ACCSCIENCE PUBLISHING

Microbes & Immunity

REVIEW ARTICLE

Integrating human microbiome profiling into precision medicine for disease prevention

Shahar Bano[1], Javeria Pervaiz[2], Muaaz Bin Waqar[3], Mah-e-Noor Abbas[4], Shamas Ul Qamar[5], Zarish Fatima[4], Zunaira Tariq[6], and Ambreen Zahra[7]*

[1]Department of Botany, University of the Punjab, Lahore, Punjab, Pakistan

[2]Institute for Specific Prophylaxis & Tropical Medicine (ISPTM), Center for Pathophysiology, Infectiology & Immunology (CePII), Medical University of Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria

[3]Department of Public Health, University of the West of Scotland, London Campus, United Kingdom

[4]Department of Biological Sciences, University of Sialkot, Punjab, Pakistan

[5]Department of Public Health, Angela Ruckson University, London, United Kingdom

[6]National Center of Genome Editing/D-8 Research Center, Center for Advanced Studies, Agriculture and Food Security, University of Agriculture, Faisalabad, Punjab, Pakistan

[7]Department of Biotechnology, Faculty of Sciences, University of Sialkot, Punjab, Pakistan

Abstract

The human microbiome is a diverse and dynamic microbial community composed of trillions of microorganisms that inhabiting various body sites, such as the gut, skin, oral cavity, respiratory tract, and urogenital system. These microbial communities sustain the host's physiological functions and health through interactions with immune, metabolic, and neurobiological processes. The microbiome has been extensively studied due to recent advancements in high-throughput genome sequencing and multi-omics technologies, which also demonstrated associations between microbial dysbiosis and a variety of metabolic, neurological, immune-mediated, cardiovascular, and infectious disorders. Simultaneously, precision medicine incorporates routine, environmental, and genetic variables to provide personalized solutions to disease prevention and treatment. The integration of microbiome research and precision medicine provide potential opportunities in early detection of diseases, risk stratification, and personalized therapeutic interventions. This narrative review identifies relevant literature from PubMed, Web of Science, and Scopus using terms like "human microbiome," "precision medicine," "microbiome profiling," "artificial intelligence," and "disease biomarkers," with emphasis on current English-language publications. The review study the diversity, structure, and functional roles of the human microbiome, and the current profiling technologies and their potential clinical uses. It also examines microbiome-directed approaches, including personalized nutrition, probiotics, and fecal microbiota transplantation. Moreover, the document addresses ethical considerations, clinical challenges, artificial intelligence, and multi-omics integration. While microbiome profiling has significant potential in precision and preventive medicine, most applications are still investigational and need more validation before being implemented in clinical practice.

Keywords: Human microbiome; Precision medicine; Microbiome profiling; Dysbiosis; Gut-brain axis; Metagenomics; Preventive healthcare

***Corresponding author:**
Ambreen Zahra
(drambreenzahra@gmail.com)

Citation: Bano S, Pervaiz J, Waqar MB, *et al*. Integrating human microbiome profiling into precision medicine for disease prevention. *Microbes & Immunity*. doi: 10.36922/MI026240065

Received: June 8, 2026

Revised: August 30, 2026

Accepted: September 1, 2026

Published online: September 9, 2026

1. Introduction and conceptual background

The human microbiome is a complex and diverse community of microorganisms, including bacteria, viruses, fungi, and archaea, that inhabit the human body. These microorganisms colonize various body sites, including the skin, oral cavity, gastrointestinal tract, respiratory tract, and urogenital tract.[1] Among these anatomical sites, the gut sustain the most overpopulated and functionally diverse microbial consortium, comprising trillions of microorganisms that synergistically contribute to host physiology and health. The microbiome is the collective genomes of these microorganisms possess significantly more genes than the human genome, highlighting their vital roles in health and disease.[2]

These microbial communities are more than mere passive dwellers, and they play an active role in ensuring physiological homeostasis. Among their main functions is in metabolism, where they aid in the digestion of complex carbohydrates and fibers, among other substrates that cannot be digested by human enzymes.[3] Gut microbes generate the necessary metabolites through fermentation, including short-chain fatty acids[4], which are reported to control inflammation, maintain intestinal health, and affect energy metabolism.[5] Moreover, the microbiome also helps in the production of essential nutrients, such as vitamin K and various B vitamins, thus improving nutritional status.[6]

The microbiome plays significant role in the development and regulation of the immune system. During early life exposure to microorganisms is important for immune maturation and helps establish the capacity of the immune system to distinguish between pathogenic and harmless antigens.[7] A healthy microbiome promotes immune tolerance and helps regulate overactive inflammatory responses. Conversely, alterations in the composition and function of the microbiome[4] have been associated with immune dysregulation and an increased risk of autoimmune and inflammatory diseases. However, how alterations in the microbiome lead to the emergence of these disorders remains to be elucidated.[8] Microbial colonization in early life is especially crucial for long-term health and immunological development. Microbial colonization initiates during the early stages of life and it triggers immune programming during birth and early infancy. Antibiotic exposure, cesarean delivery, and formula feeding are examples of disruptions during this significant developmental stage that have been associated to alterations in microbiome development and may affect a person's subsequent susceptibility to allergies, asthma, obesity, and autoimmune disorders.[9] Microbiome profiling of infants and maternal health may help identify risk factors at an early stage and proactive interventions meant to promote the production of beneficial microorganisms.

Potential strategies that may help improve the outcomes of the neonate include breastfeeding support, optimization of maternal dietary patterns, and, where required, probiotic supplementation during pregnancy.[10] Furthermore, new research emphasizes the significance of the gut-brain axis, a sophisticated network of bidirectional communication between the central nervous system and the gut bacteria. Research indicates that neuronal, endocrine, immunological, and metabolic pathways may be used by the gut bacteria to affect brain function. The causative mechanisms and clinical importance of these interactions in people are still poorly understood, and a large portion of the present information comes from experimental and preclinical research.[11] In parallel, with recent advances in microbiome research, the principle of precision medicine has becoming an increasingly significant paradigm in healthcare. In order to promote disease prevention, diagnosis, and therapy, precision medicine considers individual differences in genetics, environment, and lifestyle.[12] Precision medicine seeks to personalize interventions to individual attributes, thereby potentially improving therapeutic outcomes and minimizing side effects, in contrast to traditional techniques that are primarily based on universal treatment strategies. This personalized approach has been made possible by developments in genomics biomedical technologies, and data analytics.[13] In this context, microbiome is increasingly recognized as a potential component of precision medicine. The microbiome is dynamic and sensitive to external influences, such as nutrition, exposure to antibiotics, lifestyle, and environmental circumstances, in contrast to the comparatively stable human genome.[14] The microbiome is a potential biomarker of health and disease and a potential target for therapeutic intervention due to its adaptability. Individuals have unique microbial communities, but these communities may shift throughout time and in response to physiological and environmental conditions.[15] Recent research has highlighted the importance of dietary habits in influencing the human microbiome. Numerous clinical and observational research have shown that following a Mediterranean diet has a good impact on the generation of short-chain fatty acids, barrier function, gut microbiota composition, and markers of chronic inflammation. Nevertheless, these impacts differ in strength and consistency between populations, require further validation.[16] A wide range of diseases has been associated with disruption in this microbial balance. These include autoimmune illnesses, metabolic pathology (such as obesity and type 2 diabetes), heart disease, cancer (of

all kinds), and neuropsychiatric disorders (such as anxiety and depression).[17] Although there is still substantial variance among populations and disease situations, certain longitudinal studies have demonstrated that alterations in the microbiome appear to anticipate the onset of some diseases. As a result, while microbiological markers are being extensively studied as possible early indicators for routine clinical usage, few of them have received enough analytical and clinical validation.[18]

Microbiome profiling is an emerging research tool for prediction and prevention of disease. Its clinical applications, however, have been hindered by the interdependence of microbial signatures, as they are not always reproducible in independent cohorts due to geographic and dietary differences, and exposure to antibiotics, as well as analytical pipelines and sequencing methods. Thus, there are no common microbiome-based diagnostic signatures in use in clinical practice at present.[19] Microbiome profiling has become a promising research tool for the prediction and prevention of diseases. Microbial diversity, composition and function may be used to identify people at higher risk for certain diseases and may help guide the identification of potential preventive measures. Most microbiome markers, however, are in their investigational stages and will need more validation before they become part of the standard clinical practice. Personalized diet changes, prebiotics, probiotics and other microbiome-focused therapies are all possibilities. Despite this, the efficacy of most microbiome-directed interventions is being studied for specific clinical indications, including recurrent *Clostridioides difficile* infection.[20]

2. Literature search strategy

A narrative literature review was carried out to identify and compile information about the human microbiome, microbiome-based biomarkers, microbiome profiling, metagenomics, multi-omics, artificial intelligence (AI), and applications in precision medicine. PubMed/MEDLINE, Scopus, Web of Science Core Collection, and Google Scholar were used for literature searches. Only articles published mainly from January 1, 2015, to July 15, 2025, were considered. Foundational papers before 2015 are cited for background context. The search terms used were "human microbiome," "precision medicine," "microbiome biomarkers," "metagenomics," "artificial intelligence," and "multi-omics" which were combined using Boolean operators (AND/OR) to refine the search strategy. Where applicable, Medical Subject Headings (MeSH) were also considered to improve the identification of relevant studies in PubMed. Systematic reviews, meta-analyses, clinical studies, and good-quality experimental studies were prioritized. Studies were chosen for their relevance to disease biomarkers, disease prediction, precision medicine, disease prevention, and microbiome profiling. Peer-reviewed systematic reviews, meta-analyses, randomized controlled trials, prospective and retrospective cohort studies, and observational clinical studies with an explicit methodology were given priority. A priority was given to peer-reviewed systematic reviews, meta-analyses, randomized controlled trials, prospective and retrospective cohort studies, and observational clinical studies with an explicit methodology. Articles published in English and available in full text were included. Editorials, conference abstracts, opinion articles, duplicate publications, and studies lacking methodological detail were excluded. Animal studies were included only if they gave important mechanistic information for human microbiome studies. The selection of literature was carried out by screening the titles and abstracts and then evaluating the full text of the articles that met the criteria. Studies were included if they were scientifically relevant, used good scientific methodology, and contributed to the understanding of microbiome profiling, biomarker discovery, disease mechanisms, and precision medicine. To provide a balanced and representative synthesis of the available evidence, duplicate records and studies that were not directly related to the goals of this review were omitted. Wherever possible, high-quality evidence such as systematic reviews, meta-analyses, clinical guidelines, randomized controlled trials, and large prospective cohort studies was emphasized, with sample sizes of $n \geq 30$ per arm and mechanistic/preclinical animal studies with $n \geq 6$ per group to limit selective citation. Good-quality experimental studies included the use of suitable study design, well-described methodology, an appropriately justified sample size based on the study design, sound statistical analysis with control for relevant confounding, and reproducibility of results across independent studies. Mechanistic and preclinical studies were mainly employed to elucidate biological pathways and were not linked to evidence for clinical applications. The strength and relevance of the evidence were evaluated based on study design, methodological quality, sample size, reproducibility of results, clinical applicability, and consistency of results across independent studies. Two authors independently evaluated titles, abstracts, and full texts for scientific rigor and relevance to precision medicine, resulting in the 96 key references included in this final review.

3. The human microbiome: Structure, diversity and function

The human microbiome is highly structurally organized, microbially diverse, and functionally complex, with a distinct structure at each anatomical site. The characteristics

depend on host genetics, diet, lifestyle, environmental exposures, age, and other physiological factors, and all of them contribute to the microbiome's role in health and disease. These structural, compositional, and functional features give insights into the interactions of the host and the microbiome and their possible links to health and disease in the following sections.[21]

3.1. Structure of the human microbiome

The structural organization of the microbiome is the distribution and composition of microbial communities in various body habitats. The body sites are unique ecological niches that are influenced by a particular physiological environment, like oxygen availability, pH, nutrient composition, moisture, and immune activity.[22] The gastrointestinal tract, especially the colon, is the most densely colonized and structurally complex microbial system in the human body. It contains a higher microbial abundance than any other part of the body, and is mainly composed of the bacterial phyla of Firmicutes, Bacteroidota (previously Bacteroidetes), Actinobacteriota, and Proteobacteria.[23] These microbial communities are planktonic (free-living) and associated with the mucus, and some microorganisms are the members of localized biofilms. They interact closely with the intestinal epithelial layer, playing a role in metabolic homeostasis, barrier integrity and immune regulation.[24]

The intestinal barrier is a multi-layered defense barrier that regulates the interactions between the host and the microbiota and prevents microbial translocation and systemic inflammation, and disruption of the intestinal barrier integrity has been linked to microbial dysbiosis and is regarded as a potential contributor to the pathophysiology of various metabolic, inflammatory and autoimmune diseases. However, the causal relationships and underlying mechanisms continue to be investigated. The skin microbiome has a site-specific organization in terms of structure based on sebaceous, moist, or dry areas. Likewise, the oral cavity has organized biofilms on the tooth and mucosal surfaces, with microbial communities forming highly specialized communities.[25,26] The respiratory tract has a gradient structure, with the density of the microbes decreasing as the airways get higher.[4] The microbiome in the urogenital system and particularly in females is structurally dominated by *Lactobacillus* species, which promote a stable and protective microbial environment.[27]

3.2. Diversity of the human microbiome

Microbial diversity is the abundance of species (richness) and evenness (distribution) of the microbial communities. Microbial diversity is said to be an important measure of ecosystem stability and resilience. Although decreased microbial diversity has been linked to a variety of disease states, this association is context-specific, and more diversity does not necessarily mean better health at all body sites.[28] As illustrated in Figure 1, the most diverse microbial environment is the gut microbiome, which harbors hundreds and thousands of bacterial species.[29] Many factors contribute to microbial diversity, including diet, age, antibiotic use, geographical location, environmental factors, and host genetics. Infants are relatively homogenous, but as they grow older and have environmental exposure, their diversity rises, reaching a relatively stable adult structure.[30]

Various body sites have different degrees of diversification. There is moderate diversity in the skin microbiome, which depends on exposure to the external environment. The microbiome of the mouth is extremely diverse, as it is in constant contact with food and outside microorganisms.[31] Conversely, the urogenital microbiome, especially in healthy women, is less diverse and more stable in terms of functions, with protective *Lactobacillus* species.[32] New evidence also indicates that the vaginal microbiome is linked to reproductive and obesity health. However, the direction and causality of these relationships remain to be fully established. For women with polycystic ovary syndrome (PCOS), a higher body mass index (BMI) was correlated with decreased levels of beneficial *Lactobacillus* species and increased diversity of anaerobic bacteria, indicating that obesity may worsen vaginal dysbiosis and reproductive issues.[33] Diversity in microbes is critical to ecosystem stability and resilience. Experimental and observational studies indicate that there is some benefit to having more microbial species that could lead to increased pathogen resistance through colonization resistance, environmental stress resistance through environmental redundancy, and functional redundancy, wherein different microbial species carry out the same biological functions. However, these relationships can differ based on the body site and disease context.[34]

3.3. Functional roles of the microbiome

The functional capability of the microbiome refers to the biological processes performed by microbial communities and their interactions with the host. These functions do not just capture the individual species composition, but also capture the overall metabolic potential of the microbiome.[12] Metabolic regulation is one of the most crucial functions. Complex carbohydrates, dietary fiber, and resistant starches are broken down into short-chain fatty acids (SCFAs) in the gut by gut microbes, including acetate, propionate, and butyrate. These metabolites have important functions in energy metabolism, glucose and

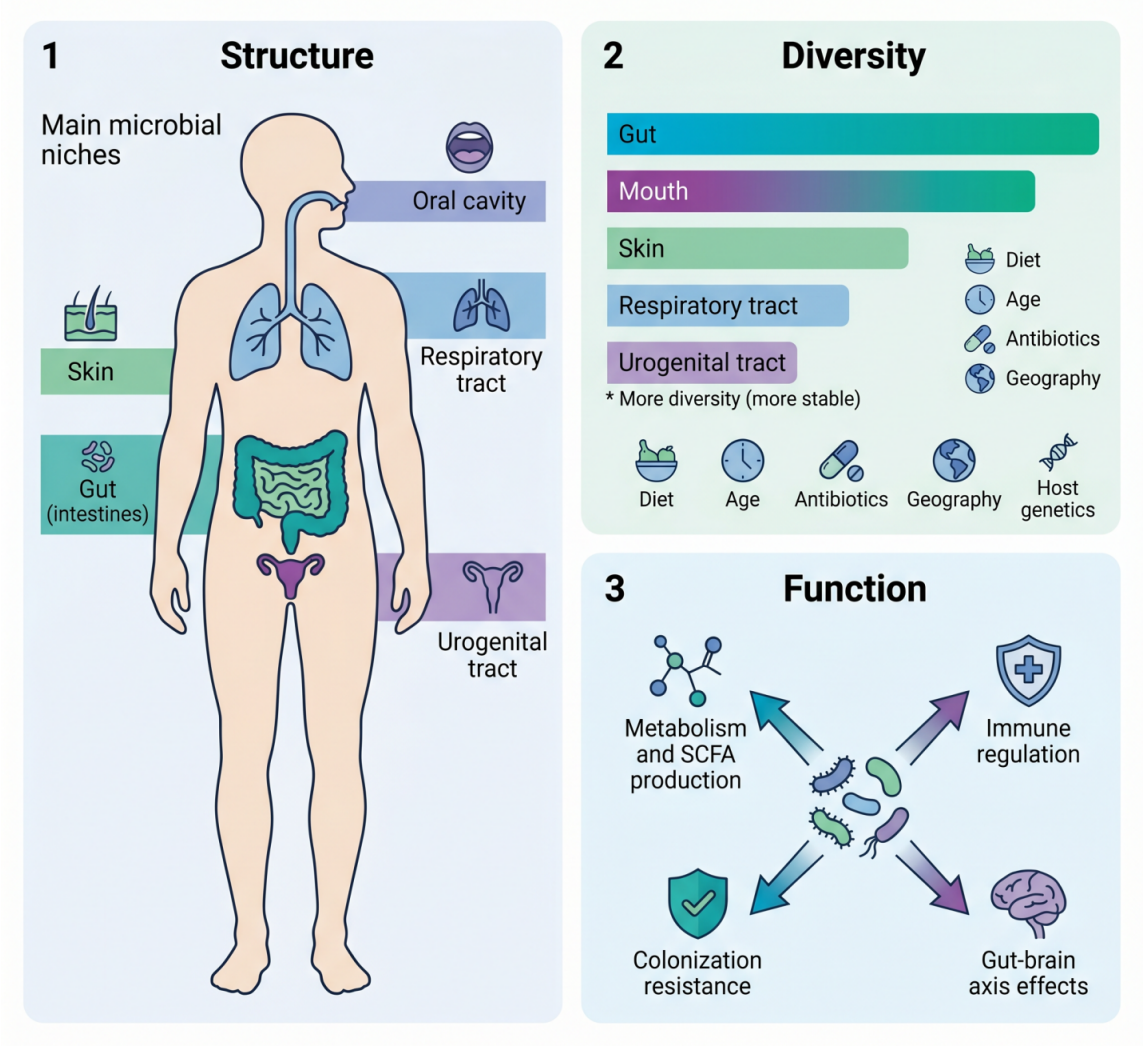

Figure 1. Structure, diversity, and functional roles of the human microbiome. Schematic created by the authors.
Abbreviations: SCFA: Short-chain fatty acid.

lipid homeostasis regulation, and protection of intestinal barrier integrity.[35] The development and modulation of the immune system also revolve around microbiomes. It educates the immune system to distinguish between dangerous pathogens and helpful microbes, thus facilitating immune tolerance. Microbial signals affect innate and adaptive immune responses, minimizing overreaction and restoring immune balance.[36] Under healthy conditions, many commensal microorganisms contribute to immune homeostasis. But some resident microorganisms, called "pathobionts," normally live in the host in a harmless manner but contribute to sites of inflammation and disease when microbial balance is altered or immune regulation is compromised. Balanced microbial ecosystem is crucial for immune health, as the onset of chronic inflammatory and autoimmune disease conditions have been linked to the expansion or activation of pathobionts.[37]

The other important role is neurobiological regulation via the gut-brain axis. The microbial metabolites, including neurotransmitter precursors and SCFAs, respond to the nervous system through neural, endocrine, and immune mechanisms. There is growing evidence that microbial metabolites can affect brain activity, stress reactions, behavior, and emotional control via neural, endocrine, immune and metabolic pathways. The current evidence is largely based on experimental and preclinical studies, but further clinical data are needed in humans.[38] The microbiome also offers colonization resistance to

pathogens due to competition for nutrients and sites of attachment, antimicrobial compounds, and enhanced host epithelial barriers. Together, these mechanisms provide colonization resistance and could potentially maintain microbial homeostasis and decrease susceptibility to pathogen colonization.

4. Microbiome profiling: Tools and technologies

Microbiome profiling includes a variety of molecular and computational methods to describe the composition, diversity, and functions of microbial communities. Based on the biological functions of the microbiome discussed in the previous section, these technologies provide a comprehensive understanding of microbial communities, beyond the traditional culture-based approach. In the last three decades, significant progress in sequencing technologies, multi-omics methods and bioinformatics has greatly facilitated the exploration of microbial communities and their interactions with health and disease.[39]

4.1. Development as an extension of culture-based methods

Traditionally, microbiological research has been based on culture-based methods, in which the microbes were cultivated in laboratory settings and identified based on morphological and biochemical features. Although these methods were fundamental to early microbiology, they are seriously limited.[40] The biggest disadvantage is that a significant percentage of microbial species, commonly known as microbial dark matter, cannot be cultured in the typical laboratory conditions. Consequently, culture-based methods provide only a partial representation of microbial diversity because many microorganisms remain difficult or impossible to culture under standard laboratory conditions.[41] With the development of the methods of molecular biology, these restrictions have been overcome, and direct analysis of microbial genetic material can be done in environmental or clinical samples without any cultivation.

4.2. Sequencing technologies

Current microbiome profiling is predominantly based on high-throughput sequencing technologies, enabling large-scale and rapid analysis of microbial DNA. One of the most popular methods of bacterial identification and classification is 16S rRNA gene sequencing. It targets the 16S ribosomal RNA gene that has conserved and variable regions, and as such, researchers can establish the bacterial taxonomy at various levels.[42] This is a low-cost technique that can be applied in the analysis of microbial diversity and community structure. 16S rRNA sequencing generally provides reliable taxonomic classification at the genus level, while species- and strain-level resolution is often limited.[43] In addition, 16S rRNA sequencing typically cannot provide high taxonomic resolution at the species or strain level and cannot directly quantify microbial functional potential. Differences in DNA extraction methods, primer selection, sequencing platforms, bioinformatics pipelines, and reference databases can introduce variability in taxonomic profiles and reduce reproducibility across studies. This method has disadvantages of measuring relatives rather than absolute microbial abundance and may lead to artificial changes in apparent abundance of other taxa that can impact biomarker discovery and interpretation. Furthermore, the functional characteristics can only be computed and not directly measured, and this could add to the uncertainty. When it comes to strain-level identification or functional characterization, however, complementary methods such as whole-genome shotgun metagenomic sequencing are often necessary (Table 1).

Table 1. Comparison of human microbiome profiling technologies

Technology	Principle	Major advantages	Major limitations	Current clinical readiness	Representative references
Culture-based methods	*In vitro* isolation and cultivation of microorganisms under controlled laboratory conditions	Enables phenotypic characterization, antimicrobial susceptibility testing, and isolation of live organisms	Most human-associated microorganisms are unculturable; low microbial diversity captured; culture bias	Limited clinical use for microbiome profiling	44
16S rRNA gene sequencing	Sequencing of conserved bacterial 16S ribosomal RNA gene variable regions	Cost-effective; high throughput; suitable for microbial community composition and diversity analysis	Limited species- and strain-level resolution; PCR and primer bias; provides relative abundance only; limited functional information	Widely used in research; limited routine clinical application	43

(*Cont'd...*)

Table 1. (***Continued***)

Technology	Principle	Major advantages	Major limitations	Current clinical readiness	Representative references
Shotgun metagenomic sequencing	Sequencing of all microbial DNA present in a sample	Species- and strain-level resolution; functional gene profiling; detects bacteria, viruses, fungi, and archaea	Higher cost; computationally intensive; host DNA contamination; requires standardized laboratory protocols, bioinformatics pipelines, and external validation	Advanced research and biomarker validation	45
Metatranscriptomics	Sequencing of total microbial RNA to identify actively expressed genes	Measures functional activity and microbial gene expression in real time	RNA instability; technically demanding; expensive; complex data analysis	Research stage	46
Metaproteomics	Mass spectrometry-based identification of microbial proteins	Direct assessment of microbial protein expression and biological function	Lower analytical depth than genomics; challenging protein identification; limited reference databases	Research stage	47
Metabolomics	Identification and quantification of microbial metabolites using mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy	Measures functional metabolic output; identifies host–microbiome interactions; detects bioactive metabolites	Metabolites originate from both host and microbiota; influenced by diet and environment; interpretation remains challenging	Emerging translational research	48
Multi-omics integration	Integration of metagenomics, metatranscriptomics, metaproteomics, and metabolomics datasets	Comprehensive characterization of microbial composition and function; improves biomarker discovery and biological interpretation	High computational complexity; requires standardized analytical pipelines and large datasets	Emerging research	43

By comparison, shotgun metagenomic sequencing is a more thorough method since it sequences all genetic material in a sample. Shotgun metagenomic sequencing allows for the identification of microorganisms at the species and, in many instances, strain level, and offers a higher taxonomic resolution than 16S rRNA gene sequencing. It sequences all microbial DNA in a sample, rather than just a subset, and thus can detect bacteria, viruses, fungi and archaea, and can characterize microbial functional genes and metabolic pathways.[49] Although highly informative, shotgun metagenomics are more expensive and computationally demanding. While shotgun metagenomics offers a greater level of taxonomic resolution than 16S rRNA sequencing, it demands a more substantial sequencing depth, computational power, and standardization of bioinformatics processing.[43]

4.3. Multi-omics approaches

To gain more insight into the activity of microbiomes, scientists are increasingly turning to multi-omics integration, a combination of numerous layers of biological data. In the analysis of RNA transcripts, Metatranscriptomics is concerned with the active expression of microbial communities. This method gives us an idea of the microbial genes that are being expressed in any given condition and offers a dynamic picture of microbial activity, not genetic capacity.[50] Metaproteomics is the study of the entire repertoire of proteins that the microbiome makes. This is because proteins are the functional molecules that may influence biological activity, and, as such, this approach assists in bridging the gap between genetic information and the real physiological effects.[51] It gives a better insight into microbial host interactions. Metabolomics is the small-molecule metabolite analysis of the activity of microbes. These metabolites include short-chain fatty acids, bile acids, and chemical precursors of neurotransmitters, which directly affect host physiology. Metabolomics gives a direct assessment of the functional

output of the microbiome, complementing genomic and transcriptomic studies, which represent the biochemical activities taking place in the host–microbiome ecosystem.[52] Collectively, these multi-omics methods offer an overarching perspective of microbial ecosystems that include genetic potential, gene expression, protein activity, and metabolic output (Figure 2).

4.4. Bioinformatics and artificial intelligence

Microbiome research is a Large and complicated datasets that requiring the use of computational techniques for diversity analysis, functional annotation, taxonomic categorization, quality control, and sequence processing. The National Center for Biotechnology Information (NCBI) databases, the Greengenes database, and the SILVA ribosomal RNA (rRNA) gene database are frequently used tools that facilitate microbial taxonomic assignment, sequence comparison, and functional annotation. Artificial intelligence (AI) and machine learning techniques have been used more frequently as microbiome datasets are increasing in order to find intricate patterns that traditional statistical methods might not be able to capture.[53] These methods have been studied for the identification of potential microbial biomarkers, feature selection, disease-risk prediction, and microbial community classification. Therefore, by combining multidimensional microbiological and clinical data to facilitate customized risk prediction and treatment stratification, AI-based methods may advance precision medicine.[54] However, the quality, representativeness, and standardization of the training data have a significant impact on the clinical reliability of such models. When applied to independent populations, models created from small, homogeneous, or poorly standardized cohorts may show overfitting and decreased generalizability.[55] Therefore, assessment of microbiome-based AI models should go beyond internal performance metrics and include external validation in independent populations, calibration evaluation, transportability assessment across patient populations and healthcare settings, and suitable data leakage prevention measures. Furthermore, evaluating the biological plausibility and clinical significance of predictions depends on the interpretability and transparency of the model. In the end, models must exhibit repeatable performance, suitable calibration, clinical utility, and, if relevant, a quantifiable net improvement over current clinical techniques before AI-assisted microbiome prediction can be included into standard precision medicine.[56] The reliability and clinical translation of microbiome profiling are still limited by a number of methodological and biological issues, despite significant technical advancements. Significant technical variability can be introduced, especially in low-biomass

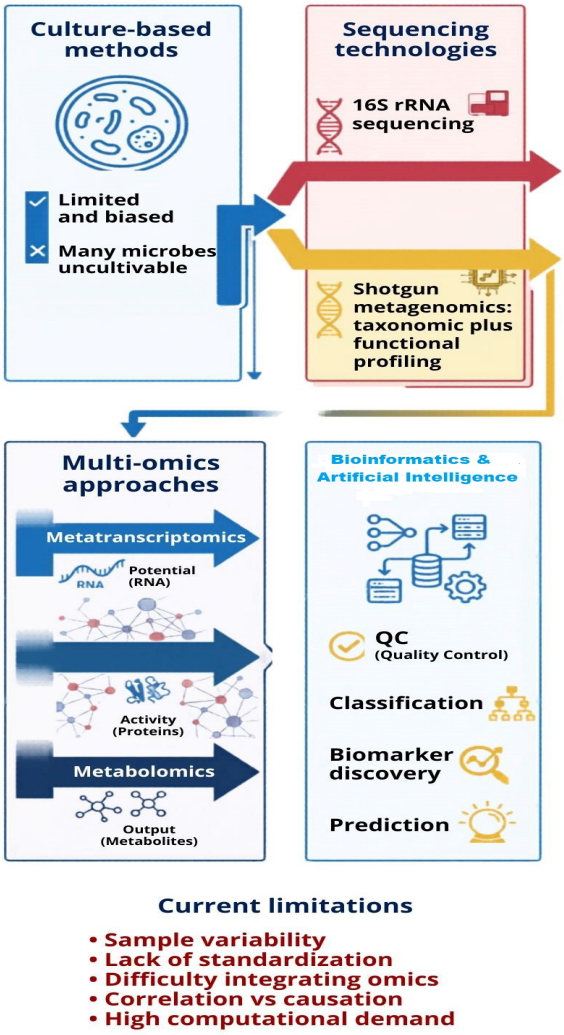

Figure 2. Overview of the principal approaches used for human microbiome profiling and downstream data analysis. Schematic created by the authors.

samples, by variations in sample collection, storage conditions, DNA extraction techniques, sequencing platforms, contaminant control, and laboratory procedures.

Variations in sequencing techniques, batch effects, bioinformatics pipelines, and reference databases, can further influence functional annotation and taxonomic classification, making direct comparison between studies difficult.[57] Moreover, microbiome data are inherently compositional, reflecting relative rather than absolute microbial abundances. Changes in one taxon's relative abundance may indicate changes in the overall microbial community, rather than a true increase or decrease in biomass. Technical challenges persist in accurate microbial

quantification and strain-level characterization. These limitations emphasize the necessity for standardized protocols, validated bioinformatics workflows, quality-control measures, and interlaboratory reproducibility to enable routine microbiome profiling in precision medicine.[58] Improving repeatability and facilitating the appropriate integration of microbiome-based testing into clinical practice will require harmonized reporting rules, external quality-assessment programs, reference materials, and clinically validated laboratory standards.

5. Microbiome and disease susceptibility

The human microbiome is vital for various physiological aspects, such as metabolism, immune regulation, and nervous system interactions. Dysbiosis, defined as changes in microbial community structure and function, can lead to numerous diseases and is not limited to reduced microbial diversity. It encompasses shifts in community composition, abundance, microbial functions, and metabolite production, impacting host metabolic, immune, and neurological processes, potentially increasing disease susceptibility.[43] According to increasing experimental and clinical data, microbiome changes may contribute to disease-related biochemical processes and are associated to many disease states. However, it is still difficult to determine whether microbial alterations are the causes, effects, or moderators of disease. Due to the observational nature of many human microbiome studies, confounding, reverse causation, and variations in food, medication usage, age, location, lifestyle, and other host characteristics can all affect the results. Therefore, without supporting longitudinal, mechanistic, and interventional evidence, relationships between microbiome characteristics and disease should not be regarded as proof of causality.[59]

5.1. Non-communicable diseases

Multiple aspects of research provide evidence connecting the microbiome to non-communicable diseases including cross-sectional and longitudinal observational studies, mechanistic experiments, animal models, and a smaller number of human intervention studies. When evaluating clinical relevance, these evidence categories should be interpreted independently because they offer distinct levels of support for biological plausibility and causality. However, the strength and direction of these relationships differ between groups and study designs.

The changes in gut microbiome, have a significant impact on non-communicable diseases like obesity, type 2 diabetes, cardiovascular diseases, and cancer. However, the strength and direction of these relationships differ between groups and study designs. In obesity, according to experimental research changes in gut microbial populations may impact intestinal barrier function, host metabolism, dietary energy harvest, and adipose tissue biology. However, research on humans continues to yield inconsistent results, and the suggested pathways are not consistently reproduced across populations.[60] Specific microbial species, such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, have been studied in relation to energy regulation, intestinal barrier integrity, metabolic health, and inflammatory pathways. They should not be seen as universal microbial indicators of health or illness, either, as their correlations with metabolic phenotypes are context-dependent. Similar to this, metabolically diverse organisms can be found in wide taxonomic groupings like Firmicutes and Bacteroidota, and changes in their relative abundance alone do not reveal anything about microbial activity. Recent population-based analysis also shows that a variety of interacting factors, such as BMI dietary habits, host genetic background, drug exposure, geography, and other environmental factors, affect gut microbial diversity and composition.[61] Variations in microbial diversity across BMI categories have not been translated into a single repeatable microbial profile highlighting the intricacy of microbiome–host interactions.[62] *Akkermansia muciniphila* and other taxa linked to the generation of SCFAs, such as *Faecalibacterium* species, are often reduced in type 2 diabetes, according to observational research. These results, however, vary among cohorts and could be impacted by food, antidiabetic drugs, obesity, and other lifestyle or metabolic factors. Modulation of host inflammatory and glucose-regulatory pathways, disturbance of intestinal barrier function, and altered microbial metabolite synthesis are some of the suggested causes. Although these processes offer biological plausibility, they do not prove that changes in the microbiome are the main cause of type 2 diabetes. In cardiovascular illness, microbial metabolites have also drawn considerable amount of attention. In both observational and experimental investigations, trimethylamine N-oxide (TMAO), a metabolite produced by a process combining microbial conversion of food nutrients and subsequent human metabolism, has been associated to atherosclerotic disease and cardiovascular risk.[63]

However, a number of factors, such as dietary consumption, renal function, drug exposure, gut bacteria composition, and host metabolic capability, affect circulating TMAO concentrations. Therefore, TMAO should currently be considered a candidate biomarker and potential mechanistic mediator rather than an established independent causal determinant of cardiovascular disease, even though elevated TMAO concentrations are linked to cardiovascular risk and may participate in disease-related pathways.[64]

In recent studies, specific microbial taxa have been identified that could be considered as potential biomarkers for metabolic disorders. Bacteria that degrade mucins (*Akkermansia muciniphila*) have been consistently linked to increased integrity of the gut barrier, increased insulin sensitivity, and decreased inflammation in the body.[65] On the other hand, decreased levels of *Faecalibacterium prausnitzii*, which is a large contributor of the anti-inflammatory SCFA, butyrate, have been reported in people with type 2 diabetes and obesity. These microorganisms are currently being extensively studied as potential biomarkers and therapeutic targets in precision medicine. Nevertheless, further prospective validation is required before they can be incorporated into routine clinical practice.[66]

The development of cancer, especially colorectal cancer, has been linked to microbial dysbiosis. Numerous cancer types have been associated with particular microbial taxa through mechanistic and observational research. Experimental studies suggest that microorganisms may contribute to carcinogenesis through a variety of pathways, including genotoxic metabolite generation, host immune response regulation, chronic inflammation, and alteration of the integrity of the epithelial barrier. However, these processes have not been identified in people for the majority of cancer types, and it is still uncertain how much microbial changes are causative rather than consequential. *Fusobacterium nucleatum* abundance in colorectal cancer has been associated with tumor formation, immunological regulation, chronic inflammation, activation of Wnt/β-catenin signaling, and adverse clinical outcomes.[67] Colorectal carcinogenesis has also been linked to other microbes. While some *Escherichia coli* strains carrying the pks genomic island produce colibactin, a genotoxic metabolite that can cause DNA damage and encourage proinflammatory signaling, enterotoxigenic *Bacteroides fragilis* can produce enterotoxin-mediated effects linked to epithelial damage, inflammation, and genomic instability.[14] The most well-known microbial risk factor for stomach cancer is *Helicobacter pylori*. Only a small percentage of infected people develop gastric cancer, indicating the role of host susceptibility, bacterial virulence factors, environmental exposures, and other cofactors. Persistent infection can cause chronic gastritis and significantly increase the risk of gastric carcinogenesis.[20] Hepatocellular carcinoma has also been linked to alterations in the gut microbiome. Proposed causes include increased intestinal permeability, microbial translocation, changed microbial metabolites, and persistent hepatic inflammation. Oral squamous cell carcinoma has also been associated to oral infections, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, albeit the majority of the information that is now available is observational and has to be confirmed in prospective research.[14] All of these results support the potential of microbial features as candidate diagnostic, predictive biomarkers, or prognostic. However, significant inter-cohort heterogeneity resulting from variations in food, exposure to antibiotics and other medications, inflammation, host genetics, geography, sample collection and processing, and analytical techniques continues to limit their application in clinical practice. Microbial fingerprints found in specific cohorts might therefore not be reliably repeatable across populations. To ascertain the reproducibility, clinical utility, and incremental value of microbiome-based biomarkers prior to their integration into routine clinical decision-making, large, well-designed, prospective multicenter studies employing standardized sampling, sequencing, bioinformatic, and statistical techniques, along with suitable external validation, are required.[59]

To categorize the representative biomarkers from the microbiome for their intended clinical application (diagnostic, prognostic, predictive, pharmacodynamic) and degree of validation, as well as potential for clinical use, the representative biomarkers are summarized in Table 2. There are few reported biomarkers currently in the investigational phase that have yet to be validated in prospective multicenter studies before they can be used in routine clinical decision-making.

Table 2. Current clinical readiness of microbiome-based applications

Application	Representative biomarker/approach	Application category	Current evidence	Clinical readiness	Major limitations	References
Fecal microbiota transplantation for recurrent *Clostridioides difficile* infection	Restoration of gut microbial diversity	Therapeutic intervention	Strong (multiple randomized controlled trials, clinical guidelines, and regulatory approval in several countries)	Established clinical practice	Donor screening, long-term safety, standardization	68,69

(*Cont'd...*)

Table 2. (*Continued*)

Application	Representative biomarker/approach	Application category	Current evidence	Clinical readiness	Major limitations	References
Inflammatory bowel disease	Reduced *Faecalibacterium prausnitzii*, metagenomic dysbiosis indices	Diagnostic / Prognostic	Moderate (multiple cohort studies and meta-analyses)	Biomarker research / early clinical validation	High inter-patient heterogeneity, lack of standardized biomarkers	51,70,58
Colorectal cancer	*Fusobacterium nucleatum*, enterotoxigenic *Bacteroides fragilis*, colibactin-producing *Escherichia coli*	Diagnostic	Moderate–strong (consistent observational studies, validation cohorts)	Validation stage	Limited specificity, geographic variation, lack of prospective validation	59
Gastric cancer	*Helicobacter pylori*	Diagnostic / Risk biomarker	Strong (extensive mechanistic and clinical evidence)	Established clinical application	Not all infected individuals develop cancer; requires integration with host risk factors	59,20
Type 2 diabetes / metabolic disease	*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*	Predictive	Emerging–moderate	Research stage	Confounding by diet, BMI, medications, geography	71
Autoimmune diseases	*Prevotella copri*, depletion of butyrate-producing bacteria	Diagnostic / Predictive	Emerging	Research stage	Mostly associative studies; limited longitudinal validation	72
Neurological disorders	Gut microbiome signatures, microbial metabolites, gut–brain axis markers	Exploratory biomarker	Emerging (primarily observational studies with limited longitudinal and mechanistic validation)	Research stage	Small cohorts, inconsistent reproducibility, uncertain causality	73
AI-based microbiome prediction models	Machine learning using multi-omics and metagenomic datasets	Predictive	Emerging	Research stage	Lack of external validation, dataset bias, limited interpretability, poor reproducibility	55

Evidence classification criteria: The evidence categories were defined according to the quality, consistency, degree of independent validation, and clinical maturity of the existing literature. When results were backed by two or more randomized controlled trials, systematic reviews or meta-analyses, clinical recommendations, or clinical applications that had received regulatory approval, they were deemed strong evidence. Findings that have not yet been extensively implemented in standard clinical practice but are backed by consistent results from several observational studies, prospective cohorts, or independent validation studies were given moderate evidence. Preliminary results from exploratory studies, small clinical cohorts, or mechanistic investigations that need additional validation in bigger and independent populations were referred to as emerging evidence. For methods that are still in the preclinical, proof-of-concept, or early translational stages and do not yet have shown clinical usefulness, experimental evidence was used. The term "validation stage" describes biomarkers or methods that have not yet been approved for routine clinical use but are undergoing analytical and clinical validation to determine their performance, repeatability, and therapeutic relevance.

Biomarker categories: Diagnostic biomarkers are used for detection or diagnosis of the disease; Prognostic biomarkers are used for disease status or outcome; Predictive biomarkers are used to determine the potential for

response to a therapeutic intervention; Pharmacodynamic biomarkers are used to reflect biological response after therapeutic intervention.

Clinical readiness classification: This refers to the classification of established clinical practice interventions or biomarkers that are supported by clinical practice or routine use. (i) Validation stage: biomedical markers which are independently clinically validated before routine use; (ii) Research stage: promising biomarkers with observational or early translational evidence but not ready for clinical use; (iii) Experimental stage: methods that are still at the proof-of-concept or preclinical developmental stage that have not yet been clinically implemented.

5.2. Immune disorders

The microbiome plays a critical role in the development and regulation of the immune system. Microbial ecosystems that are balanced contribute to immune tolerance. Although the causal relationship between microbiome changes and autoimmune disease development is not fully understood, microbial dysbiosis has been linked to immune dysregulation and immune tolerance changes. The loss of microbial diversity is closely linked with autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes.[72] A higher *Prevotella copri* abundance is linked to early rheumatoid arthritis. Experimental studies suggest that *Prevotella copri* may contribute to immune activation through Th17-mediated inflammatory pathways; however, evidence from human studies remains largely associative.[74] Depletion of other bacteria, such as *Faecalibacterium prausnitzii*, which produce butyrate, have been associated with loss of normal regulatory T-cell function and chronic gut inflammation, in contrast. While these associations are biologically plausible, existing evidence is mostly observational and prospective, long-term studies are needed to confirm taxonomic links and make these taxa good potential biomarkers.[75]

Early-life exposure to microbes plays a significant role in immune programming in allergic diseases and asthma. Lack of microbial diversity in infancy may result in inadequate immune development and predisposition to allergic sensitization. Equally, inflammatory bowel disease, comprising of Crohn's disease and ulcerative colitis, is also strongly associated with disruption of the gut microbiome.[70] Inflammatory bowel disease has been associated with the depletion of beneficial bacterial species and the increase of pro-inflammatory microbial species in patients, which may contribute to chronic intestinal inflammation. Microbial diversity is generally diminished in patients with inflammatory bowel disease, with the loss of butyrate-producing beneficial bacteria, such as *Faecalibacterium prausnitzii*, and the rise in the presence of adherent-invasive *Escherichia coli*.[51] Such microbial modulation has been linked to defective epithelial barrier function, induction of pro-inflammatory cytokines and chronic intestinal inflammation. Although biologically plausible, further longitudinal and interventional studies are required to clarify whether these changes are causal or secondary to disease.

5.3. Neurological/Psychiatric disorders

The gut–brain axis, a bidirectional communication network between the gastrointestinal tract and the central nervous system, has been shown to have potential significance. The gut microbiome may affect brain function through neuronal, endocrine, immunological, and metabolic pathways, according to emerging evidence; nevertheless, research into the underlying biological mechanisms and their clinical significance is still ongoing. Changes in microbial metabolites and neurotransmitter-related pathways, such as those involving serotonin and γ-aminobutyric acid (GABA), have been linked to alterations in gut microbial composition in a number of neurological and psychiatric illnesses. The research that is now available, however, does not prove that these microbial changes are the direct cause of disorders like anxiety or depression.[76]

Additionally, a number of studies have found that people with autism spectrum disorder have different gut microbial compositions. It is yet unknown, though, if these changes reflect variations in food, gastrointestinal symptoms, medication use, lifestyle, or other clinical and environmental factors, or if they are a part of the illness pathogenesis.[77] Microbial metabolites, especially SCFAs and tryptophan metabolites, play a role in gut–brain communication. There have also been reports of changes in taxa including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, which may be connected to immunological and neuroinflammatory pathways. However, before their biological and clinical importance can be determined, these findings need to be confirmed in larger, well-controlled, longitudinal cohorts.[66] Because observed associations may be influenced by a variety of potential confounding factors, such as diet, medication use, gastrointestinal symptoms, lifestyle, sleep, stress, socioeconomic factors, and comorbidities, it is important to take precautions when interpreting microbiome findings in neuropsychiatric disorders.[23] The information that is currently available encourages more research on microbiome brain interactions and their possible significance to neurological and psychiatric health, even though the underlying mechanisms are still unclear.

5.4. Infectious diseases

A healthy microbiome offers colonization resistance, that is, the power to ward off the settlement and flourishing of disease-causing microorganisms. This defense mechanism is accomplished by competing for nutrients and attachment sites, synthesizing antimicrobial compounds, and stimulating host immune defense. This protective barrier is compromised when the microbiome is disrupted, especially by antibiotics. Among the most thoroughly documented ones is the infection with *Clostridioides difficile*, the pathogen that may lead to serious diarrhea and colitis. In this situation, depletion of microbial diversity creates an opportunity for opportunistic pathogens to take over and cause the infection and relapse of the disease. Interventions like fecal microbiota have demonstrated clinical efficacy to bring about restoration of microbial balance, which has been promising in treating such infections.[78]

6. Applications of precision medicine and preventive healthcare

Microbiome profiling has emerged as a promising research tool in precision medicine and preventive healthcare, as it enables healthcare systems to transition to generalized treatment approaches and to practice individualized prevention and intervention. Since the human microbiome is a dynamic microbial ecosystem with unique genetically influenced characteristics, diet, environment, and lifestyle, it offers great biological information that can be applied to inform personal healthcare choices. Microbiome-based strategies have the potential to identify early signs of disease, optimize health outcomes, and decrease the burden of disease in the long term in preventive medicine.

6.1. Risk stratification

Risk stratification is one of the brightest uses of microbiome profiling that entails the grouping of people according to their predisposition to certain illnesses. Early signs of disease vulnerability can be based on microbial biomarkers, including decreased diversity, changes in bacterial ratios, or the presence of certain pathogenic organisms.[79] Indicatively, changes in gut microbiota have been linked to a higher risk of metabolic diseases, cardiovascular and inflammatory diseases.

If appropriately validated, microbiome profiling may assist healthcare providers in identifying individuals at increased disease risk and informing preventive strategies. This is a strategy that is in line with the fundamental concepts of precision medicine, where treatment is based on individual biological findings, as opposed to the population means.

6.2. Personalized nutrition

Diet is one of the main factors influencing the function and composition of gut microbes. and microbiome profiling has offered a potential basis for to formulate individualized dietary strategies. Individuals may react metabolically to the same foods in very different ways, partly reflecting by variations in the composition of gut microbes and host-related variables.[80] For instance, butyrate-producing bacteria like *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* have been related to metabolic health and lipid metabolism, whereas a greater abundance of *Prevotella* species has been linked to the metabolism of complex carbohydrates. These correlations do not, however, indicate that specific microbial taxa directly influence nutritional responses or that altering their abundance will inevitably lead to better clinical results. Increasing dietary fiber consumption, changing the composition of macronutrients, and adding food categories that may promote microbial diversity and the generation of advantageous microbial metabolites are some of the strategies being researched as microbiome-informed dietary interventions.[71] Personalized nutrition strategies have been assessed mainly for their effects on postprandial glycemic responses, inflammatory markers (CRP, IL-6, TNF-α), gastrointestinal symptoms, and body weight. While some studies suggest improved dietary predictions using microbiome-based models, their added value compared to existing clinical and lifestyle predictors is still unclear. Larger prospective studies with standardized microbiome profiling and relevant outcome measures are necessary to evaluate the true benefits of microbiome-informed dietary interventions. Currently, these approaches remain investigational rather than routinely practiced, and it is uncertain whether they surpass conventional predictors like dietary intake and BMI in forecasting health outcomes.[81]

6.3. Microbiome-based therapies

Microbiome-directed therapeutic approaches are an expanding field of research, with clinical evidence, regulatory status, and clinical indications varying substantially among interventions. These include:

- *Probiotics:* These are living microorganisms like Lactobacillus, Bifidobacterium and *Saccharomyces boulardii* that provide health benefits by maintaining a healthy balance of microorganisms and gut health.[75]
- *Prebiotics:* These are food components that are not broken down by human enzymes but are selectively fermented by microorganisms living in the gut, such as inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS).[74]

- *Synbiotics:* A combination of probiotics and prebiotics to increase the survival rate of probiotics and increase their therapeutic effect.
- *Fecal microbiota transplantation (FMT):* The aim of FMT is to restore or alter the recipient's intestinal microbial population and related functions by transferring processed fecal material from a thoroughly selected donor to a recipient. For the treatment of recurrent *Clostridium difficile* infection, FMT offers optimal clinical evidence among microbiome-based therapies. Randomized clinical trials and clinical guidelines support its use in carefully chosen patients.[68,77] Other illnesses, such as metabolic disorders and inflammatory bowel disease, have also been studied using FMT; however, the evidence for these indications is still inconsistent, and its regular clinical use has not yet been established. The generation of microbial metabolites, competition with pathogenic organisms, restoration of microbial community structure and function, and modification of host immune responses are some of the intricate mechanisms that underlie the therapeutic effects of FMT. However, it is still undetermined how much each of these pathways contributes to the microbial characteristics associated to a long-lasting therapeutic response.

Microbiome-targeted therapies, such as FMT, probiotics, prebiotics, synbiotics, postbiotics, and live biotherapeutic products, differ significantly in terms of regulatory status, quality of evidence, safety profile, and clinical indications. Appropriate donor or product screening and standardization, monitoring for adverse effects and possible pathogen transmission, regulatory supervision, and evaluation of the durability of clinical responses are all necessary for the clinical application of these strategies[82] (Table 3).

Table 3. Comparison of microbiome-based therapeutic interventions

Intervention	Mechanism of action	Primary clinical indications	Level of evidence	Potential risks / limitations	Current clinical status	Representative references
Probiotics	Administration of live microorganisms that confer health benefits by modulating microbial composition, barrier function, and immune responses	Antibiotic-associated diarrhea, selected gastrointestinal disorders, strain-specific indication	Moderate (strain- and disease-specific)	Variable efficacy, strain specificity, rare infections in immunocompromised patients	Approved as foods/ supplements in most countries; selected evidence-based clinical indications	83
Prebiotics	Selectively utilized substrates that stimulate beneficial microorganisms and increase short-chain fatty acid production	Constipation, metabolic health, selected gastrointestinal disorders	Moderate	Variable response depending on baseline microbiota; gastrointestinal bloating	Widely used as dietary interventions; evidence remains indication-specific	83
Synbiotics	Combination of probiotics and prebiotics to enhance microbial survival and activity	Selected gastrointestinal and metabolic disorders	Moderate but limited disease-specific evidence	Combination efficacy must be demonstrated; heterogeneity among formulations	Investigation for most indications	84
Postbiotics	Administration of inactivated microorganisms and/ or their bioactive components to modulate host physiology	Emerging applications in gastrointestinal and immune-related disorders	Emerging	Limited clinical trials; lack of standardized formulations	Experimental/Early translational stage	83
FMT	Restoration of microbial diversity through transfer of screened donor microbiota	Recurrent *Clostridioides difficile* infection; investigational for IBD, IBS, metabolic and neurological disorders	Strong for recurrent *C. difficile* infection; limited for other indications	Transmission of infectious agents, donor selection, regulatory oversight, long-term safety	Standard clinical therapy for recurrent *C. difficile* infection; investigational for most other diseases	85

Table 3. (*Continued*)

Intervention	Mechanism of action	Primary clinical indications	Level of evidence	Potential risks / limitations	Current clinical status	Representative references
Live biotherapeutic products	Defined live microorganisms developed and regulated as pharmaceutical products	Recurrent *C. difficile* infection and emerging precision medicine applications	Emerging–moderate	Manufacturing complexity, regulatory requirements, long-term safety under evaluation	Early clinical implementation for selected products; most remain investigational	86

Abbreviations: FMT: Fecal microbiota transplantation; IBD: Inflammatory bowel disease; IBS: Irritable bowel syndrome.

6.4. Public health applications

Microbiome profiling at the population level is one possible method for examining patterns of microbial diversity and their connections to health and disease. In addition to identifying microbial fingerprints linked to food habits, antibiotic usage, environmental exposures, and infectious or metabolic diseases, large-scale microbiome investigations may aid in characterizing population-level variance in microbial communities.[87] By enhancing knowledge of how lifestyle, environmental, and healthcare-related factors affect the human microbiome, such data may supplement traditional epidemiology and public health surveillance. For certain high-risk groups, especially those with metabolic or infectious disorders, microbiome data may improve focused preventative measures. The lack of standardized methodologies, the impact of nutrition and socioeconomic factors, the heterogeneity in microbiome composition, and the lack of sufficient evidence linking microbial signatures to health outcomes are some of the problems. Extensive validation through large, longitudinal, and diverse research is required to successfully incorporate microbiome surveillance into public health and policy.

7. Future directions, ethical, and challenges

7.1. Ethical considerations

Privacy and security of data are among the most significant ethical considerations in microbiome studies. Microbiome profiles can be highly personal and contain information that is sensitive to an individual's health status, disease susceptibility, nutrition, lifestyle, and environmental exposures. Therefore, microbiome data must be stored securely and access to this information must be controlled and used responsibly to ensure individual privacy and confidentiality.[88]

In addition to data privacy, ethical implementation of microbiome profiling should concentrate on the use of these technologies to enhance disease prevention, early diagnosis and personalized treatment, while respecting patients' rights. To ensure the safe and effective transformation of microbiome research into preventive and therapeutic care, informed consent for microbiome research subjects, responsible governance of the data created by such research, equitable access to microbiome-based care, and transparent clinical validation are crucial. Ethical principles should enable scientific innovation that also helps to optimize treatment of patients and public health care with microbiome data.[89]

7.2. Clinical challenges

The application of microbiome-based strategies in standard clinical practice is still constrained by a number of scientific, technical, and clinical obstacles despite significant advancements in microbiome research. The absence of defined and repeatable methods for sample collection, storage, DNA extraction, sequencing, data processing, and bioinformatic analysis is a significant obstacle. Variations in these methods can have a significant impact on microbial profiles and lead to conflicting research results. The majority of microbiome-based biomarkers are still being studied, and before their clinical value can be determined, they must undergo thorough validation in sizable, multicenter, prospective cohorts.[90] Despite significant advancements in technology and analysis, many microbiome-based diagnostic models are still in the research stage. Reported microbial signatures may not reliably replicate in other cohorts, and published research often involve relatively small or geographically limited populations. Geographical location, dietary habits, exposure to medications and antibiotics, host features, sample collection and processing, sequencing platforms, and bioinformatic pipelines can all contribute to this heterogeneity. Statistical association or predictive performance in a discovery cohort alone does not prove clinical utility for microbiome biomarkers. These candidates must undergo independent external validation and show added value beyond existing clinical, demographic, and laboratory predictors. Their relevance should be assessed with predefined outcomes and suitable diagnostic or prognostic performance measures.[53]

Reproducibility is another difficulty; the composition of microbiomes may considerably change depending on diet, medication, geography, and time of sampling. This variability is very high, and thus, it is not easy to develop consistent biomarkers to predict the disease. Also, strong clinical validation is required. A large number of microbiome-associated diseases are still based on observational studies, which may influence only the correlations, but not the cause-and-effect relationships.[91] Microbiome-based diagnostics and therapies must undergo large-scale and longitudinal clinical trials to establish their usefulness before they can be used on a large scale in clinical practice.[59] Suitability of microbiome-based biomarkers should be assessed with common performance metrics for biomarkers such as sensitivity, specificity, area under the receiver operating characteristic curve,[26] calibration, and external validation in independent patient cohorts. Clinical utility should also be evaluated using decision-curve analysis, cost-effectiveness, and assessment of whether biomarker-guided interventions improve patient outcomes compared with current standards of care. In addition, candidate biomarkers must be found to have incremental predictive ability over and above traditional clinical risk factors and must be found to be associated with enhanced therapeutic decision-making and/or patient outcomes. Most current microbiome models are still being developed and tested in research and have not yet been validated prospectively or proven to be clinically useful in routine clinical use.[92]

8. Future directions

The future of microbiome research is promising because of continuing advances in sequencing technologies, multi-omics integration, computational biology, and AI. Multi-omics integration will allow researchers to get past microbial composition and focus on functional activity and metabolic effects.[93] Predictive modelling that is driven by AI is another important development. Larger and more complex datasets of microbiomes are increasingly being processed using AI and machine learning algorithms. The tools have the capability of detecting microbial signatures that are related to disease, forecasting health outcomes, and assisting in the development of individualized treatment plans.[94] AI has the potential to improve the scalability and predictive performance of microbiome analyses, provided that robust external validation, model calibration, and transparent reporting standards are achieved. Complementary *in vitro*, *in vivo*, and computational studies have yielded current microbiome discoveries. *In vitro* experiments allow for mechanistic study of microbial metabolites and host cell interactions under controlled laboratory conditions, while *in vivo* animal models allow for analysis of immune responses in the host and causal relationships. However, the results obtained from experimental models need to be confirmed in a well-designed human clinical study before the models can be applied clinically.[59]

Engineered microbial therapeutics is also a quickly growing field. Synthetic biology is making it possible to design genetically modified microorganisms capable of producing target therapeutic outcomes, including the production of useful metabolites, suppression of disease-causing pathogens, or regulation of immune responses.[95] These engineered microbes are a new generation of precision therapeutics. Lastly, it is necessary to develop global microbiome databases that will accelerate research and clinical use. Big data collaborative projects gathering and pooling data on microbiomes of diverse populations will enhance knowledge about microbial diversity by geography, diet, and genetic diversity.[96] These databases will also facilitate more precise predictive models and make sure that microbiome-based medicine can be utilized in global populations. To implement population-level microbiome monitoring, there would be a need for: standardized sampling and analytical protocols, significant laboratory and bioinformatics infrastructure, streamlined workflows, data governance, patient privacy, and representative datasets of the population. At present, it is not feasible for regular public health surveillance because of these challenges.[43]

Most of the data that connects changes in the microbiome to human diseases comes from cross-sectional and observational research, which can find correlations but cannot prove causation on their own. Important insights into possible mechanisms and temporal relationships have been gained from longitudinal studies, experimental models, and interventional trials; however, there is still insufficient evidence for many disease-microbiome associations, and only a small number of findings have been validated in large, well-controlled randomized clinical trials. As a result, rather than being recognized diagnostic, prognostic, or therapeutic targets, many described microbial signatures should be considered potential biomarkers.[59] The need for longitudinal multi-omics studies, independent external validation, standardized analytical workflows, and suitably developed computational and AI approaches to enhance reproducibility, predictive performance, and clinical utility has been further highlighted by growing interest in the clinical application of microbiome profiling.[82]

9. Conclusion

The human microbiome is a dynamic and intricate ecosystem that interacts with immunological, metabolic,

and neuroregulatory systems to support host physiology. The characterization of microbial communities and their functional potential has been significantly increased by developments in high-throughput sequencing, metagenomics, metabolomics, and other multi-omics technologies. A wide range of illnesses, including metabolic, immune-mediated, neurological, and infectious disorders, have been associated to changes in the microbiome, offering an increasing number of potential indicators and treatment targets. Personalized nutrition, microbiome-directed therapies in precision medicine, and disease risk assessment are all areas where microbiome profiling holds great promise. However, methodological heterogeneity, limited reproducibility across populations, insufficient external validation, potential confounding, uncertainty regarding causality, and ethical and regulatory considerations continue to limit the application of these findings in routine clinical practice. Crucially, the discovery of a microbial connection or predictive characteristic does not prove therapeutic value or show that altering the microbiome will enhance patient outcomes on its own. Large, longitudinal, multicenter research, standardized sampling and analytical techniques, independent external validation, the integration of microbiome data with other clinical and multi-omics datasets, and a thorough assessment of clinical value and patient outcomes are all necessary for future advancements. The integration and interpretation of vast microbiome information may be made easier by AI and sophisticated computer techniques, but their clinical application will also need transparent methodology, strong validation, and evaluation in a variety of populations. Though significant data is still needed to generate repeatable biomarkers and clinically validated microbiome-guided diagnostic, prognostic, and therapeutic applications, microbiome research is generally a promising aspect of precision and preventative medicine.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Shahar Bano, Javeria Pervaiz
Writing–original draft: Zunaira Tariq, Shahar Bano, Javeria Pervaiz, Muaz Bin Waqar, Mah-e- Noor Abbas
Writing–review & editing: Ambreen Zahra, Shamas Ul Qamar, Zarish Fatima

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207-214.

   doi: 10.1038/nature11234

2. Hussain MS, Bahl G, Mishra R, *et al.* Introduction to microbiome. In: *Gut Microbiome and Environmental Toxicants*. Boca Raton, FL, USA: CRC Press; 2025:23-40.

   doi: 10.1201/9781003489221-2

3. Vieira BM, Silva GNdMe, Silva MI. Nutritional elements I: nutrients, proteins, carbohydrates, and lipids. In: *Fundamentals of Drug and Non-Drug Interactions: Physiopathological Perspectives and Clinical Approaches*. Cham: Springer; 2025:35-56.

   doi: 10.1007/978-3-031-80107-5_2

4. Astó E, Méndez I, Audivert S, Farran-Codina A, Espadaler J. The efficacy of probiotics, prebiotic inulin-type fructans, and synbiotics in human ulcerative colitis: a systematic review and meta-analysis. *Nutrients*. 2019;11(2):293.

   doi: 10.3390/nu11020293

5. Li Z, Samui S, Liu J, *et al.* Gut microbiome and metabolic health: mechanisms and precision interventions. *Gut Microbes*. 2026;18(1):2644677.

   doi: 10.1080/19490976.2026.2644677

6. Pandit NK, Sharma P, Sharma P, *et al.* Valorizing agro-food waste for microbial B vitamin biosynthesis: impacts on gut microbiota dynamics and microbial communication. *Rev Environ Sci Biotechnol*. 2026;25(1):1-24.

   doi: 10.1007/s11157-025-09753-3

7. Kim S, Ndwandwe C, Devotta H, *et al.* Role of the microbiome in regulation of the immune system. *Allergol Int*. 2025;74(2):187-196.

   doi: 10.1016/j.alit.2024.12.006

8. Graham DB, Xavier RJ. Conditioning of the immune system by the microbiome. *Trends Immunol*. 2023;44(7):499-511.

   doi: 10.1016/j.it.2023.05.002

9. Dogra SK, Kwong Chung C, Wang D, *et al.* Nurturing the early life gut microbiome and immune maturation for long term health. *Microorganisms*. 2021;9(10):2110.

doi: 10.3390/microorganisms9102110

10. Brodin P. Immune-microbe interactions early in life: a determinant of health and disease long term. *Science*. 2022;376(6596):945-950.

    doi: 10.1126/science.abk2189

11. Loh JS, Mak WQ, Tan LKS, *et al.* Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct Target Ther*. 2024;9(1):37.

    doi: 10.1038/s41392-024-01743-1

12. Shukla V, Singh S, Verma S, *et al.* Targeting the microbiome to improve human health with the approach of personalized medicine: latest aspects and current updates. *Clin Nutr ESPEN*. 2024;63:813-820.

    doi: 10.1016/j.clnesp.2024.08.005

13. Kumar A, Singh D. Evolution of traditional healthcare to modern healthcare—benefits, opportunities and challenges. In: *Artificial Intelligence in Modern Healthcare System*. Cham: Springer; 2025:303-325.

    doi: 10.1007/978-981-96-6703-1_13

14. Ratiner K, Ciocan D, Abdeen SK, *et al.* Utilization of the microbiome in personalized medicine. *Nat Rev Microbiol*. 2024;22(5):291-308.

    doi: 10.1038/s41579-023-00998-9

15. Kamel M, Aleya S, Alsubih M, *et al.* Microbiome dynamics: a paradigm shift in combatting infectious diseases. *J Pers Med*. 2024;14(2):217.

    doi: 10.3390/jpm14020217

16. Cuevas-Sierra A, Tomé-Carneiro J, Marques da Silva MM, *et al.* Inflammation and chronic disease: the Mediterranean diet in precision and personalized nutrition. *Ann Nutr Metab*. 2026;80(2):1-12.

    doi: 10.1159/000551530

17. Hou K, Wu ZX, Chen XY, *et al.* Microbiota in health and diseases. *Signal Transduct Target Ther*. 2022;7(1):135.

    doi: 10.1038/s41392-022-00974-4

18. Schmidt TSB, Raes J, Bork P. The human gut microbiome: from association to modulation. *Cell.* 2018;172(6):1198-1215.

    doi: 10.1016/j.cell.2018.02.044

19. Holmes S. Successful strategies for human microbiome data generation, storage and analyses. *J Biosci*. 2019;44(5):117.

    doi: 10.1007/s12038-019-9934-y

20. Tegegne HA, Savidge TC. Leveraging human microbiomes for disease prediction and treatment. *Trends Pharmacol Sci*. 2025;46(1):32-44.

    doi: 10.1016/j.tips.2024.11.007

21. Hacioglu O. Microbiota and microbiome. In: *Microbial World: Bacteria and Archaea*. Cham: Springer; 2026:307-331.

    doi: 10.1007/978-3-032-11438-9_9

22. Jarjees RM, Aladeeb M. The human microbiome: a comprehensive review of its role in health and disease. *Glob Multidiscip Perspect J.* 2026;3(1):23-32. Accessed May 14, 2026. https://zenodo.org/records/18471905

23. O'Riordan KJ, Moloney GM, Keane L, *et al.* The gut microbiota-immune-brain axis: therapeutic implications. *Cell Rep Med.* 2025;6(3):101982.

    doi: 10.1016/j.xcrm.2025.101982

24. Bamford NC, MacPhee CE, Stanley-Wall NR. Microbial primer: an introduction to biofilms—what they are, why they form and their impact on built and natural environments. *Microbiology*. 2023;169(8):001338.

    doi: 10.1099/mic.0.001338

25. Carmona-Cruz S, Orozco-Covarrubias L, Sáez-de-Ocariz M. The human skin microbiome in selected cutaneous diseases. *Front Cell Infect Microbiol*. 2022;12:834135.

    doi: 10.3389/fcimb.2022.834135

26. Wakabayashi J, Kimura K, Kawauchi T. The intestinal barrier: a multilayered gatekeeper against systemic disease. *Int J Microbiol*. 2026;2026:2828137.

    doi: 10.1155/ijm/2828137

27. Pérez-Cobas AE, Rodríguez-Beltrán J, Baquero F, Coque TM. Ecology of the respiratory tract microbiome. *Trends Microbiol.* 2023;31(9):972-984.

    doi: 10.1016/j.tim.2023.04.006

28. Dekaboruah E, Suryavanshi MV, Chettri D, Verma AK. Human microbiome: an academic update on human body site specific surveillance and its possible role. *Arch Microbiol*. 2020;202(8):2147-2167.

    doi: 10.1007/s00203-020-01931-x

29. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012;489(7415):220-230.

    doi: 10.1038/nature11550

30. Govender P, Ghai M. Population-specific differences in the human microbiome: factors defining the diversity. *Gene*. 2025;933:148923.

    doi: 10.1016/j.gene.2024.148923

31. Suri H, Suri H, Nagda N, *et al.* Current perspectives on the human skin microbiome: functional insights and strategies for therapeutic modulation. *Biomed Pharmacother*. 2025;193:118655.

    doi: 10.1016/j.biopha.2025.118655

32. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal

health. *Microb Cell Fact*. 2020;19(1):203.

doi: 10.1186/s12934-020-01464-4

33. Spochacz-Santoro M, Szeliga A, Durda-Masny M, *et al.* Higher BMI is associated with vaginal microbiome alterations in women with PCOS. *Reprod Fertil*. 2026;7(2):RAF260051.

doi: 10.1530/RAF-26-0051

34. Zeng J, He Z, Wang G, *et al.* Interaction between microbiota and immunity: molecular mechanisms, biological functions, diseases, and new therapeutic opportunities. *MedComm*. 2025;6(7):e70265.

doi: 10.1002/mco2.70265

35. Lee H, Song J, Lee B, *et al.* Food carbohydrates in the gut: structural diversity, microbial utilization, and analytical strategies. *Food Sci Biotechnol*. 2024;33(9):2123-2140.

doi: 10.1007/s10068-024-01648-3

36. Wang R, Lan C, Benlagha K, *et al.* The interaction of innate immune and adaptive immune system. *MedComm*. 2024;5(10):e714.

doi: 10.1002/mco2.714

37. Buret AG, Motta JP, Allain T, Ferraz J, Wallace JL. Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron? *J Biomed Sci*. 2019;26(1):1.

doi: 10.1186/s12929-018-0495-4

38. Bonanno S, Joshi NS. Engineering microbes to modulate innate immune signaling: strategies for host-microbe interactions. *Curr Opin Microbiol*. 2026;89:102695.

doi: 10.1016/j.mib.2025.102695

39. Tan Y, Huang J, Liu Y, Lai X. Progress in the detection of gut microbiota based on microfluidic technology. *Interdiscip Med*. 2026;4(1):e70069.

doi: 10.1002/inmd.70069

40. Arbefeville SS, Timbrook TT, Garner CD. Evolving strategies in microbe identification—a comprehensive review of biochemical, MALDI-TOF MS and molecular testing methods. *J Antimicrob Chemother*. 2024;79(Supplement_1):i2-i8.

doi: 10.1093/jac/dkae275

41. Nath S. Integration of microbial proteins into traditional food systems: innovations, challenges, and future perspectives. *Food Rev Int*. 2026;42(3):1532-1557.

doi: 10.1080/87559129.2025.2520453

42. Kurniawan FD, Alia D, Shiraishi M, *et al.* A systematic algorithm using 16S ribosomal RNA for accurate diagnosis of pneumonia pathogens. *Sci Rep*. 2025;15(1):29253.

doi: 10.1038/s41598-025-14841-z

43. Knight R, Vrbanac A, Taylor BC, *et al.* Best practices for analysing microbiomes. *Nat Rev Microbiol*. 2018;16(7):410-422.

doi: 10.1038/s41579-018-0029-9

44. Kuczynski J, Lauber CL, Walters WA, *et al.* Experimental and analytical tools for studying the human microbiome. *Nat Rev Genet*. 2011;13(1):47-58.

doi: 10.1038/nrg3129

45. Jovel J, Patterson J, Wang W, *et al.* Characterization of the gut microbiome using 16S or shotgun metagenomics. *Front Microbiol*. 2016;7:459.

doi: 10.3389/fmicb.2016.00459

46. Zhang Y, Thompson KN, Branck T, *et al.* Metatranscriptomics for the human microbiome and microbial community functional profiling. *Annu Rev Biomed Data Sci*. 2021;4:279-311.

doi: 10.1146/annurev-biodatasci-031121-103035

47. Wang Y, Zhou Y, Xiao X, Zheng J, Zhou H. Metaproteomics: a strategy to study the taxonomy and functionality of the gut microbiota. *J Proteomics*. 2020;219:103737.

doi: 10.1016/j.jprot.2020.103737

48. Zhang X, Li L, Butcher J, Stintzi A, Figeys D. Advancing functional and translational microbiome research using meta-omics approaches. *Microbiome*. 2019;7(1):154.

doi: 10.1186/s40168-019-0767-6

49. Nam N, Do H, Loan Trinh K, Lee N. Metagenomics: an effective approach for exploring microbial diversity and functions. *Foods*. 2023;12(11):2140.

doi: 10.3390/foods12112140

50. Jan R, Hussain A, Assad A, Khurshid S, Macha MA. Challenges with multi-omics data integration. In: *Multi-omics Technology in Human Health and Diseases*. Amsterdam, Netherlands: Elsevier; 2025:223-242.

doi: 10.1016/B978-0-443-13595-8.00010-6

51. Valdés-Mas R, Leshem A, Zheng D, *et al.* Metagenome-informed metaproteomics of the human gut microbiome, host, and dietary exposome uncovers signatures of health and inflammatory bowel disease. *Cell*. 2025;188(4):1062-1083.e36.

doi: 10.1016/j.cell.2024.12.016

52. Chen H, Kong J, Du P, *et al.* Functional metabolomics: unlocking the role of small molecular metabolites. *Front Mol Biosci*. 2025;12:1542100.

doi: 10.3389/fmolb.2025.1542100

53. Dakal TC, Xu C, Kumar A. Advanced computational tools, artificial intelligence and machine-learning approaches in gut microbiota and biomarker identification. *Front Med Technol*. 2024;6:1434799.

doi: 10.3389/fmedt.2024.1434799

54. Wang XW, Wang T, Liu YY. Artificial intelligence for microbiology and microbiome research. *Cell Syst*. 2026;17(2):101531.

doi: 10.1016/j.cels.2026.101531

55. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. *Nat Med*. 2019;25(1):44-56.

doi: 10.1038/s41591-018-0300-7

56. Marcos-Zambrano LJ, Karaduzovic-Hadziabdic K, Loncar Turukalo T, *et al.* Applications of machine learning in human microbiome studies: a review on feature selection, biomarker identification, disease prediction and treatment. *Front Microbiol*. 2021;12:634511.

doi: 10.3389/fmicb.2021.634511

57. Wang Q, Wang K, Wu W, Giannoulatou E, Ho JWK, Li L. Host and microbiome multi-omics integration: applications and methodologies. *Biophys Rev*. 2019;11(1):55-65.

doi: 10.1007/s12551-018-0491-7

58. Caminero A, Tropini C, Valles-Colomer M, *et al.* Credible inferences in microbiome research: ensuring rigour, reproducibility and relevance in the era of AI. *Nat Rev Gastroenterol Hepatol*. 2025;22(11):788-803.

doi: 10.1038/s41575-025-01100-9

59. Fischbach MA. Microbiome: focus on causation and mechanism. *Cell*. 2018;174(4):785-790.

doi: 10.1016/j.cell.2018.07.038

60. Shabani M, Mohammadi M, Norouzi S, *et al.* The relationship between gut microbiome and human diseases: mechanisms, predisposing factors and potential intervention. *Front Cell Infect Microbiol.* 2025;15:1516010.

doi: 10.3389/fcimb.2025.1516010

61. Gacesa R, Kurilshikov A, Vich Vila A, *et al.* Environmental factors shaping the gut microbiome in a Dutch population. *Nature*. 2022;604(7907):732-739.

doi: 10.1038/s41586-022-04567-7

62. Hernández-Rodríguez D, Contreras A, Melgar Lalanne G, *et al.* Fecal microbiota composition concerning body mass index and early-life factors in Mexican preschool-aged children: a cross-sectional study. *PeerJ*. 2026;14:e21253.

doi: 10.7717/peerj.21253

63. Fusco W, Adolph T, Cammarota G, *et al.* Gut microbiota and atherosclerosis. *Gut*. 2026;75(5):1067-1077.

doi: 10.1136/gutjnl-2025-335610

64. Thomas MS, Fernandez ML. Trimethylamine N-oxide (TMAO), diet and cardiovascular disease. *Curr Atheroscler Rep*. 2021;23(4):12.

doi: 10.1007/s11883-021-00910-x

65. Depommier C, Everard A, Druart C, *et al.* Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med*. 2019;25(7):1096-1103.

doi: 10.1038/s41591-019-0495-2

66. Lin W, Pu L, Qian X, *et al.* Exercise-induced modulation of gut microbiota in individuals with obesity and type 2 diabetes: a systematic review and meta-analysis. *Front Microbiol.* 2025;16:1671975.

doi: 10.3389/fmicb.2025.1671975

67. Wang N, Fang JY. Fusobacterium nucleatum, a key pathogenic factor and microbial biomarker for colorectal cancer. *Trends Microbiol.* 2023;31(2):159-172.

doi: 10.1016/j.tim.2022.08.010

68. Cubillos-Ruiz A, Guo T, Sokolovska A, *et al.* Engineering living therapeutics with synthetic biology. *Nat Rev Drug Discov.* 2021;20(12):941-960.

doi: 10.1038/s41573-021-00285-3

69. Rynikova M, Bojcukova V, Demeckova V. One therapy, many targets: redefining ulcerative colitis treatment through fecal microbiota transplantation. *Ther Adv Gastroenterol.* 2026;19:17562848261437918.

doi: 10.1177/17562848261437918

70. Bertin L, Facchin S, Barberio B, *et al.* Diet and gut microbiota in inflammatory bowel disease: a clinical and nutritional perspective. *Pharmaceuticals.* 2026;19(2):318.

doi: 10.3390/ph19020318

71. Wu X, Mu B, Li G, *et al.* Gut microbial composition, oxidative stress, and immunity in metabolic disease: toward personalized interventions. *Antioxidants*. 2026;15(2):175.

doi: 10.3390/antiox15020175

72. Liu S, Sanaie S, Abdollahi H, *et al.* The role of human microbiota in autoimmune diseases: exploring dysbiosis and mechanisms. *APMIS*. 2026;134(2):e70159.

doi: 10.1111/apm.70159

73. Rutter JW, Dekker L, Owen KA, Barnes CP. Microbiome engineering: engineered live biotherapeutic products for treating human disease. *Front Bioeng Biotechnol.* 2022;10:1000873.

doi: 10.3389/fbioe.2022.1000873

74. Nii T, Maeda Y, Motooka D, *et al.* Genomic repertoires linked with pathogenic potency of arthritogenic Prevotella copri isolated from the gut of patients with rheumatoid arthritis. *Ann Rheum Dis*. 2023;82(5):621-629.

doi: 10.1136/ard-2022-222881

75. Martín R, Rios-Covian D, Huillet E, *et al.* Faecalibacterium: a bacterial genus with promising human health applications. *FEMS Microbiol Rev*. 2023;47(4):fuad039.

doi: 10.1093/femsre/fuad039

76. Akif A, Islam MR. The microbiota-gut-brain axis in the pathophysiology of major depressive disorder: a mechanistic review. *Compr Physiol*. 2026;16(1):e70100.

doi: 10.1002/cph4.70100

77. Tingler AM, Figuereo YF, Engevik KA, *et al.* Children with autism spectrum disorder and chronic gastrointestinal symptoms have alterations in intestinal neurotransmitter pathways. *Dig Dis Sci.* 2026;71(6):2413-2431.

doi: 10.1007/s10620-026-09667-2

78. Wang R. Clostridioides difficile infection: microbe-microbe interactions and live biotherapeutics. *Front Microbiol*. 2023;14:1182612.

doi: 10.3389/fmicb.2023.1182612

79. Gilbert JA, Azad MB, Bäckhed F, *et al.* Clinical translation of microbiome research. *Nat Med*. 2025;31(4):1099-1113.

doi: 10.1038/s41591-025-03615-9

80. Malik S, Bharali R, Barbhuyan HSA, *et al.* Function of gut microbiota in longevity as a futuristic approach of personalised nutrition. In: *Microbial Approaches to Personalized Nutrition*. Hershey, PA, USA: IGI Global; 2026:303-332.

doi: 10.4018/979-8-3373-4382-2.ch010

81. Mathrani A, Yip W, Sequeira-Bisson IR, *et al.* Effect of a 12-week polyphenol rutin intervention on markers of pancreatic β-cell function and gut microbiota in adults with overweight without diabetes. *Nutrients*. 2023;15(15):3360.

doi: 10.3390/nu15153360

82. Gavanji S, Suhail M, Bencurova E, *et al.* Recent advances and clinical relevance of microbiome dynamics in health and disease. *Gut Microbes*. 2026;18(1):2679197.

doi: 10.1080/19490976.2026.2679197

83. Guarner F, Sanders ME, Szajewska H, *et al.* World Gastroenterology Organisation global guidelines: probiotics and prebiotics. *J Clin Gastroenterol*. 2024;58(6):533-553.

doi: 10.1097/MCG.0000000000002002

84. Swanson KS, Gibson GR, Hutkins R, *et al.* The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol*. 2020;17(11):687-701.

doi: 10.1038/s41575-020-0344-2

85. Kelly CR, Feuerstadt P. Diagnosis and management of Clostridioides difficile. *Am J Gastroenterol*. 2026;121(3):628-634.

doi: 10.14309/ajg.0000000000003844

86. Kandiyal B, Gupta M, Das B. Live biotherapeutics: emerging trends and future directions in microbial therapy. *Prog Mol Biol Transl Sci*. 2026;220:1-35.

doi: 10.1016/bs.pmbts.2025.12.001

87. Dera N, Kosińska-Kaczyńska K, Żeber-Lubecka N, *et al.* Impact of early-life microbiota on immune system development and allergic disorders. *Biomedicines*. 2025;13(1):121.

doi: 10.3390/biomedicines13010121

88. Huttenhower C, Finn RD, McHardy AC. Challenges and opportunities in sharing microbiome data and analyses. *Nat Microbiol*. 2023;8(11):1960-1970.

doi: 10.1038/s41564-023-01484-x

89. Choudhury A, Ortiz P, Scano C. Machine learning and multi-omics integration approaches for human microbiome data. In: Springer Handbook of Chem- and Bioinformatics. Cham: Springer; 2026:169-185.

doi: 10.1007/978-3-031-81728-1_8

90. Isali I, Wong TR, Tian S. Best practice guidelines for collecting microbiome samples in research studies. *Eur Urol Focus*. 2024;10(6):909-913.

doi: 10.1016/j.euf.2024.12.007

91. Khan SS, Greenland P, Allen NB, *et al.* Criteria to assess the predictive and clinical utility of novel models, biomarkers, and tools for risk of cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*. 2026;153(11):e953-e970.

doi: 10.1161/CIR.0000000000001401

92. Tu JB, Liao WJ, Long SP, *et al.* Construction and validation of a machine learning model for the diagnosis of juvenile idiopathic arthritis based on fecal microbiota. *Front Cell Infect Microbiol*. 2024;14:1371371.

doi: 10.3389/fcimb.2024.1371371

93. Chetty A, Blekhman R. Multi-omic approaches for host-microbiome data integration. *Gut Microbes*. 2024;16(1):2297860.

doi: 10.1080/19490976.2023.2297860

94. Kumar R, Nagraik R, Lakhanpal S, *et al.* Artificial intelligence in gut microbiome research: toward predictive diagnostics for neurodegenerative disorders. *Acta Microbiol Immunol Hung*. 2025;72(4):296-312.

doi: 10.1556/030.2025.02725

95. Heavey MK, Durmusoglu D, Crook N, Anselmo AC. Discovery and delivery strategies for engineered live biotherapeutic products. *Trends Biotechnol.* 2022;40(3):354-369.

doi: 10.1016/j.tibtech.2021.08.002

96. Arif SJ, Graham SP, Abdill RJ, Blekhman R. Analyzing human

gut microbiome data from global populations: challenges and resources. *Trends Microbiol*. 2025;33(11):1212-1223. doi: 10.1016/j.tim.2025.05.008