively, these preliminary findings indicate that probiotic supplementation may alleviate both GI and neurobehavioral manifestations in RTT, TS and DS, likely via anti-inflammatory and neuroactive metabolite pathways within the GM–brain axis. However, larger multicenter trials with longer follow-up are needed to rightly validate these promising results and identify the most effective microbial strains and treatment durations.

18.4.1.4 Prader–Willi Syndrome

Several RCTs have explored the use of probiotic supplementation to restore microbial balance and improve clinical outcomes in PWS patients.

In a randomized, double-blind, crossover study, it was evaluated the effects of *Bifidobacterium animalis* subsp. *lactis* BPL1 in children and adolescents with PWS (Amat-Bou et al., 2020). The intervention led to improvements in anthropometric parameters and insulin sensitivity, along with favorable shifts in GM composition, including increased abundance of *Bifidobacterium* and *Faecalibacterium* species—key producers of SCFAs implicated in metabolic homeostasis. These findings support the hypothesis that microbial modulation through probiotics can influence energy metabolism and adiposity regulation in PWS.

Similarly, Kong et al. (2021a, 2021b) demonstrated that supplementation with *Limosilactobacillus reuteri* LR-99 or *B. animalis* strains resulted in significant reductions in body mass index (BMI) and enhanced social communication, fine motor performance, and adaptive behavior. Notably, these improvements coincided with increased microbial diversity and elevated fecal SCFA concentrations, alongside the enrichment of *Bifidobacterium* and *Faecalibacterium*, documenting a functional restoration of the GM ecosystem. Extending this evidence, Liu and Kong (2022) observed that *B. animalis* BL-11 supplementation reshaped both gut and salivary microbiota, correlating with better growth parameters and reduced behavioral symptom severity, suggesting a systemic microbiome–host crosstalk mediated by metabolic and neuroimmune pathways.

In PWS adults, it was reported that *B. animalis* subsp. *lactis* B94 supplementation improved GI motility and laxation, mitigating constipation and improving quality of life. Together, these studies underscore the potential of probiotic therapy to address multiple domains of PWS symptomatology, including metabolic control, GI function, and behavioral regulation (Alyousif et al., 2020).

Overall, the collective data support that targeted probiotic supplementation—mainly with *Bifidobacterium* and *Limosilactobacillus* strains—can beneficially modulate GM composition and host metabolism in patients with PWS. However, given the heterogeneity of clinical response, future large-scale and longitudinal studies integrating multi-omic and behavioral analyses are required to delineate the precise microbial and molecular mechanisms underlying these therapeutic effects.

In conclusion, despite encouraging outcomes, prebiotic, probiotic, and synbiotic interventions in NDDs face several methodological and translational challenges. Considerable heterogeneity exists among studies regarding microbial strains, dosages, treatment duration, and patient characteristics, often leading to variable or modest clinical effects. Moreover, the colonization capacity of administered microbes is typically transient, suggesting that sustained benefit may require repeated or combined interventions. In addition, mechanistic understanding remains incomplete, as most studies lack integrated multi-omic analyses linking microbial, metabolic, and neurophysiological outcomes. Safety and strain specificity must be carefully evaluated, particularly in pediatric and immunocompromised populations.

Nevertheless, these microbiota-directed interventions represent a promising frontier in precision neurogastroenterology, offering the potential to modulate neurobehavioral outcomes through gut microbial ecology. Future research should prioritize personalized psychobiotic therapies, guided by baseline microbiome profiling, and integrate longitudinal, systems-level approaches to elucidate causal mechanisms and optimize therapeutic efficacy across neurodivergent populations.

Notably, as the GM is strongly influenced by nutritional patterns, dietary modulation represents the next logical and complementary strategy to sustain and potentiate the benefits of microbial interventions—an aspect explored in the following section.

18.4.2 Dietary Interventions and Nutritional Modulation

Diet is one of the most powerful determinants of GM composition and function, shaping both metabolic and neuroimmune pathways along the GM–brain axis. Through its influence on microbial diversity, metabolite production, and intestinal barrier integrity, nutrition represents a keystone for maintaining or restoring eubiosis. Dietary interventions are more attractive in NDDs because they are non-invasive, modifiable, and capable of exerting systemic effects that extend beyond the GI tract to neural, metabolic, and immune domains.

In ASD, dietary modulation has been explored primarily through ketogenic, low-FODMAP, and micronutrient-based approaches, often aimed at alleviating GI symptoms and behavioral impairments. The ketogenic diet (KD), originally developed for epilepsy management, has been shown to induce substantial microbiome remodeling and systemic metabolic effects. In a recent study, KD induced shifts in GM composition, associated with changes in inflammatory cytokines and brain-related microRNAs, suggesting that microbial and host molecular responses are tightly intertwined (Allan et al. 2024). Similarly, it was described the case of an autistic child with epilepsy and metabolic dysregulation who experienced clinical and microbiota improvement under a combined ketogenic and low-FODMAP regimen, with increased abundance of *Bifidobacterium* and *Akkermansia* (Bertuccioli et al. 2022). Complementarily, in an experimental model the supplementation with dimethylglycine and B vitamins mitigated inflammation, improved mitochondrial function, and normalized microbial composition in ASD-like conditions—emphasizing the link between nutrient cofactors, microbiota, and neuroinflammatory control (Cimmino et al., 2025). Additional functional foods, such as malted rice amazake, have shown promise in improving

constipation and microbial diversity in individuals with severe NDDs (Kageyama et al., 2021), underscoring the role of fermented and prebiotic-rich diets in supporting eubiosis.

In ADHD, nutritional interventions targeting gut–brain communication and micronutrient balance have similarly been linked to microbial modulation. The MADDY RCT demonstrated that broad-spectrum micronutrient supplementation can alter the GM composition and metabolite profiles, with specific taxa shifts correlating with clinical improvements (Stevens et al., 2019; Robinette et al., 2024; Ast et al., 2025). Recent exploratory work further indicated that dietary patterns influence reactive aggression and attention regulation through microbiome–metabolome interactions (Jakobi et al., 2024). Moreover, Long et al. showed that integrating a low-lectin diet with standard ADHD treatment improved GM diversity and symptom control, suggesting that decreasing pro-inflammatory dietary components may restore microbial balance and ameliorate behavioral outcomes (Long et al., 2024). Collectively, these findings highlight that nutritional quality and micronutrient sufficiency play a role in modulating the microbial ecology and neurobehavioral phenotype in ADHD.

In PWS fiber-enriched diets have demonstrated marked benefits through microbiota-driven mechanisms. Recently it was reported that dietary fiber supplementation improved metabolic health, reduced hyperphagic behavior, and induced favorable shifts in SCFA-producing taxa, including *Faecalibacterium* and *Bifidobacterium* (Tan et al., 2025). Mechanistic studies further elucidated that a high-fiber diet reduces microbial virulence genes and inflammatory potential (Li et al., 2021a, 2021b) and promotes strain-level genetic adaptations in *Faecalibacterium prausnitzii* that enhance butyrate metabolism and host energy balance (Li et al., 2021c). Additionally, case studies have suggested that folate-producing *Bifidobacterium longum* may mediate some of the metabolic benefits associated with high-fiber diets (Xiang et al., 2021). These observations align with broader evidence showing that dietary modulation of the microbiota can alleviate features of both genetic and simple obesity in children (Zhang et al., 2015).

From a broader perspective, these findings collectively support the concept that nutritional interventions act as potent ecological GM modulators, capable of influencing host metabolism, immune regulation, and neural function. Whether through macronutrient composition (e.g., ketogenic or low-FODMAP diets), micronutrient repletion, or prebiotic-rich fiber intake, dietary strategies represent a promising adjunct to microbial-based therapies for improving quality of life and neurobehavioral outcomes in neurodivergent populations. However, several limitations must be acknowledged. Dietary interventions are inherently complex to standardize due to individual variability in nutrient absorption, baseline microbiota composition, and metabolic phenotype. Restrictive regimens such as ketogenic or low-FODMAP diets may carry nutritional risks, mainly in pediatric populations, if not carefully monitored. Moreover, causal links between diet-induced microbial shifts and behavioral improvement remain incompletely defined, emphasizing the need for integrated nutritional–microbiome–metabolome studies. Overall, personalization of dietary interventions—guided by microbiome profiling, metabolic biomarkers, and clinical context—will be essential to optimize efficacy and safety. In the broader framework of microbiota-based therapeutics, diet represents not only a modifiable lifestyle factor but also a sustainable, synergistic approach to reinforce the benefits of psychobiotics and other microbial modulation strategies.

18.4.3 Fecal Microbiota Transplantation

FMT represents the most direct approach to restoring intestinal eubiosis by transferring a screened and processed stool suspension from a healthy donor into the GI tract of a recipient. The procedure aims to reconstitute microbial diversity, metabolic capacity, and immune balance by reintroducing a complex microbial ecosystem. Traditionally, FMT is performed via colonoscopy, naso-enteric tube, enema, or oral capsules. In recent years, an optimized variant known as washed microbiota transplantation (WMT) has emerged. In WMT, donor feces are processed through a series of high-speed microfiltration and

centrifugation steps to isolate and purify the bacterial fraction while removing debris, viruses, fungi, and pro-inflammatory molecules. This approach improves safety, reproducibility, and patient tolerability, and allows for precise microbial dosing while maintaining therapeutic efficacy comparable to conventional FMT (Pan et al., 2022; Zhang et al., 2022; Zheng et al., 2025).

Among NDDs, ASD has been the most extensively investigated target for FMT-based interventions. The pioneering open-label study by Kang et al. demonstrated that microbiota transfer therapy improved both GI and behavioral symptoms in children with ASD, with effects persisting for two years after treatment (Kang et al., 2019). Subsequent clinical and translational studies have corroborated these findings, reporting enhanced microbial diversity and enrichment of *Bifidobacterium*, *Prevotella*, and *Faecalibacterium* following treatment, together with reductions in ASD symptom severity (Li et al., 2021; Li Y. et al., 2024; Wu et al., 2025). Controlled trials have shown biochemical and metabolic correlates of these improvements —such as decreased urinary 5-hydroxyindoleacetic acid (5-HIAA) levels, indicative of serotonin pathway normalization (Wang et al., 2024a,b), and modulation of fecal metabolites linked to neurotransmitter synthesis and inflammation (Liu et al., 2023).

Two studies demonstrated that repeated WMT cycles not only ameliorated GI dysfunction and constipation but additionally led to measurable improvements in sleep quality, social responsiveness, and repetitive behaviors (Pan et al. 2022; Zhang et al. 2022). More recently, it was confirmed in prospective and retrospective analyses that both single-donor and multidonor WMT produced comparable clinical benefits, suggesting that the restoration of key microbial taxa rather than donor identity may drive therapeutic response (Wu et al., 2025; Zheng et al., 2025). Finally, Chen et al. (2024) provided mechanistic insights by showing that donor–recipient microbial compatibility at the subspecies level strongly influences engraftment success and clinical outcomes.

Collectively, these studies underscore that microbiota-based reconstitution can partially reverse the dysbiosis and metabolic derangements associated with ASD, leading to improvements in both gut and neurobehavioral symptoms.

Evidence in other NDDs is still preliminary but promising. In ADHD, a case of a child who exhibited marked improvement in attentional and behavioral symptoms following FMT was reported, agreeing with a shift toward a more eubiotic gut microbial composition (Hooi et al., 2022). Similarly, in TS, early studies documented substantial symptom decrease and GM normalization after FMT, with improvements sustained during follow-up (Zhao et al., 2017, 2020). These results suggest a potential microbiota-mediated modulation of neurotransmitter pathways and neuroinflammatory tone, although controlled trials are needed to validate these findings. Despite encouraging outcomes, several limitations and challenges temper the clinical translation of FMT and WMT in NDDs. Heterogeneity in donor selection, preparation methods, and administration routes complicates the comparison and reproducibility of results. Long-term safety, mainly regarding the transfer of antibiotic resistance genes or opportunistic pathogens, remains a concern. Moreover, interindividual differences in host genetics, immune response, and baseline microbiome composition strongly influence engraftment dynamics and therapeutic success. Nevertheless, accumulating data point to the microbiota as a modifiable therapeutic target in neurodivergent populations. Future directions should focus on standardizing protocols, integrating multi-omics profiling, and developing next-generation microbiota therapeutics, including defined microbial consortia or precision-engineered WMT, to harness the full potential of microbiome restoration in improving both GI and neurobehavioral health.

18.5 Conclusions and Future Directions

NDDs represent a highly heterogeneous group of conditions arising from complex interactions among genetic, environmental, metabolic, and immunological factors. Within this multifactorial landscape, the

human microbiome has emerged as a relevant modulatory dimension that does not redefine neurodiversity in biological terms but enriches our understanding of how neurocognitive differences may develop and manifest across the lifespan. Across conditions such as ASD, ADHD, RTT, TS, DS, and PWS, converging evidence points to condition-specific yet partially overlapping patterns of GM alteration, including decreased microbial diversity, imbalance of key commensal taxa, altered microbial metabolites, impaired mucosal integrity, and immune dysregulation. Mechanistic studies support the view of the GM–brain axis as a dynamic interface through which metabolic, immune, endocrine, and neural signals can influence neurodevelopmental trajectories. However, current evidence suggests that dysbiosis is more likely to act as a modifier of neurodevelopmental variability rather than a primary determinant, with its impact shaped by critical developmental windows, host genetics, lifestyle, and environmental exposures. In this scenario, pervasive environmental pollutants—including air pollution, heavy metals, EDCs, and M/NPs—emerge as relevant, non-isolated contributors that can perturb microbiome–brain communication during sensitive periods of development, subtly biasing risk, resilience, and phenotypic expression without implying deterministic outcomes.

Future research should therefore adopt integrative, exposome-based and longitudinal approaches to clarify cumulative and timing-specific effects, while microbiome-targeted strategies grounded in nutrition, lifestyle, and environmental burden reduction may offer promising, albeit still preliminary, avenues to enhance developmental resilience. Overall, situating the microbiome within its broader biological and environmental context supports a more holistic, person-centered framework for understanding neurodevelopmental diversity and improving quality of life though respecting the inherent variability of human cognition.

References

Aarts E, Ederveen THA, Naaijen J, Zwiers MP, Boekhorst J, Timmerman HM, Smeekens SP, Netea MG, Buitelaar JK, Franke B, *et al*. Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One* 2017, 12, e0183509. ⏎

Aburto MR, Nagpal J, Majumdar A, O'Riordan KJ, Clarke G, Cryan JF. Microbiome, exposome, and cognitive development: The known unknowns. *Annual Review of Developmental Psychology* 2025, 7, 411–438. ⏎

Adams JB, Audhya T, Geis E, Gehn E, Fimbres V, Pollard EL, Mitchell J, Ingram J, Hellmers R, Laake D, *et al*. Comprehensive nutritional and dietary intervention for autism spectrum disorder—A randomized, controlled 12-month trial. *Nutrients* 2018, 10, 369. ⏎

Agirman G, Hsiao EY. SnapShot: The microbiota–gut–brain axis. *Cell* 2021, 1849, 2524–2524.e1. ⏎

Ahmed S, Elhefnawy AM, Azouz HG, Roshdy YS, Ashry MH, Ibrahim AE, *et al*. Study of the gut microbiome profile in children with autism spectrum disorder: A single tertiary hospital experience. *Journal of Molecular Neuroscience* 2020, 70, 887–896. ⏎

Allan NP, Yamamoto BY, Kunihiro BP, Nunokawa CKL Rubas NC, Wells RK, Umeda L, Phankitnirundorn K, Torres A, Peres R, Takahashi E, Maunakea AK, Ketogenic diet induced shifts in the gut microbiome associate with changes to inflammatory cytokines and brain-related miRNAs in children with autism spectrum disorder. *Nutrients* 2024, 16(10), 1401. ⏎

Alyousif Z, Miller JL, Auger J, Sandoval M, Piano A, Tompkins TA, Dahl WJ Microbiota profile and efficacy of probiotic supplementation on laxation in adults affected by Prader–Willi syndrome: A randomized double-blind crossover trial. *Molecular Genetics & Genomic Medicine* 2020, 12, e1535. ⏎

Alyousif Z, Miller JL, Sandoval MY, MacPherson CW, Nagulesapillai V, Dahl WJ. The effects of *Bifidobacterium animalis* ssp *lactis* B94 on gastrointestinal wellness in adults with Prader–Willi syndrome: Study protocol for a randomized controlled trial. *Trials* 2018, 19(1), 256.

Amat-Bou M, Garcia-Ribera S, Climent E, Piquer-Garcia I, Corripio R, Sanchez-Infantes D, Villalta L, Elias M, Jiménez-Chillarón JC, Chenoll E, Ramón D, Ibañez L, Ramon-Krauel M, Lerin C. Effects of *Bifidobacterium animalis* subsp *lactis* BPL1 supplementation in children and adolescents with Prader–Willi syndrome: A randomized crossover trial. *Nutrients* 2020, 12, 3123. ⏎

Arnold LE, Luna RA, Williams K, Chan J, Parker RA, Wu Q, *et al*. Probiotics for gastrointestinal symptoms and quality of life in autism: A placebo-controlled pilot trial. *Journal of Child and Adolescent Psychopharmacology* 2019, 29, 659–669. ⏎

Arteaga-Henríquez G, Ramos-Sayalero C, Ibañez-Jimenez P, Karina Rosales-Ortiz S, Kilencz T, Schiweck C, Schnorr I, Siegl A, Arias-Vasquez A, Bitter I, Fadeuilhe C, Ferrer M, Lavebratt C, Matura S, Reif A, Réthelyi JM, Richarte V, Rommelse N, Antoni Ramos-Quiroga J. Efficacy of a synbiotic in the management of adults with attention-deficit and hyperactivity disorder and/or borderline personality disorder and high levels of irritability: Results from a multicenter, randomized, placebo-controlled, "basket" trial. *Brain, Behavior, and Immunity* 2024, 120, 360–371. ⏎

Ast HK, Hammer M, Zhang S, Bruton A, Hatsu IE, Leung B, McClure R, Srikanth P, Farris Y, Norby-Adams L, Robinette LM, Arnold LE, Swann JR, Zhu J, Karstens L, Johnstone JM. Gut microbiome changes with micronutrient supplementation in children with attention-deficit/hyperactivity disorder: The MADDY study. *Gut Microbes* 2025, 1, 2463570. ⏎

Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, Sears MR, Mandhane PJ, Turvey SE, Subbarao P, Becker AB, Scott JA, Kozyrskyj AL, CHILD Study Investigators. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. *BJOG: An International Journal of Obstetrics and Gynaecology* 2016, 1236, 983–993. ⏎

Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Sears MR, Becker AB, Scott JA, Kozyrskyj AL. Infant gut microbiota and the hygiene hypothesis of allergic disease: Impact of household pets and siblings on microbiota composition and diversity. *Allergy, Asthma, and Clinical Immunology: Official Journal of the Canadian Society of Allergy and Clinical Immunology* 2013, 91, 15. ⏎

Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, *et al*. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host and Microbe* 2015, 175, 690–703. ⏎

Bao C, Wei M, Pan H, Wen M, Liu Z, Xu Y, Jiang H. A preliminary study for the clinical effect of one combinational physiotherapy and its potential influence on gut microbial composition in children with Tourette syndrome. *Frontiers in Nutrition* 2023, 10, 1184311. ⏎

Bertuccioli A, Cardinali M, Di Pierro F, Zonzini GB, Matera MR. Ketogenic and low FODMAP diet in therapeutic management of a young autistic patient with epilepsy and dysmetabolism poorly responsive to therapies: Clinical response and effects of intestinal microbiota. *International Journal of Molecular Sciences* 2022, 23(15), 8829. ⏎

Bhusri B, Sutheeworapong S, Kittichotirat W, Kusonmano K, Thammarongtham C, Lertampaiporn S, Prommeenate P, Praphanphoj V, Kittitharaphan W, Dulsawat S, Paenkaew P, Cheevadhanarak S. Characterization of gut microbiota on gender and age groups bias in Thai patients with autism spectrum disorder. *Scientific Reports* 2025, 15(1), 2587. ⏎

Biagi E, Candela M, Centanni M, Consolandi C, Rampelli S, Turroni S, Severgnini M, Peano C, Ghezzo A, Scurti M, *et al*. Gut microbiome in Down syndrome. *PLoS One* 2014, 9, e112023. ⏎

Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li HD, Lieber A, Wu F, Perez-Perez GI, Chen Y, Schweizer W, Zheng X, Contreras M, Dominguez-Bello MG, Blaser MJ. Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science Translational Medicine* 2016, 8343, 343ra82. ⏎

Bonham KS, Margolis ET, Fahur Bottino G, Sobrino AC, Patel F, McCann S, Zieff MR, Miles M, Herr D, Davel L, Bosco C, Khula South African Data Collection Team, Huttenhower C, Pini N, Alexander DC, Jones DK, Williams SCR, Amso D, Gladstone M, *et al*. Codevelopment of gut microbial metabolism and visual neural circuitry over human infancy. *mBio* 2025, 168, e0083525. ⏎

Borghi E, Borgo F, Severgnini M, Savini MN, Casiraghi MC, Vignoli, A. Rett syndrome: A focus on gut microbiota. *International Journal of Molecular Science* 2017, 18, 344. ⏎

Borrego-Ruiz A, Borrego J. Neurodevelopmental disorders associated with gut microbiome dysbiosis in children. *Children (Basel, Switzerland)* 2024, 117, 796. ⏎

Botha M, Gillespie-Lynch K. Come as you are: Examining autistic identity development and the neurodiversity movement through an intersectional lens. *Human Development* 2022, 662, 93–112. ⏎

Braun JM, Kalkbrenner AE, Calafat AM, Yolton K, Ye X, Dietrich KN, Lanphear BP. Impact of early-life bisphenol A exposure on behavior and executive function in children. *Pediatrics* 2011, 1285, 873–882. ⏎

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America* 2011, 10838, 16050–16055. ⏎

Brescia P, Rescigno M. The gut vascular barrier: A new player in the gut–liver–brain axis. *Trends in Molecular Medicine* 2021, 279, 844–855. ⏎

Cai S, Lin J, Li Z, Liu S, Feng Z, Zhang Y, Zhang Y, Huang J, Chen Q. Alterations in intestinal microbiota and metabolites in individuals with Down syndrome and their correlation with inflammation and behavior disorders in mice. *Frontiers in Microbiology* 2023, 14, 1016872. ⏎

Cainelli E, Bisiacchi P. Neurodevelopmental disorders: Past, present, and future. *Children (Basel, Switzerland)* 2022, 101, 31.

Caputi V, Hill L, Figueiredo M, Popov J, Hartung E, Margolis KG, Baskaran K, Joharapurkar P, Moshkovich M, Pai N. Functional contribution of the intestinal microbiome in autism spectrum disorder, attention deficit hyperactivity disorder, and Rett syndrome: A systematic review of pediatric and adult studies. *Frontiers in Neuroscience* 2024, 18, 1341656.

Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, Thompson AL, Geng X, Gilmore JH, Knickmeyer RC. Infant gut microbiome associated with cognitive development. *Biological Psychiatry* 2018, 832, 148–159. ⏎

Cassidy-Bushrow AE, Sitarik AR, Johnson CC, Johnson-Hooper TM, Kassem Z, Levin AM, Lynch SV, Ownby DR, Phillips JM, Yong GJ, *et al*. Early-life gut microbiota and attention deficit hyperactivity disorder in preadolescents. *Pediatric Research* 2023, 93, 2051–2060. ⏎

Chambers AP, Sandoval DA, Seeley RJ. Integration of satiety signals by the central nervous system. *Current Biology* 2013, 239, R379–R388. ⏎

Chang X, Zhang Y, Chen X, Li S, Mei H, Xiao H, Ma X, Liu Z, Li R. Gut microbiome and serum amino acid metabolome alterations in autism spectrum disorder. *Scientific Reports* 2024, 14, 4037. ⏎

Chen Q, Wu C, Xu J, Ye C, Chen X, Tian H, *et al*. Donor–recipient intermicrobial interactions impact transfer of subspecies and fecal microbiota transplantation outcome. *Cell Host and Microbe* 2024, 32, 349–65.e4. ⏎

Chen YC, Lin HY, Chien Y, Tung YH, Ni YH, Gau SS. Altered gut microbiota correlates with behavioral problems but not gastrointestinal symptoms in individuals with autism. *Brain, Behavior, and Immunity* 2022, 106, 161–178. ⏎

Chen Z, Shi K, Liu X, Dai Y, Liu Y, Zhang L, Du X, Zhu T, Yu J, Fang S, *et al*. Gut microbial profile is associated with the severity of social impairment and IQ performance in children with autism spectrum disorder. *Frontiers in Psychiatry* 2021, 12, 789864. ⏎

Chu DM, Meyer KM, Prince AL, Aagaard KM. Impact of maternal nutrition in pregnancy and lactation on offspring gut microbial composition and function. *Gut Microbes* 2016, 76, 459–470. ⏎

Cimmino F, Petrella L, Cristiano C, Cavaliere G, Penna E, Pizzella A, Pirozzi C, Fogliano C, Coretti L, Lembo F, Canani RB, Avallone B, Crispino M, Trinchese G, Mollica MP. Autism spectrum disorders and nutritional interventions: Dimethylglycine and B-vitamins effects on behaviour, inflammation, microbiota and mitochondria in liver and brain synapses. *Biomed Pharmacother* 2025, 191, 118477. ⏎

Contaldo M, Lucchese A, Romano A, Della Vella F, Di Stasio D, Serpico R, Petruzzi M, Oral microbiota features in subjects with Down syndrome and periodontal diseases: A systematic review. *International Journal of Molecular Sciences* 2021, 22(17), 9251. ⏎

Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E … Dinan TG. The Microbiota–gut–brain axis. *Physiological Reviews* 2019, 994, 1877–2013. ⏎

Dahl WJ, Auger J, Alyousif Z, Miller JL, Tompkins TA Adults with Prader–Willi syndrome exhibit a unique microbiota profile. *BMC Research Notes* 2021, 14(1), 51. ⏎

Dan Z, Mao X, Liu Q, Guo M, Zhuang Y, Liu Z, Chen K, Chen J, Xu R, Tang J, *et al*. Altered gut microbial profile is associated with abnormal metabolism activity of autism spectrum disorder. *Gut Microbes* 2020, 11, 1246–1267. ⏎

De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, *et al.* Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* 2013, 8, 1–18. ⏎

De Angelis M, Francavilla R, Piccolo M, De Giacomo A, Gobbetti M. Autism spectrum disorders and intestinal microbiota. *Gut Microbes* 2015, 6, 207–213. ⏎

De Lausnay M, Verhulst S, Boel L, Van Hoorenbeeck K. Lower airway microbiota in children with Down syndrome compared to controls with similar respiratory symptomatology. *Translational Pediatrics* 2021, 7, 1818–1824. ⏎

De Sales-Millán A, Reyes-Ferreira P, Aguirre-Garrido JF, Corral-Guillé I, Barrientos-Ríos R, Velázquez-Aragón JA. Comprehensive analysis of gut microbiota composition and functional metabolism in children with autism spectrum disorder and neurotypical children: Implications for sex-based differences and metabolic dysregulation. *International Journal of Molecular Science* 2024, 25, 6701. ⏎

Ding H, Yi X, Zhang X, Wang H, Liu H, Mou WW. Imbalance in the gut microbiota of children with autism spectrum disorders. *Frontiers in Cellular and Infection Microbiology* 2021, 11, 572752. ⏎

Dwyer P. The neurodiversity approaches: What are they and what do they mean for researchers? *Human Development* 2022, 662, 73–92. ⏎

Elhossiny RM, Elshahawy HH, Mohamed HM, Abdelmageed RI. Assessment of probiotic strain *Lactobacillus acidophilus* LB supplementation as adjunctive management of attention-deficit

hyperactivity disorder in children and adolescents: A randomized controlled clinical trial. *BMC Psychiatry* 2023, 23, 823. ⏎

Evans SF, Kobrosly RW, Barrett ES, Thurston SW, Calafat AM, Weiss B, Stahlhut R, Yolton K, Swan SH. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. *Neurotoxicology* 2014, 45, 91–99. ⏎

Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, Armanini F, Truong DT, Manara S, Zolfo M, Beghini F , Bertorelli R, De Sanctis V, Bariletti I, Canto R, Clementi R, Cologna M, Crifò T, Cusumano G, *et al*. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host and Microbe* 2018, 241, 133–145.e5. ⏎

Finegold SM Summanen PH, Downes J , Corbett K, Komoriya T. Detection of *Clostridium perfringens* toxin genes in the gut microbiota of autistic children. *Anaerobe* 2017, 45, 133–137. ⏎

Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, *et al*. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* 2010, 16, 444–453. ⏎

Finegold SM, Downes J, Summanen PH. Microbiology of regressive autism. *Anaerobe* 2012, 18, 260–262. ⏎

Fitzpatrick Z, Frazer G, Ferro A, Clare S, Bouladoux N, Ferdinand J, Tuong ZK, Negro-Demontel ML, Kumar N, Suchanek O, Tajsic T, Harcourt K, Scott K, Bashford-Rogers R, Helmy A, Reich DS, Belkaid Y, Lawley TD, McGavern DB, Clatworthy MR. Gut-educated IgA plasma cells defend the meningeal venous sinuses. *Nature* 2020, 587(7834), 472–476. ⏎

Garcia-Ribera S, Amat-Bou M, Climent E, Llobet M, Chenoll E, Corripio R, Ibáñez L, Ramon-Krauel M, Lerin C, Specific dietary components and gut microbiota composition are associated with obesity in children and adolescents with Prader–Willi syndrome. *Nutrients* 2020, 12, 1063. ⏎

Gasparrini AJ, Wang B, Sun X, Kennedy EA, Hernandez-Leyva A, Ndao IM, Tarr PI, Warner BB, Dantas G. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. *Nature Microbiology* 2019, 412, 2285–2297. ⏎

Gillberg C. The ESSENCE in child psychiatry: Early symptomatic syndromes eliciting neurodevelopmental clinical examinations. *Research in Developmental Disabilities* 2010, 316, 1543–1551. ⏎

Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecular characterisation of gastrointestinal microbiota of children with autism with and without gastrointestinal dysfunction and their neurotypical siblings. *Autism Research* 2012, 5, 419–427. ⏎

Gonzalez-Santana A, Diaz Heijtz R. Bacterial peptidoglycans from microbiota in neurodevelopment and behavior. *Trends in Molecular Medicine* 2020, 268, 729–743. ⏎

Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, *et al*. A prebiotic intervention study in children with autism spectrum disorders ASDs. *Microbiome* 2018, 6, 133. ⏎

Guidetti C, Salvini E, Viri M, Deidda F, Amoruso A, Visciglia A, *et al*. Randomized double-blind crossover study for evaluating a probiotic mixture on gastrointestinal and behavioral symptoms of autistic children. *Journal of Clinical Medicine* 2022, 11, 5263. ⏎

Gulledge L, Oyebode D, Donaldson JR. The influence of the microbiome on aggressive behavior: An insight into age-related aggression. *FEMS Microbiology Letters* 2023, 370. ⏎

Harusato A, Seo W, Abo H, Nakanishi Y, Nishikawa H, Itoh Y. Impact of particulate microplastics generated from polyethylene terephthalate on gut pathology and immune microenvironments. *iScience* 2023, 264, 106474. ⏎

He J, Gong X, Hu B, Lin L, Lin X, Gong W, Zhang B, Cao M, Xu Y, Xia R, Zheng G, Wu S, Zhang Y. Altered gut microbiota and short-chain fatty acids in Chinese children with constipated autism spectrum disorder. *Scientific Reports* 2023, 13(1), 19103. ⏎

Hoban AE, Stilling RM, Ryan FJ, Shanahan F, Dinan TG, Claesson MJ, Clarke G, Cryan JF. Regulation of prefrontal cortex myelination by the microbiota. *Translational Psychiatry* 2016, 64, e774. ⏎

Hoen AG, Madan JC, Li Z, Coker M, Lundgren SN, Morrison HG, Palys T, Jackson BP, Sogin ML, Cottingham KL, Karagas MR. Sex-specific associations of infants' gut microbiome with arsenic exposure in a US population. *Scientific Reports* 2018, 81, 12627. ⏎

Hooi SL, Dwiyanto J, Rasiti H, Toh KY, Wong RKM, Lee JWJ. A case report of improvement on ADHD symptoms after fecal microbiota transplantation with gut microbiome profiling pre- and post-procedure. *Current Medical Research and Opinion* 2022, 11, 1977–1982. ⏎

Hou Y, Deng F, Guo J, Lv L, Ouyang H, Wang X, Luo Y, Chen X, Wang F. Distinct lipids profiles and associations with clinical indicators and gut microbiota in children with Prader–Willi syndrome. *Endocrinology* 2023, 164, bqad084. ⏎

Hoyo-Alvarez E, Arechavala-Lopez P, Jiménez-García M, Solomando A, Alomar C, Sureda A, Moranta D, Deudero S. *Effects of Pollutants and Microplastics Ingestion on Oxidative Stress and Monoaminergic Activity of Seabream Brains*. Aquatic Toxicology, Amsterdam, Netherlands. 2022, 242106048, 106048. ⏎

Hu D, Wang Y-X, Chen W-J, Zhang Y, Li H-H, Xiong L, Zhu H-P, Chen H-Y, Peng S-X, Wan Z-H, Zhang Y, Du Y-K. Associations of phthalates exposure with attention deficits hyperactivity disorder: A case-control study among Chinese children. *Environmental Pollution Barking, Essex* 2017, 229, 375–385. ⏎

Huang M, Liu J, Liu K, Chen J, Wei Z, Feng Z, Wu Y, Fong M, Tian R,Wang B, *et al*. Microbiome-specific statistical modeling identifies interplay between gastrointestinal microbiome and neurobehavioral outcomes in patients with autism: A case control study. *Frontiers in Psychiatry* 2021, 12, 682454. ⏎

Hughes HK, Ashwood P. Anti-*Candida albicans* IgG antibodies in children with autism spectrum disorders. *Frontiers in Psychiatry* 2018, 9, 1–5. ⏎

Hursitoglu M, Kural A, Kuras S, Akdeniz E, Sezer S, Caypinar SS, Kazezoglu C, Yaprak B, Karandere F, Guven HZ. Maternal gut microbiota in, pregnancies resulting in Down syndrome newborns—A pilot study. *Acta Clinica Croatica* 2021, 4, 722–730. ⏎

Inoue R, Sakaue Y, Kawada Y, Tamaki R, Yasukawa Z, Ozeki M, *et al*. Dietary supplementation with partially hydrolyzed guar gum helps improve constipation and gut dysbiosis symptoms and behavioral irritability in children with autism spectrum disorder. *Journal of Clinical Biochemistry and Nutrition* 2019, 64, 217–223. ⏎

Jakobi B, Cimetti C, Mulder D, Vlaming P, Franke B, Hoogman M, Arias-Vasquez A, The role of diet and the gut microbiota in reactive aggression and adult ADHD—An exploratory analysis. *Nutrients* 2024, 16(14), 2174. ⏎

Jiang HY, Zhou YY, Zhou GL, Li YC, Yuan J, Li XH, Ruan B. Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. *Behavioural Brain Research* 2018, 347, 408–413. ⏎

Jin Y, Lu L, Tu W, Luo T, Fu Z. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. *Science of the Total Environment* 2019, 649, 308–317. ⏎

Kadiyska T, Vassilev D, Tourtourikov I, Ciurinskiene S, Madzharova D, Savcheva M, Stoynev N, Mileva-Popova R, Tafradjiiska-Hadjiolova R, Mitev V. Age-dependent gut microbiome dysbiosis in autism spectrum disorder and the role of key bacterial ratios. *Nutrients* 2025, 17(11), 1775. ⏎

Kageyama S, Inoue R, Hosomi K, Park J, Yumioka H, Suka T, Kurohashi Y, Teramoto K, Syauki AY, Doi M, Sakaue H, Mizuguchi K, Kunisawa J, Irie Y Effects of malted rice amazake on constipation symptoms and gut microbiota in children and adults with severe motor and intellectual disabilities: A pilot study. *Nutrients* 2021, 13(12), 4466. ⏎

Kaluç N, Bertorello S, Tombul OK, Baldi S, Nannini G, Bartolucci G, Niccolai E, Amedei A. Gut–lung microbiota dynamics in mice exposed to nanoplastics. *NanoImpact* 2024, 36100531, 100531. ⏎

Kang DW, Adams JB, Coleman DM, *et al*. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Scientific Reports* 2019, 9, 5821. ⏎

Kang D-W, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, *et al*. Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. *Anaerobe* 2018, 49, 121–131. ⏎

Kang D-W, Park JG, Ilhan ZE, Wallstrom G, LaBaer J, Adams JB, *et al*. Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLoS One* 2013, 8, 322. ⏎

Kantarcioglu AS, Kiraz N, Aydin A. Microbiota–gut–brain Axis: Yeast species isolated from stool samples of children with suspected or diagnosed autism spectrum disorders and in vitro susceptibility against nystatin and fluconazole. *Mycopathologia* 2016, 181, 1–7. ⏎

Kong XJ, Liu K, Zhuang P, Tian R, Liu S, Clairmont C, Lin X, Sherman H, Zhu J, Wang Y, Fong M, Li A, Wang BK, Wang J, Yu Z, Shen C, Cui X, Cao H, Du T, Wan G, Cao X, The effects of *Limosilactobacillus reuteri* LR-99 supplementation on body mass index social communication fine motor function and gut microbiome composition in individuals with Prader–Willi syndrome: A randomized double-blinded placebo-controlled trial. *Probiotics and Antimicrobial Proteins* 2021a, 6, 1508–1520.

Kong XJ, Wan G, Tian R, Liu S, Liu K, Clairmont C, Lin X, Zhang X, Sherman H, Zhu J, Wang Y, Fong M, Li A, Wang BK, Wang J, Liu J, Yu Z, Shen C, Cui X, Cao H, Du T, Cao X. The effects of probiotic supplementation on anthropometric growth and gut microbiota composition in patients with Prader–Willi syndrome: A randomized double-blinded placebo-controlled trial. *Frontiers in Nutrition* 2021b, 8, 587974. ⏎

Kong X J, Liu J, Liu K, Koh M, Sherman H, Liu S, *et al*. Probiotic and oxytocin combination therapy in patients with autism spectrum disorder: A randomized, double-blinded, placebo-controlled pilot trial. *Nutrients* 2021c, 13, 1552.

Kurokawa S, Nomura K, Sanada K, Miyaho K, Ishii C, Fukuda S, Iwamoto C, Naraoka M, Yoneda S, Imafuku M, Matsuzaki J, Saito Y, Mimura M, Kishimoto T. A comparative study on dietary diversity and gut microbial diversity in children with autism spectrum disorder attention-deficit hyperactivity disorder their neurotypical siblings and non-related neurotypical volunteers: A cross-sectional study. *Journal of Child Psychology and Psychiatry* 2024, 9, 1184–1195. ⏎

Kushak RI, Winter HS, Buie TM, Cox SB, Phillips CD, Ward NL Analysis of the duodenal microbiome in autistic individuals: Association with carbohydrate digestion. *Journal of Pediatric Gastroenterology and Nutrition* 2017, 64, e110–e116. ⏎

Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-Amram H, Koren O, Forsythe P, Bienenstock J. Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *Nature Communications* 2017, 81, 15062. ⏎

Lee AG, Kang S, Yoon HJ, Im S, Oh SJ, Pak YK. Polystyrene microplastics exacerbate systemic inflammation in high-fat diet-induced obesity. *International Journal of Molecular Sciences* 2023, 2415, 12421. ⏎

Lee SH, Lin WY, Cheng TJ. Microbiota-mediated metabolic perturbations in the gut and brain of mice after microplastic exposure. *Chemosphere* 2024, 350, 141026. ⏎

Lee WT, Wong LC. Alterations of the intestinal microbiota were correlated with the severity of Tourette syndrome in children. *Movement Disorders* 2018, 33, S275. ⏎

Legault M, Bourdon JN, Poirier P. From neurodiversity to neurodivergence: The role of epistemic and cognitive marginalization. *Synthese* 2021, 1995–6, 12843–12868. ⏎

Lei M, Menon R, Manteiga S, Alden N, Hunt C, Alaniz RC, Lee K, Jayaraman A. Environmental chemical diethylhexyl phthalate alters intestinal microbiota community structure and metabolite profile in mice. *mSystems* 2019, 4, e00724-19. ⏎

Li H, Wu G, Zhao L, Zhang M. Suppressed inflammation in obese children induced by a high-fiber diet is associated with the attenuation of gut microbial virulence factor genes. *Virulence* 2021a, 1, 1754–1770.

Li H, Zhao L, Zhang M. Gut microbial SNPs induced by high-fiber diet dominate nutrition metabolism and environmental adaption of *Faecalibacterium prausnitzii* in obese children. *Frontiers in Microbiology* 2021b, 12, 683714.

Li N, Chen H, Cheng Y, Xu F, Ruan G, Ying S, *et al*. Fecal microbiota transplantation relieves gastrointestinal and autism symptoms by improving the gut microbiota in an open-label study. *Frontiers in Cellular and Infection Microbiology* 2021c, 11, 759435. ⏎

Li R, Yang J, Saffari A, Jacobs J, Baek KI, Hough G, Larauche MH, Ma J, Jen N, Moussaoui N, Zhou B, Kang H, Reddy S, Henning SM, Campen MJ, Pisegna J, Li Z, Fogelman AM, Sioutas C, *et al*. Ambient ultrafine particle ingestion alters gut microbiota in association with increased atherogenic lipid metabolites. *Scientific Reports* 2017, 71, 42906. ⏎

Li Y, Hu W, Lin B, Ma T, Zhang Z, Hu W, *et al*. Omic characterizing and targeting gut dysbiosis in children with autism spectrum disorder: Symptom alleviation through combined probiotic and medium-carbohydrate diet intervention—A pilot study. *Gut Microbes* 2024, 16, 2434675. ⏎

Li Y, Xiao P, Cao R, Le J, Xu Q, Xiao F, *et al*. Effects and microbiota changes following oral lyophilized fecal microbiota transplantation in children with autism spectrum disorder. *Frontiers in Pediatrics* 2024, 12, 1369823. ⏎

Liang Y, Wan L, Wang G, Yan H, Zhang J, Liu X, Zhang Z, Zhu G, Yang G. Clinical study of *Limosilactobacillus reuteri* for the treatment of children with chronic tic disorders/Tourette syndrome: A mid-term efficacy evaluation. *Neurology and Therapy* 2025, 1, 279–290. ⏎

Lin C, Zeng T, Lu C, Li D, Liu Y, Li B, *et al*. Efficacy and safety of *Bacteroides fragilis* BF839 for pediatric autism spectrum disorder: A randomized clinical trial. *Frontiers in Nutrition* 2024, 11, 1447059. ⏎

Liu K, Kong XJ. Altered salivary microbiota following *Bifidobacterium animalis* subsp *lactis* BL-11 supplementation are associated with anthropometric growth and social behavior severity in individuals with Prader–Willi syndrome. *Probiotics and Antimicrobial Proteins* 2022, 14, 699–711. ⏎

Liu NH, Liu HQ, Zheng JY, Zhu ML, Wu LH, Pan HF, *et al*. Fresh washed microbiota transplantation alters gut microbiota metabolites to ameliorate sleeping disorder symptom of autistic children. *Journal of Microbiology* 2023, 61, 741–753. ⏎

Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, Yu Y, Mei L, Yang P, Tang Y, Zheng P. Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. *Scientific Reports* 2019, 9, 287. ⏎

Long L, Peng H, Chen X, Wang F, Long W, Cheng M, Ma J. The impact of integrating a low-lectin diet with traditional ADHD treatments on gut microbiota composition and symptom improvement in children—A cohort study. *Neuropsychiatric Disease and Treatment* 2024, 20, 535–549. ⏎

Luna RA, Oezguen N, Balderas M, Venkatachalam A, Runge JK, Versalovic J, *et al*. Distinct microbiome–neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum

disorder. *Cellular and Molecular Gastroenterology and Hepatology* 2017, 3, 218–230. ⏎

Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, Zhuo N, Wang H, Wang L, Wu D. Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: A study of 91 term infants. *Scientific Reports* 2020, 101, 15792. ⏎

Marklund U. Diversity, development and immunoregulation of enteric neurons. *Nature Reviews. Gastroenterology and Hepatology* 2022, 192, 85–86. ⏎

Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, Kubota H, Swinkels S, Sakai T, Oishi K, Kushiro A, Knol J. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PLoS One* 2016, 116, e0158498. ⏎

Mazzone L, Dooling SW, Volpe E, Uljarević M, Waters JL, Sabatini A, *et al*. Precision microbial intervention improves social behavior but not autism severity: A pilot double-blind randomized placebo-controlled trial. *Cell Host Microbe* 2024, 32, 106–116.e6. ⏎

McLennan H, Aberdein R, Saggers B, *et al*. Thirty years on from Sinclair: A scoping review of neurodiversity definitions and conceptualisations in empirical research. *Review Journal of Autism and Developmental Disorders* 2025. ⏎

Mehra A, Arora G, Sahni G, Kaur M, Singh H, Singh B, Kaur S. Gut microbiota and autism spectrum disorder: From pathogenesis to potential therapeutic perspectives. *Journal of Traditional and Complementary Medicine* 2023, 132, 135–149. ⏎

Mercado-Perez A, Beyder A. Gut feelings: Mechanosensing in the gastrointestinal tract. *Nature Reviews. Gastroenterology and Hepatology* 2022, 195, 283–296. ⏎

Mitsuhata C, Kado N, Hamada M, Nomura R, Kozai K. Characterization of the unique oral microbiome of children with Down syndrome. *Scientific Reports* 2022, 12(1), 14150. ⏎

Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxidants, Redox Signaling* 2014, 207, 1126–1167. ⏎

Monda V, Villano I, Messina A, Valenzano A, Esposito T, Moscatelli F, Viggiano A, Cibelli G, Chieffi S, Monda M, Messina G. Exercise modifies the gut microbiota with positive health effects. *Oxidative Medicine and Cellular Longevity* 2017, 20171, 3831972. ⏎

Morishima S, Takeda K, Greenan S, Maki Y. Salivary microbiome in children with Down syndrome: A case-control study. *BMC Oral Health* 2022, 22(1), 438. ⏎

Nam W, Kim H, Bae C, Kim J, Nam B, Lee Y, Kim J, Park S, Lee J, Sim J. *Lactobacillus* HY2782 and *Bifidobacterium* HY8002 decrease airway hyperresponsiveness induced by chronic PM2.5 inhalation in mice. *Journal of Medicinal Food* 2020, 236, 575–583. ⏎

Neurodivergent 2023, August 31. Cleveland Clinic. https://my.clevelandclinic.org/health/symptoms/23154-neurodivergent ⏎

Niu M, Li Q, Zhang J, Wen F, Dang W, Duan G, *et al*. Characterization of intestinal microbiota and probiotics treatment in children with autism spectrum disorders in China. *Frontiers in Neurology* 2019, 10, 1084. ⏎

Novau-Ferré N, Papandreou C, Rojo-Marticella M, Canals-Sans J, Bulló M. Gut microbiome differences in children with attention deficit hyperactivity disorder and autism spectrum disorder and effects of probiotic supplementation: A randomized controlled trial. *Research in Developmental Disability* 2025, 161, 105003. ⏎

Nunez H, Nieto PA. Mars RA. Ghavami M, Sew Hoy C, Sukhum K. Early life gut microbiome and its impact on childhood health and chronic conditions. *Gut Microbes* 2025, 171, 2463567. ⏎

Olsson LM, Poitou C, Tremaroli V, Coupaye M, Aron-Wisnewsky J, Bäckhed F, Clément K, Caesar R. Gut microbiota of obese subjects with Prader–Willi syndrome is linked to metabolic health. *Gut* 2020, 69,

1229–1238. ⏎

Ouyang L, Li Q, Rao S, Su R, Zhu Y, Du G, Xie J, Zhou F, Feng C, Fan G. Cognitive outcomes caused by low-level lead, cadmium, and mercury mixture exposure at distinct phases of brain development. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 2023, 175, 113707. ⏎

Pan ZY, Zhong HJ, Huang DN, Wu LH, He XX. Beneficial effects of repeated washed microbiota transplantation in children with autism. *Frontiers in Pediatrics* 2022, 10, 928785. ⏎

Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of Medical Microbiology* 2005, 54, 987–991. ⏎

Pärtty A, Kalliomäki, M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. *Pediatric Research* 2015, 77, 823–828. ⏎

Peng Y, Tan Q, Afhami S, Deehan EC, Liang S, Gantz M, Triador L, Madsen KL, Walter J, Tun HM, Haqq AM. The gut microbiota profile in children with Prader–Willi syndrome. *Genes (Basel)* 2020, 118, 904. ⏎

Perera F, Vishnevetsky J, Herbstman JB, Calafat AM, Xiong W, Rauh V, Wang S. Prenatal bisphenol a exposure and child behavior in an inner-city cohort. *Environmental Health Perspectives* 2012, 1208, 1190–1194. ⏎

Phan J, Calvo DC, Nair D, Jain S, Montagne T, Dietsche S, *et al*. Precision synbiotics increase gut microbiome diversity and improve gastrointestinal symptoms in a pilot open-label study for autism spectrum disorder. *mSystems* 2024, 9, e0050324. ⏎

Prehn-Kristensen A, Zimmermann A, Tittmann L, Lieb W, Schreiber S, Baving L, Fischer A. Reduced microbiome alpha diversity in young patients with ADHD. *PLoS One* 2018, 13, e0200728. ⏎

Pulikkan J, Maji A, Dhakan DB, Saxena R, Mohan B, Anto MM, *et al*. Gut microbial dysbiosis in Indian children with autism spectrum disorders. *Microbial Ecology* 2018, 76, 1102–1114. ⏎

Raghavan K, Dedeepiya VD, Ikewaki N, Sonoda T, Iwasaki M, Preethy S, *et al*. Improvement of behavioural pattern and alpha-synuclein levels in autism spectrum disorder after consumption of a beta-glucan food supplement in a randomised, parallel-group pilot clinical study. *BMJ Neurology* 2022, Open 4, e000203. ⏎

Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S, D'Amore E, Rinaldo D, Matta M, Giorgini E. Plasticenta: First evidence of microplastics in human placenta. *Environment International* 2021, 146, 106274. ⏎

Ramsperger AFRM, Bergamaschi E, Panizzolo M, Fenoglio I, Barbero F, Peters R, Undas A, Purker S, Giese B, Lalyer CR, Tamargo A, Moreno-Arribas MV, Grossart HP, Kühnel D, Dietrich J, Paulsen F, Afanou AK, Zienolddiny-Narui S, Eriksen Hammer S, Kringlen Ervik T, Graff P, Brinchmann BC, Nordby KC, Wallin H, Nassi M, Benetti F, Zanella M, Brehm J, Kress H, Löder MGJ, Laforsch C. Nano- and microplastics: A comprehensive review on their exposure routes, translocation, and fate in humans. *NanoImpact* 2023, 29, 100441. ⏎

Rao M, Gershon MD. The bowel and beyond: The enteric nervous system in neurological disorders. *Nature Reviews. Gastroenterology and Hepatology*. 2016, 139, 517–528. ⏎

Ratsika A, Codagnone MC, O'Mahony S, Stanton C, Cryan JF. Priming for life: Early life nutrition and the microbiota–gut–brain axis. *Nutrients* 2021, 132, 423. ⏎

Ren S, Wang X, Qin J, Mu Q, Ye S, Zhang Y, Yu W, Guo J. Altered gut microbiota correlates with cognitive impairment in Chinese children with Down's syndrome. *European Child & Adolescent*

Psychiatry 2022, 1, 189–202. ⏎

Rezazadegan M, Forootani B, Hoveyda Y, Rezazadegan N, Amani R. Major heavy metals and human gut microbiota composition: A systematic review with nutritional approach. *Journal of Health, Population, and Nutrition* 2025, 441, 21. ⏎

Robertson RC, Manges AR, Finlay BB, Prendergast AJ. The human microbiome and child growth—First 1000 days and beyond. *Trends in Microbiology*, 2019, 272, 131–147. ⏎

Robinette LM, Hatsu IE, Johnstone JM, Bruton AM, Leung BMY Arnold LE. Treatment response to supplemental nutrients for ADHD is independent of diet quality: The MADDY study RCT. *Nutritional Neuroscience* 2024, 4, 319–328. ⏎

Roen EL, Wang Y, Calafat AM, Wang S, Margolis A, Herbstman J, Hoepner LA, Rauh V, Perera FP, Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age. *Environmental Research* 2015, 142, 739–745. ⏎

Rosas HD, Morgan XC, Tao Y, Lai F, Mercaldo ND. Gut dysbiosis in Down syndrome: A potentially unexplored culprit for early Alzheimer's disease. *Alzheimer's and Dementia* 2025, 6, e70330. ⏎

Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M, *et al*. Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain, Behavior, and Immunity* 2018, 70, 354–368. ⏎

Roswall J, Olsson LM, Kovatcheva-Datchary P, Nilsson S, Tremaroli V, Simon M.-C, Kiilerich P, Akrami R, Krämer M, Uhlén M, Gummesson A, Kristiansen K, Dahlgren J, Bäckhed F. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host and Microbe* 2021, 295, 765–776.e3. ⏎

Ryan BC, Vandenbergh JG. Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Hormones and Behavior* 2006, 501, 85–93. ⏎

Saha S, Nair MR, Rai K, Shetty V, Anees TMM, Shetty AK, D'souza N. A novel sugar-free probiotic oral rinse influences oral *Candida albicans* in children with Down syndrome post complete oral rehabilitation: A pilot randomized clinical trial with 6-month follow-up. *Probiotics and Antimicrobial Proteins* 2025, 18(1):175–188. ⏎

Sangsefidi ZS, Sangsefidi ZS, Moharreri F, Heydari Yazdi AS, Eslami S, Emadzadeh B, Ghorani B, Sarabi-Jamab M, Farahmand A, Modiri Dovom A, Ghanaei A, Emadzadeh M. Effect of probiotics as an adjunctive therapy with Ritalin among ADHD children and adolescents: A triple-blind randomized controlled trial. *Nutritional Neuroscience* 2025, 4, 522–531. ⏎

Sanmarco LM, Wheeler MA, Gutiérrez-Vázquez C, Polonio CM, Linnerbauer M, Pinho-Ribeiro FA, Li Z, Giovannoni F, Batterman KV, Scalisi G, Zandee S, EJ, Heck ES, Alsuwailm M, Rosene DL, Becher B, Chiu IM, Prat A, Quintana FJ. Gut-licensed IFNγ^+ NK cells drive LAMP1$^+$TRAIL$^+$ anti-inflammatory astrocytes. *Nature* 2021, 590(7846), 473–479. ⏎

Santocchi E, Guiducci L, Prosperi M, Calderoni S, Gaggini M, Apicella F, *et al*. Effects of probiotic supplementation on gastrointestinal, sensory and core symptoms in autism spectrum disorders: A randomized controlled trial. *Frontiers in Psychiatry* 2020, 11, 550593. ⏎

Schnell A, Huang L, Singer M, Singaraju A, Barilla RM, Regan BML, Bollhagen A, Thakore PI, Dionne D, Delorey TM, Pawlak M, Meyer Zu Horste G, Rozenblatt-Rosen O, Irizarry RA, Regev A, Kuchroo VK. Stem-like intestinal Th17 cells give rise to pathogenic effector T cells during autoimmunity. *Cell* 2021, 18426, 6281–6298.e23. ⏎

Scribante A, Appendino P, Maiorani C, Fontanarosa P, Pascadopoli M, Cammisuli S, Azouz B, Buttiglieri S, Butera A. Home efficacy of a postbiotic-based gel compared with a gel without active ingredients for

the treatment of gingival inflammation in patients with Down syndrome: A randomized controlled study. *Dentistry Journal (Basel)* 2025, 132, 62. ⏎

Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-Feki HSA, Saad K, *et al*. The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. *Nutritional Neuroscience* 2018, 21, 676–681. ⏎

Shah PJ, Boilson M, Rutherford M, Prior S, Johnston L, Maciver D, Forsyth K. Neurodevelopmental disorders and neurodiversity: Definition of terms from Scotland's National Autism Implementation Team. *The British Journal of Psychiatry: The Journal of Mental Science* 2022, 2213, 577–579. ⏎

Shao Y, Forster S, Tsaliki EC, Vervier K, Strang A, Simpson N, *et al*. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* 2019, 574, 117–121. ⏎

Sharma P, Chauhan N, Gupta N. Diverse brains, shared humanity: Neurodiversity and neurodevelopmental disorders. *Journal of the Indian Association for Child and Adolescent Mental Health* 2025, 21(1), 7–9. ⏎

Sichel J. Improvements in gastrointestinal symptoms among children with autism spectrum disorder receiving the Delpro® probiotic and immunomodulator formulation. *Journal of Probiotics and Health* 2013, 1, 102. ⏎

Skott E, Yang LL, Stiernborg M, Söderström Å, Rüegg J, Schalling M, *et al*. Effects of a synbiotic on symptoms and daily functioning in attention deficit hyperactivity disorder: A double-blind randomized controlled trial. *Brain, Behavior and Immunity* 2020, 89, 9–19. ⏎

Soltysova M, Tomova A, Paulinyova M, Lakatosova S, Trebaticka J, Ostatnikova D. Gut microbiota in children and adolescents with autism, ADHD and anorexia nervosa, and its link to the levels of satiety hormones. *Neuroscience* 2025, 585, 394–407. ⏎

Son J, Zheng S, Rowehl L, Tian XM, Zhang Y, Zhu W, *et al*. Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the Simons simplex collection. *PLoS One* 2015, 10, e0137725. ⏎

Stenning A, Bertilsdotter-Rosqvist H. Neurodiversity studies: Mapping out possibilities of a new critical paradigm. *Disability and Society* 2021, 36(9), 1532–1537. ⏎

Stevens AJ, Purcell RV, Darling KA, Eggleston MJF, Kennedy MA, Rucklidge JJ. Human gut microbiome changes during a 10-week randomized control trial for micronutrient supplementation in children with attention deficit hyperactivity disorder. *Scientific Reports* 2019, 9, 10128. ⏎

Stewart C, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, *et al*. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 2018, 562, 583–588. ⏎

Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, *et al*. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* 2017, 5, 24. ⏎

Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, *et al*. Altered gut microbiota in Rett syndrome. *Microbiome* 2016, 4, 41. ⏎

Sun Y, Deng G, Fan J, Feng F, Ge Q, Song Y, *et al*. Associations of air PM2.5 level with gut microbiota in Chinese Han preschoolers and effect modification by oxytocin receptor gene polymorphism. *Environmental Research* 2022, 214(Pt 4), 114123. ⏎

Tan Q, Peng Y, Deehan EC, Vieira FT, Ho BWP, Afhami S, *et al*. Fiber intervention study in Prader–Willi syndrome: Insights into metabolic and microbiota shifts. *Journal of Clinical Endocrinology and Metabolism* 2025, 110(11), 3120–3132. ⏎

Thapa S, Venkatachalam A, Khan N, Naqvi M, Balderas M, Runge JK, *et al*. Assessment of the gut bacterial microbiome and metabolome of girls and women with Rett syndrome. *PLoS One* 2021, 16, e0251231. ⏎

Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, *et al*. Gastrointestinal microbiota in children with autism in Slovakia. *Physiology and Behavior* 2015, 138, 179–187. ⏎

Toscano de Oliveira M, Gonzalez MAS, Rosetto Boiate JV, Mesa V, Gonçalves DH, Pinto MGRS, *et al.* Altered gut microbiota composition and feeding behaviours in children with autism spectrum disorder: A comparative pilot study. *Annals of Human Biology* 2025, 52(1), 2535430. ⏎

Trezzi S, Scaccabarozzi G, Nossa R, Piazza C, Bianchi AR, Rosi E, *et al*. Behavioural, cognitive, and neurophysiological effects of a synbiotic supplementation in drug-naïve children with ADHD. *Clinical Nutrition ESPEN* 2025, 65, 408–417. ⏎

Valencia-Buitrago M, Oliveira-Carvalho RD, Cardoso V, Triviño-Valencia J, Salamanca-Duque LM, Martínez-Díaz V, *et al*. Metagenomic characterization of gut microbiota in children with autism spectrum disorder. *Pharmaceuticals (Basel)* 2025, 18(9), 1376. ⏎

Wan Y, Zhang L, Xu Z, Su Q, Leung TF, Chan D, *et al*. Alterations in fecal virome and bacteriome interplay in children with autism spectrum disorder. *Cell Reports Medicine* 2024, 5(2), 101409. ⏎

Wan L, Ge WR, Zhang S, Sun YL, Wang B, Yang G. Case-control study of the effects of gut microbiota composition on neurotransmitter metabolic pathways in children with attention deficit hyperactivity disorder. *Frontiers in Neuroscience* 2020, 14, 127. ⏎

Wang LJ, Tsai CS , Chou WJ, Kuo HC, Huang YH, Lee SY, *et al*. Add-on *Bifidobacterium bifidum* supplement in children with attention-deficit/hyperactivity disorder: A 12-week randomized double-blind placebo-controlled clinical trial. *Nutrients* 2024a, 16(14), 2260. ⏎

Wang H, Liu S, Xie L, Wang J. Gut microbiota signature in children with autism spectrum disorder who suffered from chronic gastrointestinal symptoms. *BMC Pediatrics* 2023a, 23, 476. ⏎

Wang L, Yu L, Liu Z, Che C, Wang Y, Zhao Y, *et al*. FMT intervention decreases urine 5-HIAA levels: A randomized double-blind controlled study. *Frontiers in Medicine* 2024b, 11, 1411089.

Wang LJ, Li SC, Li SW, Kuo HC, Lee SY, Huang LH, *et al*. Gut microbiota and plasma cytokine levels in patients with attention-deficit/hyperactivity disorder. *Translational Psychiatry* 2022, 12, 76. ⏎

Wang LJ, Yang CY, Chou WJ, Lee MJ, Chou MC, Kuo HC, *et al*. Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. *European Child and Adolescent Psychiatry* 2020a, 29, 287–297. ⏎

Wang Q, Yang Q, Liu X. The microbiota–gut–brain axis and neurodevelopmental disorders. *Protein and Cell* 2023b, 14(10), 762–775.

Wang W, Zhou J, Chen M, Huang X, Xie X, Li W, *et al*. Exposure to concentrated ambient PM2.5 alters the composition of gut microbiota in a murine model. *Particle and Fibre Toxicology* 2018, 15(1), 17. ⏎

Wang Y, Li N, Yang J, Zhao D, Chen B, Zhang G, *et al*. Probiotics and fructo-oligosaccharide intervention modulate the microbiota–gut brain axis to improve autism spectrum disorder, reducing also the hyper-serotonergic state and the dopamine metabolism disorder. *Pharmacology Research* 2020b, 157, 104784.

Wernroth ML, Peura S, Hedman A, Hetty S, Vicenzi S, Kennedy B, *et al*. Development of gut microbiota during the first 2 years of life. *Scientific Reports* 2022, 12(1), 9080. ⏎

Wild CP. Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology, Biomarkers and Prevention* 2005, 14(8), 1847–1850. ⏎

Wong LC, Hsu CJ, Wu YT, Chu HF, Lin JH, Wang HP, *et al*. Investigating the impact of probiotic on neurological outcomes in Rett syndrome: A randomized double-blind and placebo-controlled pilot study. *Autism* 2024, 28(9), 2267–2281. ⏎

Wu CC, Wong LC, Hsu CJ, Yang CW, Tsai YC, Cheng FS, *et al*. Randomized controlled trial of probiotic PS128 in children with Tourette syndrome. *Nutrients* 2021, 13(11), 3698. ⏎

Wu R, Teng X, Guo Y, Cai Y, Lv Y, Gao H, *et al*. Gut microbiota characteristics and therapeutic effects of fecal microbiota transplantation in children with autism spectrum disorder in central China: A combined cross-sectional and prospective study. *Frontiers in Pediatrics* 2025, 13, 1648471. ⏎

Wu T, Wang H, Lu W, Zhai Q, Zhang Q, Yuan W, *et al*. Potential of gut microbiome for detection of autism spectrum disorder. *Microbial Pathogenesis* 2020, 149, 104568. ⏎

Xi W, Gao X, Zhao H, Luo X, Li J, Tan X, *et al*. Depicting the composition of gut microbiota in children with tic disorders: An exploratory study. *Journal of Child Psychology and Psychiatry* 2021, 62, 1246–1254. ⏎

Xia Y, Wang P, Vincent J. Why we need neurodiversity in brain and behavioral sciences. *Brain-X* 2024, 22, e70. ⏎

Xiang B, Zhao L, Zhang M. Metagenome-scale metabolic network suggests folate produced by *Bifidobacterium longum* might contribute to high-fiber-diet-induced weight loss in a Prader–Willi syndrome child. *Microorganisms* 2021, 9(12), 2493. ⏎

Xiang F, Zhang M, Wei X, Chang J. Gut microbiota composition and phylogenetic analysis in autism spectrum disorder: A comparative study. *Frontiers in Psychiatry* 2025, 16, 1609638. ⏎

Xu X, Yang Y, Wang R, Wang Y, Ruan Q, Lu Y. Perinatal exposure to di-2-ethylhexyl phthalate affects anxiety- and depression-like behaviors in mice. *Chemosphere* 2015, 124, 22–31. ⏎

Yang LL, Stiernborg M, Skott E, Xu J, Wu Y, Landberg R, *et al*. Effects of a synbiotic on plasma immune activity markers and short-chain fatty acids in children and adults with ADHD: A randomized controlled trial. *Nutrients* 2023a, 15(5), 1293. ⏎

Yang G, Gong C, Zheng X, Hu F, Liu J, Wang T, *et al*. Early clues and molecular mechanism involved in neurodegenerative diseases induced in immature mice by combined exposure to polypropylene microplastics and DEHP. *Environmental Pollution* 2023b, 336, 122406.

Yang YN, Yang YC, Lin IH, Chen YY, Lin HY, Wu CY, *et al*. Phthalate exposure alters gut microbiota composition and IgM vaccine response in human newborns. *Food and Chemical Toxicology* 2019, 132, 110700. ⏎

Yang Z, Zhang Y, Ran S, Zhang J, Gao Y, Zhang Y, *et al*. Exposure to ambient air pollution over developmental stages induced neurodevelopmental impairment in mice offspring via microbiome–gut–brain axis. *Particle and Fibre Toxicology* 2025, 22(1), 20. ⏎

Yap CX, Henders A, Alvares GA, Wood DLA, Krause L, Tyson GW, *et al*. Autism-related dietary preferences mediate autism–gut microbiome associations. *Cell* 2021, 184, 5916–5931. ⏎

Yuan M, Wang Q, Lu Y, Xu P, Pan C, Zhang W, *et al*. Comparison of gut viral communities between autism spectrum disorder and healthy children. *Frontiers in Cellular and Infection Microbiology* 2025, 15, 1660970. ⏎

Zeng Q, Hu Y, Xie L, Zhang X, Huang Y, Ye J, *et al*. Gut microbiota diversity and composition in children with autism spectrum disorder: Associations with symptom severity. *PeerJ* 2025, 13, e19528. ⏎

Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. *Environmental Research* 2019, 171, 501–509. ⏎

Zhang C, Yin A, Li H, Wang R, Wu G, Shen J, *et al*. Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. *EBioMedicine* 2015, 2(8), 968–984. ⏎

Zhang M, Ma W, Zhang J, He Y, Wang J. Analysis of gut microbiota profiles and microbe–disease associations in children with autism spectrum disorders in China. *Scientific Report* 2018, 8, 1–9. ⏎

Zhang Y, Zhang J, Pan Z, He X. Effects of washed fecal bacteria transplantation in sleep quality, stool features and autism symptomatology: A Chinese preliminary observational study. *Neuropsychiatric*

Disease and Treatment 2022, 18, 1165–1173. ⏎

Zhao H, Shi Y, Luo X, Peng L, Yang Y, Zou L. The effect of fecal microbiota transplantation on a child with Tourette syndrome. *Case Reports in Medicine* 2017, 2017, 6165239. ⏎

Zhao HJ, Luo X, Shi YC, Li JF, Pan F, Ren RR, *et al*. The efficacy of fecal microbiota transplantation for children with Tourette syndrome: A preliminary study. *Frontiers in Psychiatry* 2020, 11, 554441. ⏎

Zheng YM, Ye MM, Zhang HY, Luo DP, Liu T, He XX, *et al*. Retrospective review: Single- and multidonor washed microbiota transplantation have equivalent efficacy in the treatment of autism. *Frontiers in Cellular and Infection Microbiology* 2025, 15, 1606417. ⏎

Zhong ML, Cai YQ, Tang YF, Dai YL, Jiang YH, Ni Y, *et al*. Gut microbiota: A potential cause of higher insulin sensitivity in children with Prader–Willi syndrome. *Journal of Endocrinological Investigation* 2024, 47(4), 1029–1036. ⏎

Zhou X, Guo Z, Ling Y, Teng W, Cui J, Yan Z, *et al*. Causal effect of air pollution on the risk of brain health and potential mediation by gut microbiota. *Ecotoxicology and Environmental Safety* 2024, 285, 117080. ⏎

Zou R, Xu F, Wang Y, Duan M, Guo M, Zhang Q, *et al*. Changes in the gut microbiota of children with autism spectrum disorder. *Autism Research* 2020, 13, 1614–1625. ⏎

Zuffa S, Schimmel P, Gonzalez-Santana A, Belzer C, Knol J, Bölte S, *et al*. Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. *Translational Psychiatry* 2023, 13, 257. ⏎

19

Circadian Rhythm and the Microbiome

Olivia Lutz and Sunil Thomas

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19.1 Introduction

The earth's consistent 24-hour rotation has driven the evolution of circadian oscillators across all forms of life, from prokaryotes to eukaryotes (Loudon, 2012). In response to predictable daily environmental fluctuations, animals exhibit rhythmic changes in behavior and physiology. These rhythms encompass well-recognized patterns such as the sleep-wake cycle, as well as subtler oscillations in neurological, metabolic, endocrine, cardiovascular, and immune processes. Circadian rhythmicity is generated endogenously by genetically encoded molecular clocks, in which interacting components produce self-sustaining cycles of gene expression and protein activity, resulting in oscillations with an approximately 24-hour periodicity (Patke et al., 2020). In mammals, the circadian system is hierarchically organized, with a central pacemaker regulating sleep-wake behavior and synchronizing peripheral tissue clocks through humoral and neuronal signaling pathways. At the cellular level, circadian clocks consist of interlocking core clock genes that generate tissue-specific transcriptional programs of clock-

controlled genes (CCGs). Through these CCGs, circadian clocks regulate a wide range of physiological functions, including metabolism, gastrointestinal transit time, mucus secretion, antimicrobial peptide production, immune defense, and intestinal barrier integrity (Heddes et al., 2022). By coordinating behavior, organ function, and cellular processes with environmental cycles, circadian rhythms optimize physiological performance and overall health. Disruption of this temporal organization increases susceptibility to numerous diseases, including metabolic syndrome, cancer, and cardiovascular disease (CVD) (Voigt et al., 2016).

Beyond host physiology, accumulating evidence demonstrates that the intestinal microbiome is also subject to circadian regulation. Microbial rhythmicity arises through both intrinsic microbial oscillatory mechanisms and rhythmic host-derived cues, particularly feeding behavior. Dietary composition and, critically, the timing of food intake strongly influence microbial community structure and metabolic activity. Disruption of feeding rhythms can therefore alter microbial oscillations, with significant downstream consequences for host immune function and metabolic homeostasis (Voigt et al., 2016).

The recognition that gut microbial communities exhibit robust daily rhythms in both composition and function has fundamentally reshaped our understanding of host-microbe symbiosis. Once considered a relatively static ecosystem, the gut microbiome is now understood to be a highly dynamic and temporally organized system that both responds to and influences host circadian physiology. A landmark study by Thaiss et al. in 2014 provided the first compelling evidence of this relationship, demonstrating that approximately 15% of bacterial operational taxonomic units (OTUs) display time-of-day-dependent oscillations in abundance. Notably, members of the phyla *Bacteroidetes* and *Firmicutes* exhibited antiphasic rhythmicity across the diurnal cycle, indicating a structured and coordinated microbial temporal program. Importantly, these oscillations were abolished in mice lacking functional circadian clocks, establishing host circadian machinery as a primary driver of microbial rhythmicity (Thaiss et al., 2014).

These findings revealed that two biological systems long studied independently—the circadian timing system and the gut microbiome—are deeply interconnected and mutually regulatory. The circadian clock coordinates temporal organization across multiple biological scales, from molecular oscillations in gene transcription to organism-wide behaviors, such as feeding, activity, and sleep. In turn, the gut microbiome acts as both an effector and modulator of circadian signaling through the rhythmic production of metabolites and microbial-associated molecular patterns that influence host metabolism, immunity, and intracellular signaling pathways. Through this bidirectional communication, circadian-microbiome coordination supports metabolic efficiency, immune surveillance, and tissue homeostasis (Banno et al., 2018).

Modern lifestyles pose unprecedented challenges to the integrity of circadian-microbiome coordination. Shift work, affecting approximately 15–20% of the workforce in industrialized countries, represents one of the most potent sources of chronic circadian disruption and is strongly associated with metabolic syndrome, type 2 diabetes, and CVD (Vyas et al., 2012). Sleep disorders further exacerbate this burden; projections estimate that obstructive sleep apnea will affect nearly 77 million US adults by 2050, representing a 35% relative increase from 2020 levels (Boers et al., 2025; Senaratna et al., 2017). Concurrently, widespread adoption of Western dietary patterns—characterized by high fat and refined sugar intake and low dietary fiber—has markedly reduced microbial diversity and promoted dysbiotic community configurations. The convergence of circadian misalignment and diet-induced microbial disruption produces synergistic effects that amplify metabolic and inflammatory dysfunction across the lifespan.

The pathophysiological consequences of impaired circadian-microbiome communication extend across nearly every organ system. Disruptions in one or both systems have been linked to obesity, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), CVD, inflammatory bowel disease (IBD), cancer, and neuropsychiatric disorders. Increasing evidence suggests these associations are mechanistically interconnected, with circadian

dysregulation and microbial imbalance reinforcing one another through shared metabolic, immune, and inflammatory pathways (Bautista and López-Cortés, 2025). Importantly, this relationship evolves across the lifespan. Early life represents a critical window during which circadian rhythms and microbial communities undergo coordinated maturation, shaping long-term metabolic and immune trajectories. In contrast, aging is characterized by an attenuation of circadian amplitude alongside shifts toward pro-inflammatory and less diverse microbial profiles, changes that may synergistically accelerate biological aging, cognitive decline, and disease susceptibility (Cobarro et al., 2025).

Understanding the mechanisms governing circadian-microbiome interactions offers significant opportunities for therapeutic intervention. Behavioral strategies, such as time-restricted feeding (TRF), can restore microbial rhythmicity and improve metabolic outcomes even under obesogenic dietary conditions. Physical activity functions as a potent *Zeitgeber* for peripheral circadian clocks while simultaneously promoting beneficial shifts in gut microbial composition and function (Du et al., 2025). In addition, microbiome-targeted approaches—including probiotics, prebiotics, and dietary fiber supplementation—may influence circadian function through metabolite-mediated signaling pathways. Pharmacologic and hormonal interventions, such as melatonin supplementation, further underscore the therapeutic potential of targeting this axis.

This review synthesizes current knowledge on bidirectional circadian-microbiome communication from early development through aging. We examine underlying molecular and cellular mechanisms, explore links to disease pathogenesis—including cancer and neurodegeneration—and evaluate emerging therapeutic strategies aimed at restoring temporal and microbial homeostasis within this fundamental axis of physiological regulation.

19.2 The Circadian–Microbiome Axis in Early Life

The infant is born with a weak and immature sleep-wake rhythm that is closely coupled to the maternal circadian system. After birth, the newborn's circadian rhythm must develop independently. This process is initiated by environmental light cues: light enters the eye, is detected by the retina, and the signal is transmitted via the retinohypothalamic tract to the suprachiasmatic nucleus (SCN). The SCN functions as the central circadian clock of the brain, coordinating rhythmic signals to peripheral tissues and organ systems. Through this central regulation, circadian rhythms govern melatonin and cortisol secretion, body temperature, motor activity, blood pressure, digestion, and the consolidation of sleep. These components of circadian organization emerge progressively during early life (Brooks and Canal, 2013).

Postnatal maturation of circadian rhythms follows a defined temporal sequence. A circadian rhythm of cortisol becomes detectable at approximately 8 weeks of age, followed by the emergence of melatonin secretion and improvements in sleep efficiency around 9 weeks. Rhythms in core body temperature and the expression of circadian clock genes typically appear by about 11 weeks of age (Joseph et al., 2015). Together, these milestones reflect the gradual establishment of a functional endogenous circadian system in the newborn.

The development of circadian rhythms occurs in parallel with the maturation of the gut microbiome, beginning during critical developmental windows in early life. During the first year, both systems undergo coordinated and interdependent development, representing a foundational period in which environmental inputs shape circadian timing and microbial colonization with long-term consequences for health. Recent studies demonstrate that circadian maturation in infancy is closely associated with gut microbiota composition, highlighting a critical window during which the circadian system and microbiome coevolve (Mühlematter et al., 2025; Heppner et al., 2024).

Gut bacteria in infants exhibit circadian rhythmicity, with cycles of activity and quiescence influenced by environmental factors, such as light exposure and feeding schedules. This interaction is bidirectional: the host

circadian clock regulates microbial rhythms, while microbial signals feedback to influence host circadian function (Heppner et al., 2024; Voigt et al., 2016). Longitudinal studies reveal that increasing gut bacterial diversity—an indicator of microbiome maturation—is associated with the development of behavioral sleep patterns, including nighttime sleep preference, as well as neurophysiological markers of sleep homeostasis, such as slow-wave activity (Schoch et al., 2022; Wang et al., 2022b).

The functional significance of this synchronized development extends beyond sleep regulation to broader aspects of neurodevelopment. Sleep quality, timing, and duration in infancy are closely linked to executive function, neural connectivity, synaptic stability, and overall cognitive maturation (Kurth et al., 2015; LeBourgeois et al., 2013). Disruptions during this sensitive developmental period may therefore have cascading effects across multiple domains of brain and behavioral development.

Emerging evidence indicates that the long-term consequences of disrupted circadian-microbiome synchronization extend well beyond infancy. Exposure to artificial light, irregular feeding schedules, and other modern environmental stressors during early life may predispose individuals to metabolic dysfunction, immune dysregulation, and neurocognitive impairments later in life. These findings underscore the importance of supporting circadian-microbiome health beginning in prenatal care and continuing through pediatric practice, with careful attention to sleep hygiene, feeding timing, and light exposure during sensitive developmental periods (Wu et al., 2026).

Maternal circadian health plays a critical role in shaping neonatal outcomes. Maternal circadian rhythm disruption has been shown to exacerbate inflammatory disorders in offspring, including necrotizing enterocolitis and neonatal sepsis, through reduced maternal production of docosahexaenoic acid (DHA). Notably, perinatal DHA supplementation can mitigate neonatal inflammation, highlighting a potential protective intervention during this vulnerable period (Cui et al., 2024).

Breast milk further contributes to circadian entrainment and microbiome development through pronounced diurnal variation in its bioactive

components. Melatonin concentrations peak at night and are undetectable during the day, while cortisol exhibits a morning peak and evening nadir. Breastfed infants achieve consolidated circadian rest-activity rhythms significantly earlier than mixed-fed infants. In addition, human milk oligosaccharides (HMOs), present at concentrations of 5–15 g/L in mature breast milk, selectively promote the growth of *Bifidobacterium* species. Meta-analyses demonstrate substantial mother-to-infant transmission of these bacteria, with specific strains persisting in the infant gut for up to 12 months (Häusler et.. al., 2024).

Remarkably, infant circadian rhythmicity involving the gut microbiome emerges very early in life. Heppner et al. (2024) identified circadian oscillations in the infant gut microbiome as early as 2 weeks after birth, with rhythms becoming more robust over time. Importantly, these bacteria retain rhythmicity when cultured ex vivo, indicating the presence of intrinsic bacterial circadian oscillators independent of the host environment.

Modern environmental conditions pose significant challenges to normal circadian-microbiome development. The high prevalence of infant sleep problems may reflect misalignment between environmental cues and endogenous biological rhythms during prenatal and postnatal development (Wong et al., 2022; Jenni and LeBourgeois, 2006). Human fetuses and infants increasingly develop in environments saturated with artificial light, yet the acute and long-term developmental consequences of this circadian disruption remain poorly understood (Wong et al., 2022; Kok et al., 2024).

In later childhood, circadian misalignment continues to affect sleep health. When children attempt to sleep at inappropriate internal circadian times, they experience longer sleep latencies, reduced sleep efficiency, and shorter total sleep duration. Consistently placing children in bed too early relative to their circadian phase may induce conditioned arousal responses, potentially contributing to the development of insomnia (LeBourgeois et al., 2013). These issues have substantial public health implications: approximately 24% of children aged 5–15 years in Australia sleep less than 9 hours per night (Olds et al., 2010; Evans-Whipp and Gasser, 2019), and

short sleep duration is a strong predictor of obesity, independent of diet and physical activity (Miller et al., 2018; Cappuccio et al., 2008).

19.3 Architecture of the Mammalian Circadian System

The mammalian circadian system is organized as a hierarchical, multi-oscillator network designed to coordinate physiology and behavior within the 24-hour day. At the apex of this system lies the central pacemaker located in the SCN of the anterior hypothalamus, which synchronizes a constellation of peripheral clocks distributed throughout nearly all tissues and organ systems. This central–peripheral architecture allows organisms to integrate environmental time cues with internal metabolic and physiological demands, ensuring temporal coherence across the body.

The SCN itself is a highly specialized bilateral structure situated above the optic chiasm. It contains approximately 20,000 neurons in rodents and an estimated 50,000–100,000 neurons in humans (van den Pol, 1980). These neurons are arranged into two major functional subdivisions: a ventrolateral "core" region and a dorsomedial "shell" region. The core primarily receives photic input and is critical for entrainment to the external light-dark cycle, whereas the shell is more involved in maintaining robust endogenous rhythmicity and distributing timing signals to downstream targets. Individual SCN neurons are intrinsically rhythmic, generating near-24-hour oscillations in membrane excitability, intracellular calcium levels, gene expression, and metabolic activity. Remarkably, these rhythms persist even when SCN neurons are isolated in vitro, underscoring the cell-autonomous nature of the circadian clock.

Light information reaches the SCN directly through the retinohypothalamic tract, which originates from a specialized subset of retinal neurons known as intrinsically photosensitive retinal ganglion cells (ipRGCs). These cells express the photopigment melanopsin, which is maximally sensitive to short-wavelength blue light at approximately 480 nm (Bailes and Lucas, 2013; Berson et al., 2002; Hattar et al., 2002). Unlike

rods and cones, which support image-forming vision, melanopsin-expressing ipRGCs are dedicated to non-image-forming visual functions, including circadian entrainment and pupillary reflexes. Light exposure resets the circadian clock according to a well-characterized phase response curve: light during the early subjective night induces phase delays, whereas light exposure during the late subjective night produces phase advances. This mechanism enables precise daily alignment of the internal clock with the external environment.

Beyond the SCN, peripheral circadian clocks are present in virtually all metabolically active tissues, including the liver, adipose tissue, skeletal muscle, pancreas, heart, kidney, and gastrointestinal tract. These peripheral oscillators are built from the same core molecular clock machinery as the SCN but differ in their primary entraining cues. Whereas the SCN is predominantly synchronized by light, peripheral clocks are highly sensitive to non-photic signals, such as feedingb-fasting cycles, fluctuations in body temperature, autonomic nervous system activity, and hormonal cues, particularly glucocorticoids and insulin. Cellular metabolic state, reflected in factors such as nicotinamide adenine dinucleotide (NAD+/NADH) ratios, also plays a critical role in modulating peripheral clock timing and amplitude.

Among peripheral clocks, the intestinal epithelium occupies a unique and central position in circadian organization due to its constant exposure to environmental inputs, including nutrients and microbes. The gut exhibits robust circadian rhythms that regulate CCGs involved in nutrient transport, epithelial barrier integrity, immune surveillance, and mucus and antimicrobial peptide secretion (Hunter and Bechtold, 2025). These rhythmic processes not only optimize digestive efficiency across the day-night cycle but also create temporal niches that shape microbial activity and composition. As a result, the intestinal clock serves as a key interface linking host circadian biology with the gut microbiome, integrating metabolic, immune, and environmental signals to maintain systemic homeostasis.

Together, this layered architecture—comprising a light-entrained central pacemaker and metabolically sensitive peripheral clocks—allows the mammalian circadian system to achieve both stability and flexibility. The SCN provides a unifying temporal reference, while peripheral oscillators fine-tune local physiology in response to tissue-specific demands, enabling adaptive responses to changing environmental and metabolic conditions.

19.4 The Transcription-Translation Feedback Loop

At the molecular level, circadian rhythmicity is generated by a set of interlocking transcription-translation feedback loops (TTFLs) that produce self-sustaining oscillations with an approximately 24-hour period. These loops are cell-autonomous and conserved across mammalian tissues, forming the fundamental timing mechanism that underlies circadian control of physiology and behavior. The core oscillator is organized into positive and negative regulatory limbs that interact in a precisely timed sequence of gene transcription, protein synthesis, post-translational modification, and protein degradation (Liu et al., 2024b).

The positive limb of the core loop is driven by the transcription factors Circadian Locomotor Output Cycles Kaput (CLOCK) and Brain and Muscle ARNT-Like Protein 1 (BMAL1). During the subjective day, CLOCK and BMAL1 heterodimerize and bind to E-box enhancer elements (CACGTG) located in the promoters of numerous CCGs. Among their most important targets are the *Period* (*Per1, Per2*, and *Per3*) and *Cryptochrome* (*Cry1* and *Cry2*) genes, which together constitute the negative limb of the circadian feedback loop (Albrecht, 2020; Bellengier et al., 2022; Xiang et al., 2021). Activation of these genes initiates molecular events that ultimately lead to the repression of their own transcription, establishing rhythmic gene expression.

CLOCK plays a dual role as both a transcription factor and an epigenetic regulator. It possesses intrinsic histone acetyltransferase activity, which acetylates histones and facilitates chromatin remodeling at clock gene

promoters, thereby enhancing transcriptional accessibility (Doi et al., 2006; Hirayama et al., 2007). As transcription proceeds, PER and CRY proteins accumulate in the cytoplasm during the mid-to-late subjective day. Their abundance and timing are finely tuned by extensive post-translational modifications, particularly phosphorylation by casein kinase 1δ/ε and other kinases, which regulate protein stability, subcellular localization, and degradation rates.

During the late subjective day and early subjective night, PER and CRY proteins form multimeric complexes that translocate into the nucleus. Once inside the nucleus, these PER–CRY complexes interact directly with the CLOCK:BMAL1 heterodimer, inhibiting its transcriptional activity and thereby repressing the expression of *Per, Cry*, and other CCGs. This repression constitutes the negative feedback phase of the cycle. Over time, PER and CRY proteins are targeted for ubiquitination and proteasomal degradation, leading to a gradual decline in their levels. As repression is relieved, CLOCK:BMAL1 activity is restored, allowing the next round of transcriptional activation to begin and completing one circadian cycle.

Stability and precision of the core oscillator are further enhanced by a second interlocking feedback loop involving nuclear receptors of the REV-ERB and Receptor Tyrosine Kinase-Like Orphan Receptor (ROR) families. REV-ERBα and REV-ERBβ act as transcriptional repressors, whereas RORα, RORβ, and RORγ function as transcriptional activators. These factors compete for binding to ROR response elements (ROREs) within the promoter region of the *Bmal1* gene. Rhythmic expression of REV-ERB and ROR proteins generates oscillations in *Bmal1* transcription, reinforcing the amplitude and robustness of the core TTFL. This auxiliary loop serves as a stabilizing mechanism that buffers the circadian clock against environmental and metabolic perturbations.

Importantly, the REV-ERB/ROR regulatory axis provides a direct molecular link between circadian timing and cellular metabolism. REV-ERB proteins integrate signals related to heme availability and cellular redox state, while RORs respond to lipid-derived ligands, enabling the clock to sense and respond to metabolic cues. Through their downstream

targets, these nuclear receptors influence lipid metabolism, glucose homeostasis, mitochondrial biogenesis, and oxidative metabolism, thereby aligning metabolic processes with the time of day (Cho et al., 2012; Kojetin and Burris, 2014; Duez and Staels, 2009).

These interconnected TTFLs form a resilient and adaptable molecular clock. By coupling gene regulation, epigenetic modification, protein turnover, and metabolic sensing, the TTFL architecture enables circadian systems to generate precise daily rhythms while remaining responsive to environmental and physiological signals.

19.5 The Gut Microbiome: Composition and Temporal Dynamics

19.5.1 Community Structure and Metabolic Functions

The human gastrointestinal tract hosts a highly complex and densely populated microbial ecosystem that plays a central role in host metabolism, immunity, and physiological regulation. This community comprises trillions of microorganisms, including bacteria, archaea, viruses, and fungi, with bacteria representing the most extensively characterized component (Das, 2022). At the phylum level, the bacterial microbiome is dominated by *Bacteroidetes* and *Firmicutes*, which together account for approximately 80–90% of total bacterial abundance. Other important but less abundant phyla include *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, each contributing distinct metabolic and immunomodulatory functions that enhance overall ecosystem resilience and functional redundancy.

Microbial density and composition vary markedly along the length of the gastrointestinal tract, reflecting gradients in pH, oxygen tension, nutrient availability, bile acids, and host immune activity. The stomach harbors relatively sparse microbial populations, typically ranging from 10^3 to 104 cells per gram, due to its acidic environment and rapid transit time. Microbial density increases through the small intestine and reaches its peak

in the colon, where bacterial concentrations exceed 10^{11} cells per gram of luminal content. This extraordinary density makes the colonic microbiome one of the most concentrated microbial ecosystems on Earth, rivaling even the most densely populated natural environments. The colon thus serves as the primary site of microbial metabolic activity and host-microbe interactions (Sekirov et al., 2010).

A defining metabolic function of the colonic microbiome is the fermentation of indigestible dietary components, particularly complex carbohydrates and dietary fibers that escape digestion in the upper gastrointestinal tract. Through coordinated enzymatic activity, gut bacteria convert these substrates into short-chain fatty acids (SCFAs), with acetate, propionate, and butyrate as the principal end products. Distinct microbial taxa specialize in the production of specific SCFAs: butyrate is predominantly produced by members of the *Lachnospiraceae* and *Ruminococcaceae* families; propionate is largely generated by *Bacteroides* species; and acetate is produced by a wide range of bacterial taxa. This metabolic specialization supports cross-feeding interactions, in which metabolic byproducts of one group serve as substrates for another, enhancing ecosystem stability.

Beyond serving as metabolic waste products, SCFAs are key energy sources and signaling molecules for the host. Butyrate alone supplies approximately 70% of the energy requirements of colonocytes, supporting epithelial integrity and barrier function. Acetate and propionate enter the circulation and influence peripheral tissues, contributing to hepatic gluconeogenesis, lipid metabolism, and appetite regulation. Importantly, SCFAs also act as signaling molecules through G-protein coupled receptors such as GPR41 and GPR43, as well as through histone deacetylase inhibition, thereby modulating immune responses, inflammation, and gene expression across multiple organ systems (Mann et al., 2024; LaBouyer et al., 2022; Blaak et al., 2020).

In addition to carbohydrate fermentation, the gut microbiome plays a critical role in bile acid metabolism. Primary bile acids synthesized in the liver are secreted into the intestine, where they are enzymatically modified

by gut bacteria into secondary bile acids, including deoxycholic acid and lithocholic acid. These secondary bile acids function as potent signaling molecules that activate nuclear receptors, such as the farnesoid X receptor (FXR) and membrane-bound G-protein-coupled receptors, like TGR5. Through these pathways, bile acid signaling influences lipid absorption, glucose metabolism, energy expenditure, and inflammatory tone, further integrating microbial activity with host metabolic regulation.

Gut microbes also extensively metabolize the essential amino acid tryptophan through multiple biochemical pathways. These pathways yield a range of bioactive indole derivatives that act as ligands for the aryl hydrocarbon receptor (AhR). Activation of AhR signaling plays a pivotal role in maintaining intestinal barrier integrity, regulating mucosal immune responses, and shaping host-microbe tolerance. Emerging evidence suggests that tryptophan-derived microbial metabolites may also interact with circadian regulatory pathways, linking microbial metabolic activity to host temporal physiology (Yan et al., 2023; Han et al., 2023).

Collectively, the community structure and metabolic functions of the gut microbiome extend far beyond simple digestion. Through the production of SCFAs, transformation of bile acids, and generation of signaling metabolites, the microbiome acts as a dynamic biochemical organ that communicates with host metabolic, immune, and circadian systems, exerting profound influence on health and disease.

19.5.2 Discovery of Microbiome Diurnal Rhythmicity

The recognition that the gut microbiome exhibits diurnal rhythmicity emerged from advances in high-throughput sequencing and systems-level analytical approaches. Early studies employing 16S rRNA gene sequencing demonstrated that the composition of the intestinal microbiota is not static but instead fluctuates predictably across the 24-hour day. Approximately 15–20% of identified OTUs display statistically significant oscillations in relative abundance over the daily cycle, indicating that a substantial fraction of the microbial community is under temporal regulation.

At higher taxonomic resolution, clear rhythmic patterns are evident at the phylum level. In particular, *Bacteroidetes* and *Firmicutes*—the two dominant bacterial phyla in the mammalian gut—exhibit antiphasic oscillations. In nocturnal mice, Bacteroidetes abundance peaks during the light phase, corresponding to the host's rest and fasting period, whereas *Firmicutes* abundance peaks during the dark phase, which coincides with active feeding and locomotor behavior. These reciprocal oscillations suggest functional partitioning of microbial metabolism across the day and underscore the close coupling between host behavior, nutrient availability, and microbial community structure. In addition to compositional changes, total bacterial load also fluctuates diurnally, with fecal bacterial counts showing approximately two-fold variation between peak and nadir across the daily cycle (Froy and Weintraub, 2025).

Beyond changes in taxonomic composition, multi-omics approaches have revealed pronounced circadian regulation of microbial function. Metagenomic and metatranscriptomic analyses demonstrate that bacterial gene expression patterns vary substantially over the course of the day. Genes involved in carbohydrate transport and metabolism show peak expression during host feeding periods, reflecting increased substrate availability and metabolic activity. In contrast, genes associated with stress resistance, nutrient scavenging, and maintenance functions tend to peak during fasting periods, when microbial communities must adapt to reduced nutrient input and altered luminal conditions. These findings indicate that microbial communities dynamically reprogram their functional output in synchrony with host behavioral rhythms.

Metabolomic profiling has further substantiated the existence of microbiome diurnal rhythmicity by revealing time-of-day-dependent oscillations in microbial-derived metabolites. SCFAs, key products of bacterial fermentation, exhibit distinct diurnal patterns. In nocturnal rodents, butyrate and propionate concentrations peak around Zeitgeber time 2 (ZT2), corresponding to the early light phase following nocturnal feeding. Acetate displays a bimodal oscillatory pattern, suggesting regulation by multiple microbial pathways and host absorption dynamics ((Wang et al.

2022; Segers et al. 2019; Thaiss et al. 2014; Pickel & Sung, 2020; Litichevskiy & Thaiss, 2022). These rhythmic metabolite profiles provide a direct biochemical link between microbial activity and host circadian physiology.

Collectively, these discoveries established that the gut microbiome is a temporally organized ecosystem, exhibiting coordinated daily rhythms in composition, gene expression, and metabolic output. This diurnal organization reflects a tight coupling between microbial communities and host circadian behavior, particularly feeding-fasting cycles, and provides a mechanistic foundation for understanding how microbial rhythms contribute to daily oscillations in host metabolism, immune function, and overall physiological homeostasis.

19.6 Bidirectional Circadian–Microbiome Communication

19.6.1 Host Clock Control of Microbiome Rhythmicity

Genetic and environmental perturbation studies provide compelling evidence that host circadian clocks play a central role in shaping microbiome rhythmicity. Mouse models with targeted disruption of core clock genes have been particularly informative in disentangling host-driven temporal regulation from microbe-intrinsic dynamics. Mice lacking the core clock gene *Bmal1* exhibit a complete loss of behavioral circadian rhythms, including locomotor activity and feeding-fasting cycles. Microbiome profiling in these animals revealed that, although the overall taxonomic composition of the gut microbiota remains broadly similar to that of wild-type controls, the characteristic diurnal oscillations in key bacterial phyla—most notably *Bacteroidetes* and *Firmicutes*—are entirely abolished (Thaiss et al., 2014). These findings demonstrate that host circadian timing, rather than static community structure, is essential for generating microbial rhythmicity.

Consistent results have been observed in mice with the deletion of other core clock components. Per1/Per2 double knockout mice also display a loss of circadian organization in the gut microbiome, further reinforcing the conclusion that an intact TTFL in the host is required to impose temporal structure on microbial communities. Importantly, these effects are not merely secondary to global circadian disruption but can arise from tissue-specific clock dysfunction. Mice with intestinal epithelial cell-specific deletion of *Bmal1* retain functional circadian clocks in the brain and other peripheral tissues, yet they exhibit a marked disruption of microbiome oscillations. This finding demonstrates that the local clock within the intestinal epithelium plays a dominant and autonomous role in organizing microbial community dynamics, independent of central circadian control (Fan et al., 2026).

The intestinal epithelial clock exerts its influence on the microbiome by regulating multiple aspects of the luminal environment that define microbial niches. These include the rhythmic secretion of antimicrobial peptides, such as REG3γ, oscillations in mucus production and glycosylation patterns, daily changes in epithelial permeability and barrier integrity, and time-of-day-dependent variation in nutrient absorption and availability. Together, these processes create a temporally structured habitat in which different microbial taxa are favored at different times of day, thereby driving predictable oscillations in microbial abundance and function.

Among environmental cues, the timing of food intake has emerged as a particularly powerful driver of microbiome rhythmicity. TRF experiments have shown that feeding-fasting cycles can override other circadian signals in shaping microbial oscillations. When nocturnal mice are restricted to feeding exclusively during the light phase—opposite to their normal feeding schedule—diurnal microbiome oscillations are preserved but undergo a phase shift, realigning with the new feeding time (Zarrinpar et al., 2014). These findings highlight feeding behavior as a dominant *Zeitgeber* for the gut microbiome and underscore the tight coupling between host behavior, intestinal physiology, and microbial dynamics.

Chronic disruption of circadian rhythms produces more profound and pathological effects on the microbiome. Experimental models of chronic jet lag, which induce persistent circadian misalignment, result in severely blunted or arrhythmic microbial oscillations along with substantial changes in overall community composition. These alterations are accompanied by metabolic dysfunction, including weight gain, insulin resistance, and impaired glucose tolerance. Parallel observations have been made in humans: shift workers exhibit distinct microbiome profiles compared to day workers, characterized by reduced alpha diversity and a decreased abundance of SCFA-producing bacteria (Zhang et al., 2025; Inokawa et al., 2020).

The causal role of circadian-disrupted microbiota in metabolic disease has been demonstrated through fecal microbiota transplantation (FMT) experiments. Transfer of microbiota from jet-lagged humans into germ-free mice leads to increased weight gain and impaired glucose tolerance compared with the transplantation of microbiota collected from the same donors before jet-lag exposure or after circadian recovery (Thaiss et al., 2014). These findings provide direct evidence that circadian misalignment reshapes the microbiome in ways that can drive metabolic pathology, firmly establishing host circadian clocks as critical regulators of microbiome rhythmicity and host metabolic health.

19.6.2 Microbiome Influence on Host Circadian Rhythms

Evidence from germ-free and microbiota-manipulated animal models demonstrates that the gut microbiome actively contributes to the regulation of host circadian rhythms, particularly in peripheral tissues. Germ-free mice, which develop in the complete absence of microorganisms, exhibit marked alterations in the expression of core clock genes in multiple peripheral organs, including the liver, white adipose tissue, and intestinal epithelium. In contrast, the SCN, which functions as the central circadian pacemaker, remains largely intact and rhythmic, indicating that microbial

signals are especially important for peripheral clock regulation rather than for central clock function (Bafna et al., 2023).

The causal role of the microbiome in shaping host circadian gene expression is further supported by recolonization studies. The introduction of a conventional microbiota into adult germ-free mice partially restores normal rhythmic expression of clock genes in peripheral tissues, demonstrating that microbial signals remain capable of modulating circadian programs even after early developmental windows. Similarly, perturbation of the microbiome in conventionally raised mice through broad-spectrum antibiotic treatment induces changes in peripheral clock gene expression, particularly in the liver, although these effects are generally less severe than those observed in germ-free animals (Ge et al., 2017). Together, these findings indicate that both the presence and composition of the gut microbiota influence the amplitude and phase of peripheral circadian clocks.

FMT experiments provide direct evidence that microbiomes shaped by circadian disruption acquire functional properties capable of altering host physiology. When microbiota from mice with intestinal epithelial cell-specific deletion of *Bmal1* are transplanted into germ-free recipient mice, the recipients develop altered intestinal gene expression profiles despite possessing intact host clocks. Notably, these changes include the reduced recruitment of Th17 cells and inflammatory monocytes to the intestinal mucosa, highlighting a microbiome-mediated pathway linking circadian regulation to immune function (Thaiss et al., 2016).

Collectively, these studies demonstrate a bidirectional relationship between the host circadian system and the gut microbiome. While host clocks impose temporal structure on microbial communities, microbial-derived signals feed back to fine-tune peripheral circadian gene expression and immune homeostasis. This reciprocal interaction underscores the role of the microbiome as an active participant in circadian regulation rather than a passive responder to host-driven rhythms.

19.6.3 SCFAs as Circadian Regulators

SCFAs are the most extensively studied class of microbial metabolites shown to influence host circadian rhythms. Experimental studies demonstrate that SCFAs can directly modulate the phase and amplitude of peripheral circadian clocks. Oral administration of mixed SCFAs induces phase shifts in clock gene expression in multiple tissues. In the kidney and submandibular gland, SCFA treatment advances the rhythmic expression of *Per2* and *Bmal1*, whereas effects in the liver are more variable and depend on metabolic context and feeding state (Tahara et al., 2018). These findings highlight the tissue-specific nature of SCFA-clock interactions.

At the cellular level, SCFAs exert direct regulatory effects on clock gene expression. Leone et al. (2015) demonstrated that butyrate modulates the expression of *Per2* and *Bmal1* in cultured mouse hepatocytes and alters the hepatic *Per2:Bmal1* expression ratio following intraperitoneal administration in vivo. These results provide strong evidence that SCFAs act not only indirectly through systemic metabolic changes but also directly at the level of clock gene regulation within target cells.

SCFAs influence circadian regulation through multiple molecular mechanisms. Butyrate and propionate are potent inhibitors of class I histone deacetylases (HDACs), leading to increased histone acetylation, chromatin relaxation, and enhanced transcriptional accessibility (Davie, 2003; Koh et al., 2016). This epigenetic mechanism is particularly relevant to circadian control, as HDAC3 forms a repressive complex with the nuclear receptor corepressor and is rhythmically recruited by REV-ERBα to suppress *Bmal1* transcription. Inhibition of HDAC3 by butyrate and propionate can therefore alter the amplitude and phase of circadian gene expression by disrupting this repressive arm of the clock (Feng et al., 2011).

Direct experimental evidence for this mechanism has been provided by Fawad and colleagues, who showed that SCFA-induced phase shifts in intestinal epithelial circadian rhythms depend on HDAC activity. In intestinal enteroid models, knockout of HDAC3 abolished the effects of butyrate on *Per2* expression, confirming that HDAC-mediated epigenetic

regulation is essential for SCFA-driven circadian modulation (Fawad et al., 2022). These findings underscore the importance of epithelial epigenetic machinery in translating microbial metabolic signals into circadian outcomes.

In addition to epigenetic regulation, SCFAs signal through specific G-protein-coupled receptors, including FFAR3 (GPR41), FFAR2 (GPR43), and HCAR2 (GPR109A), which are expressed across a wide range of tissues. Activation of these receptors initiates intracellular signaling cascades involving cAMP, MAPK, and calcium pathways, which may converge on circadian transcriptional networks and modulate clock gene expression (Brown et al., 2003; Le Poul et al., 2003; Thangaraju et al., 2009). This receptor-mediated signaling provides an additional layer through which microbial metabolites can influence host temporal physiology.

Clinical evidence further supports the role of SCFAs as circadian regulators in humans. A randomized controlled trial in patients with active ulcerative colitis evaluated the effects of oral sodium butyrate supplementation (600 mg/kg body weight daily for 12 weeks). Butyrate treatment significantly upregulated the expression of core circadian clock genes, including *CRY1, CRY2, PER1*, and *BMAL1*, in peripheral blood cells. These molecular changes were accompanied by reductions in inflammatory markers, such as calprotectin and high-sensitivity C-reactive protein (hs-CRP), as well as improvements in subjective sleep quality (Firoozi et al., 2024). Together, these findings position SCFAs—particularly butyrate—as key microbial-derived regulators linking gut metabolism, circadian biology, inflammation, and sleep.

19.7 Disruption of the Circadian–Microbiome Axis in Disease

19.7.1 Metabolic Disorders

Both circadian disruption and alterations in the gut microbiome contribute to the development of metabolic disease through interconnected and mutually reinforcing mechanisms. Early experimental work demonstrated that the gut microbiome of genetically obese mice has an enhanced capacity for dietary energy harvest. When this "obese microbiome" is transplanted into germ-free mice, the recipients accumulate significantly more body fat than animals receiving microbiota from lean donors, despite comparable caloric intake (Turnbaugh et al., 2006). Dietary factors further exacerbate this interaction: high-fat diet feeding in rodents disrupts circadian clock gene expression while simultaneously abolishing normal microbiome rhythmicity, producing synergistic effects that accelerate weight gain, insulin resistance, and metabolic dysfunction (Kohsaka et al., 2007; Leone et al., 2015).

Type 2 diabetes mellitus (T2DM) is similarly associated with combined circadian and microbiome abnormalities. Individuals with T2DM display altered gut microbial composition characterized by reduced overall diversity and a marked depletion of butyrate-producing bacteria, including *Faecalibacterium, Roseburia*, and certain *Clostridium* species (Qin et al., 2012; Karlsson et al., 2013). Circadian disruption further increases diabetes risk: meta-analyses indicate that shift workers have an approximately 1.1- to 1.3-fold higher risk of developing T2DM compared with day workers, with risk increasing in proportion to the duration of rotating night shift exposure (Pan et al., 2011; Gan et al., 2015). Interventional studies underscore the functional importance of the microbiome in metabolic control. FMT from lean donors to individuals with metabolic syndrome leads to significant improvements in peripheral insulin sensitivity, accompanied by an increased abundance of butyrate-producing bacteria in the recipient gut (Vrieze et al., 2012).

Non-alcoholic fatty liver disease (NAFLD), represents another major metabolic disorder at the intersection of circadian biology and the microbiome. NAFLD affects approximately 25% of adults worldwide, with prevalence estimates ranging from 17% to over 50% in Western populations (Younossi et al., 2016). The liver is a highly circadian organ, exhibiting

robust daily regulation of genes involved in lipid metabolism, gluconeogenesis, and detoxification. Disruption of core clock genes such as *Clock* and *Bmal1* promotes hepatic lipid accumulation and steatosis in mouse models, demonstrating a direct role for circadian machinery in liver health (Saran et al., 2020). The gut microbiome contributes to NAFLD via the gut-liver axis: increased intestinal permeability allows bacterial products, including lipopolysaccharide (LPS), to translocate to the liver through the portal circulation, where they activate inflammatory pathways and exacerbate hepatic injury (Tripathi et al., 2018; Jiang et al., 2020).

19.7.2 Inflammatory Bowel Disease

IBD provides a compelling example of how circadian disruption and microbiome dysbiosis converge on immune-mediated pathology. Patients with IBD frequently report sleep disturbances, and molecular analyses of intestinal biopsies reveal a profound disruption of circadian clock gene expression. Core clock genes—including *CLOCK, CRY1, CRY2, PER1*, and *PER2*—are significantly downregulated in intestinal tissues from IBD patients, affecting both inflamed and non-inflamed regions (Weintraub et al., 2020; Liu et al., 2017). The presence of clock gene disruption even in non-inflamed tissue suggests that circadian dysregulation may precede overt inflammation and potentially serve as an early biomarker of disease susceptibility.

Concurrently, the gut microbiome of individuals with IBD exhibits characteristic dysbiosis, marked by reduced microbial diversity and the depletion of key butyrate-producing taxa such as *Faecalibacterium prausnitzii* and *Roseburia hominis* (Machiels et al., 2014; Sokol et al., 2008). The loss of these bacteria compromises epithelial energy supply, barrier integrity, and anti-inflammatory signaling, further destabilizing intestinal homeostasis.

Mechanistic studies have revealed that circadian regulation of intestinal immunity plays a pivotal role in IBD pathogenesis. Group 3 innate lymphoid cells (ILC3s), which are essential for mucosal defense and

epithelial repair, are highly enriched in circadian clock gene expression and require intact circadian machinery for proper function. Recent landmark work demonstrated that the nuclear receptors REV-ERBα and REV-ERBβ maintain ILC3 identity through epigenetic regulation of the transcription factor RORγt. Combined deletion of these clock components drives the conversion of ILC3s into pro-inflammatory ILC1-like cells, reduces protective interleukin-22 (IL-22) production, and increases interferon-γ (IFN-γ)-mediated inflammation (Bhattarai et al., 2025). This finding establishes clock genes as direct regulators of immune cell identity rather than passive timing elements.

This immune-circadian pathway is tightly integrated with microbial signaling. Microbial ligands activate toll-like receptors, inducing IL-23 secretion, which stimulates IL-22 production by ILC3s and activates STAT3 signaling in intestinal epithelial cells. Specific commensals, such as segmented filamentous bacteria, generate diurnal rhythms in REG3γ antimicrobial peptide expression via ILC3-mediated pathways, producing time-of-day variation in pathogen resistance (Brooks et al., 2021). In IBD, disruption of this finely tuned system creates a vicious cycle: circadian misalignment impairs epithelial barrier function and immune regulation, while reduced microbial production of SCFAs further suppresses clock gene expression, reinforcing inflammatory pathology (Tahara et al., 2018; Liu et al., 2017).

19.7.3 Cardiovascular Disease

CVD exhibits strong circadian patterns, with acute events such as myocardial infarction and stroke occurring most frequently during the morning hours. These temporal patterns reflect underlying molecular clock control of cardiac physiology. Germline deletion of *Bmal1* in mice leads to age-associated dilated cardiomyopathy, characterized by ventricular dilation, thinning of the myocardial wall, and systolic dysfunction (Lefta et al., 2012). Similarly, cardiomyocyte-specific deletion of *Bmal1* produces age-dependent dilated cardiomyopathy with diastolic dysfunction, adverse

remodeling of the extracellular matrix, and a shortened lifespan (Chen et al., 2014). These studies demonstrate that circadian regulation is essential for maintaining normal cardiac structure and function throughout life.

The gut microbiome is a key mediator of cardiovascular health, acting through both metabolic and circadian-dependent pathways. One of the most well-characterized microbial metabolites in this context is trimethylamine N-oxide (TMAO), produced from phosphatidylcholine by gut bacterial metabolism. TMAO promotes atherosclerosis by facilitating macrophage foam cell formation and impairing reverse cholesterol transport, establishing a mechanistic link between diet, microbiota, and CVD (Wang et al., 2011). Clinical studies have confirmed this association: in patients undergoing elective coronary angiography, elevated plasma TMAO levels independently predicted major adverse cardiovascular events, including myocardial infarction, stroke, and death, with a hazard ratio of 2.54 for the highest versus lowest quartile of TMAO (Tang et al., 2013).

Beyond TMAO, SCFAs produced by microbial fermentation influence blood pressure and vascular function through circadian-dependent mechanisms. SCFAs act via G-protein-coupled receptors expressed in vascular tissues. The olfactory receptor Olfr78, found in vascular smooth muscle and the juxtaglomerular apparatus, responds to propionate and acetate by stimulating renin secretion, whereas GPR41 (FFAR3) in the vascular endothelium mediates SCFA-induced vasodilation (Pluznick et al., 2013; Natarajan et al., 2016). Genetic studies confirm opposing roles: *Olfr78* knockout mice are hypotensive with reduced plasma renin, while *Gpr41* knockout mice develop isolated systolic hypertension.

Human cohort studies have revealed complex interactions between SCFA-producing bacteria and blood pressure. The HELIUS study of 4,672 participants across six ethnic groups found that bacteria such as *Roseburia* species were associated with lower systolic blood pressure. However, paradoxically, individuals with higher blood pressure often exhibit elevated fecal SCFA levels, suggesting impaired intestinal absorption of SCFAs in hypertensive individuals. Animal studies corroborate this, showing defective butyrate transport in spontaneously hypertensive rats. Thus,

reduced systemic SCFA availability despite adequate microbial production may contribute to hypertension by limiting GPR41-mediated vasodilatory signaling (Verhaar et al., 2020; Yang et al., 2019a).

Circadian disruption, particularly from shift work, dramatically increases cardiovascular risk by perturbing microbiome composition and function. Meta-analyses of 21 studies encompassing 173,010 participants show that shift workers face a 17–26% increased risk of ischemic heart disease and cardiovascular mortality (Torquati et al., 2018). Risk increases with duration: each additional 5 years of shift work raises CVD risk by 5% and cardiovascular mortality by 4% (Wang et al., 2018). The UK Biobank confirmed that shift workers have an 11% higher risk of incident CVD and a 25% higher risk of fatal cardiovascular events compared to non-shift workers, with smoking, sleep disturbances, and adiposity as key mediators (Ho et al., 2022a).

Mechanistically, circadian disruption alters gut microbial composition and rhythmicity. Abnormal light–dark cycles in mice downregulate bacterial genes for butyrate production while upregulating LPS synthesis and transport genes, accompanied by increased intestinal permeability (Voigt et al., 2014). Increased permeability facilitates LPS translocation into the systemic circulation, promoting chronic low-grade inflammation that contributes to metabolic and cardiovascular dysfunction (Summa et al., 2013). Fecal transplantation from circadian-disrupted, metabolically impaired mice into healthy recipients is sufficient to induce metabolic dysfunction, confirming the causal role of dysbiotic microbiota (Cao et al., 2026).

Sex differences play a critical role in cardiovascular–circadian–microbiome interactions. In a mouse model of shift work, female mice maintained behavioral and transcriptional rhythmicity despite chronic circadian misalignment, whereas male mice developed pronounced metabolic dysfunction when fed a high-fat diet. Dysbiosis emerged only in male mice, with an increase in bacteria producing diabetogenic branched-chain amino acids (BCAAs). Antibiotic ablation of the microbiota mitigated these effects, confirming microbiome involvement (Anderson et al., 2023).

Human data mirror these findings: female shift workers exhibit stronger circadian rhythmicity in physical activity and a lower incidence of metabolic syndrome than male shift workers, suggesting that female resilience to circadian disruption may be evolutionarily conserved (Duffy et al., 2011).

Together, these data highlight a tightly integrated cardiovascular-circadian-microbiome axis in which circadian misalignment and microbial dysbiosis synergistically drive metabolic and vascular pathology, with sex-specific differences modulating susceptibility and outcomes.

19.7.4 Cancer

The relationship between circadian disruption and cancer risk has gained increasing recognition from international health authorities. In 2019, the International Agency for Research on Cancer (IARC) classified shift work involving circadian disruption as "probably carcinogenic to humans" (Group 2A) (IARC Monographs Vol 124 Group, 2019; Erren and Lewis, 2019), based on epidemiological and mechanistic evidence. This designation underscores the clinical relevance of chronotherapeutic approaches in oncology, as the same circadian pathways implicated in carcinogenesis can be leveraged or restored therapeutically.

Mechanistically, circadian disruption influences both tumor initiation and progression through multiple interconnected pathways (Masri and Sassone-Corsi, 2018). Core clock genes regulate key cellular processes, including cell cycle progression, the DNA damage response, and apoptosis (Gaucher et al., 2018; Farshadi et al., 2020). Circadian misalignment can increase cancer risk via melatonin suppression, the dysregulation of clock genes such as *CLOCK* and *BMAL1*, reduced natural killer cell activity, and chronic inflammation resulting from metabolic imbalance (Logan and McClung, 2019; Scheiermann et al., 2018). Importantly, these pathways also provide therapeutic targets for chronotherapy, where restoring or exploiting circadian regulation may enhance treatment efficacy while minimizing systemic toxicity.

The tumor-suppressive versus oncogenic roles of clock genes are highly context-dependent, with significant implications for chronotherapy design (Chang and Lai, 2019). For example, *BMAL1* expression correlates with improved overall survival in colorectal cancer, nasopharyngeal carcinoma, and metastatic melanoma (Tang et al., 2018; de Assis et al., 2018a), supporting strategies to enhance *BMAL1* function, such as ROR agonists currently in clinical trials for cancer immunotherapy (Cash et al., 2021). Conversely, in prostate cancer cells, *CLOCK* and *PER2* are downregulated while *BMAL1* is upregulated relative to normal epithelial cells, and melatonin administration can resynchronize these deregulated clock genes (Jung-Hynes et al., 2010). These context-specific differences emphasize that chronotherapeutic protocols must be tailored to tumor-specific circadian profiles rather than applied universally (Pariollaud and Lamia, 2020).

Genetic variation in clock genes further illustrates the circadian–cancer connection and may explain interindividual differences in response to chronotherapy. Mutations in *CLOCK, PER1, PER2, CRY1*, and *CRY2* can influence tumor progression, with effects often depending on tissue context (Fu and Lee, 2003; Masri and Sassone-Corsi, 2018). Single-nucleotide polymorphisms in *CLOCK* genes are associated with estrogen receptor/progesterone receptor-negative breast cancer (Reszka et al. 2017; Reszka & Zienolddin., 2018), highlighting the need for personalized chronotherapy approaches that account for patient genotype (Ozturk et al., 2017).

Experimental evidence from animal models supports causality between circadian disruption and tumor development. Chronic jet lag accelerates lung tumorigenesis in non-small-cell lung cancer models (Papagiannakopoulos et al., 2016) and induces hepatocellular carcinoma through extensive rewiring of hepatic circadian gene expression and metabolic dysfunction (Kettner et al., 2016). These findings mirror epidemiological observations in shift workers (Walker et al., 2020) and suggest that chronic circadian misalignment creates a tumor-permissive environment. Conversely, interventions that restore circadian rhythms—

through light therapy, TRF, or exercise—may reduce tumor permissiveness while optimizing cytotoxic therapy timing (Sulli et al., 2019).

The immune system represents another critical link between circadian disruption and cancer progression, with implications for immunotherapy timing. Circadian misalignment impairs immune surveillance by affecting $CD8^+$ T cells and tumor-associated macrophages, key mediators of anti-tumor immunity (Wang et al., 2024; Hadadi et al., 2020; Aiello et al., 2020). Mechanistic data align with clinical observations showing that higher *BMAL1* expression correlates with an improved response to anti-PD1 immunotherapy in melanoma patients (de Assis et al., 2018b). Moreover, the release of circulating tumor cells is temporally regulated, occurring predominantly during sleep under circadian control of hormone receptors (Diamantopoulou et al., 2022). This suggests that circadian timing influences not only tumor initiation but also metastatic potential, emphasizing the importance of optimizing the timing of surgical interventions and systemic therapies to minimize disease progression.

19.7.4.1 The Circadian Microbiome–Cancer Axis

The gut microbiome plays a pivotal role in the intersection of circadian biology and cancer, representing an emerging area with implications for both prevention and chronotherapy (Tognini et al., 2018). Microbiome composition modulates systemic inflammation, immune function, and metabolic health, all of which influence cancer risk and therapeutic response. Circadian disruption can perturb microbiome composition and function, providing an additional pathway by which lifestyle factors may contribute to oncogenesis. Notably, the microbiome itself exhibits circadian rhythms in both composition and activity (Thaiss et al., 2014; Zarrinpar et al., 2014), which can affect drug metabolism, immune surveillance, and the production of bioactive metabolites that modulate tumor growth. These rhythmic microbial functions are particularly relevant in the context of interventions such as TRF, which can reinforce microbial oscillations.

Despite strong evidence from animal models, translating circadian–microbiome findings to humans remains challenging. Rodent studies

demonstrate that sleep deprivation induces notable microbiome alterations, whereas human studies often show modest changes, with many effects failing to reach statistical significance after correction for multiple testing. For example, while reduced microbial richness has been observed in sleep-restricted humans, rodent findings linking circadian disruption to increased intestinal permeability are frequently not replicated. This translational gap underscores the need for caution when extrapolating mechanistic insights from preclinical models to clinical chronotherapy applications (Karl et al., 2023; Shan et al., 2025; Fishbein et al., 2021).

Time-restricted eating (TRE) illustrates the complexities of translating circadian–microbiome interventions from rodents to humans. In mice, TRF robustly modifies microbiome composition and enhances rhythmicity (Chaix et al., 2014; Hatori et al., 2012; Zarrinpar et al., 2014). However, secondary analyses of human TRE trials often find minimal effects on α-diversity, β-diversity, or microbial composition despite metabolic improvements such as weight loss and enhanced glucose tolerance (Sutton et al., 2018; Parr et al., 2020). Meta-analyses further quantify this discrepancy, showing substantial reductions in Shannon diversity in rodents under sleep deprivation or TRF, while human effects are far smaller and often non-significant. These observations highlight the necessity for well-powered human studies specifically designed to assess microbiome responses to circadian interventions in cancer patients, as some metabolic benefits may occur independently of microbial changes.

Methodological rigor is a critical challenge in circadian–microbiome research. Many studies fail to report stool sample collection times, despite evidence that microbial composition fluctuates significantly over hours. Within-individual, day-to-day variation often exceeds between-individual variation, with factors such as stool moisture content contributing substantially to observed differences (Thaiss et al., 2016). Such variability has profound implications for studies linking circadian interventions to microbiome dynamics and therapeutic outcomes. Future chronotherapy trials incorporating microbiome analyses should standardize sample collection timing, control for confounding variables, and employ robust

statistical frameworks capable of accounting for high temporal and interindividual variability.

19.7.4.2 Host Tolerance Rhythms in Cancer Chronochemotherapy

Chronochemotherapy leverages the intrinsic temporal organization of both normal and malignant tissues to optimize the therapeutic index of anticancer treatments. Over the past decades, research has demonstrated that the efficacy and toxicity of more than 30 widely used chemotherapy drugs can vary by over 50% depending on the time of administration (Lévi, 2001; Innominato et al., 2014). This temporal variation arises from circadian oscillations in drug metabolism, the DNA repair response, and cellular defense mechanisms, which collectively shape windows of enhanced host tolerance.

At the molecular level, drug-metabolizing enzymes exhibit robust circadian rhythms that directly influence the pharmacokinetics of chemotherapeutic agents. For example, 5-fluorouracil (5-FU) metabolism is governed by two key enzymes with opposing circadian patterns. Dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme responsible for 5-FU clearance, reaches peak activity in the late afternoon (~4:00 PM) and dips to its lowest in the early morning (~4:00 AM) (Harris et al., 1990). In contrast, thymidylate synthase (TS), the molecular target of 5-FU, oscillates in an anti-phasic pattern relative to DPD (Johnston et al., 1995; Lincoln et al., 2000; Salonga et al., 2000; Pullarkat et al., 2001). Clinical studies confirm that this circadian orchestration of metabolism and target availability modulates 5-FU's time-dependent bioavailability and efficacy, providing a mechanistic basis for optimized dosing schedules (Jacobs et al., 2016).

Endogenous antioxidant systems also contribute to time-of-day-dependent host tolerance. Glutathione, a tripeptide critical for the cellular detoxification of chemotherapeutic agents, exhibits daily fluctuations, peaking in the afternoon (~4:00 PM). This rhythm has been successfully exploited to reduce toxicity from platinum-based drugs such as cisplatin and oxaliplatin, allowing clinicians to align drug administration with

periods of maximal antioxidant protection in normal tissues (Zeng et al., 2005).

Early clinical evidence strongly supports the benefits of chronotherapy in improving safety and tolerance. In advanced ovarian cancer, morning administration of doxorubicin (6:00 AM) combined with evening cisplatin (6:00 PM) resulted in fewer complications and reduced renal toxicity compared to the reverse schedule (Hrushesky, 1985; Sothern et al., 1989; Lévi et al., 1990). Similarly, chronomodulated combination therapy in metastatic colorectal cancer—timing irinotecan, oxaliplatin, 5-FU, and leucovorin to the host's circadian rhythms—produced delayed disease progression, improved overall survival, and enhanced tolerance relative to conventional chemotherapy schedules (Lévi et al., 1997; Gholam et al., 2006; Innominato et al., 2020).

Computational modeling has provided further mechanistic insight, showing that appropriately timed drug delivery maximizes cytotoxicity in tumor cells while minimizing damage to normal tissues (Altinok et al., 2009). These analyses underscore the dual advantages of chronotherapy: enhancing therapeutic efficacy (chrono-efficacy) while simultaneously reducing systemic toxicity (chrono-tolerance). By harnessing predictable circadian rhythms in metabolism, DNA repair, and antioxidant defenses, chronochemotherapy represents a rational, evidence-based approach to improving both the safety and effectiveness of cancer treatment.

19.7.4.3 Circadian Clock Control of the Cell Cycle

The circadian clock exerts profound control over the cell cycle, providing a mechanistic foundation for time-of-day-dependent therapeutic effects in cancer. Direct regulation of key cell cycle genes occurs at multiple levels through the core CLOCK/BMAL1 transcriptional machinery (Fu et al., 2002; Matsuo et al., 2003; Granda et al., 2005; Gaucher et al., 2018). Critical regulators of cell cycle progression, such as c-MYC and cyclin D1, which drive entry into S-phase, and *Wee1*, which gates entry into M-phase, are under circadian control (Gaucher et al., 2018; Lee et al., 2019). Additionally, circadian regulation extends to checkpoint controllers

including p21, p16/INK4a, p27, and p57, as well as associated signaling pathway components (Sotak et al., 2014).

The circadian clock also modulates the DNA damage response, repair mechanisms, and apoptotic pathways, all of which intersect with cell cycle checkpoints (Antoch et al., 2005; Gauger and Sancar, 2005; Gorbacheva et al., 2005; Kang et al., 2009, 2010; Sancar et al., 2010; Gaddameedhi et al., 2011). Perturbations of clock genes in animal and cellular models alter rhythmic mitosis, cell proliferation, and tumor growth (Wood et al., 2008; Taniguchi et al., 2009; Yang et al., 2009,; Kang et al., 2010; Zhao et al., 2013; Puram et al., 2016; Kiessling et al., 2017; Chang and Lai, 2019; Dong et al., 2019; Shafi et al., 2021). Enhancing intratumor circadian rhythms using synchronizing agents such as dexamethasone, forskolin, or heat shock reduces the proportion of cells in S-phase, increases G1-phase arrest, and inhibits tumor growth, suggesting that the tumor-suppressive function of the clock is mediated, in part, through cell cycle arrest (Kiessling and Cermakian, 2017).

Time-of-day-specific anticancer efficacy has been linked directly to circadian clock gene expression. For instance, temozolomide and irinotecan display rhythmic cytotoxicity in glioblastoma and colorectal cancer cells, with maximal sensitivity coinciding with peaks in *Bmal1* expression (Ballesta et al., 2011; Dulong et al., 2015; Slat et al., 2017). Silencing *Bmal1* abolishes these rhythms, demonstrating a direct mechanistic role. Elevated *Bmal1* also increases sensitivity to oxaliplatin and paclitaxel in colorectal cancer and tongue squamous cell carcinoma cells (Zeng et al., 2014; Tang et al., 2017). Similarly, *Per2* acts as a circadian modulator of DNA-damaging agents; timed administration of oxaliplatin at the peak of PER2 expression significantly enhances efficacy in human oral squamous cell carcinoma tumor grafts by periodically suppressing PCNA transcription and thereby limiting DNA repair (Tang et al., 2019).

Beyond DNA-targeting agents, time-of-day-dependent anticancer effects extend to synthetic compounds such as SR9009, bortezomib, and aldehyde dehydrogenase inhibitors, as well as natural products like curcumin, with efficacy influenced by circadian metabolic factors in glioblastoma, liver,

and breast cancer models (Sarma et al., 2016; Matsunaga et al., 2018; Wagner et al., 2019). Systematic cell-based screens reveal that the cytotoxicity of multiple drugs is abolished in *Bmal1* knockout cancer cells, including heat shock protein 90 (HSP90) inhibitors, highlighting the critical role of intact circadian rhythms in mediating time-of-day-dependent drug sensitivity (Lee, 2021). Interestingly, HSP90 inhibitors require both synchronized circadian rhythms and coordinated cell cycle progression, emphasizing that the temporal regulation of cellular dynamics in tumors dictates optimal timing for anticancer therapies.

19.7.4.4 Tumor Cell Cycle Rhythms

The existence of circadian rhythms in tumor cell proliferation has been recognized for over half a century. Early work by Garcia-Sainz and Halberg in 1966 first reported a 24-hour rhythm in the cell cycle of human mammary cancer biopsy samples, providing initial evidence that cancer cells exhibit time-of-day-dependent behavior. Subsequent studies across multiple tumor types confirmed that the timing of mitotic activity varies throughout the day, with distinct circadian patterns dependent on tumor origin (Klevecz et al., 1987; Smaaland et al., 1991; Hrushesky et al., 1998). These findings laid the foundation for chronotherapy approaches that exploit temporal vulnerabilities in cancer cells.

A pivotal clinical observation came from a flow cytometry study in ovarian cancer patients, which demonstrated that tumor cell proliferation—quantified as the proportion of cells in S-phase—follows a robust 24-hour rhythm, peaking in the mid-to-late morning (Klevecz et al., 1987). Notably, this rhythm was almost 12 hours out of phase with proliferation in normal, non-tumor tissues. This temporal misalignment between tumor and healthy cells presents a unique opportunity: chemotherapy agents that target specific cell cycle phases can be administered at times when tumor cells are most vulnerable while minimizing damage to normal tissues.

Preclinical and clinical studies have confirmed that timing chemotherapy according to tumor cell cycle rhythms enhances efficacy and reduces toxicity. Agents targeting the S-phase, such as cisplatin and 5-FU, as well as

M-phase-specific drugs like docetaxel, and G1-phase-specific agents like seliciclib, display maximal anti-tumor effects and minimal systemic toxicity when administered at optimized times (Tampellini et al., 1998; Iurisci et al., 2006, 2009; Bernard et al., 2010). These findings underscore the principle that temporal targeting of the cell cycle can improve the therapeutic index of anticancer drugs.

Recent research has extended these concepts to targeted therapies, particularly cyclin-dependent kinase inhibitors (CDK). CDK4/6 inhibitors, such as palbociclib, which block the G1-to-S transition in the cell cycle, have been shown to reduce proliferation in cultured cells and suppress tumor growth in mouse models in a time-of-day-dependent manner (Lee et al., 2019). These studies indicate that even modern, precision-targeted therapies retain sensitivity to circadian timing, reinforcing the potential of chronotherapy in contemporary oncology.

The integration of tumor cell cycle rhythms with circadian clock biology provides a compelling framework for optimizing cancer treatment. By aligning drug administration with both intrinsic tumor rhythms and host circadian biology, clinicians can potentially maximize tumor kill, minimize toxicity, and improve patient outcomes. Future research is needed to map tumor-specific cell cycle oscillations across cancer types and to incorporate circadian biomarkers into personalized chemotherapy scheduling.

19.7.4.5 Radiation Therapy and Circadian Timing

Beyond chemotherapy, radiation therapy—a cornerstone treatment for many brain and peripheral tumors—also appears to exhibit time-of-day-dependent effects, though the evidence remains relatively limited (Mazzola et al., 2021). Emerging data suggest that administering radiation at specific circadian phases can improve tumor control while minimizing damage to healthy tissue, potentially enhancing the therapeutic index of radiotherapy.

Clinical observations provide early support for circadian optimization. For example, radiation administered in the morning has been associated with a reduced symptom burden in patients with adenocarcinomas, with minimal adverse effects on normal mucosal tissue and skin (Shuboni-

Mulligan et al., 2019). Such findings are consistent with the concept that normal tissues possess circadian-regulated DNA repair and antioxidant defenses, which may create windows of enhanced tolerance that can be exploited clinically.

Sex-specific differences further influence circadian responses to radiotherapy. In a study of patients with painful bone metastases, female patients showed significantly improved pain relief and response rates when treatment was delivered between 11:00 AM and 2:00 PM, whereas male patients did not demonstrate the same temporal pattern (Chan et al., 2017). This highlights the importance of considering both circadian phase and patient sex when designing time-optimized radiation protocols.

Mechanistically, these effects are supported by the circadian regulation of the DNA damage response and repair pathways. Core clock genes, including *PER1, PER2*, and *BMAL1*, orchestrate the timing of DNA repair enzymes, checkpoint activation, and apoptosis in response to genotoxic stress (Sancar et al., 2010; Gaddameedhi et al., 2011). By aligning radiation delivery with periods of maximal DNA repair capability in normal cells and heightened tumor cell vulnerability, clinicians can theoretically maximize tumor-cell killing while sparing healthy tissue.

In addition to DNA repair, the circadian modulation of cell cycle progression, apoptosis, and reactive oxygen species (ROS) handling in both tumor and normal tissues may further contribute to time-dependent differences in radiation response. Preclinical studies in rodent models indicate that radiation administered at specific circadian phases produces greater tumor regression and fewer gastrointestinal or hematologic toxicities, consistent with observations in humans (Mazzola et al., 2021).

Collectively, these findings support the notion that circadian-informed radiation therapy could complement chemotherapy chronotherapy, offering a holistic, time-optimized treatment strategy. While additional large-scale, randomized clinical trials are needed, the integration of circadian biology into radiotherapy planning represents a promising avenue to improve patient outcomes and reduce side effects in cancer treatment.

19.7.4.6 Circadian Clock Targeting for Cancer Immunotherapy

The circadian clock has emerged as a promising target for cancer immunotherapy, reflecting growing recognition of its role in modulating the tumor microenvironment and immune surveillance (Cash et al., 2021). Over the last decade, immunotherapy has revolutionized cancer treatment, with multiple US Food and Drug Administration (FDA)-approved therapies now harnessing or engineering the immune system to attack tumors. Mounting evidence suggests that circadian timing may influence immunotherapy efficacy, as molecular clocks within tumors regulate immune cell infiltration, antigen presentation, and the broader tumor-immune landscape (Cash et al., 2021; Zhang et al., 2021).

Across diverse cancer types, core circadian genes, including *BMAL1*, *CLOCK*, and *PER2*, have been associated with tumor antigen processing, presentation, and immunogenicity, although these effects are often tumor-type-dependent (Liu et al., 2019). For instance, analysis of 340 metastatic melanomas from The Cancer Genome Atlas revealed that higher *BMAL1* expression correlates with increased T-lymphocyte infiltration, elevated expression of the immune checkpoint protein programmed cell death 1 (PD-1) and its ligand programmed cell death ligand 1 (PD-L1), and improved response to anti-PD1 therapy (de Assis et al., 2018a). Patients with higher *BMAL1* levels exhibited better overall survival, suggesting a direct link between tumor clock function and immunotherapy efficacy.

Similarly, multi-omics studies in lung cancers, including adenocarcinomas and squamous cell carcinomas, demonstrate that $CD8^+$ T-cell infiltration positively correlates with *CLOCK* and *BMAL1* expression, highlighting the broader relevance of tumor clocks in shaping the anti-tumor immune response (Yang et al., 2019b). These findings support the concept that enhancing circadian clock activity in tumors may potentiate immune surveillance and improve outcomes for checkpoint inhibitor therapies.

Pharmacological modulation of the circadian clock is being explored as a novel immunotherapeutic strategy. ROR agonists such as LYC-55716,

which activate *BMAL1* transcription, are currently in preclinical and early clinical trials. These agents have demonstrated favorable safety, pharmacokinetics, and tolerability, and show potential for synergistic use with existing immunotherapies to enhance T-cell-mediated tumor clearance (Cash et al., 2021). By synchronizing or boosting tumor circadian activity, these drugs aim to improve immune cell recruitment and anti-tumor responses, providing a mechanistic basis for combination chronotherapy approaches.

However, the circadian clock's role in cancer immunity is highly context-dependent. In glioblastoma stem cells (GSCs), *CLOCK* and *BMAL1* can promote tumor progression by upregulating olfactomedin-like protein 3 (OLFML3), a proangiogenic chemokine that recruits immunosuppressive microglia into the tumor microenvironment (Chen et al., 2020). Similarly, prognostic models indicate that circadian clock genes can enhance glioma growth and proliferation while modulating immune infiltration (Wang et al., 2021). These findings highlight that in some cancers, clock activation may paradoxically support tumor survival and immune evasion, emphasizing the need for tumor-specific circadian interventions.

The dual and sometimes opposing roles of circadian genes underscore that effective clock-based immunotherapy requires precision. Strategies must account for tumor type, microenvironment composition, and immune contexture, integrating clock gene expression profiling into personalized treatment planning. By tailoring the timing and type of immunotherapeutic interventions to the tumor-specific circadian landscape, clinicians may optimize T-cell activation, checkpoint blockade efficacy, and overall treatment outcomes.

19.7.4.7 Chronotherapeutic Approaches

The growing recognition that circadian timing modulates drug absorption, efficacy, and toxicity has led to the development of chronotherapy, an approach that aligns medication administration with endogenous biological rhythms. Antihypertensive therapy was among the earliest areas of investigation. Several studies suggested that bedtime dosing improved

nocturnal blood pressure control and reduced cardiovascular risk, most notably the Ambulatory Blood Pressure Monitoring for Prediction of Cardiovascular Events (MAPEC) study, which reported significantly fewer cardiovascular events with nighttime administration (Hermida et al., 2010). However, these findings were later challenged by the large, pragmatic Treatment in Morning versus Evening (TIME) trial, which found no significant difference in cardiovascular outcomes between evening and morning dosing (Mackenzie et al., 2022), highlighting the complexity of translating circadian principles into clinical practice.

Cancer chemotherapy represents one of the most established and compelling applications of chronotherapy. Extensive preclinical and clinical evidence demonstrates that the timing of cytotoxic drug delivery can markedly influence both therapeutic efficacy and toxicity profiles. Chronomodulated chemotherapy regimens, designed to synchronize drug delivery with circadian rhythms in cell cycle progression, DNA repair, and drug metabolism, have shown improved tolerability and, in some cases, superior outcomes compared with conventional schedules (Lévi et al., 1997; Mormont and Lévi, 2003; Innominato et al., 2014; Lévi et al., 2024). Similarly, emerging evidence indicates that vaccine responses may be enhanced by morning administration, potentially reflecting circadian variation in antigen presentation and immune responsiveness (Long et al., 2016; Zhang et al., 2022; Ince et al., 2022).

Chronotherapeutic principles extend beyond CVD and oncology to a wide range of drug classes. In rheumatoid arthritis, corticosteroid chronotherapy has shown clear benefits. In the CAPRA trials, modified-release prednisone administered at 10:00 PM—designed to release the drug at approximately 2:00 AM, coinciding with the nocturnal peak in pro-inflammatory cytokines—produced superior symptom control compared with standard morning dosing (Buttgereit et al., 2011). Asthma therapy provides another well-characterized example: inhaled corticosteroids are most effective when administered in the late afternoon or early evening (~3:00–5:30 PM), aligning with the circadian pattern of airway

inflammation and nocturnal symptom worsening (Pincus et al., 1995; Smolensky et al., 1999).

Statin chronotherapy illustrates the importance of integrating circadian biology with pharmacokinetic properties. Short-half-life statins, such as simvastatin and lovastatin, achieve greater lipid-lowering efficacy when taken in the evening, coinciding with peak nocturnal hepatic cholesterol synthesis. In contrast, long-acting statins, including atorvastatin and rosuvastatin, maintain consistent efficacy regardless of dosing time, reflecting their prolonged half-lives and sustained pharmacodynamic effects (Awad et al., 2017; Izquierdo-Palomares et al., 2016). This distinction underscores that chronotherapeutic optimization must be individualized to specific drug characteristics.

Recent advances have highlighted striking time-of-day effects in cancer immunotherapy. Observational and prospective analyses suggest that early daytime administration of immune checkpoint inhibitors is associated with substantially improved survival—up to fourfold in some studies—compared with later dosing. These benefits are thought to arise from circadian regulation of immune cell trafficking, antigen presentation, and tumor infiltration, positioning circadian timing as a potentially critical variable in immuno-oncology treatment protocols (Lévi et al., 2024).

Psychiatric chronotherapy represents an emerging frontier. Given the central role of circadian systems in mood regulation, sleep, and neurotransmitter dynamics, optimizing the timing of antidepressants, anxiolytics, and mood stabilizers may enhance efficacy while reducing adverse effects. Incorporating chronotype assessment into psychiatric care offers a promising strategy for personalized treatment, aligning medication timing with individual biological rhythms (Colita et al., 2024).

The apparent contradiction between earlier antihypertensive chronotherapy studies and the TIME trial provides a cautionary example of how methodological rigor and bias assessment shape clinical recommendations. While earlier trials reported large reductions in cardiovascular events with bedtime dosing, the TIME trial, involving 21,104 participants, found no significant difference between evening and

morning administration, with primary endpoints occurring in 3.4% and 3.7% of participants, respectively (hazard ratio (HR) 0.95; 95% confidence interval (CI) 0.83–1.10; p = 0.53) (Mackenzie et al., 2022; Maqsood et al., 2023).

This controversy underscores the necessity of large, well-designed, randomized controlled trials to validate chronotherapeutic claims. The failure of high-quality trials to replicate earlier findings suggests that the cardiovascular benefits of antihypertensive chronotherapy may have been overstated, reinforcing the need for careful, evidence-based integration of circadian principles into clinical practice.

19.7.4.8 Clinical Challenges and Future Directions

Despite the growing promise of chronochemotherapy, much of the supporting evidence still derives from preclinical and early clinical studies, and significant challenges remain for widespread clinical implementation. Interindividual variability in chronotype, sex, age, disease stage, and genetic background contribute substantially to differences in pharmacodynamic responses among cancer patients, complicating the standardization of timed treatment protocols (Ozturk et al., 2017; Pariollaud and Lamia, 2020). Genetic heterogeneity in circadian clock function—including polymorphisms in core clock genes such as *CLOCK*, *PER*, and *CRY*—may help explain why some patients derive pronounced benefit from chronotherapeutic strategies while others show minimal or no response (Fu and Lee, 2003; Reszka et al., 2019).

Beyond genetic factors, environmental and physiological disruptions of circadian rhythms further amplify interindividual variability in cancer progression and treatment response. Factors such as shift work, irregular sleep–wake schedules, psychosocial stress, and systemic illness can profoundly alter circadian timing, influencing tumor biology as well as drug metabolism and toxicity (Lee et al., 2019; Walker et al., 2020; Koritala et al., 2021). These disruptions complicate the identification of an optimal treatment window and may blunt the benefits of otherwise well-designed chronotherapeutic regimens.

Circadian misalignment is also closely linked to the chronic sleep disturbances, fatigue, and depression experienced by many patients following cancer diagnosis and treatment (Innominato et al., 2014; Telias and Wilcox, 2019). This relationship can establish a vicious cycle in which cancer and its therapies disrupt circadian rhythms, and the resulting circadian dysfunction in turn accelerates tumor progression, worsens quality of life, and reduces treatment efficacy (Hadadi et al., 2020). Addressing circadian health, therefore, is not merely an adjunct consideration but a potentially integral component of effective cancer care.

Future directions in cancer chronotherapy are likely to emphasize integrative and personalized approaches aimed at restoring or reinforcing circadian organization. Interventions such as scheduled light exposure, structured meal timing, and appropriately timed physical activity may help strengthen endogenous rhythms and enhance responsiveness to time-optimized chemotherapy (Gabriel and Zierath, 2019; Lewis et al., 2020). When combined with individualized chronotherapeutic drug scheduling, these strategies hold promise for improving both patient well-being and antitumor efficacy. Ultimately, the convergence of circadian biology, behavioral interventions, and precision oncology represents a compelling path forward for the next generation of personalized cancer chronotherapy.

19.7.5 Neuropsychiatric Disorders

Neuropsychiatric disorders are frequently accompanied by profound disruptions in circadian organization and gut microbial ecology. Individuals with major depressive disorder commonly exhibit abnormalities in circadian rhythms, including blunted or flattened cortisol secretion profiles, delayed or fragmented sleep–wake cycles, and a reduced amplitude of daily physiological rhythms (Hickie et al., 2013). Parallel to these circadian alterations, the gut microbiome of depressed individuals differs markedly from that of healthy controls, with consistent reductions in butyrate-producing taxa and overall microbial diversity.

Causal evidence for microbiome involvement in mood regulation comes from FMT studies. Transfer of gut microbiota from patients with depression into germ-free mice induces depression-like phenotypes, including anhedonia, anxiety-like behavior, and altered stress responsivity, demonstrating that microbial communities can directly influence affective behavior (Kelly et al., 2016; Zheng et al., 2016). Mechanistically, microbiome-brain communication in depression involves multiple converging pathways, including dysregulated tryptophan metabolism and serotonergic signaling, reduced production of SCFAs, increased intestinal permeability with systemic inflammation, and bidirectional signaling through the vagus nerve (Cryan et al., 2019).

Importantly, microbiome alterations are not confined to a single psychiatric diagnosis but instead reveal transdiagnostic patterns across multiple mental health conditions. Systematic reviews and large cohort analyses consistently report the depletion of *Faecalibacterium* and *Coprococcus*—both key SCFA-producing genera—and the enrichment of *Eggerthella* in patients with depression, bipolar disorder, schizophrenia, and anxiety disorders. These changes are accompanied by reduced α-diversity compared with healthy controls, suggesting a shared microbial signature of psychiatric vulnerability rather than disorder-specific dysbiosis (Valles-Colomer et al., 2019; Pan et al., 2022).

Together, these findings support a model in which circadian disruption and microbiome dysregulation interact to influence neuropsychiatric health. Altered microbial metabolism may impair circadian signaling and neuroimmune balance, while disrupted circadian rhythms further destabilize microbial community structure, creating self-reinforcing loops that contribute to mood and cognitive dysfunction. This emerging framework highlights the gut-brain-circadian axis as a promising target for novel therapeutic strategies in psychiatric disorders.

19.7.5.1 Aging and Its Associated Disorders

The convergence of circadian disruption, gut microbiome dysbiosis, and aging represents a central axis in understanding age-related physiological

decline and neurodegenerative disease. Aging is accompanied by a progressive weakening of circadian rhythmicity and marked alterations in microbiome composition, and growing evidence suggests that these parallel changes may reinforce one another, accelerating biological aging and vulnerability to disease.

Factors associated with healthy longevity include the maintenance of a diverse and resilient gut microbiome, the production of beneficial microbial metabolites, efficient nutrient-sensing pathways, strong social engagement, psychological well-being, robust circadian regulation, balanced nutrition, and regular physical activity (Acosta-Rodríguez et al., 2021; Hassan et al., 2025). In contrast, accelerated aging is driven by chronic low-grade inflammation, epigenetic drift, oxidative stress, mitochondrial dysfunction, circadian misalignment, and genomic instability (Verma et al., 2025; Kondratov et al., 2006). Within this framework, circadian integrity and microbiome health emerge as core determinants of successful aging rather than secondary modifiers.

Advancing age is associated with characteristic changes in the circadian system, including reduced rhythm amplitude, increased fragmentation of sleep–wake cycles, and a tendency toward phase advancement. Behavioral and physiological rhythms become less stable, and circadian misalignment contributes to metabolic dysfunction that may itself hasten aging processes (Duffy et al., 2015; Kondratova and Kondratov, 2012). Although direct causal links between altered clock gene expression and aging pathology remain under investigation, many CCGs regulate pathways central to age-related diseases, including metabolism, inflammation, and cellular stress responses (Park et al., 2021; Logan and McClung, 2019). These circadian changes often coincide with shifts toward pro-inflammatory microbiome configurations, characterized by reduced microbial diversity and the diminished production of beneficial metabolites.

With increasing age, the disruption of natural biological rhythms becomes more common, frequently manifesting as sleep disturbances, impaired energy homeostasis, and the reduced stability of the gut microbiome (Hood and Amir, 2017; Ghosh et al., 2022). Distinguishing the

features of healthy aging from those of pathological aging remains a major challenge, and recognition of the interdependence between circadian regulation and microbial ecology is essential for addressing this question.

The brain-gut-microbiome axis plays a particularly prominent role in neurodegenerative diseases, where circadian dysfunction and dysbiosis may act synergistically to drive pathology. Disruption of circadian rhythms and alterations in gut microbial composition are increasingly linked to age-associated neurodegenerative disorders, with bidirectional communication between the brain and gut microbiota mediated through endocrine, immune, and neural pathways (Peng et al., 2022; Seo and Holtzman, 2021).

In Alzheimer's disease (AD), a bidirectional relationship exists between circadian disruption and disease pathology. Amyloid-β accumulation promotes the degradation of core circadian regulators such as *BMAL1* and CREB-binding protein (CBP), leading to circadian rhythm impairment (Song et al., 2015; Kress et al., 2018). Conversely, clock gene expression patterns in key brain regions differ significantly between patients with AD and healthy controls (Cermakian et al., 2011; Oh et al., 2018), suggesting that circadian dysfunction may both result from and contribute to disease progression. This reciprocal relationship creates a vicious cycle in which pathology and circadian disruption mutually reinforce one another.

The gut microbiome contributes to neurodegenerative processes through multiple mechanisms, including the altered production of neuroactive metabolites, increased intestinal permeability with the systemic dissemination of inflammatory mediators, and the modulation of neuroinflammation. Dysbiosis in aging is associated with reduced levels of beneficial microbial metabolites, particularly SCFAs, which are increasingly implicated in the pathogenesis of age-related neurodegenerative disorders (Ho et al., 2022b; Bostick et al., 2024). During pathological aging, rhythmic cortisol expression in the hippocampus may remain relatively preserved compared with melatonin rhythms (Waller et al., 2016; Ferrari et al., 1997), while aging is associated with marked reductions in hypocretin/orexin signaling, contributing to sleep

fragmentation and cognitive decline (Kessler et al., 2011; Porkka-Heiskanen et al., 2004).

Parkinson's disease (PD) provides a compelling example of circadian–microbiome–brain interaction. The Braak hypothesis proposes that α-synuclein pathology originates in the gut and spreads to the brain via the vagus nerve. Experimental studies have shown that the injection of α-synuclein preformed fibrils into the gut muscularis layer of mice results in the progressive propagation of pathology to the dorsal motor nucleus of the vagus, followed by the locus ceruleus and ultimately the substantia nigra over several months. Truncal vagotomy or the genetic deletion of α-synuclein prevents this gut-to-brain transmission, supporting a causal role for the vagal pathway.

Epidemiological data reinforce this mechanism. Large registry studies from Denmark and Sweden demonstrate that truncal vagotomy is associated with a reduced long-term risk of PD, whereas selective vagotomy confers no protection, suggesting that complete vagal denervation is required to interrupt disease propagation.

Circadian dysfunction is pervasive in PD, with sleep disorders affecting 60–98% of patients and insomnia prevalence ranging from 30% to 80%. Rapid eye movement (REM) sleep behavior disorder often precedes motor symptoms by 5–20 years or more and carries a high risk of phenoconversion to Parkinsonism or dementia, underscoring the early involvement of circadian and sleep systems in disease pathogenesis.

Consistent and reproducible microbiome alterations characterize PD. Meta-analyses reveal an increased abundance of *Akkermansia*, *Lactobacillus*, *Bifidobacterium*, *Verrucomicrobiaceae*, and *Desulfovibrio*, alongside reductions in anti-inflammatory and SCFA-producing taxa such as *Prevotella*, *Faecalibacterium*, *Lachnospiraceae*, *Roseburia*, *Blautia*, and *Coprococcus*. These shifts reflect a transition toward a pro-inflammatory microbial milieu. Constipation, a common prodromal symptom affecting over 60% of patients with PD, often predates motor manifestations and highlights the early enteric nervous system involvement in the disease process.

Together, these observations position age-related disorders as emergent properties of disrupted circadian-microbiome-brain interactions, suggesting that preserving rhythmic integrity and microbial resilience may be central to strategies aimed at promoting healthy aging and preventing neurodegenerative diseases.

19.8 Melatonin: A Multifunctional Chronobiotic and Microbiome Modulator

Melatonin (N-acetyl-5-methoxytryptamine) is a pleiotropic indoleamine with diverse physiological roles. It acts through multiple receptor pathways: MT_1 and MT_2 are G-protein-coupled membrane receptors, while MT_3 corresponds to the cytosolic enzyme quinone reductase 2, which exhibits potent antioxidant activity when bound to melatonin. In mammals, melatonin is primarily synthesized by the pineal gland in the brain, but substantial production also occurs in extrapineal sites, including the retina, gastrointestinal tract, and immune system (Bonmatí-Carrión and Rol, 2023).

19.8.1 Circadian Function and Chronobiotic Properties

As the principal chronobiotic hormone, pineal-derived melatonin conveys time-of-day information to the body, acting as a chemical signal of darkness. Its secretion follows a robust circadian rhythm, with nocturnal peaks and undetectable daytime levels, and is acutely suppressed by light at night. This rhythmic release enables the synchronization of peripheral and central circadian clocks, regulating sleep–wake cycles and other time-dependent physiological processes. While melatonin synthesized in extrapineal tissues exhibits its own circadian dynamics, the interplay between pineal and local rhythms remains incompletely understood (Bonmatí-Carrión and Rol, 2023).

19.8.2 Gastrointestinal Melatonin and Microbiome Interactions

The gastrointestinal tract produces melatonin in concentrations approximately 400 times higher than the pineal gland, primarily via enterochromaffin cells, but also through contributions from gut bacteria (Raikhlin et al., 1975; Kvetnoy et al., 2002; Zimmermann et al., 2024; Bubenik, 2002). Gut-derived melatonin regulates intestinal motility, protects the mucosa from oxidative stress, modulates immune function, and interacts with the microbiome. Its effects on the gut are both direct, via melatonin receptors expressed on intestinal epithelial cells, and indirect, through the circadian entrainment of microbial activity. This bidirectional communication supports gut homeostasis and overall health (Gupta et al., 2026).

The microbiome actively modulates intestinal melatonin synthesis. Certain bacterial species, such as *Lactobacillus reuteri* and *Escherichia coli*, stimulate melatonin production via the TLR2/4–MyD88–NF-κB signaling pathway, promoting the expression of arylalkylamine N-acetyltransferase (AANAT), the rate-limiting enzyme in melatonin biosynthesis. MyD88 deficiency in colonic epithelial cells abolishes this microbial influence, highlighting a direct mechanistic link between the gut microbiota and host circadian signaling (Liu et al., 2024a; Reigstad et al., 2015).

19.8.3 Melatonin Effects on Microbiome Composition

Melatonin also influences microbial community structure, creating positive feedback loops that reinforce gut and systemic homeostasis. It decreases the Firmicutes-to-Bacteroidetes ratio and reduces Proteobacteria abundance while increasing beneficial genera such as *Akkermansia*, *Lactobacillus*, and *Faecalibacterium* (Xu et al., 2017; Kim et al., 2020; Gao et al., 2019; Park et al., 2020). These shifts are associated with improved metabolic health, reduced inflammation, and enhanced circadian stability, indicating that melatonin supplementation may promote a favorable microbiome independently of its central chronobiotic functions.

19.8.4 Therapeutic Implications

Melatonin's dual role as a circadian regulator and microbiome modulator positions it as a promising intervention for conditions involving circadian-microbiome disruption, including metabolic disorders, IBD, mood disorders, and sleep disturbances (Iesanu et al., 2022; Zimmermann et al., 2024). Its safety profile, even at relatively high doses, supports broad translational potential. Optimal timing, dosing, and patient stratification remain key areas for future research.

19.8.5 Melatonin in Drug Metabolism and Chronotherapy

The gut microbiome plays a critical role in circadian drug metabolism. Microbial β-glucuronidases can reactivate hepatic glucuronide-conjugated drugs in the intestinal lumen, affecting chemotherapeutic and Non-steroidal anti-inflammatory drugs (NSAID) bioavailability. Bacterial tyrosine decarboxylases convert levodopa to dopamine pre-absorption, influencing PD treatment, while *Eggerthella lenta* reduces digoxin efficacy. Circadian rhythms in hepatic blood flow and drug-metabolizing gene expression further modulate absorption and metabolism, providing the rationale for chronotherapy to optimize efficacy and tolerability in cancer treatment and other diseases (Levi et al., 1997; Levi et al., 2024).

19.8.6 Neuroprotective and Anti-Inflammatory Actions

Melatonin exerts additional protective effects beyond circadian regulation. In spinal cord injury models, melatonin reduces oxidative stress by regulating malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and myeloperoxidase (MPO) levels. It also inhibits apoptosis, attenuates edema, and mitigates secondary tissue injury (Yang et al., 2016; Ghaisas et al., 2011).

19.8.7 Integration of Circadian and Microbiome Functions

Overall, melatonin serves as a central molecular messenger linking circadian timing with metabolic, immune, and microbial regulation. By synchronizing peripheral clocks, modulating microbial composition, and exerting antioxidant and immunomodulatory effects, melatonin integrates temporal and environmental cues to maintain systemic homeostasis. Understanding these multifaceted roles opens avenues for therapeutic interventions targeting circadian-microbiome axes in a variety of diseases.

19.9 Exercise as a Circadian-Microbiome Modulator

Physical activity is a powerful, integrative intervention that simultaneously enhances circadian rhythmicity and gut microbiome health through interconnected biological pathways. Beyond its well-established cardiovascular and metabolic benefits, exercise exerts profound regulatory effects on the circadian-microbiome axis, with broad implications for disease prevention, health maintenance, and therapeutic intervention across multiple physiological systems.

Regular moderate- to high-intensity exercise is consistently associated with increased gut microbial diversity, abundance, resilience, and functional versatility (Mailing et al., 2019; Cataldi et al., 2022). Both circadian rhythmicity and habitual physical activity directly shape microbial adaptation and stability (Liang and FitzGerald, 2017). Exercise-induced modulation of the microbiome is mediated through several mechanisms, including accelerated intestinal transit time, immune system remodeling, altered bile acid metabolism, and changes in intestinal pH and oxygen tension (Mailing et al., 2019). When combined with a balanced diet, regular physical activity promotes the production of beneficial microbial metabolites, supports intestinal environmental stability, and strengthens epithelial barrier integrity (Clauss et al., 2021; Clarke et al., 2014).

Exercise also directly influences circadian timing systems at both central and peripheral levels. Notably, exercise can induce phase shifts in peripheral clocks, particularly within skeletal muscle, without altering the

master clock located in the SCN (Wolff and Esser, 2012; Martin and Esser, 2022). This indicates that exercise timing serves as a potent *Zeitgeber* for peripheral tissues, allowing local clocks to be entrained independently. Such tissue-specific entrainment is thought to optimize metabolic efficiency, physical performance, and overall health, and may be especially beneficial for resynchronizing circadian rhythms disrupted by shift work, jet lag, or irregular lifestyles (Gabriel and Zierath, 2019; Kemler et al., 2020).

The sleep-enhancing effects of exercise further highlight its role in circadian regulation. Physical activity improves sleep quality through multiple mechanisms, including thermoregulatory effects, reductions in psychological stress, improvements in mood, and the reinforcement of circadian alignment (Banno et al., 2018; Yang et al., 2021). Emerging evidence suggests that exercise-induced alterations in gut microbiota composition may partially mediate these sleep benefits, underscoring the bidirectional nature of circadian-microbiome communication (Dalton et al., 2019; Wang et al., 2022b). In this context, the microbiome may act as an intermediary through which exercise enhances circadian stability and sleep architecture.

The protective role of exercise against cancer may also be mediated, in part, through circadian and microbiome-dependent pathways. Large-scale meta-analyses demonstrate that regular physical activity is associated with reduced cancer incidence and improved survival across multiple cancer types (Friedenreich et al., 2020; McTiernan et al., 2019). Proposed mechanisms include systemic anti-inflammatory effects, exercise-induced metabolite signaling, and enhanced immune surveillance (Pedersen and Febbraio, 2008; Severinsen and Pedersen, 2020). Given that both circadian disruption and microbiome dysbiosis are independently linked to cancer risk, the cancer-preventive effects of exercise likely arise from coordinated regulation across these interconnected systems (Chen et al., 2025).

Therapeutic strategies targeting circadian clocks increasingly emphasize the restoration of rhythmic homeostasis through interventions such as controlled light exposure, TRF, the minimization of shift work, and scheduled physical activity (Potter and Wood, 2020; Lowden et al., 2019).

These approaches operate in a bidirectional relationship with the gut microbiome, reinforcing metabolic and immune balance. Incorporating structured exercise prescriptions into treatment paradigms for metabolic disorders, mood disturbances, and other conditions associated with circadian-microbiome dysregulation represents a mechanistically informed, evidence-based strategy that addresses multiple pathogenic pathways simultaneously.

19.10 Chrononutrition and TRF

Chrononutrition examines how the timing of food intake interacts with circadian biology to regulate metabolism, gut microbial dynamics, and overall health. Most metabolic pathways oscillate with circadian rhythms, making the alignment of eating patterns with the body's internal clock, a powerful strategy for improving metabolic resilience, and reducing disease risk. TRF, a core chrononutrition approach, consolidates daily caloric intake into defined windows—typically 4–12 hours—without necessarily altering total caloric consumption.

Preclinical studies provide compelling evidence that TRF protects against metabolic disease by restoring circadian alignment. In mice fed high-fat diets, restricting food access to an 8- to 12-hour window prevents obesity, hepatic steatosis, and glucose intolerance despite equivalent caloric intake compared with ad libitum feeding (Hatori et al., 2012; Chaix et al., 2014). These benefits are accompanied by the restoration of robust diurnal oscillations in the gut microbiome, which are otherwise blunted by high-fat diets. Importantly, FMT from TRF-fed mice to metabolically compromised recipients partially transfers these protective effects, indicating that rhythmic microbial activity is a key mediator of TRF-induced metabolic improvements (Zarrinpar et al., 2014).

Human studies increasingly support these findings with TRE interventions associated with modest but consistent weight loss, improved insulin sensitivity and glucose tolerance, reductions in blood pressure, and

improvements in sleep quality (Wilkinson et al., 2020). Early time-restricted feeding (eTRF), in which food intake is confined to the early or mid-day hours, appears particularly effective. This pattern aligns food intake with circadian rhythms in insulin sensitivity, pancreatic β-cell responsiveness, and energy expenditure, which peak earlier in the day (Sutton et al., 2018). Observational and interventional studies of Ramadan fasting—a natural model of intermittent, TRE—also demonstrate significant shifts in gut microbiome composition and metabolic markers, suggesting that temporal eating patterns can reshape host-microbe interactions with downstream physiological consequences (Adawi et al., 2017).

Meal timing itself functions as a powerful *Zeitgeber* for peripheral clocks, particularly in the liver, gut, and adipose tissue. Disruption of microbial rhythmicity has been strongly linked to metabolic disease; arrhythmic gut microbiome signatures are predictive of type 2 diabetes (T2D) risk. Late-night eating, especially during periods of elevated melatonin, suppresses insulin secretion and impairs glucose tolerance, exacerbating circadian misalignment between central and peripheral clocks. Meta-analyses of TRE interventions in individuals with T2D reveal significant reductions in body weight and hemoglobin A_{1c} (HbA_{1c}), reinforcing the clinical relevance of aligning food intake with circadian timing (Reytor-Gonzalez et al., 2025).

Diet composition further interacts with circadian biology. Ketogenic diets, for example, exert strong effects on endogenous clocks. Experimental studies show that ketogenic feeding induces de novo circadian oscillations in the gut, mediated in part by the ketone body β-hydroxybutyrate, which acts as a signaling metabolite. This dietary pattern dramatically increases rhythmic gene expression across metabolic tissues. Similarly, SCFAs such as butyrate—produced by gut microbial fermentation—directly modulate clock gene expression in hepatocytes, rapidly influencing core clock components such as *Per2* and *Bmal1*. These findings underscore the role of microbial metabolites as molecular links between diet and circadian regulation (Gangitano et al., 2021).

Bioactive dietary compounds also influence circadian clocks. Polyphenols have emerged as potent modulators of clock gene function. Nobiletin, a citrus-derived flavonoid, enhances circadian clock amplitude and protects against high-fat diet-induced obesity and metabolic dysfunction in diabetic mouse models; notably, these effects are abolished in the absence of functional clock genes, demonstrating clock dependence. Resveratrol, another well-studied polyphenol, acts through SIRT1, a nutrient-sensing deacetylase that regulates circadian timing by deacetylating PER2 to promote its degradation while simultaneously facilitating *Bmal1* transcription. Through these mechanisms, dietary polyphenols reinforce circadian robustness and metabolic homeostasis (Man et al., 2020).

Collectively, these findings position chrononutrition—and TRF in particular—as a central strategy for restoring circadian alignment, stabilizing the gut microbiome, and improving metabolic health. Rather than focusing solely on caloric quantity or macronutrient composition, circadian-aligned eating emphasizes when food is consumed as a determinant of physiological function, offering a scalable and biologically grounded approach to disease prevention and healthy aging.

19.11 Microbiome-Based Interventions

Targeted manipulation of the gut microbiome has emerged as a promising therapeutic strategy for metabolic, inflammatory, and neurobehavioral disorders. Among the most accessible and evidence-based approaches is increased dietary fiber intake, which promotes microbial diversity and supports the production of beneficial metabolites such as SCFAs (Fu et al., 2022). High-fiber diets consistently enrich saccharolytic bacteria and enhance SCFA production, particularly butyrate, leading to improved gut barrier integrity, reduced systemic inflammation, and enhanced metabolic control. Clinical and preclinical studies demonstrate that prebiotic supplementation—non-digestible fibers selectively utilized by beneficial microbes—improves glucose tolerance, insulin sensitivity, lipid

metabolism, and inflammatory markers, highlighting the therapeutic potential of diet-driven microbiome modulation (Makki et al., 2018).

Probiotic supplementation represents another widely studied intervention, with strain-specific benefits observed across gastrointestinal, metabolic, and mental health conditions. Well-characterized probiotic strains, including *Lactobacillus* and *Bifidobacterium* species, have been shown to improve symptoms of irritable bowel syndrome (IBS), reduce antibiotic-associated diarrhea, modulate immune responses, and influence host metabolism and mood via gut-brain signaling pathways (Hill et al., 2014). However, the growing recognition of interindividual variability in probiotic responsiveness has shifted attention toward more personalized and next-generation probiotic approaches.

Next-generation probiotics are defined as commensal organisms with well-characterized functional roles in host physiology. Among these, *Akkermansia muciniphila* has attracted significant interest due to its inverse association with obesity, insulin resistance, and low-grade inflammation. Clinical trials demonstrate that supplementation with live or pasteurized *A. muciniphila* improves insulin sensitivity, lipid profiles, and markers of metabolic inflammation in individuals with metabolic syndrome (Depommier et al., 2019). These findings suggest that the targeted restoration of keystone microbial species may offer more precise therapeutic benefits than traditional probiotics.

Microbial metabolites themselves are increasingly recognized as therapeutic agents. Butyrate, a major SCFA produced by fiber-fermenting bacteria, exerts potent anti-inflammatory and epigenetic effects by serving as an energy source for colonocytes and acting as a histone deacetylase inhibitor. Direct butyrate supplementation and butyrate-enhancing dietary strategies have shown efficacy in IBD, where they reduce mucosal inflammation, strengthen epithelial barrier function, and upregulate circadian clock gene expression. This link between microbial metabolites and circadian regulation underscores the interconnected nature of the microbiome and host biological timing systems.

FMT represents the most direct method of microbiome manipulation and provides compelling proof-of-principle for microbiome-based therapies. FMT has demonstrated remarkable efficacy in treating recurrent *Clostridioides difficile* infection, with cure rates exceeding those of conventional antibiotic therapies. Beyond infectious diseases, metabolic applications of FMT have shown promise. The transplantation of microbiota from lean donors into individuals with metabolic syndrome resulted in improved peripheral insulin sensitivity and increased microbial diversity, although effects varied among recipients (Vrieze et al., 2012; Quraishi et al., 2017). These findings highlight both the therapeutic potential and the complexity of microbiome transfer, emphasizing the importance of donor selection, microbial engraftment, and host factors.

Collectively, microbiome-based interventions—including dietary fiber enrichment, prebiotics, probiotics, next-generation commensals, microbial metabolites, and FMT—represent a rapidly evolving therapeutic landscape. When integrated with circadian-aligned lifestyle strategies such as timed feeding, sleep optimization, and physical activity, these approaches offer a systems-level framework for restoring host-microbe homeostasis and improving health across a wide range of chronic diseases.

19.12 Chronotherapeutic Approaches

The recognition that circadian timing modulates drug absorption, metabolism, efficacy, and toxicity has led to the development of chronotherapy, an approach that aligns medication administration with endogenous biological rhythms. One of the most widely studied examples is antihypertensive therapy, where blood pressure regulation follows a strong circadian pattern. Early observational and interventional studies suggested that bedtime administration of antihypertensive agents produced superior nocturnal blood pressure control and reduced cardiovascular events. The MAPEC study reported a significant reduction in cardiovascular outcomes with bedtime dosing (Hermida et al., 2010). In contrast, the large, pragmatic

TIME trial found no significant difference between evening and morning dosing, highlighting important inconsistencies in the evidence base (Mackenzie et al., 2022).

Cancer chemotherapy represents one of the most established and biologically grounded applications of chronotherapy. Extensive experimental and clinical research has demonstrated that the timing of chemotherapeutic drug delivery can substantially influence both antitumor efficacy and toxicity profiles, reflecting circadian variation in cell cycle progression, DNA repair capacity, and drug metabolism (Lévi et al., 1997; Mormont and Lévi, 2003; Innominato et al., 2014; Lévi et al., 2024). Similarly, vaccination responses appear to be modulated by circadian timing, with multiple studies reporting enhanced antibody responses following morning administration, a phenomenon that likely reflects circadian regulation of innate and adaptive immune function (Long et al., 2016; Zhang et al., 2022; Ince et al., 2022).

Beyond cardiovascular and oncological contexts, chronotherapeutic principles extend to a wide range of pharmacological classes. Corticosteroid chronotherapy has shown promise in inflammatory diseases such as rheumatoid arthritis. In the CAPRA trials, modified-release prednisone administered at 10:00 PM—designed to release the drug at approximately 2:00 AM, coinciding with the nocturnal peak in pro-inflammatory cytokine production—demonstrated superior efficacy compared with conventional morning dosing (Buttgereit et al., 2011). Asthma management similarly benefits from timed drug delivery, as airway inflammation follows a circadian pattern. Inhaled corticosteroids show optimal efficacy when administered in the late afternoon, between 3:00 PM and 5:30 PM, aligning treatment with peak inflammatory activity (Pincus et al., 1995; Smolensky et al., 1999).

Statin chronotherapy illustrates the importance of pharmacokinetic considerations in chronotherapeutic optimization. Short half-life statins, such as simvastatin and lovastatin, are most effective when administered in the evening to coincide with nocturnal hepatic cholesterol synthesis. In contrast, long-acting statins, including atorvastatin and rosuvastatin,

maintain consistent efficacy regardless of dosing time due to their prolonged half-lives (Awad et al., 2017; Izquierdo-Palomares et al., 2016). This distinction underscores the necessity of integrating circadian biology with drug-specific properties when designing chronotherapeutic strategies.

Recent advances in cancer immunotherapy have revealed striking time-of-day effects in immune checkpoint inhibitor (ICI) treatment. Early daytime administration of ICIs has been associated with up to a fourfold improvement in survival compared with later administration, potentially mediated by circadian regulation of immune cell trafficking, activation, and tumor infiltration (Lévi et al., 2024). These findings suggest that circadian timing may become a critical variable in optimizing immuno-oncological treatment protocols.

Psychiatric pharmacotherapy represents an emerging frontier for chronotherapeutic applications. Given the central role of the circadian system in mood regulation, sleep architecture, and neurotransmitter dynamics, the timing of antidepressants, anxiolytics, and mood stabilizers may substantially influence both therapeutic efficacy and adverse effect profiles. Incorporating chronotype assessment into psychiatric treatment selection offers a promising pathway toward more personalized and effective mental health care (Colita et al., 2024).

The discrepancy between the TIME trial and earlier studies of antihypertensive chronotherapy provides a paradigm example of how study design and bias assessment shape clinical recommendations. While earlier trials reported dramatic reductions in cardiovascular events with bedtime dosing, the TIME trial, which enrolled 21,104 participants, observed no significant difference in primary outcomes between evening (3.4%) and morning (3.7%) dosing groups (HR 0.95; 95% CI 0.83–1.10; $p = 0.53$) (Mackenzie et al., 2022; Maqsood et al., 2023).

This controversy highlights the necessity of rigorous methodology, large-scale randomized trials, and careful bias assessment when evaluating chronotherapeutic claims. The failure of well-conducted randomized studies to replicate earlier high-effect estimates suggests that the benefits of antihypertensive chronotherapy may have been overstated, reinforcing the

need for cautious interpretation and evidence-based integration of circadian principles into clinical practice.

19.13 Personalized Medicine Approaches

The integration of chronotype assessment with microbiome profiling represents a pivotal advance in precision medicine, enabling interventions that are tailored to an individual's biological timing and microbial ecology. Chronotype can be assessed using validated questionnaire-based tools such as the Munich ChronoType Questionnaire (MCTQ), as well as objective measures including actigraphy. The gold-standard dim-light melatonin onset (DLMO) assessment reveals substantial interindividual variability, with phase differences of up to 4 hours between early and late chronotypes (Kantermann et al., 2015). Recent advances in wearable technology now allow continuous, real-world circadian monitoring, reducing reliance on labor-intensive laboratory protocols (Kim et al., 2024; Kang et al., 2023).

In parallel, microbiome profiling technologies have progressed from 16S rRNA gene sequencing toward shotgun metagenomics, enabling species- and strain-level resolution. Complementary metabolomics platforms quantify microbiome-derived metabolites, including SCFAs, secondary bile acids, and tryptophan derivatives, thereby capturing the functional outputs of microbial activity. Multi-omic integration approaches increasingly employ machine learning to simulate dietary, prebiotic, and probiotic interventions in silico, facilitating personalized strategies aimed at enhancing beneficial taxa such as the butyrate-producing *Faecalibacterium prausnitzii* (Thaiss et al., 2016; Liu et al., 2025). Together, these tools support the development of individualized chronotherapy and microbiome-optimized nutrition.

The personalized medicine paradigm for circadian-microbiome health extends beyond static profiling toward comprehensive systems biology frameworks. Wearable devices have transformed the capacity for continuous circadian assessment in everyday environments, with

accelerometry-based algorithms now capable of extracting digital circadian biomarkers from passively collected data (Kim et al., 2024). These biomarkers correlate with treatment response in mood disorders and may enable the dynamic, real-time adjustment of chronotherapeutic interventions (Kang et al., 2023).

Machine learning methods are increasingly applied to predict optimal intervention timing from complex multi-omic datasets. Algorithms trained on transcriptomic, metabolomic, and microbiome data can infer the individual circadian phase from single biological samples, potentially eliminating the need for repeated DLMO testing (Hesse et al., 2020; Braun et al., 2018; Hughey et al., 2017). The identification of compact gene expression signatures sufficient for accurate phase estimation represents a critical step toward scalable clinical implementation.

Plasma-metabolome-based biomarkers for circadian phase have emerged as particularly promising clinical tools, offering minimally invasive alternatives to melatonin-based assessments (Depner et al., 2022). At the conceptual level, the recognition that multiple clocks operate concurrently within individuals—biological time, social time, and solar time—has important implications for personalized intervention design, as misalignment among these clocks may drive disease risk and therapeutic variability (Klerman et al., 2020).

Systems biology approaches integrating circadian and microbiome data have also revealed sex-specific differences in host-microbiome rhythmicity, underscoring the importance of incorporating sex as a biological variable in personalized medicine (Weger et al., 2019). Experimental evidence indicates that the microbiome is required for sex-specific diurnal rhythms of host gene expression and metabolism, suggesting that chronotype, microbiome composition, and sex jointly shape optimal intervention strategies. Multi-omic pipelines combining 16S rRNA sequencing, shotgun metagenomics, metabolomics, and host transcriptomics are now providing unprecedented resolution of circadian-microbiome dynamics across health and disease states (Segers and Depoortere, 2021; Pearson et al., 2020).

Wearable devices increasingly provide validated circadian biomarkers with clinical relevance. Accelerometry-based measures of circadian phase correlate with biological aging and can predict mortality risk in large population cohorts. Although consumer-grade devices show systematic deviation from the DLMO gold standard, they remain valuable for longitudinal monitoring and population-scale research (Shim et al., 2024).

Ingestible microbiome monitoring technologies are also advancing rapidly. Self-powered biosensing capsules enable real-time measurement of intestinal metabolites, while smart pills incorporating engineered bacteria can detect inflammatory biomarkers in situ. pH-responsive polymer coatings further allow site-specific sampling of the gastrointestinal tract, opening new avenues for spatially resolved microbiome assessment (Min et al., 2025).

Finally, artificial intelligence (AI) and machine learning underpin the emerging field of personalized chronotherapy. Computational models can classify circadian versus non-circadian transcripts directly from DNA sequence features, while integrated chronotherapy algorithms synthesize timing information across molecular and physiological layers. This convergence of technologies is encapsulated in the concept of the "chronobiome," wherein remote sensors, physiological data streams, metagenomics, metabolomics, and transcriptomics are combined to detect and leverage temporal patterns across biological systems (Liang and FitzGerald, 2017).

19.14 Circadian Rhythm and Public Health Implications

At the population level, growing recognition of circadian biology as a determinant of long-term health has major implications for public policy. Evidence identifying critical developmental windows, together with the classification of shift work involving circadian disruption as a probable carcinogen, underscores the need for coordinated interventions across maternal–child health programs, workplace regulation, and urban planning

—particularly with respect to artificial light exposure. Approximately one-quarter of children in developed countries obtain insufficient sleep, a deficit associated with increased obesity risk independent of diet and physical activity. These findings suggest that public health strategies must incorporate circadian and microbiome optimization as preventive measures beginning early in life (Miller, 2018; Wang et al., 2022). In parallel, the elevated risks of cancer, metabolic disease, and accelerated biological aging observed in chronically disrupted shift workers demand serious consideration of work-schedule redesign, enhanced disease screening, and targeted preventive interventions for vulnerable occupational groups (IARC, 2019; Kettner, 2016; Kecklund & Axelsson, 2016).

Among population-level interventions, school start time reform has one of the strongest evidence bases. The American Academy of Pediatrics recommends that middle and high schools begin no earlier than 8:30 AM, aligning institutional schedules with adolescent circadian biology. California Senate Bill 328, implemented in July 2022, marked the first statewide mandate in the United States, affecting nearly three million students. Economic modeling suggests that later school start times could generate tens of billions of dollars in societal benefits through improved academic performance, better long-term health outcomes, and reductions in motor vehicle accidents caused by drowsy driving (Miller et al., 2018; Cappuccio et al., 2008).

Beyond school settings, social jetlag—the chronic mismatch between biological time and socially imposed schedules—affects a substantial proportion of working and studying adults in industrialized societies. Individuals experiencing pronounced social jetlag exhibit higher rates of mood and anxiety symptoms, metabolic dysregulation, and cardiometabolic risk. Importantly, exposure to circadian disruption is not evenly distributed across society (Roenneberg et al., 2023). Shift work follows clear socioeconomic gradients, with lower educational attainment and income associated with a higher likelihood of night or rotating shifts, thereby amplifying existing health inequities (Herrmann et al., 2023).

Racial and ethnic sleep disparities further compound circadian and microbiome disruption at the population level. African Americans experience a disproportionately higher risk of AD and related dementias compared with non-Hispanic White populations. Sleep research consistently shows that Black individuals, on average, spend less time in bed, obtain shorter sleep duration, and experience poorer sleep efficiency, with neighborhood safety, noise, and environmental stressors mediating many of these disparities (Johnson et al., 2019). At a broader ecological scale, geographic variation in microbiome composition reflects degrees of modernization: traditional hunter-gatherer populations exhibit markedly higher microbial richness than urbanized populations, with pronounced seasonal cycling that tracks dietary availability and lifestyle patterns (Ghosh et al., 2022). Together, these findings highlight how circadian disruption, social determinants of health, and microbiome alterations intersect to shape population health, reinforcing the need for equity-focused circadian public health strategies.

19.15 Conclusion

The bidirectional interplay between circadian rhythms and the gut microbiome represents a central organizing principle of human physiology. This deeply conserved partnership, shaped over hundreds of millions of years of coevolution, orchestrates biological processes across molecular, cellular, and systemic levels, creating a temporal framework essential for health from development through aging. Evidence from diverse experimental and clinical studies demonstrates that circadian and microbial systems are intimately interdependent; their synchronized function is crucial for optimal metabolic, immune, cognitive, and overall physiological health.

The integration of chronotype assessment with microbiome profiling offers a transformative avenue for precision medicine. Individual differences in circadian phase preference, as measured by DLMO, can vary

by up to 4 hours, while microbiome composition is highly personalized. These variations suggest that interventions tailored to individual temporal and microbial profiles—such as optimized medication timing, diet composition, exercise scheduling, and probiotic or prebiotic selection—may outperform generalized approaches. Personalized circadian-microbiome strategies thus hold promise for maximizing therapeutic efficacy and promoting long-term wellness.

Future research priorities include longitudinal studies tracking the development and evolution of circadian–microbiome interactions from prenatal stages through older age, mechanistic investigations into how exercise and dietary interventions modulate both systems, and clinical trials assessing whether preserving circadian-microbiome synchrony can delay cognitive decline, reduce cancer risk, or improve cardiometabolic outcomes. The refinement of chronotherapeutic protocols, the development of next-generation probiotics, and the practical translation of TRF and chrononutrition strategies represent critical steps toward clinical implementation.

By recognizing the circadian-microbiome relationship as a cornerstone of human health and applying evidence-based, temporally aligned interventions at molecular, cellular, and organismal levels, we can leverage these ancient biological rhythms and microbial partnerships to prevent disease, enhance resilience, and extend healthspan.

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References

Acosta-Rodríguez VA, Rijo-Ferreira F, Green CB, and Takahashi JS (2021) Importance of circadian timing for aging and longevity. *Nature Communications* 12:2862. ⏎

Adawi M, Watad A, Brown S, Aazza K, Aazza H, Zouhir M, Sharif K, Ghanayem K, Farah R, Mahagna H, and Amital H (2017) Ramadan fasting exerts immunomodulatory effects: insights from a systematic review. *Frontiers in Immunology* 8:1144. ⏎

Aiello I, Mul Fedele ML, Roman F, Marpegan L, Caldart C, Chiesa JJ, Golombek DA, Finkielstein CV, and Paladino N (2020) Circadian disruption promotes tumor-immune microenvironment remodeling favoring tumor cell proliferation. *Science Advances* 6:eaaz4530. ⏎

Albrecht U (2020) Molecular connections between circadian clocks and mood-related behaviors. *Journal of Molecular Biology* 432:3714–3721. ⏎

Altinok A, Lévi F, and Goldbeter A (2009) Identifying mechanisms of chronotolerance and chronoefficacy for the anticancer drugs 5-fluorouracil and oxaliplatin by computational modeling. *European Journal of Pharmaceutical Sciences* 36(1):20–38. ⏎

Anderson ST, Meng H, Brooks TG, Tang SY, Lordan R, Sengupta A, Nayak S, Mřela A, Sarantopoulou D, Lahens NF, Weljie A, Grant GR, Bushman FD, FitzGerald GA (2023, May 17) Sexual dimorphism in the response to chronic circadian misalignment on a high-fat diet. *Science Translational Medicine* 15(696):eabo2022. ⏎

Antoch MP, Gorbacheva VY, Vykhovanets O, Toshkov IA, Kondratov RV, Kondratova AA, Lee C, and Nikitin AY (2005) Disruption of the circadian clock due to the Clock mutation has discrete effects on aging and carcinogenesis. *Cell Metabolism* 2(4):233–241. ⏎

Awad K, Mikhailidis DP, Katsiki N, Muntner P, and Banach M (2017) Effect of ezetimibe monotherapy on plasma lipoprotein(a) concentrations in patients with primary hypercholesterolemia: a systematic review and meta-analysis of randomized controlled trials. *Drugs* 78(4):453–462. ⏎

Bafna A, Lau P, Banks G, and Nolan PM (2023) Harvesting mouse suprachiasmatic nucleus by vibrating microtome for diurnal transcriptome analysis. *STAR Protocols* 4(4):102618. ⏎

Bailes HJ and Lucas RJ (2013) Human melanopsin forms a pigment maximally sensitive to blue light supporting activation of G protein-coupled signaling cascades. *Proceedings of the Royal Society B: Biological Sciences* 280:20122987. ⏎

Ballesta A, Dulong S, Abbara C, Cohen B, Okyar A, Clairambault J, and Lévi F (2011) A combined experimental and mathematical approach for molecular-based optimization of irinotecan circadian delivery. *PLoS Computational Biology* 7(9):e1002143. ⏎

Banno M, Harada Y, Taniguchi M, Tobita R, Tsuda H, Takahashi K, Yagyu O, Tanigawa Y, Takeda R, and Yamamoto M (2018) Exercise can improve sleep quality: a systematic review and meta-analysis. *PeerJ* 6:e5172. ⏎

Bautista J and López-Cortés A (2025, Nov 4) Chronobiome medicine: circadian regulation of host–microbiota crosstalk in systemic physiology. *Front Endocrinol (Lausanne)* 16:1691172. ⏎

Bellengier C, Duez H, and Pourcet B (2022) Chapter 8: NLRP3 inflammasome pathway and the circadian clock. In: *Circadian Rhythms in Bacteria and Microbiomes*. Elsevier Inc. ⏎

Bernard S, Cajavec Bernard B, Lévi F, and Herzel H (2010) Tumor growth rate determines the timing of optimal chronomodulated treatment schedules. *PLoS Computational Biology* 6(3):e1000712. ⏎

Berson DM, Dunn FA, and Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295:1070–1073. ⏎

Bhattarai B, *et al*. (2025) Circadian circuits control plasticity of group 3 innate lymphoid cells by sustaining epigenetic configuration of RORγt. *Nature Immunology* 26:1527–1539. ⏎

Blaak EE, Canfora EE, Theis S, Frost G, Groen AK, Mithieux G, Nauta A, Scott K, Stahl B, van Harsselaar J, van Tol R, Vaughan EE, and Verbeke

K (2020, Sep 1) Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes* 11(5):411–455. ⏎

Boers E, *et al.* (2025) Projecting the 30-year burden of obstructive sleep apnoea in the USA: a prospective modelling study. *Lancet Respiratory Medicine*. ⏎

Bonmatí-Carrión MÁ and Rol MA (2023, Dec 23) Melatonin as a mediator of the gut microbiota–host interaction: implications for health and disease. *Antioxidants (Basel)* 13(1):34. ⏎

Bostick JW, Schonfeld M, and DaSilva NA (2024) Gut microbiota-derived short chain fatty acids: impact on cellular bioenergetics and metabolic homeostasis. *Critical Reviews in Food Science and Nutrition* 64:3700–3717. ⏎

Braun R, Kath WL, Iwanaszko M, Kula-Eversole E, Abbott SM, Reid KJ, Zee PC, and Allada R (2018) Universal method for robust detection of circadian state from gene expression. *Proceedings of the National Academy of Sciences USA* 115(39):E9247–E9256. ⏎

Brooks E and Canal MM (2013) Development of circadian rhythms: role of postnatal light environment. *Neuroscience and Biobehavioral Reviews* 37(4):551–560. ⏎

Brooks JF, *et al*. (2021) The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *Cell* 184:4154–4167.e12. ⏎

Brown AJ, Goldsworthy SM, Barnes AA, *et al*. (2003) The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *Journal of Biological Chemistry* 278(13):11312–11319. ⏎

Bubenik GA (2002) Gastrointestinal melatonin: localization, function, and clinical relevance. *Digestive Diseases and Sciences* 47:2336–2348. ⏎

Buttgereit F, Mehta D, Kirwan J, Szechinski J, Boers M, Alten RE, Supronik J, Szombati I, Romer U, Witte S, Saag KG, and CAPRA-2 Study Group (2011) Low-dose prednisone chronotherapy for rheumatoid arthritis: a randomised clinical trial (CAPRA-2). *Annals of the Rheumatic Diseases* 72(2):204–210. ⏎

Cao P, Li Y, Zhang S, Li C, Sun Y, and An B (2026) Study on the efficacy and mechanism of fecal microbiota transplantation for depression based on circadian rhythm. *Brain, Behavior, and Immunity* 131:106186. ⏎

Cappuccio FP, Taggart FM, Kandala NB, Currie A, Peile E, Stranges S, and Miller MA (2008) Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* 31:619–626. ⏎

Cash E, Sephton SE, Chagpar AB, Spiegel D, Rebholz WN, Zimmaro LA, Tillie JM, and Dhabhar FS (2021) Circadian disruption and biomarkers of tumor-promoting inflammation in breast cancer survivors. *Brain, Behavior, and Immunity* 91:539–548. ⏎

Cataldi S, Bonavolontà V, Poli L, Clemente FM, De Candia M, Carvutto R, Bellapianta F, Palumbo A, João PV, and Fischetti F (2022) The relationship between physical activity and human gut microbiome: a systematic review. *Biology* 11:479. ⏎

Cermakian N, Lamont EW, Boudreau P, and Boivin DB (2011) Circadian clock gene expression in brain regions of Alzheimer's disease patients and control subjects. *Journal of Biological Rhythms* 26:160–170. ⏎

Chaix A, Zarrinpar A, Miu P, and Panda S (2014) Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metabolism* 20(6):991–1005. ⏎

Chan S, Zhang L, Rowbottom L, McDonald R, Bjarnason GA, Tsao M, Danjoux C, Barnes E, Popovic M, Pulenzas N, and Chow E (2017) Does the time of radiotherapy affect treatment outcomes? A review of the literature. *Clinical Oncology* 29(4):231–238. ⏎

Chang WH and Lai AG (2019) The pan-cancer mutational landscape of the PPAR pathway reveals universal patterns of dysregulated metabolism and interactions with tumor immunity and hypoxia. *Annals of the New York Academy of Sciences* 1448(1):65–82. ⏎

Chen J, Li Y, Wang L, and Liu Q (2025) The molecular mechanisms of exercise in cancer prevention and management. *European Journal of Cancer Prevention* doi: 10.1097/CEJ.0000000000000989. ⏎

Chen P, Hsu WH, Chang A, Tan Z, Lan Z, Zhou A, Spring DJ, Lang FF, Wang YA, and DePinho RA (2020) Circadian regulator CLOCK recruits immune-suppressive microglia into the GBM tumor microenvironment. *Cancer Discovery* 10(3):371–381. ⏎

Chen PS, Chen LS, Fishbein MC, Lin SF, and Nattel S (2014, Apr 25) Role of the autonomic nervous system in atrial fibrillation: pathophysiology and therapy. *Circulation Research* 114(9):1500–1515. ⏎

Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, Chong LW, DiTacchio L, Atkins AR, Glass CK, *et al*. (2012) Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* 485:123–127. ⏎

Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R, *et al*. (2014) Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63:1913–1920. ⏎

Clauss M, Gérard P, Mosca A, and Leclerc M (2021) Interplay between exercise and gut microbiome in the context of human health and performance. *Frontiers in Nutrition* 8:637010. ⏎

Cobarro CG, Soares LI, Kutsenko Y, Tomas-Loba A (2025, Oct 17) Circadian system and aging: where both times interact. *Frontiers in Aging* 6:1646794. ⏎

Colita A, Colita D, Apostol A, Azoicai A, Cheles O, Popescu MC, Udristoiu T, and Zgura A (2024) Chronotype and psychiatric disorders. *Current Health Sciences Journal* 47(2):157–172. ⏎

Cui Z, Xu H, Wu F, *et al*. (2024) Maternal circadian rhythm disruption affects neonatal inflammation via metabolic reprograming of myeloid cells. *Nature Metabolism* 6:899–913. ⏎

Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S,

Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, and Dinan TG (2019, Oct 1) The microbiota–gut–brain axis. *Physiological Reviews* 99(4):1877–2013. ⏎

Dalton A, Mermier C, and Zuhl M (2019) Exercise influence on the microbiome–gut–brain axis. *Gut Microbes* 10:555–568. ⏎

Das B (2022) An introduction to human microbiome. *Progress in Molecular Biology and Translational Science* 191(1):1–28. ⏎

Davie JR (2003) Inhibition of histone deacetylase activity by butyrate. *Journal of Nutrition* 133(7 Suppl):2485S–2493S. ⏎

de Assis LVM, Kinker GS, Moraes MN, Markus RP, Fernandes PA, and Castrucci AML (2018a) Expression of the circadian clock gene BMAL1 positively correlates with antitumor immunity and patient survival in metastatic melanoma. *Frontiers in Oncology* 8:185. ⏎

de Assis LVM, Moraes MN, Castrucci AML, and Menaker M (2018b) The effect of white light on normal and malignant murine melanocytes: a link between opsins, clock genes, and melanogenesis. *Biochimica et Biophysica Acta—Molecular Cell Research* 1865(8):1119–1133.

Depner CM, Cogswell DT, Bisesi PJ, Markwald RR, Cruickshank-Quinn C, Quinn K, Melanson EL, Reisdorph N, and Wright KP Jr (2022) Developing preliminary blood metabolomics-based biomarkers of insufficient sleep in humans. *Sleep* 43(7):zsz321. ⏎

Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Falony G, Raes J, Maiter D, Delzenne NM, de Barsy M, Loumaye A, Hermans MP, Thissen JP, de Vos WM, and Cani PD (2019) Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nature Medicine* 25(7):1096–1103. ⏎

Diamantopoulou Z, Castro-Giner F, Schwab FD, Foerster C, Saini M, Budinjas S, Strittmatter K, Krol I, Seifert B, Heinzelmann-Schwarz V, *et al*. (2022) The metastatic spread of breast cancer accelerates during sleep. *Nature* 607:156–162. ⏎

Doi M, Hirayama J, and Sassone-Corsi P (2006) Circadian regulator CLOCK is a histone acetyltransferase. *Cell* 125:497–508. ⏎

Dong Z, Zhang G, Qu M, Gimple RC, Wu Q, Qiu Z, Prager BC, Wang X, Kim LJY, Morton AR, Dixit D, Zhou W, Huang Z, Mack SC, Serrano DM, Wang L, Hawes J, Prolo LM, Barrett LE, Gerner M, Bagley SJ, O'Rourke DM, Binder ZA, Nakano I, Koumenis C, Furnari F, Yang YM, and Rich JN (2019) Targeting glioblastoma stem cells through disruption of the circadian clock. *Cancer Discovery* 9(11):1556–1573. ⏎

Du D, Yuan Y, Guan X, Xie Q, Sun X, and Dong Z (2025, Dec) Gut microbiota, circadian rhythms and their interactions: implications for the pathogenesis and treatment of depression. *Annals of Medicine* 57(1):2561222. doi: 10.1080/07853890.2025.2561222

Duez H and Staels B (2009) Rev-erbα: an integrator of circadian rhythms and metabolism. *Journal of Applied Physiology* 107:1972–1980. ⏎

Duffy JF, Zitting KM, and Chinoy ED (2015) Aging and circadian rhythms. *Sleep Medicine Clinics* 10:423–434. ⏎

Duffy JF, Cain SW, Chang AM, Phillips AJ, Münch MY, Gronfier C, Wyatt JK, Dijk DJ, Wright KP Jr, and Czeisler CA (2011) Sex difference in the near-24-hour intrinsic period of the human circadian timing system. *Proceedings of the National Academy of Sciences USA* 108(Suppl 3):15602–8. ⏎

Dulong S, Ballesta A, Okyar A, and Lévi F (2015) Identification of circadian determinants of cancer chronotherapy through in vitro chronopharmacology and mathematical modeling. *Molecular Cancer Therapeutics* 14(9):2154–2164. ⏎

Erren TC and Lewis P (2019) Can yesterday's lung cancer hazards inform today's electro-magnetic field and night shift work research? *Journal of Occupational Medicine and Toxicology* 14:31. ⏎

Evans-Whipp T and Gasser C (2019) *Are Children Getting Enough Sleep? Growing Up in Australia: The Longitudinal Study of Australian Children*. Australian Institute of Family Studies. ⏎

Fan Y, Luo H, Zheng N, Li L, Zhang J, Li B, and Feng H (2026) Circadian clock genes Per1/Per2 deficiency induces premature age-related motor function decline in mice. *Physiology and Behavior* 308:115245. ⏎

Farshadi E, Yan J, Leclere P, Goldbeter A, Chaves I, and van der Horst GTJ (2020) The positive circadian regulators CLOCK and BMAL1 control G2/M cell cycle transition through Cyclin B1. *Cell Cycle* 18(1):16–33. ⏎

Fawad JA, Luzader DH, Hanber GF, *et al*. (2022) Histone deacetylase inhibition by gut microbe-generated short-chain fatty acids entrains intestinal epithelial circadian rhythms. *Gastroenterology* 163(5):1377–1390.e11. ⏎

Feng D, Liu T, Sun Z, *et al*. (2011) A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. *Science* 331(6022):1315–1319. ⏎

Ferrari E, Arcaini A, Gornati R, Pelanconi L, Cravello L, Fioravanti M, Solerte SB, and Magri F (1997) Pineal and pituitary–adrenocortical function in physiological aging and in senile dementia. *Chronobiology International* 14:385–396. ⏎

Firoozi D, Masoumi SJ, Mohammad-Kazem Hosseini Asl S, *et al*. (2024) Effects of short-chain fatty acid-butyrate supplementation on expression of circadian-clock genes, sleep quality, and inflammation in patients with active ulcerative colitis: a double-blind randomized controlled trial. *Lipids in Health and Disease* 23(1):216. ⏎

Fishbein AB, Knutson KL, and Zee PC (2021) Circadian disruption and human health. *Journal of Clinical Investigation* 131(19):e148286. ⏎

Friedenreich CM, Stone CR, Cheung WY, and Hayes SC (2020) Physical activity and mortality in cancer survivors: a systematic review and meta-analysis. *JNCI Cancer Spectrum* 4:pkz080. ⏎

Fu J, Zheng Y, Gao Y, and Xu W (2022) Dietary fiber intake and gut microbiota in human health. *Microorganisms* 10(12):2507. ⏎

Fu L and Lee CC (2003) The circadian clock: pacemaker and tumor suppressor. *Nature Reviews Cancer* 3:350–361. ⏎

Fu L, Pelicano H, Liu J, Huang P, and Lee CC (2002) The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 111(1):41–50. ⏎

Gabriel BM and Zierath JR (2019) Circadian rhythms and exercise: re-setting the clock in metabolic disease. *Nature Reviews Endocrinology* 15:197–206. ⏎

Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC, and Sancar A (2011) Control of skin cancer by the circadian rhythm. *Proceedings of the National Academy of Sciences USA* 108(46):18790–18795. ⏎

Gan Y, Yang C, Tong X, *et al*. (2015) Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occupational and Environmental Medicine* 72(1):72–78. ⏎

Gangitano E, Gnessi L, Lenzi A, and Ray D (2021) Chronobiology and metabolism: Is ketogenic diet able to influence circadian rhythm? *Frontiers in Neuroscience* 15:756970. ⏎

Gao T, Wang Z, Dong Y, Cao J, Lin R, Wang X, Yu Z, and Chen Y (2019) Role of melatonin in sleep deprivation-induced intestinal barrier dysfunction in mice. *Journal of Pineal Research* 67:e12574. ⏎

Garcia-Sainz M and Halberg F (1966) Mitotic rhythms in human cancer, reevaluated by electronic computer programs—evidence for temporal pathology. *Journal of the National Cancer Institute* 37(2):279–292. ⏎

Gaucher J, Montellier E, and Sassone-Corsi P (2018) Molecular cogs: interplay between circadian clock and cell cycle. *Trends in Cell Biology* 28(5):368–379. ⏎

Gauger MA and Sancar A (2005) Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. *Cancer Research* 65(15):6828–6834. ⏎

Ge X, Ding C, Zhao W, *et al.* (2017) Antibiotics-induced depletion of mice microbiota induces changes in host serotonin biosynthesis and intestinal motility. *Journal of Translational Medicine* 15:13. ⏎

Ghaisas MM, Ahire YS, Dandawate PR, Gandhi SP, Mule M (2011) Effects of combination of thiazolidinediones with melatonin in dexamethasone-

induced insulin resistance in mice. *Indian Journal of Pharmaceutical Science* 73:601–607. ⏎

Gholam D, Giacchetti S, Brézault-Bonnet C, Bouchahda M, Hauteville D, Adam R, Ducot B, Ghémard O, Kustlinger F, Jasmin C, Lévi F, and Innominato PF (2006) Chronomodulated irinotecan, oxaliplatin, and leucovorin-modulated 5-fluorouracil as ambulatory salvage therapy in patients with irinotecan- and oxaliplatin-resistant metastatic colorectal cancer. *Oncologist* 11(10):1072–1080. ⏎

Ghosh TS, Shanahan F, and O'Toole PW (2022) The gut microbiome as a modulator of healthy ageing. *Nature Reviews Gastroenterology and Hepatology* 19:565–584. ⏎

Gorbacheva VY, Kondratov RV, Zhang R, Cherukuri S, Gudkov AV, Takahashi JS, and Antoch MP (2005) Circadian sensitivity to the chemotherapeutic agent cyclophosphamide depends on the functional status of the CLOCK/BMAL1 transactivation complex. *Proceedings of the National Academy of Sciences USA* 102(9):3407–3412. ⏎

Granda TG, Liu XH, Smaaland R, Cermakian N, Filipski E, Sassone-Corsi P, and Lévi F (2005) Circadian regulation of cell cycle and apoptosis proteins in mouse bone marrow and tumor. *FASEB Journal* 19(2):304–306. ⏎

Gupta P, Debnath B, Ashique S, Ramzan M, Yasmin S, Shorog E, Mantry S, Tariq M, Sridhar SB, Panigrahy UP, Sharma H, Iqbal A, Prabhakar PK, and Ansari MY (2026, Jan) The melatonin–microbiome axis: a new frontier in gut health for the immunomodulatory, antioxidant and anti-inflammatory properties. *Inflammopharmacology* 34(1):227–242. ⏎

Hadadi E, Taylor W, Li XM, Aslan Y, Villote M, Rivière J, Duvallet G, Auriau C, Dulong S, Raymond-Letron I, *et al.* (2020) Chronic circadian disruption modulates breast cancer stemness and immune microenvironment to drive metastasis in mice. *Nature Communications* 11:3193. ⏎

Han B, Lv X, Liu G, Li S, Fan J, Chen L, Huang Z, Lin G, Xu X, Huang Z, Zhan L, Lv X (2023, Jan–Dec) Gut microbiota-related bile acid

metabolism-FXR/TGR5 axis impacts the response to anti-α4β7-integrin therapy in humanized mice with colitis. *Gut Microbes* 15(1):2232143. ⏎

Harris BE, Song R, Soong SJ, and Diasio RB (1990) Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Research* 50(1):197–201. ⏎

Hassan NU, Smith WK, Nawaz HA, and Wang H (2025) Resetting the circadian clock: implications for preventing age-related diseases and extending healthy lifespan. *Biogerontology* 26:156. ⏎

Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, BushongEA, Gill S, Leblanc M, Chaix A, Joens M, Fitzpatrick JA, Ellisman MH, and Panda S (2012) Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism* 15(6):848–860. ⏎

Hattar S, Liao HW, Takao M, Berson DM, and Yau KW (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295:1065–1070. ⏎

Häusler S, Lanzinger E, Sams E, Fazelnia C, Allmer K, Binder C, Reiter RJ, and Felder TK (2024) Melatonin in human breast milk and its potential role in circadian entrainment: a nod towards chrononutrition? *Nutrients* 16(10):1422. ⏎

Heddes M, Altaha B, Niu Y, *et al.* (2022) The intestinal clock drives the microbiome to maintain gastrointestinal homeostasis. *Nature Communications* 13:6068. ⏎

Heppner N, Reitmeier S, Heddes M, Kleigrewe K, Haller D, and Kiessling S (2024) Infant microbiota exhibits diurnal rhythms in expression of microbial molecular clocks and butyrate production but not composition. *Cell Host and Microbe* 32:573–587. ⏎

Herrmann AM, Zaal PM, Chappin MMH, Schemmann B, and Lühmann A (2023) “We don’t need no (higher) education”—How the gig economy

challenges the education-income paradigm. *Technological Forecasting and Social Change* 186(Part A):122136. ⏎

Hermida RC, Ayala DE, Mojón A, and Fernández JR (2010) Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiology International* 27(8):1629–1651. ⏎

Hesse J, Malhan D, Yalçin M, Aboumanify O, Basti A, and Relógio A (2020) An optimal time for treatment—predicting circadian time by machine learning and mathematical modelling. *Cancers* 12(11):3103. ⏎

Hickie IB, Naismith SL, Robillard R, Scott EM, and Hermens DF (2013, Mar 22) Manipulating the sleep–wake cycle and circadian rhythms to improve clinical management of major depression. *BMC Medicine* 11:79. ⏎

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, and Sanders ME (2014) Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology* 11(8):506–514. ⏎

Hirayama J, Sahar S, Grimaldi B, Tamaru T, Takamatsu K, Nakahata Y, and Sassone-Corsi P (2007) CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature* 450:1086–1090. ⏎

Ho FK, Celis-Morales C, Gray SR, Demou E, Mackay D, Welsh P, Katikireddi SV, Sattar N, and Pell JP (2022, a) Association and pathways between shift work and cardiovascular disease: a prospective cohort study of 238 661 participants from UK Biobank. *International Journal of Epidemiology*51(2):579–590. ⏎

Ho L, Ono K, Tsuji M, Mazzola P, Singh R, and Pasinetti GM (2022ba) Protective roles of intestinal microbiota-derived short chain fatty acids in Alzheimer's disease-type dementia. *Aging and Disease* 13:1252–1267.

Hood S and Amir S (2017) The aging clock: circadian rhythms and later life. *Journal of Clinical Investigation* 127:437–446. ⏎

Hrushesky WJM (1985) Circadian timing of cancer chemotherapy. *Science*, 228(4695):73–75. ⏎

Hrushesky WJM, Roemeling RV, Lanning RM, and Rabatin JT (1998) Circadian-shaped infusions of floxuridine for progressive metastatic renal cell carcinoma. *Journal of Clinical Oncology* 8(9):1504–1513. ⏎

Hughey JJ (2017) Machine learning identifies a compact gene set for monitoring the circadian clock in human blood. *Genome Medicine* 9(1):19. doi:10.1186/s13073-017-0406-4 ⏎

Hunter AL, Bechtold DA (2025, Mar 26) The metabolic significance of peripheral tissue clocks. *Communications Biology* 8(1):497. ⏎

IARC Monographs Vol 124 Group (2019) Carcinogenicity of night shift work. *Lancet Oncology* 20:1058–1059. ⏎

Iesanu MI, Zahiu CDM, Zagrean AM, and Zagrean L (2022) Melatonin–microbiome two-sided interaction in dysbiosis-associated conditions. *Antioxidants* 11:2244. ⏎

Ince LM, Zhang Z, Beesley S, Vonslow RM, Saer BRC, Matthews LC, Begley N, Gibbs JE, Ray DW, and Loudon ASI (2022) Circadian variation in pulmonary inflammatory responses is independent of rhythmic glucocorticoid signaling in airway epithelial cells. *FASEB Journal* 33(1): 126–139. ⏎

Innominato PF, Giacchetti S, Moreau T, Bjarnason GA, Focan C, Garufi C, Iacobelli S, Tampellini M, Tumolo S, Carvalho C, Karaboué A, Poncet A, and Lévi F (2020) Fatigue and weight loss predict survival on circadian chemotherapy for metastatic colorectal cancer. *Cancer Medicine* 9(23):8952–8963.

Innominato PF, Roche VP, Palesh OG, Ulusakarya A, Spiegel D, and Lévi FA (2014) The circadian timing system in clinical oncology. *Annals of Medicine* 46(4):191–207. ⏎

Inokawa H, Umemura Y, Shimba A, *et al.* (2020) Chronic circadian misalignment accelerates immune senescence and abbreviates lifespan in mice. *Scientific Reports* 10:2569. ⏎

Iurisci I, Filipski E, Reinhardt J, Bach S, Gianella-Borradori A, Iacobelli S, Meijer L, and Lévi, F (2006) Improved tumor control through circadian clock induction by Seliciclib, a cyclin-dependent kinase inhibitor. *Cancer Research* 66(22):10720–10728. ⏎

Iurisci I, Cumashi A, Sherman AA, Tsvetkov PO, Tinari N, Piccolo E, D'Egidio M, Adlouni M, Mammola CL, Natoli C, Iacobelli S, and Lévi F (2009) Synthetic inhibition of circadian clock protein cryptochrome enhances INF-alpha-driven apoptosis in human renal cancer cells. *Chronobiology International* 26(6):1062–1079. doi:10.3109/07420520903218962 ⏎

Izquierdo-Palomares JM, Fernandez-Tabera JM, Plana MN, Fernandez-Fontecha E, Fernandez-Perez C, Azcona L, and Gomez A (2016) Atorvastatin should be administered at bedtime to have a more significant effect on lipids: a pilot study. *Revista Española de Cardiología (English Edition)* 69(3):265–271. doi:10.1016/j.recesp.2015.09.029 ⏎

Jenni OG and LeBourgeois MK (2006) Understanding sleep–wake behavior and sleep disorders in children: the value of a model. *Current Opinion in Psychiatry* 19:282–287. ⏎

Jiang X, Zheng J, Zhang S, Wang B, Wu C, Guo X (2020) Advances in the involvement of gut microbiota in pathophysiology of NAFLD. *Frontiers in Medicine* 7:361. ⏎

Johnson DA, Jackson CL, Williams NJ, and Alcántara C (2019) Are sleep patterns influenced by race/ethnicity—a marker of relative advantage or disadvantage? Evidence to date. *Nature and Science of Sleep* 11:79–95. ⏎

Johnston PG, Lenz HJ, Leichman CG, Danenberg KD, Allegra CJ, Danenberg PV, and Leichman L (1995) Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Research* 55(7):1407–1412. ⏎

Joseph D, Chong NW, Shanks ME, *et al.* (2015) Getting rhythm: how do babies do it? *Archives of Disease in Childhood Fetal and Neonatal Edition* 100:F50–F54. ⏎

Jung-Hynes B, Huang W, Reiter RJ, and Ahmad N (2010) Melatonin resynchronizes dysregulated circadian rhythm circuitry in human prostate cancer cells. *Journal of Pineal Research* 49(1):60–68. ⏎

Kang TH, Reardon JT, Kemp M, and Sancar A (2009) Circadian oscillation of nucleotide excision repair in mammalian brain. *Proceedings of the National Academy of Sciences USA* 106(8):2883–2888. doi:10.1073/pnas.0812638106 ⏎

Kang TH, Lindsey-Boltz LA, Reardon JT, and Sancar A (2010) Circadian control of XPA and excision repair of cisplatin-DNA damage by cryptochrome and HERC2 ubiquitin ligase. *Proceedings of the National Academy of Sciences USA* 107(11):4890–4895. ⏎

Kang SG, Seo BK, Choi SJ, Jang S, Kim SJ, Cho HS, and Park YM (2023) Association between sleep and circadian rhythms disruption and major depressive disorder. *Journal of Clinical Sleep Medicine* 19(8):1495–1503. ⏎

Kantermann T, Sung H, and Burgess HJ (2015) Comparing the Morningness-Eveningness Questionnaire and Munich ChronoType Questionnaire to the dim light melatonin onset. *Journal of Biological Rhythms* 30(5):449–453. ⏎

Karl JP, Whitney CC, Wilson MA, Fagnant HS, Radcliffe PN, Chakraborty N, Campbell R, Hoke A, Gautam A, Hammamieh R, and Smith TJ (2023) Severe, short-term sleep restriction reduces gut microbiota community richness but does not alter intestinal permeability in healthy young men. *Scientific Reports* 13:27463. ⏎

Karlsson FH, Tremaroli V, Nookaew I, *et al.* (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498(7452):99–103. ⏎

Kelly JR, Borre Y, O' Brien C, Patterson E, el Aidy S, Deane J, Kennedy PJ, Beers S, Scott K, Moloney G, Hoban AE, Scott L, Fitzgerald P, Ross

P, Stanton C, Clarke G, Cryan JF, and Dinan TG (2016) Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research* 82:109–18. doi: 10.1016/j.jpsychires.2016.07.019 ⏎

Kemler D, Wolff CA, and Esser KA (2020) Time-of-day dependent effects of contractile activity on the phase of the skeletal muscle clock. *Journal of Physiology* 598:3631–3644. ⏎

Kessler BA, Stanley EM, Frederick-Duus D, and Fadel J (2011) Age-related loss of orexin/hypocretin neurons. *Neuroscience* 178:82–88. ⏎

Kettner NM, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, Katchy CA, Lee C, Moore DD, and Fu L (2016) Circadian homeostasis of liver metabolism suppresses hepatocarcinogenesis. *Cancer Cell* 30:909–924. ⏎

Kiessling S, Beaulieu-Laroche L, Blum ID, Landgraf D, Welsh DK, Storch KF, Labrecque N, and Cermakian N (2017) Enhancing circadian clock function in cancer cells inhibits tumor growth. *BMC Biology* 15(1):13. ⏎

Kim SW, Kim S, Son M, Cheon JH, and Park YS (2020) Melatonin controls microbiota in colitis by goblet cell differentiation and antimicrobial peptide production through Toll-like receptor 4 signalling. *Scientific Reports* 10:2232. ⏎

Kim S, Sohn J, Kim Y, and Kim JW (2024) Validation of wrist-worn consumer sleep and activity trackers in adults and elderly in real-life settings. *Scientific Reports* 14(1):8239. doi:10.1038/s41598-024-58741-9 ⏎

Klerman EB, Brager A, Carskadon MA, Depner CM, Foster R, Goel N, Harrington M, Holloway PM, Knauth P, Lear SA, Qian J, Sletten TL, and Smith MR (2020) Keeping an eye on circadian time in clinical research and medicine. *Clinical and Translational Medicine* 10(2):e116. doi:10.1002/ctm2.116 ⏎

Klevecz RR, Shymko RM, Blumenfeld D, and Braly PS (1987) Circadian gating of S phase in human ovarian cancer. *Cancer Research* 47(24 Pt 1):6267–6271. ⏎

Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 165(6):1332–1345. ⏎

Kohsaka A, Laposky AD, Ramsey KM, *et al.* (2007) High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism* 6(5):414–421. ⏎

Kojetin DJ and Burris TP (2014) REV-ERB and ROR nuclear receptors as drug targets. *Nature Reviews Drug Discovery* 13:197–216. ⏎

Kok EY, Kaur S, Mohd Shukri NH, Teo BW, Lim SB, Badon SE, and Lee YS (2024) The effect of light exposure on infant circadian rhythm and sleep at 6 months: insights from a prospective Asian birth cohort study. *European Journal of Pediatrics* 184:112. ⏎

Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, and Antoch MP (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes and Development* 20:1868–1873. ⏎

Kondratova AA and Kondratov RV (2012) The circadian clock and pathology of the aging brain. *Nature Reviews Neuroscience* 13:325–335. ⏎

Koritala BSC, Porter KI, Arshad OA, Gajula RP, Mitchell HD, Arman T, Manrique C, Newman R, Gonzalez RJ, Reiter RJ, and Chaudhari A (2021) Night shift schedule causes circadian dysregulation of DNA repair genes and elevated DNA damage in humans. *Journal of Pineal Research* 70(3):e12726. ⏎

Kress GJ, Liao F, Dimitry J, Cedeno MR, FitzGerald GA, Holtzman DM, and Musiek ES (2018) Regulation of amyloid-β dynamics and pathology by the circadian clock. *Journal of Experimental Medicine* 215:1059–1068. ⏎

Kurth S, Olini N, Huber R, and LeBourgeois MK (2015) Sleep and early cortical development. *Current Sleep Medicine Reports* 1:64–73. ⏎

Kvetnoy IM, Ingel IE, Kvetnaia TV, Malinovskaya NK, Rapoport SI, Raikhlin NT, Trofimov AV, and Yuzhakov VV (2002) Gastrointestinal

melatonin: cellular identification and biological role. *Neuroendocrinology Letters* 23:121–132. ⏎

LaBouyer M, Holtrop G, Horgan G, *et al.* (2022) Higher total faecal short-chain fatty acid concentrations correlate with increasing proportions of butyrate and decreasing proportions of branched-chain fatty acids across multiple human studies. *Gut Microbiome* 3:e2. ⏎

Le Poul E, Loison C, Struyf S, *et al.* (2003) Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *Journal of Biological Chemistry* 278(28):25481–25489. ⏎

LeBourgeois MK, Wright KP Jr, LeBourgeois HB, and Jenni OG (2013) Dissonance between parent-selected bedtimes and young children's circadian physiology influences nighttime settling difficulties. *Mind, Brain, and Education* 7:234–242. ⏎

Lee Y (2021) Roles of circadian clocks in cancer pathogenesis and treatment. *Experimental and Molecular Medicine* 53(10):1529–1538. doi:10.1038/s12276-021-00681-0 ⏎

Lee Y, Chun SK, and Kim K (2019) Sumoylation of the circadian clock protein PER2 regulates the circadian period. *Experimental and Molecular Medicine* 51(9):1–11. ⏎

Leone V, Gibbons SM, Martinez K, *et al.* (2015) Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 17(5):681–689. ⏎

Lewis P, Korf HW, Kuffer L, Groß JV, and Erren TC (2020) Exercise time cues (zeitgebers) for human circadian systems can foster health and improve performance: a systematic review. *BMJ Open Sport and Exercise Medicine* 6(1):e000443. ⏎

Lévi F, Okyar A, Dulong S, Innominato PF, and Clairambault J (2024) Circadian timing in cancer treatments. *Annu Rev Pharmacol Toxicol*. 50:377–421. doi: 10.1146/annurev.pharmtox.48.113006.094626 ⏎

Lévi F, Zidani R, and Misset JL (1997) Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in

metastatic colorectal cancer. *International Organization for Cancer Chronotherapy Lancet* 350:681–686. ⏎

Lévi F (2001) Circadian chronotherapy for human cancers. *Lancet Oncology* 2(5):307–315. ⏎

Lévi F, Misset JL, Brienza S, Adam R, Metzger G, Itzakhi M, Caussanel JP, Kunstlinger F, Lecouturier S, and Descorps-Declere A (1990) Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil and folinic acid in metastatic colorectal cancer. *European Journal of Cancer* 28A(2):341–346. ⏎

Liang X and FitzGerald GA (2017) Timing the microbes: the circadian rhythm of the gut microbiome. *Journal of Biological Rhythms* 32:505–515. ⏎

Lincoln DW, Hrushesky WJM, and Wood PA (2000) Circadian organization of thymidylate synthase activity in normal tissues: a possible basis for 5-fluorouracil chronotherapeutic advantage. *International Journal of Cancer* 88(3):479–485. ⏎

Liu B, Yu D, Sun J, Wu Y, and Xia K (2024a) Microbiota regulates peripheral clock through MyD88-dependent mechanism. *Gut Microbes* 16:2313769. ⏎

Liu J, Zou Y, Tang Y, Xi M, Xie L, Zhang Q, and Gong J (2019) Circadian clock gene regulatory networks and therapeutic targets for cancers. *Signal Transduction and Targeted Therapy* 4:15. ⏎

Liu L, Chang X, Liu Y, Hang D, Li X, Wu Y, Liu J, He Y, Wang C, Wang J, Pan A, and Giovannucci EL (2025) Joint association of metabolic syndrome and genetic susceptibility with colorectal cancer incidence and survival: a prospective cohort study. *American Journal of Clinical Nutrition* 121(1):29–39. ⏎

Liu X, *et al.* (2017) Bidirectional regulation of circadian disturbance and inflammation in inflammatory bowel disease. *Inflammatory Bowel Diseases* 23:1741–1751. ⏎

Liu X-L, Duan Z, Yu M, and Liu X (2024b) Epigenetic control of circadian clocks by environmental signals. *Trends in Cell Biology* 34(12):992–

1006.

Logan RW and McClung CA (2019) Rhythms of life: circadian disruption and brain disorders across the lifespan. *Nature Reviews Neuroscience* 20:49–65. ⏎

Long JE, Drayson MT, Taylor AE, Toellner KM, Lord JM, and Phillips AC (2016) Morning vaccination enhances antibody response over afternoon vaccination: a cluster-randomised trial. *Vaccine* 34(24):2679–2685. ⏎

Loudon AS (2012, Jul 24) Circadian biology: a 2.5 billion year old clock. *Current Biology* 22(14):R570–R571. ⏎

Lowden A, Moreno C, Holmbäck U, Lennernäs M, and Tucker P (2019) Eating and shift work: effects on habits, metabolism, and performance. *Industrial Health* 57:213–227. ⏎

Machiels K, *et al.* (2014) A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* 63:1275–1283. ⏎

Mackenzie IS, Rogers A, Poulter NR, Williams B, Brown MJ, Webb DJ, Ford I, Collier D, Findlay E, Grieve K, *et al.* (2022a) Cardiovascular outcomes in adults with hypertension with evening versus morning dosing of usual antihypertensives in the UK (TIME study): a prospective, randomised, open-label, blinded-endpoint clinical trial. *Lancet* 400:1417–1425. ⏎

Mailing LJ, Allen JM, Buford TW, Fields CJ, and Woods JA (2019) Exercise and the gut microbiome: a review of the evidence, potential mechanisms, and implications for human health. *Exercise and Sport Sciences Reviews* 47:75–85. ⏎

Makki K, Deehan EC, Walter J, and Bäckhed F (2018) The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host and Microbe* 23(6):705–715. ⏎

Man AWC, Xia N, Daiber A, and Li H (2020) The roles of gut microbiota and circadian rhythm in the cardiovascular protective effects of polyphenols. *British Journal of Pharmacology* 177(6):1278–1293. ⏎

Mann ER, Lam YK, and Uhlig HH (2024, Aug) Short-chain fatty acids: linking diet, the microbiome and immunity. *Nature Reviews Immunology* 24(8):577–595. ⏎

Maqsood MH, Messerli FH, Skolnick AH, Newman JD, Berger JS, and Bangalore S (2023) Timing of antihypertensive drug therapy: a systematic review and meta-analysis of randomized clinical trials. *Hypertension* 80:1544–1554. ⏎

Martin RA and Esser KA (2022) Time for exercise? Exercise and its influence on the skeletal muscle clock. *Journal of Biological Rhythms* 37:579–592. ⏎

Masri S and Sassone-Corsi P (2018) The emerging link between cancer, metabolism, and circadian rhythms. *Nature Medicine* 24:1795–1803. ⏎

Matsunaga N, Ikeda M, Takiguchi T, Koyanagi S, and Ohdo S (2018) The molecular mechanism regulating 24-hour rhythm of CYP2E1 expression in the mouse liver. *Hepatology* 48(1):240–251. ⏎

Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, and Okamura H (2003) Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 302(5643):255–259. ⏎

Mazzola R, Cuccia F, Figlia V, Rigo M, Nicosia L, Giaj-Levra N, Ricchetti F, Tomasini D, Vigo A, Di Paola G, Ruggieri R, Cavalleri S, Fiorentino A, and Alongi F (2021) Stereotactic body radiotherapy for oligometastatic castration sensitive prostate cancer: a propensity score matching analysis between testosterone-targeting therapy and hormonal castration-sparing therapy. *Radiation Oncology* 16(1):72. ⏎

McTiernan A, Friedenreich CM, Katzmarzyk PT, Powell KE, Macko R, Buchner D, Pescatello LS, Bloodgood B, Tennant B, Vaux-Bjerke A, *et al.* (2019) Physical activity in cancer prevention and survival: a systematic review. *Medicine and Science in Sports and Exercise* 51:1252–1261. ⏎

Lefta M, Campbell KS, Feng H-Z, Jin J-P, and Esser KA (2012) *American Journal of Physiology—Heart and Circulatory Physiology* 303:4. ⏎

Miller MA, Kruisbrink M, Wallace J, Ji C, and Cappuccio FP (2018) Sleep duration and incidence of obesity in infants, children, and adolescents: a systematic review and meta-analysis of prospective studies. *Sleep* 41:zsy018. ⏎

Min J, Ahn H, Lukas H, *et al.* (2025) Continuous biochemical profiling of the gastrointestinal tract using an integrated smart capsule. *Nature Electronics* 8:844–855. ⏎

Mormont MC and Lévi F (2003) Cancer chronotherapy: principles, applications, and perspectives. *Cancer* 97(1):155–169. ⏎

Mühlematter C, Schoch SF, Schmid A, Lauber F, Huwiler A, Koller T, Zbinden J, Meier N, Huber R, Zimmermann P, *et al.* (2025) Gut microbiota rhythmicity: a 24-h fecal sampling study in healthy infants. *Scientific Reports* 15:25424. ⏎

Natarajan N, Hori D, Flavahan S, Steppan J, Flavahan NA, Berkowitz DE, Pluznick JL (2016, Nov 1) Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiological Genomics* 48(11):826–834. ⏎

Oh ST, Seo J, Lee JH, Song JH, and Kang JS (2018) DNA methylation of the CLOCK gene promoter is associated with age-related circadian dysregulation in Alzheimer's disease. *Alzheimer's and Dementia* 14:899–909. ⏎

Olds T, Maher C, Blunden S, and Matricciani L (2010) Normative data on the sleep habits of Australian children and adolescents. *Sleep* 33:1381–1388. ⏎

Froy O and Weintraub Y (2025, Jul 9) The circadian clock, metabolism, and inflammation—the holy trinity of inflammatory bowel diseases. *Clinical Science (London)* 139(13):777–790. ⏎

Ozturk N, Lee JH, Gaddameedhi S, and Sancar A (2017) Loss of cryptochrome reduces cancer risk in p53 mutant mice. *Proceedings of the National Academy of Sciences USA* 114(12):3185–3190. ⏎

Pan A, Schernhammer ES, Sun Q, and Hu FB (2011) Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in

women. *PLoS Medicine* 8(12):e1001141. ⏎

Pan S, Ali K, Kahathuduwa C, *et al.* (2022, Feb 5) Meta-analysis of positive psychology interventions on the treatment of depression. *Cureus* 14(2):e21933.

Papagiannakopoulos T, Bauer MR, Davidson SM, Heimann M, Subbaraj L, Bhutkar A, Bartlebaugh J, Vander Heiden MG, and Jacks T (2016) Circadian rhythm disruption promotes lung tumorigenesis. *Cell Metabolism* 24:324–331. ⏎

Pariollaud M and Lamia KA (2020) Cancer in the fourth dimension: What is the impact of circadian disruption? *Cancer Discovery* 10(11):1694–1710. ⏎

Park S, Lee SK, Park Y, Lee S, and Yi S (2021) Circadian regulation in aging. *Translational Medicine of Aging* 5:1–9. ⏎

Park YS, Kim SH, Park JW, Kho Y, Seok PR, Shin JH, Choi YJ, Jun JH, and Kim SI (2020) Melatonin in the colon modulates intestinal microbiota in response to stress and sleep deprivation. *Intestinal Research* 18:325–336. ⏎

Patke A, Young MW, and Axelrod S (2020, Feb) Molecular mechanisms and physiological importance of circadian rhythms. *Nature Reviews Molecular Cell Biology* 21(2):67–84.

Pedersen BK and Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological Reviews* 88:1379–1406. ⏎

Peng W, Song R, Yu H, Sun Y, Wu X, and Fang J (2022) Circadian rhythms and the gut–brain axis in Alzheimer's disease: a bidirectional regulation. *Ageing Research Reviews* 78:101620. ⏎

Pincus DJ, Humeston TR, and Martin RJ (1995) Further studies on the chronotherapy of asthma with inhaled steroids: the effect of dosage timing on drug efficacy. *Journal of Allergy and Clinical Immunology* 95(6):1172–1178. ⏎

Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JI,

Eichmann A, Peti-Peterdi J, and Caplan MJ (2013, Mar 12) Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proceedings of the National Academic Science USA* 110(11):4410–4415. ⏎

Porkka-Heiskanen T, Alanko L, Kalinchuk A, and Stenberg D (2004) Adenosine and sleep deprivation. *Progress in Neurobiology* 73:151–152. ⏎

Potter GDM and Wood TR (2020) Greater freedom of choice regarding sleep and work times: a promising approach to managing circadian disruption, fatigue, and safety in shift work. *Frontiers in Nutrition* 7:116. ⏎

Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, Warren R, Tsao-Wei D, Groshen S, and Lenz HJ (2001) Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics Journal* 1(1):65–70. ⏎

Puram RV, Kowalczyk MS, de Boer CG, Schneider RK, Miller PG, McConkey M, Tothova Z, Tejero H, Heckl D, Järås M, Chen MC, Li H, Tamayo A, Cowley GS, Rozenblatt-Rosen O, Al-Shahrour F, Regev A, and Ebert BL (2016) Core circadian clock genes regulate leukemia stem cells in AML. *Cell* 165(2):303–316. ⏎

Qin J, Li Y, Cai Z, *et al.* (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490(7418):55–60. ⏎

Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, and Iqbal TH (2017) Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory *Clostridium difficile* infection. *Alimentary Pharmacology and Therapeutics* 46(5):479–493. doi:10.1111/apt.14201 ⏎

Raikhlin NT, Kvetnoy IM, and Tolkachev VN (1975) Melatonin may be synthesised in enterochromaffin cells. *Nature* 255:344–345. ⏎

Reigstad CS, Salmonson CE, Rainey JF 3rd, Szurszewski JH, Linden DR, Sonnenburg JL, Farrugia G, and Kashyap PC (2015) Gut microbes

promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB Journal* 29:1395–1403. ⏎

Reszka E, Przybek M, Muurlink O, and Pepłońska B (2019) Circadian gene variants and breast cancer. *International Journal of Molecular Sciences* 20:5673. ⏎

Reytor-González C, Simancas-Racines D, Román-Galeano NM, Annunziata G, Galasso M, Zambrano-Villacres R, Verde L, Muscogiuri G, Frias-Toral E, and Barrea L (2025) Chrononutrition and energy balance: how meal timing and circadian rhythms shape weight regulation and metabolic health. *Nutrients* 17(13):2135. ⏎

Roenneberg T (2023) How can social jetlag affect health? *Nature Reviews Endocrinology* 19:383–384. ⏎

Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB, and Danenberg PV (2000) Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clinical Cancer Research* 6(4):1322–1327. ⏎

Sancar A, Lindsey-Boltz LA, Kang TH, Reardon JT, Lee JH, and Ozturk N (2010) Circadian clock control of the cellular response to DNA damage. *FEBS Letters* 584(12):2618–2625. ⏎

Saran AR, Dave S, and Bhardwaj A (2020) Circadian rhythms in the pathogenesis and treatment of fatty liver disease. *Gastroenterology* 158(7):1948–1966. ⏎

Sarma A, Sharma VP, Sarkar AB, Sekar MC, Srivastava RK, and Mohanty S (2016) The circadian clock modulates anti-cancer properties of curcumin. *BMC Cancer* 16:759. doi:10.1186/s12885-016-2789-9 ⏎

Schoch SF, Castro-Mejía JL, Krych L, Lück S, Heisel T, Kirchner A, Meng C, Jakobsen RR, Nielsen DS, and Jenni OG (2022) Infant sleep, caregiving, and family functioning: complex interactions during a critical developmental period. *Progress in Neurobiology* 209:102208. ⏎

Sekirov I, Russell SL, Antunes LCM, and Finlay BB (2010) Gut microbiota in health and disease. *Physiological Reviews* 90(3):859–904. ⏎

Senaratna CV, Perret JL, Lodge CJ, Lowe AJ, Campbell BE, Matheson MC, Hamilton GS, and Dharmage SC (2017) Prevalence of obstructive sleep apnea in the general population: a systematic review. *Sleep Medicine Reviews* 34:70–81. ⏎

Seo DO and Holtzman DM (2021) Gut microbiota: from the forgotten organ to a potential key player in the pathology of Alzheimer's disease. *Gut Microbes* 13:1957407. ⏎

Severinsen MCK and Pedersen BK (2020) Muscle-organ crosstalk: the emerging roles of myokines. *Endocrine Reviews* 41:594–609. ⏎

Shafi AA, McNair CM, McCann JJ, Alshalalfa M, Shostak A, Severson TM, Zhu Y, Bergman A, Gordon N, Mandigo AC, Chand SN, Gallagher P, Dylgjeri E, Lal A, Leiby B, Greenfield EA, McCue P, Gomella LG, Lanchbury JS, Sailer V, Rbiger T, Feng FY, Tewari A, FitzGerald GA, Knudsen KE, and Astsaturov I (2021) The circadian cryptochrome, CRY1, is a pro-tumorigenic factor that rhythmically modulates DNA repair. *Nature Communications* 12(1):401. ⏎

Shan W, Zang W, and Zuo Z (2025) Sleep deprivation disrupts diurnal rhythmicity of gut microbiota and blood inflammatory cytokines in mice. *PLoS One* 20(11):e0335754. ⏎

Shim J, Fleisch E, and Barata F (2024) Circadian rhythm analysis using wearable-based accelerometry as a digital biomarker of aging and healthspan. *npj Digital Medicine* 7:146. ⏎

Shuboni-Mulligan DD, Breton G, Smart D, Gilbert M, and Armstrong TS (2019) Radiation chronotherapy—clinical impact of treatment time-of-day: a systematic review. *Journal of Neuro-Oncology* 145(3):415–427. ⏎

Slat EA, Sponagel J, Marpegan L, Simon T, Kfoury N, Kim A, Binz A, Herzog ED, Greeley S, Mrugala MM, Oliveira AM, Simon SM, Nicchitta CV, FitzGerald GA, and Hogenesch JB (2017) Cell-intrinsic, Bmal1-dependent circadian regulation of temozolomide sensitivity in glioblastoma. *Journal of Biological Rhythms* 32(2):121–129. ⏎

Smaaland R, Laerum OD, Lote K, Sletvold O, Sothern RB, and Bjerknes R (1991) DNA synthesis in human bone marrow is circadian stage dependent. *Blood* 77(12):2603–2611. ⏎

Smolensky MH, Lemmer B, and Reinberg AE (1999) Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. *Advanced Drug Delivery Reviews* 33(1–2):147–161. ⏎

Sokol H, *et al.* (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *PNAS* 105:16731–16736. ⏎

Song H, Moon M, Choe HK, Han DH, Jang C, Kim A, Cho S, Kim K, and Mook-Jung I (2015) Aβ-induced degradation of BMAL1 and CBP leads to circadian rhythm disruption in Alzheimer's disease. *Molecular Neurodegeneration* 10:13. ⏎

Sotak M, Sumová A, and Pácha J (2014) Cross-talk between the circadian clock and the cell cycle in cancer. *Annals of Medicine* 46(4):221–232. ⏎

Sothern RB, Levi F, Haus E, Halberg F, and Hrushesky WJ (1989) Control of a murine plasmacytoma with doxorubicin-cisplatin: dependence on circadian stage of treatment. *Journal of the National Cancer Institute* 81(2):135–145. ⏎

Summa KC, Voigt RM, Forsyth CB, Shaikh M, Cavanaugh K, *et al.* (2013) Disruption of the circadian clock in mice increases intestinal permeability and promotes alcohol-induced hepatic pathology and inflammation. *PLoS One* 8(6):e67102. ⏎

Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, and Peterson CM (2018) Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metabolism* 27(6):1212–1221e3. ⏎

Tahara Y, Yamazaki M, Sukigara H, *et al.* (2018) Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue. *Scientific Reports* 8(1):1395. ⏎

Tampellini M, Filipski E, Liu XH, Lemaigre G, Li XM, Vrignaud P, Francois E, Bissery MC, and Lévi F (1998) Docetaxel

chronopharmacology in mice. *Cancer Research* 58(17):3896–3904. ⏎

Tang Q, Cheng B, Xie M, Chen Y, Zhao J, Zhou X, and Chen L (2018) Circadian clock gene Bmal1 inhibits tumorigenesis and increases paclitaxel sensitivity in tongue squamous cell carcinoma. *Chronobiology International* 35:414–423. ⏎

Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, and Hazen SL (2013, Apr 25) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *New England Journal of Medicine* 368(17):1575–1584. ⏎

Tang Q, Cheng B, Xie M, Chen Y, Zhao J, Zhou X, and Chen L (2017) Circadian clock gene Bmal1 inhibits tumorigenesis and increases paclitaxel sensitivity in tongue squamous cell carcinoma. *Cancer Research* 77(2):532–544. ⏎

Tang Q, Hann SS, Chen Y, Xie M, and Cheng B (2019) Targeting circadian PER2 degradation to improve cancer therapy: a translational link from bench to clinic. *Molecular Pharmacology* 95(4):430–438. ⏎

Taniguchi H, Fernández AF, Setién F, Ropero S, Ballestar E, Villanueva A, Yamamoto H, Imai K, Shinomura Y, and Esteller M (2009) Epigenetic inactivation of the circadian clock gene BMAL1 in hematologic malignancies. *Cancer Research* 69(21):8447–8454. ⏎

Telias I and Wilcox ME (2019) Sleep and circadian rhythm in critical illness. *Critical Care* 23(1):82. ⏎

Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, Abramson L, Katz MN, Korem T, Zmora N, Kuperman Y, Biton I, Gilad S, Harmelin A, Shapiro H, Halpern Z, Segal E, Elinav E. (2018) Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 159(3): 514–29. doi: 10.1016/j.cell.2014.09.048.

Thaiss CA, Levy M, Korem T, Dohnalová L, Shapiro H, Jaitin DA, David E, Winter DR, Gury-BenAri M, Tatirovsky E, *et al.* (2016) Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167:1495–1510. ⏎

Thangaraju M, Cresci GA, Liu K, *et al.* (2009) GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Research* 69(7):2826–2832. ⏎

Torquati L, Mielke GI, Brown WJ, and Kolbe-Alexander T (2018, May 1) Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose–response relationship. *Scandinavian Journal of Work, Environment and Health* 44(3):229–238. ⏎

Tripathi A, Debelius J, Brenner DA, *et al.* (2018) The gut–liver axis and the intersection with the microbiome. *Nature Reviews Gastroenterology and Hepatology* 15(7):397–411. ⏎

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, and Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444(7122):1027–1031. ⏎

Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, Claes S, Van Oudenhove L, Zhernakova A, Vieira-Silva S, and Raes J (2019, Apr) The neuroactive potential of the human gut microbiota in quality of life and depression. *Nature Microbiology* 4(4):623–632. ⏎

van den Pol AN (1980) The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *Journal of Comparative Neurology* 191:661–702. ⏎

Verhaar BJH, Collard D, Prodan A, Levels JHM, Zwinderman AH, Bäckhed F, Vogt L, Peters MJL, Muller M, Nieuwdorp M, van den Born BJH (2020, Nov 21) Associations between gut microbiota, faecal short-chain fatty acids, and blood pressure across ethnic groups: the HELIUS study. *European Heart Journal* 41(44):4259–4267. ⏎

Verma AK, Singh S, and Rizvi SI (2025) Disruption of circadian clock and accelerated aging: role of oxidative stress and inflammation. *Biogerontology* 26:132. ⏎

Voigt RM, Forsyth CB, Green SJ, Engen PA, and Keshavarzian A (2016) Circadian rhythm and the gut microbiome. *International Review of Neurobiology* 131:193–205. ⏎

Voigt RM, Forsyth CB, Green SJ, Mutlu E, Engen P, *et al.* (2014) Circadian disorganization alters intestinal microbiota. *PLoS One* 9(5):e97500. ⏎

Vrieze A, Van Nood E, Holleman F, *et al.* (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143(4):913–916. ⏎

Vyas MV, Garg AX, Iansavichus AV, Costella J, Donner A, Laugsand LE, Janszky I, Mrkobrada M, Parraga G, and Hackam DG (2012) Shift work and vascular events: systematic review and meta-analysis. *BMJ* 345:e4800. ⏎

Wagner PM, Monjes NM, and Guido ME (2019) Chemotherapeutic effect of SR9009, a REV-ERB agonist, on the human glioblastoma T98G cells. *ASN Neuro* 11:1759091419892713.

Walker WH, Walton JC, DeVries AC, and Nelson RJ (2020) Circadian rhythm disruption and mental health. *Translational Psychiatry* 10(1):28. ⏎

Waller KL, Mortensen EL, Avlund K, Osler M, Fagerlund B, Lauritzen M, and Jennum P (2016) Melatonin and cortisol profiles in late midlife and their association with age-related changes in cognition. *Nature and Science of Sleep* 8:47–53. ⏎

Wang C, Zeng Q, Gül ZM, Zhang Y, Han I, Xu Y, Tao Q, Beutel AK, Lam E, Lin C, *et al*. (2024) Circadian regulation governs tumor cell infiltration dynamics into host tissues and dictates responsiveness to CD8+ T cell immunotherapy. *Cell* 187:2690–2702. ⏎

Wang D, Ruan W, Chen Z, Peng Y, and Li W (2018, Aug) Shift work and risk of cardiovascular disease morbidity and mortality: a dose–response meta-analysis of cohort studies. *European Journal of Preventive Cardiology* 25(12):1293–1302. ⏎

Wang Y, van de Wouw M, Drogos L, Fergusson G, Godbout R, Mandhane PJ, Moraes TJ, Simons E, Turvey SE, Subbarao P, *et al.* (2022a) Associations between sleep and gut microbiota in Canadian preschool children: results from the CHILD cohort study. *Sleep* 45:zsac020. ⏎

Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, and Hazen SL (2011, Apr 7) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472(7341):57–63.

Wang Z, Wang Z, Lu T, Chen W, Yan W, Yuan K, Shi L, Liu X, Zhou X, and Shi J (2022b) The microbiota–gut–brain axis in sleep disorders. *Sleep Medicine Reviews* 65:101691.

Wang Z, Li X, Zhou L, and Liu J (2021) Construction and validation of prognostic signature for glioma based on circadian rhythm-related genes. *Translational Neuroscience* 12(1):374–391. ⏎

Weintraub Y, *et al*. (2020) Clock gene disruption is an initial manifestation of inflammatory bowel diseases. *Clinical Gastroenterology and Hepatology* 18:115–122.e1. ⏎

Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, Wang X, Fleischer JG, Navlakha S, Panda S, and Taub PR (2020) Ten-hour time-restricted eating reduces weight, blood pressure, and atherogenic lipids in patients with metabolic syndrome. *Cell Metabolism* 31(1):92–104.e5. ⏎

Wolff CA and Esser KA (2012) Scheduled exercise phase-shifts the circadian clock in skeletal muscle. *Medicine and Science in Sports and Exercise* 44:1663–1670. ⏎

Wong SD, Wright KP Jr, Spencer RL, Vetter C, Hicks LM, Jenni OG, and LeBourgeois MK (2022) Development of the circadian system in early life: maternal and environmental factors. *Journal of Physiological Anthropology* 41:22. ⏎

Wood PA, Yang X, Taber A, Oh EY, Ansell C, Ayers SE, Al-Assaad Z, Carnevale K, Berger FG, Peña MM, and Hrushesky WJ (2008) Period 2 mutation accelerates ApcMin/+ tumorigenesis. *Molecular Cancer Research* 6(11):1786–1793. ⏎

Wu G, Zhang J, Yan S, Liu N, Chen W, Wu F, Wang Z, Zhang J, Yang Y, Deng Y, Qiu X, Liu J, Shi L, Cui X, Wan R, Li X, Han Y, Yang G.

Circadian rhythms and microbiota: molecular crosstalk and its implications for health and disease. *Biol Direct*. 21(1):47. doi: 10.1186/s13062-026-00748-w.

Xiang K, Xu Z, Hu YQ, He YS, Wu GC, Li TY, Wang XR, Ding LH, Zhang Q, Tao SS, Ye DQ, Pan HF, and Wang DG (2021, Aug) Circadian clock genes as promising therapeutic targets for autoimmune diseases. *Autoimmunity Reviews* 20(8):102866. ⏎

Xu P, Wang J, Hong F, Wang S, Jin X, Xue T, Jia L, and Zhai Y (2017) Melatonin prevents obesity through modulation of gut microbiota in mice. *Journal of Pineal Research* 62:e12399. ⏎

Yan M, Man S, Sun B, *et al*. (2023) Gut liver brain axis in diseases: the implications for therapeutic interventions. *Signal Transduction and Targeted Therapy* 8:443. ⏎

Yang PY, Ho KH, Chen HC, and Chien MY (2021) Exercise training improves sleep quality in middle-aged and older adults with sleep problems: a systematic review. *Frontiers in Psychiatry* 12:664499. ⏎

Yang T, Magee KL, Colon-Perez LM, Larkin R, Liao YS, Balazic E, Cowart JR, Arocha R, Redler T, Febo M, Vickroy T, Martyniuk CJ, Reznikov LR, and Zubcevic J (2019a, Jun) Impaired butyrate absorption in the proximal colon, low serum butyrate and diminished central effects of butyrate on blood pressure in spontaneously hypertensive rats. *Acta Physiology (Oxford)* 226(2):e13256. ⏎

Yang X, Wood PA, Ansell CM, Ohmori M, Oh EY, Xiong Y, Berger FG, Peña MM, and Hrushesky WJ (2009) Beta-catenin induces beta-TrCP-mediated PER2 degradation altering circadian clock gene expression in intestinal mucosa of ApcMin/+ mice. *Journal of Biochemistry* 145(3):289–297.

Yang Y, Yuan G, Xie H, Wei T, Zhu D, Cui J, Liu X, Gu J, and Xie Y (2019b) Circadian clock associates with tumor microenvironment in thoracic cancers. *Aging* 11(24):11814–11828. ⏎

Yang L, Yao M, Lan Y, Mo W, Sun YL, Wang J, Wang YJ, and Cui XJ (2016) Melatonin for spinal cord injury in animal models: a systematic

review and network meta-analysis. *Journal of Neurotrauma* 33:290–300. ⏎

Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, and Wymer M (2016) Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64(1):73–84. ⏎

Zarrinpar A, Chaix A, Yooseph S, and Panda S (2014) Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metabolism* 20(6):1006–1017. ⏎

Zeng ZL, Wu MW, Sun J, Sun YL, Cai YC, Huang YJ, and Xue L (2005) Effects of the biological clock gene Bmal1 on tumour growth and anti-cancer drug activity. *Journal of Biochemistry* 148(3):319–326. ⏎

Zeng ZL, Luo HY, Yang J, Wu WJ, Chen DL, Huang P, and Xu RH (2014) Overexpression of the circadian clock gene Bmal1 increases sensitivity to oxaliplatin in colorectal cancer. *Clinical Cancer Research* 20(4):1042–1052. ⏎

Zhang H, Telfer BA, Tonkin JL, Woodcock M, Shaw A, Skornyakov S, Truszkowski S, Lord JM, Ahiante BO, James ND, Inman M, O'Donnell VB, Williams S, Fielding JWL, and Sharma R (2022) Time-of-day influences immune response to an inactivated vaccine against SARS-CoV-2. *Journal of Biological Chemistry* 298(6):101946. ⏎

Zhang Q, Litwin C, Dietert K, Tsialtas I, Chen WH, Li Z, and Koronowski KB (2025) Frequent shifts during chronic jet lag uncouple liver rhythms from the light cycle in male mice. *Journal of Biological Rhythms* 40(2):194–207. ⏎

Zhang Z, Hunter L, Wu G, Maidstone R, Mizoro Y, Vonslow R, Fife M, Hopwood T, Begley N, Saer B, Wang P, Cunningham P, Baxter M, Durrington H, Blaikley JF, Hussell T, Rattray M, Hogenesch JB, Gibbs JE, Ray DW, and Loudon ASI (2021) Genome-wide effect of pulmonary airway epithelial cell-specific Bmal1 deletion. *FASEB Journal* 35(3):e21286. ⏎

Zhao N, Yang K, Yang G, Chen D, Tang H, Zhao D, and Zhao C (2013) Aberrant expression of clock gene Period2 promotes growth and metastasis of renal cell carcinoma. *Oncology Letters* 5(5):1547–1551. ⏎

Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, Zeng L, Chen J, Fan S, Du X, Zhang X, Yang D, Yang Y, Meng H, Li W, Melgiri ND, Licinio J, Wei H, and Xie P (2016, Jun) Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry* 21(6):786–796. ⏎

Zimmermann P, Kurth S, Pugin B, and Bokulich NA (2024) Microbial melatonin metabolism in the gut microbiome. *npj Biofilms and Microbiomes* 10:139. ⏎

20

Pitfalls in Microbiome Research

Ulrike Löber

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20.1 Introduction

Microbiome research has undergone a dramatic transformation since the early 2000s. The launch of the Human Microbiome Project marked a conceptual shift in biology: humans were no longer defined solely by their own genomes, but by the composite of host and microbial genetic material, a "supraorganism" composed of both somatic and symbiotic life (Turnbaugh et al., 2007). With this came the promise of new insights into immunity, metabolism, development, and disease.

As scientific momentum built, so too did commercial and public fascination. Microbiome analysis became a consumer product. Gut health turned into a marketable lifestyle. Influencers promoted unvalidated fasting routines and probiotics. Personalized nutrition companies promised insights derived from stool samples, often backed by limited or absent clinical evidence. A parallel narrative emerged in academic research, suggesting microbiome shifts could explain conditions as complex as obesity, cardiovascular disease, depression, or Parkinson's. Recent work has

proposed microbiota-based interventions as adjunctive strategies for treating or even preventing central nervous system disorders (Loh et al., 2024).

Researchers have begun working toward these aspirations, though consistently reproducible findings remain a challenge in many areas. Several large-scale studies have failed to replicate earlier associations. Others have revealed that methodological choices, sample collection, DNA extraction, sequencing depth, or normalization can introduce biases larger than the biological effects being studied (Costea et al., 2017). Metadata are often inconsistently recorded, contamination controls are frequently omitted, and definitions of "dysbiosis" vary with each paper.

Despite years of discussion, standardization remains elusive. Extraction protocols differ from lab to lab. Reference genomes are incomplete. Metadata reporting is patchy. Efforts to define a roadmap for robust microbiome science highlight the need for shared standards, validated controls, and coordinated metadata (Amos et al., 2020; McGuinness et al., 2024).

Technological progress is helping. Long-read sequencing approaches now enable the assembly of nearly complete genomes from complex microbial communities, thereby improving our understanding of strain-level functional diversity (Albertsen, 2023). Nevertheless, critical conceptual challenges remain, including how we define ecological baselines, determine causality, and translate microbial signals into diagnostics or therapies.

After two decades of microbiome research, many of the most-cited findings remain associations rather than mechanisms. The field must move beyond taxonomy to explore function, context, and interaction across ecological scales (Clavel et al., 2022).

This chapter critically examines the most consequential pitfalls across microbiome pipelines, from study design and sample handling to sequencing, annotation, statistics, and interpretation. Each section blends literature-based critique with real-world examples, including work from our own studies. The goal is not to discourage innovation, but to advocate for

methodological precision, humility, and reproducibility as the foundation for lasting impact.

Note: Throughout this chapter, we refer to several bioinformatic tools and analytical pipelines as examples to illustrate specific methodological points. These references are not intended as endorsements or a comprehensive review. They rather reflect tools I am familiar with or have found illustrative in practice. Many other excellent alternatives exist, and the field continues to evolve rapidly.

20.2 Pitfalls in Study Design

A recurring misconception in microbiome science is that sequencing DNA equates to observing microbial life. However, this assumption falters in low-biomass samples, where relic DNA can outweigh the live microbial signal. Standard nucleic acid-based workflows rarely differentiate between viable and dead cells, unless complemented by techniques like PMA treatment, RNA-based analysis, or BONCAT labeling (Emerson et al., 2017).

Technical variables in microbiome studies, such as sample collection protocols, DNA extraction methods, and sequencing batches, can introduce significant variation into microbial community profiles. In a controlled human study by Bartolomaeus et al. (2020) the impact of several commonly used collection and extraction protocols on the gut microbiome of healthy donors was systematically compared. Their analysis revealed that the choice of extraction method alone explained a comparable proportion of variation in microbial profiles as individual identity. At the same time, biological covariates such as age or body mass index (BMI) contributed substantially less (Bartolomaeus et al., 2020).

Notably, even when all other parameters were held constant, different protocols yielded divergent taxonomic abundances, leading to inconsistent enterotype classification and variation in the detection of health-associated taxa. These shifts were not limited to low-abundance organisms but also

included changes in the dominant phyla. In some cases, the technical effects mimicked or masked associations with clinical variables, raising concerns about the reproducibility and generalizability of findings drawn from studies using different pipelines.

Associations between specific microbial taxa and clinical metadata were consistent in effect size and direction with those introduced by the extraction method. This highlights the importance of carefully controlling for technical variability when interpreting such associations.

To minimize these risks, comprehensive metadata documentation, including technical factors such as the extraction protocol and sequencing batch, is essential. Equally important are balanced study designs, where technical steps are randomized across groups, and post hoc statistical controls are applied where harmonization is not possible. Without these precautions, biologically plausible findings may, in fact, reflect methodological bias.

When such technical artifacts are not accounted for through randomization, blocking, and comprehensive metadata collection, they risk becoming confounded with biological variables of interest. For example, if all treatment group samples are extracted in one batch while controls are handled in another, any batch-specific bias may be misinterpreted as a treatment effect.

To mitigate this, metadata must go beyond clinical phenotype or diet. It should include the processing batch, extraction date, technician ID, reagent lot numbers, and, in the case of animal studies, the cage ID. Randomization of samples across processing steps and balanced representation across sequencing runs are also essential to reduce bias. Without such safeguards, even well-intentioned designs risk concluding that the technical structure is more important than the biological signal.

Equally problematic is the widespread neglect of controls. Studies often lack sufficient extraction blanks, mock communities, or spike-in standards. Without these, it is nearly impossible to benchmark sensitivity, detect contamination, or calibrate absolute abundance.

Lastly, statistical power and study purpose are frequently mismatched. Overly broad hypotheses applied to underpowered studies invite p-hacking and post hoc narratives. Better microbiome study design hinges on hypothesis-driven sampling, rich metadata, and honest appraisals of analytical limits.

20.3 Contamination and DNA Extraction Bias

Low-biomass samples, such as brain, sputum, and nasal swabs, are especially vulnerable to contamination, often from reagents, kits, or sequencing flow cells. In a benchmark metagenomic study of Neonatal Intensive Care Unit (NICU) swabs, a combination of prevalence scoring, genome coverage, and external evidence revealed that over half of the detected taxa were reagent-derived (Ayala-Montaño et al., 2026,).

This challenge became especially apparent in our own work with respiratory microbiome samples from a Ugandan HIV-positive cohort (Kayongo et al., 2023). At the time, we conducted 16S rRNA gene sequencing under the reasonable assumption that amplification targeting the 16S region would be inherently specific to bacterial DNA, making contamination unlikely or irrelevant. The standard narrative is that since humans and other eukaryotes lack 16S rRNA genes, any amplification product should reflect microbial content. However, our initial results, which appeared to show associations between microbial community composition in sputum and lung function, prompted closer scrutiny during peer review, particularly regarding the potential influence of contamination in these low-biomass samples.

Although we had not initially included extraction blanks, we undertook a detailed reassessment of the data. This revealed high levels of host-derived DNA, which likely contributed to misleading amplification and taxonomic profiling. In the absence of wet-lab contamination controls, we addressed the issue through custom bioinformatic approaches, including host contamination filtering and confounder analysis. This process was

technically challenging but ultimately effective, as key microbial associations shifted substantially following decontamination, underscoring how off-target amplification and host DNA interference can distort results, even in 16S-based studies.

Our experience highlights that bioinformatic rigor and contamination-aware workflows are not just enhancements but necessities in low-biomass microbiome research. This is especially pronounced in low-biomass samples. Further scrutiny suggested that in the context of high host DNA content, typical in sputum or other human-derived samples, 16S primers may exhibit off-target binding, either to mitochondrial DNA or other conserved regions of the eukaryotic genome. We hypothesize that this may occur not just through direct mispriming but also due to competitive inhibition during amplification. In such cases, the high abundance of host nucleic acid may reduce primer availability or selectively amplify even rare off-target products.

These findings highlight how technical artifacts can closely mimic biological signals, particularly when sample biomass is low, host DNA content is high, and controls are not sufficiently integrated. Without rigorous design, including preemptive negative controls and awareness of host DNA interference, the resulting microbial profiles risk reflecting lab artefacts more than the true respiratory microbiome.

Such issues have led to high-profile misinterpretations. In a study by Bedarf et al. (2021), purported microbial signatures in brain tissue from Parkinson's patients were attributed to off-target amplification, rather than genuine colonization (Bedarf et al., 2021).

Host DNA can also dominate sequencing libraries, obscuring microbial reads. When host DNA exceeded 90%, non-specific amplification and chimeric alignments increased. In this setting, post-clustering OTU filtering was more effective than decontamination or masked reference-based filtering (Birkner et al. in preparation).

Best practices now demand rigorous, contamination-aware workflows, including multiple negative controls, quantification of microbial versus host DNA, the use of spike-ins, and careful tracking of lab batches and reagents.

20.4 Bioinformatics and Annotation Pitfalls

The way we annotate microbiome data plays a central role in how we interpret microbial presence, diversity, and function. Yet, the foundations on which annotation is built, reference databases and genome assemblies, are often flawed. Many widely used genome references contain errors, such as chimeric assemblies, where sequences from different organisms are mistakenly merged. These artifacts can mislead taxonomic classifiers and functional assignment tools, resulting in incorrect or inflated predictions of community composition. Contaminants in reference databases may further complicate analysis by propagating false matches across studies. Even high-quality assemblies may still suffer from incomplete curation, lateral gene transfer artifacts, or taxonomic mislabeling. These issues remain difficult to detect without the use of dedicated quality control tools (Orakov et al., 2021).

A second challenge arises from efforts to infer function from marker gene data, particularly 16S rRNA sequencing. Tools like PICRUSt or Tax4Fun attempt to project gene content based on phylogenetic proximity to known genomes. While attractive in terms of cost and speed, this approach is prone to error in non-gut, non-human environments or when the reference genomes do not adequately represent the microbial diversity present. In many cases, predicted gene profiles appear consistent across samples even when the sample labels are shuffled, indicating that predictions are primarily driven by average community structure rather than true sample-specific inference. These tools tend to perform best when applied to human stool data, but their generalizability beyond that remains limited (Sun et al., 2020).

One particularly sensitive application is the profiling of antibiotic resistance genes (ARGs). Functional annotation pipelines often rely on curated ARG databases such as ResFinder, CARD, or AMRFinder. These are based primarily on known, sequence-characterized resistance genes, which makes them excellent for detecting well-studied resistance elements,

but poor at identifying novel or divergent genes. Functional metagenomics, by contrast, reveals many ARGs that lack homology to known sequences and are entirely missed by annotation-based approaches. Even among homologs, short-read alignment can fail when ARGs are truncated, fragmented, or embedded in mobile genetic elements. Expanding the reference space with resources like ResFinderFG or utilizing specialized tools, such as AmrProfiler, can improve sensitivity; however, few studies integrate these resources into routine workflows. Most pipelines still default to canonical databases, thereby underestimating the diversity of resistomes (Gschwind et al., 2023).

Even when correct genes are identified, pathway-level annotation introduces another layer of complexity. Many functional pipelines, particularly those using the MetaCyc database or the HUMAnN framework, infer complete metabolic pathways based on partial gene matches. This process, often driven by scoring systems that reward pathway presence regardless of completeness, can result in over annotation. Entire pathways may be reported as "present" based on one or two genes, even when essential enzymes are missing. Gene context, operon structure, and pathway redundancy are often ignored. This leads to inflated functional profiles and misleading conclusions, especially in comparative or clinical studies where subtle differences may be overinterpreted. Careful validation of pathway completeness, ideally with genome-resolved data or transcript-level confirmation, is essential (Sun et al., 2020). These issues underscore that annotation is not a neutral step but a source of systematic bias, with recurring patterns and practical countermeasures outlined in Table 20.1.

TABLE 20.1

Key Pitfalls of Phylogenetic Misassignment and Incomplete Pathway Annotation ⏎

Pitfall	Impact	Suggested Mitigation
Incomplete or chimeric reference genomes	False taxa or gene detection	Use GUNC, filter low-quality refs

Pitfall	Impact	Suggested Mitigation
Marker-based functional inference	Poor performance outside human samples	Use with caution; validate with MGS
ARG databases miss functionally novel genes	Underestimated resistome	Include ResFinderFG, AmrProfiler
Overannotation of pathways	Inflated functional claims	Enforce pathway completeness checks
Misclassification of reads	Incorrect taxonomic/functional links	Prefer long-read + phylogenetic placement

20.5 Statistical Interpretation and Normalization

Compositionality and sparsity in microbiome data complicate standard statistical analysis. Applying t-tests or unadjusted correlations to relative abundance data can yield spurious results. Rarefaction, although controversial, remains defensible when used for diversity metrics (Saary et al., 2017).

More sophisticated methods, centered log-ratio transformations, DESeq2 (Love et al., 2014), or zero-inflated models, address these constraints but are often misused or underreported. Tools like LongDat (Chen et al., 2023), metadeconfoundR (Fromentin et al., 2022), or MaAsLin (Nickols et al., 2024) offer frameworks for correcting confounders, batch effects, and multiple testing in longitudinal studies.

Measures like Shannon and Simpson diversity are among the most widely reported statistics in microbiome studies. Their appeal lies in their simplicity and apparent interpretability: higher diversity is often assumed to signal better health or ecosystem resilience. Yet this assumption is rarely scrutinized. These indices simplify complex community structures into a single number. While they can detect gross shifts in richness and evenness, they do not capture function, interaction, or strain-level dynamics. Moreover, slight differences in diversity are frequently overinterpreted,

reported as biologically meaningful without validation or consideration of statistical power.

Beyond univariate summaries, multivariate techniques such as principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) are frequently used to visualize beta diversity. These ordination plots, often generated from distance matrices such as Bray–Curtis or UniFrac, are visually compelling but can be misleading. The apparent separation between groups can be an artifact of sample size, scaling, or the chosen distance metric, rather than a robust biological distinction. Without proper hypothesis testing, such visualizations risk serving more as rhetorical devices than as analytical tools.

Confounding remains a central concern in microbiome analysis. Factors such as medication use, body mass index, diet, and disease severity can shape microbial communities in ways that mimic or mask associations of interest. For example, in an analysis of type 2 diabetes and the gut microbiome, microbial signatures initially attributed to disease were later found to correlate more strongly with metformin treatment. This distinction only became apparent through careful statistical control (Forslund et al., 2015). Similarly, when exploring the metabolome–microbiome interface, controlling for comorbidities and pharmacological interventions was essential to isolate meaningful signals from cardiometabolic phenotypes (Fromentin et al., 2022).

The high dimensionality of microbiome data, often involving thousands of taxa or genes, makes multiple hypothesis testing a core statistical challenge. Without appropriate correction, the likelihood of false positives increases significantly, particularly in exploratory studies. Yet, false discovery rate (FDR) control remains inconsistently applied. While procedures like the Benjamini–Hochberg method are widely available, many published studies either omit correction or report unadjusted p-values without context. Worse, significance thresholds (e.g., FDR < 0.1 or < 0.25) are sometimes applied post hoc, only when convenient for narrative support. In this landscape, p-hacking becomes a serious concern, manifesting as repeated testing of different variable subsets, redefinition of

outcomes, or selective reporting of results that cross arbitrary significance thresholds.

Crucially, p-values without corresponding effect sizes are meaningless. A statistically significant result (even one with a very low p-value) offers little scientific value unless paired with an estimate of the effect size. Without that, readers cannot judge biological relevance, clinical applicability, or whether the finding could have arisen by chance in large datasets. Microbiome studies are particularly vulnerable to this because the noise is high, the effects are often subtle, and the data are compositional and sparse.

Robust analysis requires more than declaring what is "significant." It requires demonstrating the magnitude of the difference, in which direction, under what assumptions, and whether that signal remains stable under resampling or in independent cohorts. Some tools build these principles into their frameworks, emphasizing effect-size reporting, confounder adjustment, and false discovery control alongside interpretability. But these tools are only as effective as the mindset of the analyst using them.

Addressing this requires more than just statistical tools; it demands transparent reporting of how many tests were performed, what correction strategy was applied, and whether the analysis was exploratory or confirmatory. Still, methodological awareness and a cautious interpretation of marginal findings remain essential to guard against false discovery in microbiome research.

Rigorous microbiome statistics require more than software and p-values. They demand thoughtful study design, the use of appropriate models for compositional and sparse data, explicit attention to confounding, and clear, transparent reporting of assumptions and limitations.

20.6 Interpretation Pitfalls, Outlook, and Conclusion

Interpretation is the bridge between data and meaning. Unfortunately, it is also where microbiome research is most prone to exaggeration. Correlation

is often mistaken for causation, diversity metrics are overvalued, and visualization aesthetics usually take precedence over statistical support.

Artificial intelligence and machine learning are increasingly used in microbiome prediction, but often lack interpretability. Many published models are "black boxes" with limited generalizability and should be taken with caution (Rozera et al., 2025). Without benchmarking, validation, and biological grounding, these tools risk compounding noise rather than resolving it.

Despite this, the future of microbiome science is bright. Standards like STORMS, FAIR data practices, and explainable AI are on the rise. Longitudinal studies and the integration of multi-omics are growing in importance. Interventions are becoming more personalized and mechanistically informed.

This chapter has highlighted pitfalls not to diminish the field but to strengthen it. If microbiome science is to fulfill its promise, it must embrace methodological transparency, cross-disciplinary collaboration, and a deepened commitment to rigor.

Allow me to end this chapter with a personal note. As researchers working with high-dimensional, computationally intensive microbiome data, we hold both scientific and environmental responsibilities. Thoughtful planning of study design, analytical pipelines, and interpretation strategies not only improves the clarity and impact of our work but also minimizes unnecessary computational waste. In a time of global ecological stress, even decisions made in silico matter. Let us approach our bioinformatics workflows not only with rigor but also with intention, because we only have one world.

References

Albertsen, M. (2023). Long-read metagenomics paves the way toward a complete microbial tree of life. *Nature Methods*, *20*(1), 30–31. https://doi.org/10.1038/s41592-022-01726-6 ⏎

Amos, G. C. A., Logan, A., Anwar, S., Fritzsche, M., Mate, R., Bleazard, T., & Rijpkema, S. (2020). Developing standards for the microbiome field. *Microbiome*, *8*(1). https://doi.org/10.1186/s40168-020-00856-3 ⏎

Ayala-Montaño S, Afolayan AO, Kociurzynski R, Loeber U, Reuter S. (2026). Mitigation and detection of putative microbial contaminant reads from long-read metagenomic datasets. *Microb Genom*. 12(1):001609. doi: 10.1099/mgen.0.001609

Bartolomaeus, T. U. P., Birkner, T., Bartolomaeus, H., Löber, U., Avery, E. G., Mähler, A., Weber, D., Kochlik, B., Balogh, A., Wilck, N., Boschmann, M., Müller, D. N., Markó, L., & Forslund, S. K. (2020). Quantifying technical confounders in microbiome studies. *Cardiovascular Research*, cvaa128. https://doi.org/10.1093/cvr/cvaa128 ⏎

Bedarf, J. R., Beraza, N., Khazneh, H., Özkurt, E., Baker, D., Borger, V., Wüllner, U., & Hildebrand, F. (2021). Much ado about nothing? Off-target amplification can lead to false-positive bacterial brain microbiome detection in healthy and Parkinson's disease individuals. *Microbiome*, *9*(1), 75. https://doi.org/10.1186/s40168-021-01012-1 ⏎

Chen, C.-Y., Löber, U., & Forslund, S. K. (2023). LongDat: An R package for covariate-sensitive longitudinal analysis of high-dimensional data. *Bioinformatics Advances*, *3*(1), vbad063. https://doi.org/10.1093/bioadv/vbad063 ⏎

Clavel, T., Horz, H.-P., Segata, N., & Vehreschild, M. (2022). Next steps after 15 stimulating years of human gut microbiome research. *Microbial Biotechnology*, *15*(1), 164–175. https://doi.org/10.1111/1751-7915.13970 ⏎

Costea, P. I., Zeller, G., Sunagawa, S., Pelletier, E., Alberti, A., Levenez, F., Tramontano, M., Driessen, M., Hercog, R., Jung, F.-E., Kultima, J. R., Hayward, M. R., Coelho, L. P., Allen-Vercoe, E., Bertrand, L., Blaut, M., Brown, J. R. M., Carton, T., Cools-Portier, S., … Bork, P. (2017). Towards standards for human fecal sample processing in metagenomic

studies. *Nature Biotechnology*, *35*(11), 1069–1076. https://doi.org/10.1038/nbt.3960 ⏎

Emerson, J. B., Adams, R. I., Román, C. M. B., Brooks, B., Coil, D. A., Dahlhausen, K., Ganz, H. H., Hartmann, E. M., Hsu, T., Justice, N. B., Paulino-Lima, I. G., Luongo, J. C., Lymperopoulou, D. S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A., Vaishampayan, P., & Rothschild, L. J. (2017). Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. *Microbiome*, *5*(1), 86. https://doi.org/10.1186/s40168-017-0285-3 ⏎

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Chatelier, E. L., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H. K., Arumugam, M., Kristiansen, K., Voigt, A. Y., Vestergaard, H., Hercog, R., Costea, P. I., Kultima, J. R., Li, J., Jørgensen, T., … Pedersen, O. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, *528*(7581), 262–266. https://doi.org/10.1038/nature15766 ⏎

Fromentin, S., Forslund, S. K., Chechi, K., Aron-Wisnewsky, J., Chakaroun, R., Nielsen, T., Tremaroli, V., Ji, B., Prifti, E., Myridakis, A., Chilloux, J., Andrikopoulos, P., Fan, Y., Olanipekun, M. T., Alves, R., Adiouch, S., Bar, N., Talmor-Barkan, Y., Belda, E., … Pedersen, O. (2022). Microbiome and metabolome features of the cardiometabolic disease spectrum. *Nature Medicine*, *28*(2), Article 2. https://doi.org/10.1038/s41591-022-01688-4 ⏎

Gschwind, R., Ugarcina Perovic, S., Weiss, M., Petitjean, M., Lao, J., Coelho, L. P., & Ruppé, E. (2023). ResFinderFG v2.0: A database of antibiotic resistance genes obtained by functional metagenomics. *Nucleic Acids Research*, *51*(W1), W493–W500. https://doi.org/10.1093/nar/gkad384 ⏎

Kayongo, A., Bartolomaeus, T. U. P., Birkner, T., Markó, L., Löber, U., Kigozi, E., Atugonza, C., Munana, R., Mawanda, D., Sekibira, R., Uwimaana, E., Alupo, P., Kalyesubula, R., Knauf, F., Siddharthan, T.,

Bagaya, B. S., Kateete, D. P., Joloba, M. L., Sewankambo, N. K., … Forslund, S. K. (2023). Sputum microbiome and chronic obstructive pulmonary disease in a Rural Ugandan Cohort of Well-Controlled HIV Infection. *Microbiology Spectrum*, *0*(0), e02139-21. https://doi.org/10.1128/spectrum.02139-21 ⏎

Loh, J. S., Mak, W. Q., Tan, L. K. S., Ng, C. X., Chan, H. H., Yeow, S. H., Foo, J. B., Ong, Y. S., How, C. W., & Khaw, K. Y. (2024). Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduction and Targeted Therapy*, *9*(1), 37. https://doi.org/10.1038/s41392-024-01743-1 ⏎

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8 ⏎

McGuinness, A. J., Stinson, L. F., Snelson, M., Loughman, A., Stringer, A., Hannan, A. J., Cowan, C. S. M., Jama, H. A., Caparros-Martin, J. A., West, M. L., & Wardill, H. R. (2024). From hype to hope: Considerations in conducting robust microbiome science. *Brain, Behavior, and Immunity*, *115*, 120–130. https://doi.org/10.1016/j.bbi.2023.09.022 ⏎

Nickols, W. A., Kuntz, T., Shen, J., Maharjan, S., Mallick, H., Franzosa, E. A., Thompson, K. N., Nearing, J. T., & Huttenhower, C. (2024). MaAsLin 3: Refining and extending generalized multivariable linear models for meta-omic association discovery. *bioRxiv*. https://doi.org/10.1101/2024.12.13.628459 ⏎

Orakov, A., Fullam, A., Coelho, L. P., Khedkar, S., Szklarczyk, D., Mende, D. R., Schmidt, T. S. B., & Bork, P. (2021). GUNC: Detection of chimerism and contamination in prokaryotic genomes. *Genome Biology*, *22*, 178. https://doi.org/10.1186/s13059-021-02393-0 ⏎

Rozera, T., Pasolli, E., Segata, N., & Ianiro, G. (2025). Machine learning and artificial intelligence in the multi-omics approach to gut microbiota. *Gastroenterology*, *169*(3), 487–501. https://doi.org/10.1053/j.gastro.2025.02.035 ⏎

Saary, P., Forslund, K., Bork, P., & Hildebrand, F. (2017). RTK: Efficient rarefaction analysis of large datasets. *Bioinformatics*, *33*(16), 2594–2595. https://doi.org/10.1093/bioinformatics/btx206 ⏎

Sun, S., Jones, R. B., & Fodor, A. A. (2020). Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. *Microbiome*, *8*(1), 46. https://doi.org/10.1186/s40168-020-00815-y ⏎

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*(7164), 804–810. https://doi.org/10.1038/nature06244 ⏎

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