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Microbiome-Based Diagnostics: Current Progress

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ABSTRACT

Microbiome-based diagnostics is an emerging field that leverages microbial communities and their functional profiles to support disease detection, monitoring, and personalized healthcare. Advances in next-generation sequencing, metabolomics, and proteomics have enabled high-resolution characterization of the microbiome, uncovering its associations with conditions such as cancer, inflammatory bowel disease, diabetes, obesity, and mental health disorders. Diagnostic applications span infectious disease detection, chronic disease management, and individualized interventions, with platforms such as MYcrobiota demonstrating promise for culture-free profiling of low-biomass clinical samples. Metabolomic and proteomic approaches further reveal the metabolic and functional roles of microbial communities, offering novel biomarkers for clinical use. The gut microbiome, in particular, has emerged as a critical determinant of metabolic, immune, and neurological health, with evidence supporting its role in shaping responses to therapy and influencing host physiology. Despite these advances, significant challenges remain, including inter-individual variability, lack of standardized methodologies, and limited clinical validation. Ethical concerns surrounding privacy and data ownership also require attention. Integration of multi-omics, artificial intelligence, and standardized clinical pipelines is expected to advance the field toward routine application. Microbiome-based diagnostics thus holds transformative potential for precision medicine, offering new opportunities for early detection, risk assessment, and personalized therapeutic strategies.

Keywords: Microbiome-based diagnostics**,** Next-generation sequencing (NGS)**,** Metabolomics and proteomics**,** Gut microbiota and Precision medicine

INTRODUCTION

Microbiome-based diagnostics is an emerging approach that uses the microorganisms selectively associated with disease to guide clinical decision-making [1]. Advances in next-generation sequencing, metabolomics, and proteomics provide unparalleled opportunities for translation of the microbiome to clinical applications. Microbiome-based diagnostics can indicate the risk of developing a certain condition, determine the severity of disease, or predict the response to a certain therapy. Many studies have documented microbiome biomarkers associated with a wide range of diseases, including cancer, inflammatory bowel diseases, diabetes, and others. Development of the microbiome into a clinically valuable diagnostic tool, however, requires navigation of a not inconsiderable number of difficulties and challenges.

Understanding the Microbiome

A microbiome encompasses the total collection of microbes and their genetic information present in a defined environment along with the spectrum of environmental conditions integral to the microbe's existence. It represents an ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space [1]. Microbiome-based diagnostics refer to the methods applying state-of-the-art technologies such as next-generation sequencing, enabling rapid analysis of the microbiota to identify the causative pathogens. Label-free technologies for microbial metabolite detection have seen applications in bacteria identification for antibiotic susceptibility testing. Similarly, emerging tools from metaproteomics and metatranscriptomics reveal functional roles of the gut microbiome that enable its use in disease diagnosis. Among these, the gut microbiome holds a preeminent position in regulating human health. Evidence points to the association of alteration in the gut

microbial population with the outbreak of many metabolic disorders [1]. Clinical studies have displayed the therapeutic potential of probiotics and psychobiotics defined by their beneficial impact on host health related to their gut-brain axis interactions in mental stability and emotional behaviour. The high-throughput analysis of intestinal microbial communities is revealing relevance to other aspects of human health and has now being incorporated as a standard practice in clinical studies involving any systemic disorder. Guidelines dealing with ethical concerns involving privacy and informed consent of the donors represent an essential framework for studies involving human gut microbiome. Nevertheless, issues pertaining to the irreproducibility of analytical data due to the lack of standardization in sampling and analytical procedures, in addition to cohort size and inter-individual variability, often present a serious challenge in establishing the clinical use of these findings [1].

Definition and Components

The microbiome is defined as the collective genome of the microbiota, the microorganisms that naturally exist within the human body, including bacteria, viruses, fungi, and archaea [2]. These microorganisms create an internal ecosystem of mutualistic symbionts and commensals that is deeply intertwined with the body's metabolic and physiologic state [3]. Some microorganisms are key for biological processes such as nutrient digestion, immune modulation, pathogen displacement, and metabolite exchange. The microbiome and microbiota are closely linked, and the terms are often used interchangeably. Any medical or scientific testing and analysis that involves the microbiome for healthcare applications including clinical diagnostic, personalized treatment, and health-associated monitoring is considered microbiome-based diagnostic analysis [2, 3].

Role in Human Health

The human body hosts a diverse microbiome, comprising both symbiotic and pathogenic microorganisms that inhabit various sites, including the skin, oral cavity, gut, and respiratory and urogenital tracts [4]. The microbiome is recognized as a "second genome," playing a vital role in maintaining immune, metabolic, endocrine, and neurological homeostasis, especially in the gastrointestinal tract [2]. The gut harbors the most abundant microbial ecosystem composed of bacteria, fungi, archaea, viruses, and eukaryotic organisms, where bacteria account for more than 90%. Its formation begins early in life and can be altered throughout the lifespan by various influencing factors [4, 2].

Technological Advances in Microbiome Analysis

A deeper understanding of the microbiome became possible by advanced technologies such as next-generation sequencing from 2005 onward. Additionally, new omics methods such as metabolomics and proteomics constitute important additional sources of information. Complementary methods to analyse microbiome data have been developed, which greatly catalysed the emergence of microbiome-based diagnostics [1].

Next-Generation Sequencing

Next-generation sequencing (NGS) offers high-throughput, sensitive analysis of microbial communities and genomic characteristics [1]. It generates millions of nucleotide sequence reads from DNA/RNA extracted from biological samples. NGS provides more detailed taxonomic information than quantitative PCR or targeted sequencing; it overcomes limitations such as equipment cost and primer design constraints in 16S rRNA NGS. MYcrobiota0a culture-free microbiota profiling platform fulfilling clinical diagnostic requirements0employs a validated 16S rRNA gene NGS pipeline. This methodology minimizes PCR amplification of contaminating 16S rRNA gene molecules, a common issue with conventional 16S rRNA gene NGS methods in low-biomass samples. MYcrobiota accurately profiles microbial compositions in clinical samples; in culture-negative cases, it confirms the absence of 16S rRNA gene copies unless fastidious or anaerobic organisms are present [2]. The platform09s sensitivity to detect bacterial operational taxonomic units at very low abundances makes it suitable for characterizing sterile sites such as synovial fluids, cerebrospinal fluids, and blood samples. Extensive validation studies remain necessary prior to routine clinical implementation.

Metabolomics and Proteomics

The metabolic activity of microbiota profoundly influences human health. A method was recently proposed to characterize microorganisms and their interactions with hosts via small metabolites [4]. Metabolomics studies the collection of metabolites in a biological unit; it plays a crucial role at the interface between gut microbiota and the host. Applied to the gut microbiota, it assesses the metabolites released by microbial communities and represents potential biomarkers for clinical diagnosis. Studies profiling fecal and urinary metabolites in obese mice demonstrated that gut microflora modulates host metabolism, revealing obesity-related metabolite profiles and new perspectives for diagnosis and treatment [4]. Proteomics provides insights into the phenotype of microbial communities hosted by humans and, thus, addresses the molecular mechanisms underpinning clinical problems. It emerged as a promising alternative for modeling relevant pathogens, especially in the analysis of infections. Despite attempts to transition metaproteomics from research to clinical applications, no practical in-house method currently exists. The challenges stem from intrinsic sample heterogeneity, limited instrumentation, and the

complexities of processing and integrating multi-omics data. Advanced metaproteomic methodologies are essential for progression in this area [4].

Current Applications in Diagnostics

Microbiome-based diagnostics rely on the identification and quantification of microbial species and their products within individuals, enabling assessment of health status or identification of dysbiosis associated with disease [2]. Diagnostic applications fall broadly into three categories: screening and diagnostic support for infective conditions, management and therapeutic targeting of chronic and non-infectious conditions, and personalization of interventions and formulation through pre-, pro-, and synbiotic approaches. Culture-independent techniques can reduce turnaround times, increase reproducibility, and detect low-abundance and anaerobic bacteria; however, clinical interpretation remains constrained by an incomplete understanding of many human microbiota-pathology relationships [1]. The frequent identification of bacterial contamination in culture-negative clinical samples from sterile body sites led to the development of a microbiota profiling methodology, which avoids the amplification of contaminant DNA introduced during sample processing. The MYcrobiota platform was validated and evaluated on various clinical samples, existing culture-negative specimens included, to demonstrate its potential as a culture-free microbiota profiling tool for clinical diagnostics[1]. Sensitivity analyses revealed that the platform can detect bacterial operational taxonomic units at very low abundances, making it suitable for investigation of sterile body sites. While the absence of DNA-based species identification and antibiotic susceptibility information limits some applications, MYcrobiota offers a standardized workflow providing highly accurate overviews of microbial composition, thereby confirming the absence of 16S rRNA gene copies in culture-negative samples and reducing the risk of false negatives. Extensive clinical and financial validation studies remain necessary before routine implementation of microbiome-based diagnostics can be considered [1, 2].

Infectious Disease Detection

Infectious disease diagnosis represents one of the early areas in which microbiome analysis demonstrated immediate applications. The approach provides rapid and comprehensive assessment of the combined physiological state of the host, associated microbes, and potential pathogens. This capability is crucial for informing optimal treatment decisions and guiding the implementation of tailored therapeutic strategies [2]. Microbiome analysis remaining independent of cultivation processes constitutes an advantage that circumvents selection biases commonly encountered with traditional culturing methods. Additionally, the evaluated sample volume is markedly larger than that used in standard culture settings, thereby enriching the diagnostic examination. Detection of bacterial operational taxonomic units (OTUs) at markedly low relative abundances extends the applicability of the diagnostic platform to normally sterile body sites, including synovial fluids, cerebrospinal fluids, and blood specimens. The provision of a robust, standardized, and validated framework for microbial composition assessment further enhances diagnostic reliability, including the capacity to substantiate the absence of 16S rRNA gene sequences in culture-negative samples [5]. Although limitations such as the absence of species-level identification and accompanying antimicrobial susceptibility profiles persist, the established methodology delineates a foundational step towards integrating microbiota research into routine clinical diagnostics. Comprehensive validation studies remain requisite to fully ascertain its prospective clinical utility [2, 5].

Chronic Disease Management

Management of chronic disease is another important area in which the microbiome is used for diagnostic purposes. The composition of the gut microbiota influences the host's metabolic phenotype, resulting in chronic metabolic diseases such as obesity, type 2 diabetes, and liver-steatosis [6]. A meta-analysis conducted by Boers et al. shows suggestions that the microbiome is sufficiently discriminatory to be used by diagnostic classifiers for certain diseases [2].

Personalized Medicine

Microbiome-based diagnostics enable an unbiased molecular characterization of clinical specimens, facilitating the identification and exploitation of microbiome signatures for tailored diagnostic paradigms [2]. These approaches show considerable promise in personalized medicine platforms, where the individual microbial fingerprint can predict the propensity for specific disorders and guide therapeutic interventions. Nutritional recommendations tailored to an individual's microbial composition and the administration of live microorganisms as psychobiotics exemplify such applications. The analytical characterization of the human microbiome will inevitably become integral to the development and implementation of next-generation personalized medicine [7].

Microbiome and Gut Health

The gut microbiota commonly contains 500–1,000 diverse bacterial species, with variations related to host genotype, diet, age, gender and geographical location. High-throughput sequencing and metabolomic analyses have generated growing evidence of microbiota influences on chronic disease[8]. Changes in gut microbiota composition and fermentation products are important factors in the development of metabolic disorders such as

obesity and type 2 diabetes. Alterations in the gut microbiota may therefore be diagnostic or prognostic markers for pathology and valuable targets for novel therapeutics. Despite many metagenomic and metabolomic association studies, the link between gut microbiota and host metabolism remains poorly characterised [8]. Microbiome-based diagnostics promise to revolutionise healthcare at levels ranging from pathogen identification to cancer transcriptome-based screening, within a personalised medicine approach. Worldwide efforts aim to solve the global challenge of how the microbiome informs human health, disease susceptibility and drug responses [9]. The size and complexity of the information available in an individual's microbiomes have yet to be fully exploited in precision nutrition. Ensemble-learning algorithms produce long lists of microbes, microbial pathways and metabolites whose relevance to the studied outcome requires further assessment [10]. Many mechanisms are not well understood because microbiomes from healthy individuals vary extensively and universally applicable microbial signatures are thus difficult to define. Large-scale cause-and-effect interventional studies addressing the functional characteristics of a healthy individual's microbiomes are needed. In addition, current microbiome-disease association knowledge remains limited to sequencing correlations, and the utility of microbiome analysis to promote better health is restricted [10].

Gut Microbiota Composition

Not surprisingly, the gut microbiota is the part of the human microbiome reported most frequently in diagnostic applications. The gut's community composition largely determines the microbial functionality maintained across the lower intestine, exhibited in areas of enrichment or depletion relative to the human gut metagenome (HMP). The gut microbiota, dominated by Firmicutes and Bacteroidetes on the taxonomic level of phylum, is exposed to a constant flux of both exogenous (nutrition) and endogenous (mucosal secretions) substrates playing an important role in host health. Hence, alterations in this community have been associated with conditions as diverse as obesity, diabetes, intestinal bowel syndrome, and several chronic inflammatory diseases. Furthermore, control of microbial abundance and variety likely plays a key role in the age-related loss of physiological and immunological function, with long-lived elderly individuals exhibiting microbiota compositions and diversity levels more akin to younger adults [11]. Within this context, individual-specific edge-based features of the microbiota network's dynamics have been used to segment pre-disease and disease classes, highlighting the analytical value that personalized microbiota-dynamics characterization holds for patient stratification [11].

Impact on Metabolic Disorders

Metabolic disorders, including obesity and diabetes, represent major health challenges of the twenty-first century [8]. Advances in microbiome sequencing and omics techniques such as metabolomics and proteomics have revealed that the gut microbiota contributes essential functions that influence host lipid and glucose metabolism, thereby affecting health outcomes [4]. In a shared context of microbiota dysbiosis, metabolite concentrations undergo alterations with corresponding functional effects across the spectrum of metabolic health, underscoring the important role of metabolite signaling in the progression of metabolic disease [12].The human gastrointestinal tract hosts a vast and abundant microbial community that constitutes the gut microbiota. Evidence increasingly emphasizes its vital role in human health. Perturbations can lead to a dysbiotic state associated with various chronic human pathologies. Preliminary gut microbiota studies performed in metabolic disease patients revealed a significant dysbiotic signature. Early studies documented a marked depletion in key functional butyrate-producing Firmicutes from the Clostridiales order, such as Faecalibacterium prausnitzii and Roseburia intestinalis.

Microbiome in Mental Health

A complex interplay exists between the gut microbiome and the brain along the gut–brain axis. This interrelation is significant because it offers opportunities to affect brain function through gut interventions in disorders such as depression, anxiety, and autism spectrum disorder [13]. Probiotics, known as psychobiotics, have been shown to benefit patients withrological and psychiatric disorders and may be applied as a probiotic treatment or adjuvant therapy in gastrointestinal-related psychological disorders [14].

Gut-Brain Axis

The value of gut metabolites for the development of psychobiotics was highlighted by the beneficial effect on major depressive patients reported in a recent nutritional interventional study [15]. Growing interest in the interplay between microbiota and the brain has encouraged investigation of the role of microbiome metabolism in the gut-brain axis. The gut and the brain communicate via endocrine, immune, and humoral links [16]. Numerous studies show that psychoneurological and neurodegenerative illnesses are involved in microbiota–brain communication and suggest microbiota-based therapeutics.

Psychobiotics

The term "psychobiotics" was introduced in 2013 to describe a class of probiotics capable of producing antimicrobials and neuroactive molecules such as acetylcholine, catecholamines, γ-aminobutyric acid (GABA), and serotonin (5-hydroxytryptamine or, 5-HT). These probiotics mediate anxiolytic and antidepressant effects through a bidirectional pathway known as the microbiota–gut–brain axis (MGBA). This axis provides a mechanistic link between the gut microbiota and the brain and offers a promising framework for understanding the potential of psychobiotics to promote mental health and counteract neuropsychiatric disorders. Psychobiotics constitute a novel class of psychotropic agents that are believed to exert antidepressant effects [17]. They are under active investigation as potential future interventions for the management of depression and anxiety. Comprehensive, multi-level analyses of the gut–brain axis and microbiome are being conducted to enhance the understanding of their influence on a spectrum of neuropsychiatric conditions. Certain probiotics, including GABA-producing Lactobacillus plantarum and Bifidobacterium adolescentis, have demonstrated antidepressant effects. Knowledge of the gut microbiota has been extensively applied to both the diagnosis and treatment of depression, with emerging interest in other neuropsychiatric conditions. Innovative approaches, such as metagenomics combined with machine learning, are employed to identify biomarkers associated with mental health disorders. These methodologies facilitate the discovery of metabolic signatures and specific microbiota profiles linked to depression and other neuropsychiatric conditions, thereby illuminating the evolving role of psychobiotics in mental health treatment. Advances in investigative technologies and data analysis techniques are progressively elucidating the functional roles of microbial communities and their host interactions [13]. Extensive characterization of established probiotic strains has been achieved, and new strains targeting particular mechanisms are emerging. MGBA-based interventions present a potential strategy for the prevention or treatment of a broad array of conditions, though their implementation remains complex due to strain-specific activities, intricate host networks, and industrial-scale production challenges. Future research directions emphasize innovative methodologies aimed at deepening the understanding of psychobiotic function, addressing existing knowledge gaps, and broadening their applicability. High-throughput sequencing and metabolomics constitute promising tools for the personalization of psychobiotic treatments by detailed analysis of individual microbial communities, enabling targeted therapies that could alleviate mental health symptoms and serve a preventative role in at-risk populations. The integration of artificial intelligence in microbiome research stands to create significant opportunities for the discovery and functional enhancement of psychobiotics [17, 13].

Ethical Considerations in Microbiome Research

Microbiome-based diagnostics are highly desirable and widely anticipated, but such applications are still in their infancy [2]. Certain ethical issues are of particular concern. Microbiome data can reveal an individual's predisposition to obesity or other conditions, and when combined with genetic information, can be more revealing than genetic data alone [18]. Protection against discrimination is therefore essential. Unlike human data, microbiome data may also disclose personal socio-economic details such as birthplace and travel history, possibly exposing individuals to even greater prejudice. Contemporary research and policy efforts emphasize the establishment of clear rules, standards and procedures for collection of samples and legitimate access to materials 19. Informed consent, data sharing and ownership of specimens must be carefully respected. The question of truly informed consent, in which the potential scope of future research is fully understood, is liable to become increasingly difficult to address as methods advance and potentially useful applications multiply. Maintaining transparency remains high priority throughout [18].

Data Privacy Issues

Microbiome research is advancing rapidly and raising novel questions and uncertainties for research ethics and research governance. Data that seem benign may harbour hidden risks. Data or samples collected for one research question may be used to answer different, unanticipated questions [1]. Participants may consent to participate in initial projects but be unaware of the future uses of samples and data. The rapid development of genomic techniques means that data deemed uninteresting today could be highly sensitive tomorrow. Public questions about protection against unforeseen, potentially invasive uses of data naturally grow along with public awareness of microbiome research and its future possibilities [1]. Privacy concerns are also likely to be accentuated, as the microbiome metabolites are more and more frequently identified and it will be possible to link a given microbiome profile to a subject's particular habitat, behaviour, lifestyle, diet and pathological disorders. The benefits of microbiome research can only be realised if there is appropriate legal, ethical and regulatory oversight. The specific ethical issues in microbiome research vary from project to project. Further development of the existing oversight frameworks, involving ethics and review boards, is the most logical way forward, rather than creating new and additional bodies. The current trend is at least to create curated central repositories of human microbiomes that can be used in translational and population health studies [2]. In the absence of adequate

oversight, the collection and subsequent use of data and samples that travel beyond the initial location can increase the actual or perceived risks for research participants or groups. At the same time, overly restrictive policies may prevent data and samples from being shared with scientists who will advance human health.

Informed Consent

The formulation of valid informed consent for human microbiome research is complex and raises numerous ethical issues. Potentially, the privacy interests in microbiome data may be significant [18]. Microbial data alone can reveal individuals' predispositions to obesity, and when combined with genetic data, can uncover sensitive personal details such as socioeconomic status. Microbiome profiles have been shown to predict, often more accurately than genetic information, the susceptibility to conditions like asthma or inflammatory bowel disease, heightening concerns over potential discrimination [18]. The possibility of stigma based on socio-demographic attributes, such as class, caste, birthplace, or travel history, may be even more disturbing than genetic discrimination. Conversely, microbiome research holds promise for reducing stigma; for example, identifying microbial predispositions to obesity might foster greater understanding and acceptance [18]. The issue of consent is further complicated by the challenge of whether participants can provide fully informed consent amid uncertain research outcomes and unspecified future uses.

Challenges in Microbiome Diagnostics

Various challenges exist in the application of microbiome-based diagnostics. The standardisation of sample collection and storage, DNA extraction, and data analysis methods remain unachieved despite attempts, affecting the comparability of results across studies. Most current knowledge on the diagnostic power of microbiome data derives from 16S rRNA gene amplicon surveys or low-resolution metagenomic datasets [2]. While they provide important resources for translational researchers and lay a foundation for clinical applications, they are unlikely to capture the full potential of the microbiome as a biomarker. The resolution required to effectively characterise particular communities will likely vary by body site and disease type. Diseases associated with microbiomes dominated by a limited number of closely related species would require higher-resolution approaches to distinguish between important individual members. Data derived from stool, the most commonly sampled body site, may not reflect accurately the state of the mucosal microbiome in the colon or that of other relevant sites, such as the small intestine. Sample collection and storage for blood- or fluid-derived diagnoses will be critical. Inter-individual variation in the microbiome will continue to represent another major challenge [2]. Large-scale microbiome-wide association studies may be necessary to overcome this issue, and contextualising an individual's clinical phenotype with matched whole-genome host and microbiome sequence data will represent an important route to diagnosis and insight. More generally, clinicians and researchers must consider broader ethical issues related to the microbiome, including those associated with more rudimentary efforts to characterise the microbiome within and between sample types [2].

Standardization of Methods

Microbiome-based diagnostics herald a paradigm shift in medical research, with exciting diagnostic applications already underway thanks to significant advances in m oiome profile correlation diagnostics. These applications range from assisting in the diagnosis of infectious diseases to supporting clinicians in managing chronic diseases, exemplifying the potential for greater diagnostic accuracy. In the future, such applications will become integral components of personalized approaches to clinical care. The advancement of microbiome analysis, microbiomics or microbiome research is fundamentally a result of developments in next-generation sequencing, as well as metabolomics and proteomics [1]. Why does the microbiome matter? Essential to human health is a complement of millions of bacteria, fungi, viruses, and parasites that inhabit our bodies; collectively, these dependencies are known as the microbiome. In humans, the microbiome plays an important role in metabolism by interacting with the digestion of food. Gut microbiota composition is more than a mere reflection of diet; the gut biome actively influences the development of metabolic diseases such as obesity and type 2 diabetes. Evidence also supports a two-way interaction between gut microbiota and brain function. Certain probiotics, known as psychobiotics, can confer mental health benefits (e.g., by decreasing anxiety and depression). Ethical challenges do arise: microbiome datasets can reveal health and disease information, raising concerns about data privacy and informing participants during the informed consent process of microbiome-based research [1, 2].

Inter-individual Variability

The human microbiota exhibits substantial inter-individual variability, often attributable to differences in lifestyle and other undocumented factors. Over time, each individual develops a distinct, personalized microbiota, contributing to unique microbiota compositional dynamics and related host phenotypes, such as varying disease susceptibilities. This high degree of variation complicates disease classification among multiple phenotypic classes [11]. Therefore, reducing the influence of inter-individual variability and extracting personalized features from microbiota data are crucial for classification purposes. By considering individual differences, analysis approaches

can overcome confounding effects and leverage inter-individual variation to identify personalized biomarkers. Exploring individual-specific signatures in microbiota dynamics facilitates the development of personalized diagnostics and precision medicine strategies. These signatures offer insights into host-pathogen interactions and prevailing micro-environmental conditions for each individual. Consequently, characterizing personalized microbiota dynamics and extracting individual-specific markers have become a research focus [11].

Future Directions in Microbiome Research

Conclusion During the past two decades, there has been an unprecedented explosion in microbiome research that has led to a plethora of discoveries and a complete change in our view of the models of human health and disease. The findings demonstrated the importance of the microbiome and its contribution to metabolic activities and immune responses controlling various biological functions in a healthy human body [4]. These recent observations led to further investigations on possible therapeutic and diagnostic applications demonstrating the significant potential of microbiome research that is still largely unexplored [6]. The development of new molecular and multi-omics technology in recent years enables rapid, personalized parallel profiling of complex microbial communities and transforms microbiome research from hypothesis-driven academia to technological platforms that open up new avenues for clinical translation. Research in the microbiome field is expected to develop at a rapid pace and will change healthcare approaches worldwide in the coming years. To realize the potential applications of these newly developed technologies, it is important to understand the complexity of the human microbiome and to establish the cause–effect relationship between the increased disease risk or altered health status, which is mainly limited due to the complexity of host environments and gut microbiome composition [4]. The recent developments of in vivo and in vitro models provided valuable means to recapitulate the microbiome microenvironment and to explore its effect on the host in the progression of various diseases. Nevertheless, validation of the current findings in epidemiological studies or human trials is necessary to uncover the potential of these therapeutic and diagnostic tools [2, 6, 4].

Emerging Technologies

Microbiome-based diagnostics is a rapidly developing field that employs various profiling methodologies for the quantitative analysis of microbial communities to address clinical questions [2]. The increasing availability of next-generation sequencing platforms has already facilitated the transition of microbiota analysis from a research tool to routine diagnostic use and has fueled expectations that microbiota profiling will become a valuable asset of diagnostic medicine. Conventional culture-based laboratory investigations of infected body sites or clinical samples originating from normally sterile sites remain the current gold standard, but the sensitivity of such methods is suboptimal, and they do not provide any quantitative information about microbial community dynamics [2]. An alternative approach is therefore required to overcome these limitations.

Potential Therapeutic Applications

The ability of MYcrobiota to detect bacterial OTUs at very low abundances makes it suitable for investigating normally sterile body sites such as synovial fluids, cerebrospinal fluids, and blood samples. The construction of MYcrobiota is a step toward integrating quantitative microbiota profiling into clinical diagnostics. The method offers a comprehensive overview of microbial composition and confirms the absence of 16S rRNA gene copies in culture-negative samples using a validated 16S rRNA gene NGS workflow. However, due to limitations like lack of species identification and detailed antibiotic susceptibility information, further validation studies are necessary to justify its routine clinical use. Extensive clinical and financial validation will be needed before molecular microbiota profiling can be widely adopted in diagnostic laboratories [2]. The numerous microbiome-disease associations have generated hope that understanding host-microbe interactions will lead to therapeutic applications. Microbiome-based therapies are viewed as more natural and may address root causes of disease, such as microbial dysbiosis. The most successful application has been fecal microbiota transplantation (FMT), which involves transplanting stool from a healthy donor to a patient. FMT aims to correct microbial imbalances and has shown success, especially in treating Clostridioides difficile infection (CDI). Results for other diseases like inflammatory bowel disease (IBD) have been mixed. Challenges include variability among donors and recipients, safety concerns, unclear mechanisms, and regulatory issues. Future therapies are expected to involve refined bacterial cocktails, single strains, and microbial metabolites [2]. Several companies are developing such treatments, and microbiome therapeutics are poised to enter the clinic. Significant hurdles remain in developing effective next-generation microbiome-based therapies [6]. Global human microbiome therapeutics is expected to grow substantially by 2027, reaching a market size of USD 1,731 million. Although research has demonstrated the efficacy of microbiome therapeutics, further understanding of the microbiome and its interaction with the host is needed before moving into clinical trials [2]. Strategies to avoid contamination when using bacterial suspensions are essential. Live therapeutics requires proper genomic characterization to distinguish disease-specific microbes from healthy ones. Companies should increase production of bacteria-specific products and develop pills with

single-microbe species to improve immune response and treatment efficacy. Collaboration between microbiome therapeutic companies and pharmaceutical industries is necessary to enhance treatment outcomes. Clinical trial results should be further explored for applications in autoimmunity and neurological disorders [20].

Case Studies in Microbiome Diagnostics

Microbiome-based diagnostics capitalize on the microbiota's one-to-many relationship with disease, thereby exploiting the microbiome as a biological fingerprint to identify and classify specific health statuses [1]. Approaches to diagnostic interpretation rely on microbiome analysis of physiological fluids often blood, urine, or respiratory secretions from multiple bodily niches and employ predictive models to estimate the probability of candidate infections. Diagnostic platforms have quickly progressed from experimental protocols for research and epidemiology to commercial systems that serve national-scale health systems, and rapidly evolving technologies promise continued expansion and the possibility of widespread integration at lower infrastructural thresholds and cost [2].

Successful Implementations

Currently, only a few microbiome-based diagnostic tests have reached routine medical practice, despite several promising developments. MYcrobiota represents a notable example of a standardized 16S rRNA gene next-generation sequencing (NGS) platform developed for routine clinical microbiological diagnostics [21]. The workflow incorporates an innovative micPCR/NGS methodology that substantially reduces chimera formation and prevents PCR competition effects, thereby enabling more precise detection of bacterial operational taxonomic units (OTUs), particularly at low abundances. The inclusion of an internal calibrator facilitates quantification of 16S rRNA gene copies and subtraction of contaminant copies, enhancing accuracy and lowering the limit of detection. Applied to low-biomass clinical samples from patients suspected of infection, MYcrobiota enables reliable detection and quantitative profiling of bacterial OTUs even when traditional cultures are negative [1].Although the platform has yet to undergo extensive validation and clinical implementation studies, it demonstrates the potential of molecular microbiota profiling to provide comprehensive insights into microbial composition or to confirm the absence of bacteria in culture-negative samples within a fully validated 16S rRNA gene NGS framework. MYcrobiota exemplifies an emerging generation of diagnostic tools intended to support the detection, quantification, and detailed characterization of bacterial DNA derived from complex clinical specimens. These developments underscore the ongoing translation of microbiota research from a scientific instrument into a clinical diagnostic modality. Future investigations will be required to fully establish the clinical utility and economic case for routine adoption of such technologies in medical laboratories [2].

Lessons Learned

MYcrobiota facilitates the confirmation of culture-negative outcomes, thereby enhancing the reliability of diagnostic results. The platform's sensitivity allows for the detection of bacterial OTUs present at very low abundances, rendering it suitable for the examination of typically sterile bodily fluids, such as synovial and cerebrospinal fluids, as well as blood [2]. The development of MYcrobiota constitutes an initial step toward the integration of microbiota research into clinical diagnostics; however, comprehensive validation studies are necessary to substantiate its routine application. The methodology provides a highly accurate depiction of microbial composition or verifies the absence of 16S rRNA gene copies in culture-negative samples through a standardized workflow. Although it does not yield species-level identification or information regarding antibiotic susceptibility, MYcrobiota nonetheless exhibits promise for clinical diagnostic use. Further investigations are requisite to assess the clinical relevance of 16S rRNA gene NGS findings. MYcrobiota employs a novel micPCR/NGS approach that mitigates chimera formation and PCR competition, complemented by a dedicated bioinformatics pipeline that automates the analytical process from raw sequence data to final reporting [21]. The platform enables precise detection of bacterial OTUs, quantification of 16S rRNA gene copies, and subtraction of contamination attributable to reagents or the laboratory environment. It ensures the identification of bacteria at minimal abundance levels and the confirmation of bacterial DNA absence in culture-negative specimens. An extensive array of quality control metrics is made available throughout data analysis, facilitating rigorous evaluation. Considerations encompassing operational costs, personnel training requirements, quality assurance protocols, and seamless integration within existing systems are central to the successful deployment of such diagnostic tools in routine clinical laboratories. The adoption of platforms akin to MYcrobiota represents a critical advancement toward the widespread implementation of 16S rRNA gene NGS in clinical microbiological diagnostics [2 , 21].

Regulatory Landscape for Microbiome Diagnostics

Clostridium difficile infections (CDI) are among the most common types of hospital-acquired infections globally. Traditional treatments, including antibiotic therapy, often fail to prevent the high rate of recurrence. To address this, the live biotherapeutic product RBX2660 developed by Rebiotix has emerged as a medical approach targeting

the recovery of the gut microbiome following CDI. RBX2660 is an investigational microbiome restoration therapy containing a broad consortium of live microbes derived from human stool. Currently in phase 3 trials, it demonstrated a favorable safety profile across more than 1,000 administrations during phase 2 studies [6]. Similarly, in the diagnostics domain, microbiota analysis studies the host organism and its associated microbiota as a unified meta-organism, investigating changes in the microbial community associated with various diseases. However, the diversity and spatial distribution of microbes on and inside the human body complicate the identification of diagnostic microbial signatures and the interpretation of microbiota profiles in clinical settings [2]. As a pioneering company in commercial clinical microbiome diagnostics, Microba offers the Microba Community Profiler as a globally available service for clinicians and researchers. This service utilizes sequencing and taxonomic classification of microbial DNA extracted from biological samples to determine the species present and their relative abundances. The diagnostic report incorporates the patient's microbial composition, including opportunistic pathogens, antimicrobial resistance genes, species richness, diversity, and network connections within the microbiome [22].

Current Regulations

Regulatory agencies have not yet issued official guidelines specifically addressing microbiome-based diagnostics. Therefore, laboratories seeking to develop such assays may encounter uncertainty regarding the appropriate regulatory pathway. For diagnostic devices, both the regulatory classification of the test and the classification of the underlying technology must be considered [1, 2, 21]. Microbiome signatures can manifest as multiple analytes or patterns of analytes of various types, including nucleic acids, proteins, and small molecules. The diversity of these signatures creates complexity when mapping to regulatory classification. Moreover, many microbiome features cannot be considered individual entities but rather constitute collections of analytes. Traditionally, the US Food and Drug Administration (FDA) has regulated multiple-analyte signatures only if they operate on a fixed algorithm that weighs each analyte's contribution to a final result. This approach has limited applicability for microbiome signatures, since taxonomic abundance data are compositional and cannot be weighed independently [1]. Microbiome profiling can be performed by sequencing and separate computational analysis or by hybridization techniques. Because both methods produce similar data, the selection of a single regulatory pathway is challenging [2]. Again, data analysis presents a parallel difficulty: in some platforms, correspondence between a particular sequence and a signature feature is readily evident, whereas in others, conversion of raw instrument output into signature elements requires lengthy bioinformatics procedures [21].

Future Regulatory Challenges

The availability of regulatory frameworks for microbiome-based diagnostics varies globally. Western countries such as the United States and the United Kingdom have regulatory bodies that provide oversight, although challenges remain in establishing specific guidelines for these products [22]. Developing guidelines tailored to the unique aspects of microbiome diagnostics will contribute to their integration into healthcare practice. Consequently, commercialization may initially focus on markets where the regulatory landscape is better defined. Even within jurisdictions with comprehensive regulatory systems, establishing acceptable criteria for microbiome diagnostics requires further effort. Recognizing the opportunity, developers have pursued strategies to expedite approval in the absence of explicit regulations; still, numerous questions persist in a rapidly evolving field. Requirements concerning the rigor of scientific literature, validation of molecular and bioinformatics methods, assessment of within-cycle reproducibility, and evaluation of preanalytical variables all demand clarification. Thus, despite growing recognition of the potential of microbiome-based diagnostics, the path toward wide clinical adoption remains complex [22].

Market Trends and Economic Impact

The commercial utilization of microbiome-based diagnostics has expanded with the industry's growth. New types of gut-health-related feed additives based on microbiome research include microbially processed co-fermented rapeseed meal and seaweed, which enhance colon mucosal development and reduce intestinal inflammation in weaned piglets. Replacing in-feed zinc oxide with these additives also improves piglet performance, intestinal development, and health indicators. Xylooligosaccharides prebiotics represent another novel category of affordable feed additives for modulating gut health in pigs at scale. Probiotics in poultry feed serve as microbiome modulators to combat infections and intoxications. Multi-omics analyses of traditional East Balkan meat products revealed amino acid metabolism as a key microbial driver in sensory attributes, highlighting the importance of applying autochthonous microbiomes in precision meat fermentation to ensure quality and safety [23]. The microbiota of home-made and industrial kefir from Greece was characterized to differentiate these sources. In investigations of the pink discoloration defect in cheese, Thermus microbes were identified as causative agents, leading to the development of PCR- and qPCR-based detection assays. Characterization of microbial ecosystems in meat processing facilities demonstrates how microbiome analyses can support improved hygiene and food safety

management [24]. D-tryptophan was discovered as a microbiome-modulating prebiotic capable of mitigating asthma [2].

Industry Growth

According to recent analyses, the number of companies involved in microbiome-based diagnostics has grown substantially over the past decade, particularly since 2017, with investments also exhibiting a notable upward trend. The development of a culture-free microbiota profiling platform (MYcrobiota) for clinical diagnostics illustrates progress in the field [2]. This platform is capable of detecting bacterial operational taxonomic units at very low abundances and is therefore suited for investigating normally sterile body sites, such as synovial fluids and blood samples. MYcrobiota provides a highly accurate overview of the microbial composition or confirms the absence of 16S rRNA gene copies in culture-negative samples, following a standardized workflow. The method lacks species-level identification and detailed information on antibiotic susceptibility but nevertheless holds promising applications in clinical diagnostics. Extensive validation studies, however, are necessary to justify routine clinical use. The development of MYcrobiota represents a step towards integrating quantitative microbiota profiling into diagnostic laboratories [2]. The expanding industrial microbiome sector also benefits from advances in bioinformatics and data science. Routine analyses can verify the presence of desired strains or identify potential pathogens in end products. Although not yet common in microbiome studies, long-read sequencing platforms, such as PacBio and Oxford Nanopore Technologies, open avenues for applications like fermentation studies, which remain unfeasible with short-read amplicon sequencing. Nanopore's on-demand sequencing appears suitable for quality-control applications targeting pathogen detection, yet its high error rate limits accuracy at the strain level. Assemblies generated from short-read datasets often remain fragmented within complex communities, but integrated long-read sequencing is expected to become a standard for acquiring complete microbial genomes. The decline in sequencing costs has led to increased adoption of machine-learning and data-science techniques in microbiome research, facilitating larger datasets and deeper sequencing. These technologies have a growing impact on disease diagnostics, personalized-health recommendations, and probiotic screening. Tools such as USEARCH incorporate machine-learning methods designed for researchers lacking specialized bioinformatics training [24].

Investment Opportunities

A key aspect of the ongoing worldwide focus on the microbiome field is increased investment to support the implementation of microbiome-based applications within the human and animal healthcare sectors. Microbiome-based diagnostics is particularly lucrative owing to the straightforward integration of microbiome assays into established healthcare settings. The global microbiome analysis market and microbiome-based diagnostics market are growing rapidly, propelled by an upsurge in demand from research laboratories, food and pharmaceutical companies, hospitals, diagnostics laboratories, and academic research institutions. Microbiome diagnostics combines next-generation sequencing approaches with machine learning for data interpretation and disease association studies [24]. Regulatory agencies like the U.S. Food and Drug Administration have issued guidance on microbiome-based applications, underscoring both potential investment opportunities and challenges. Various microbiome diagnostic platforms are commercially available, many of which remain unvalidated [2]. MYcrobiota, a culture-free microbiota profiling platform, offers a stepwise development path toward introducing quantitative microbiota profiling into clinical diagnostics. Its ability to detect bacterial OTUs at very low abundances renders it suitable for investigating normally sterile body sites such as synovial fluids, cerebrospinal fluids, and blood samples. Extensive clinical and financial validation studies are necessary to justify the routine introduction of molecular profiling methods into clinical laboratories. While the method does not yet support species identification or detailed antibiotic susceptibility predictions, it provides a highly accurate overview of microbial composition or confirms the absence of 16S rRNA gene copies in culture-negative samples using a standardized and validated workflow. Yet, owing to the complexity of typical microbiota samples, complementary assays and the support of well-trained specialists remain important; nevertheless, the overall approach appears promising and merits further investigation to evaluate clinical relevance [2, 24].

Public Perception and Awareness

Public awareness and understanding of microbiome-based diagnostics remain largely limited, primarily confined to individuals with a scientific background [2]. Consequently, the broader public often harbours misconceptions regarding this subject area. Developing appropriate means to inform and disseminate the potential of microbiome-based diagnostics in healthcare is crucial [25]. Medical and healthcare organisations therefore face the imperative to make efforts to enhance public understanding. Increasing public knowledge and acceptance of the microbiome's potential for predicting microbiome-associated diseases continues to be a pivotal priority. This undertaking holds particular significance given the swift expansion of microbiome-based diagnostic solutions worldwide, with such

applications becoming available to diverse segments of the population including, for instance, VIPs and celebrities who seek to proactively manage their health or directly confirm the nature of their microbiome [2, 23].

Education and Outreach

Microbiome-based diagnostics have gained considerable attention in the scientific community. Widespread publicity in popular scientific journals, such as Nature or Science, has helped increase awareness among the general public [2]. Moreover, dedicated workshops organized by groups like the Else Kröner-Fresenius-Foundation or Goethe-University, which bring together academic, clinical, and commercial participants, are utilized to disseminate current knowledge to healthcare professionals, the scientific community, and the public. In contrast, social media campaigns should be used cautiously, as the general public's understanding of the complexity of microbiome-based applications is limited. However, such campaigns are suited for education and outreach targeted towards scientists and clinicians [2].

Misconceptions about Microbiome

The human microbiome represents a complex community of commensal and pathogenic microoganisms, including viruses, bacteria, fungi, and other eukaryotes residing in and on the body [2]. Numerous studies have shown that the microbiome exerts a profound impact on human health and well-being [4], as well as on the progression of chronic and infectious diseases. An awareness of the microbiome has grown exponentially during the past decade, together with new advanced technologies that enable a comprehensive analysis of the microbiota and their functional activities. Among others, next-generation sequencing (NGS) and derivates, metabolomics and proteomics studies, as well as multiplexed analyses are the most important technologies facilitating microbiome-based diagnostic applications and enhancing research in this field. Microbiome-based diagnostics is the testing of various sample types (human-derived, animal-derived, or environmental) in a clinical or monitoring setting in order to characterize the complete microbiota of these samples; as such, the obtained information allows for a more in-depth interrogation of the microbiome of the environmental/host ecosystem [2,4].

CONCLUSION

Microbiome-based diagnostics represents a transformative approach to modern healthcare, enabling clinicians to move beyond traditional symptom-based assessments toward molecular-level insights into disease risk, progression, and treatment response. Advances in next-generation sequencing, metabolomics, and proteomics have provided unprecedented opportunities to characterize microbial communities and their functional contributions to health and disease. Applications in infectious disease detection, chronic disease management, and personalized medicine highlight the diagnostic value of microbiome research, while psychobiotics and gut–brain axis investigations underscore its relevance to mental health. Despite these advances, challenges such as lack of standardization, inter-individual variability, and limited clinical validation remain barriers to routine implementation. Nevertheless, the integration of multi-omics data, artificial intelligence, and standardized clinical pipelines promises to revolutionize diagnostics in the coming decades. With careful navigation of scientific, ethical, and practical considerations, microbiome-based diagnostics has the potential to reshape precision medicine and improve healthcare outcomes worldwide.

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