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Exploring the role of gut microbiota in human health

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Abstract

The study explores the intricate relationship between the human gut microbiota and health. It analyzes the gut microbiota's roles in digestion, metabolism, immune responses, and overall well-being. The review discusses the composition and diversity of gut microbial communities, emphasizing their symbiotic relationship with the host. It also examines how gut dysbiosis, or microbial imbalance, relates to health conditions like inflammatory bowel diseases and metabolic disorders. The review highlights research methodologies like metagenomics and metabolomics that deepen our understanding of gut microbiota function. It also explores external factors, such as diet and antibiotic use, in shaping the gut microbiome. The review discusses potential therapeutic interventions like probiotics and fecal microbiota transplantation, suggesting a future for personalized medicine. By synthesizing existing knowledge, the review aims to advance understanding of the gut microbiota's role in health and suggest future research and interventions.

Keywords: Dysbiosis; Gut microbiota; Human health; Microbiome; Therapeutic interventions

1. Introduction

The human gut microbiota, composed of trillions of microorganisms, plays a crucial role in maintaining host health. The gut microbiota is a complex ecosystem consisting of bacteria, viruses, fungi, and archaea, with the bacterial component being the most extensively studied. This microbial community interacts with the host in various ways, influencing nutrient metabolism, immune system development and function, and even behavior through the gut-brain axis. Disruptions to the microbiome have been associated with severe pathologies of the host, including metabolic disease, cancer and inflammatory bowel disease. Understanding the composition and function of the gut microbiota is essential for elucidating its role in human health and disease.

The gut microbiota exerts profound influences on human health through multiple mechanisms. One of its fundamental roles lies in nutrient metabolism, particularly the fermentation of dietary fibers and complex carbohydrates that are resistant to digestion by host enzymes. This process generates short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which serve as an energy source for colonic epithelial cells and play a crucial role in maintaining gut barrier integrity and regulating immune responses [1]. Furthermore, the gut microbiota contributes to the synthesis of essential vitamins, including vitamin K and certain B vitamins, which are pivotal for various physiological processes, such as blood clotting and energy metabolism [2]. Additionally, microbial metabolites, such as neurotransmitters and neuroactive compounds produced in the gut, can influence brain function and behavior through the gut-brain axis, highlighting the intricate connection between the gut microbiota and central nervous system [3].

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Given the multifaceted roles of the gut microbiota in human health this review aims to provide a comprehensive overview of its functions and implications for disease pathogenesis and treatment. It will discuss the composition and function of the gut microbiota, its influence on host physiology and metabolism, and its role in the pathogenesis of various diseases. It will also explore potential therapeutic strategies targeting the gut microbiota and highlight future directions for research in this rapidly evolving field.

2. Composition of gut microbiota

The gut microbiota is a diverse and dynamic community of microorganisms that colonize the gastrointestinal tract, with the majority residing in the colon. The composition of the gut microbiota can vary widely among individuals and is influenced by various factors, including diet, age, genetics, and environmental exposures [4]. While bacteria are the predominant members, the gut microbiota also includes viruses (bacteriophages), fungi, and archaea, each contributing to the overall ecosystem [5].

2.1. Diversity of Microorganisms

The gut microbiota is primarily composed of bacteria, which belong to several phyla, including Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia, and Proteobacteria. Firmicutes and Bacteroidetes are the two dominant phyla in the gut, comprising up to 90% of the total bacterial population [6]. Within these phyla, there are thousands of different species, with each individual harboring a unique combination of microbial species, known as their "microbiota fingerprint" [7]. These species exhibit considerable variability across individuals, influenced by factors such as age, diet, geography, and host genetics. Besides genera from phyla Firmicutes and Bacteroidetes, human colon also harbors primary pathogens, e.g., species such as *Campylobacter jejuni*, *Salmonella enterica*, *Vibrio cholera* and *Escherichia coli*, and *Bacteroides fragilis*, but with a low abundance (0.1% or less of entire gut microbiome) [8]. Besides this longitudinal difference, there also exists an axial difference from the lumen to the mucosal surface of the intestine. While *Bacteroides*, *Bifidobacterium*, *Streptococcus*, Enterobacteriacae, *Enterococcus*, *Clostridium*, *Lactobacillus,* and *Ruminococcus* are the predominant luminal microbial genera (which can be identified in stool), only *Clostridium, Lactobacillus, Enterococcus* and *Akkermansia* are the predominant mucosa and mucus associated genera (detected in the mucus layer and epithelial crypts of the small intestine).

Recent advances in high-throughput sequencing technologies, such as metagenomics and 16S rRNA gene sequencing, have enabled comprehensive profiling of the gut microbiota composition at various taxonomic levels. These studies have revealed substantial inter-individual variation in gut microbial communities, termed microbial "enterotypes," characterized by differences in the relative abundance of key bacterial taxa [9]. Metabolomics is another rapidly expanding field of gut microbiota research that evaluates small molecules associated with the interrelationship of host-bacterial metabolism that has implications in health and disease. Composite data from the gut microbiota and the metabolome currently provides the most powerful evidence that can demonstrate the closest association with health and diseased states. However, with advances in large-scale sequencing, artificial intelligence (AI)-related machine learning can serve as a means to analyze large-scales of data related to microorganisms along with determinations regarding the type and status of diseases [10].

In addition to bacteria, the gut microbiota also includes viruses (predominantly bacteriophages), fungi (e.g., *Candida*, *Saccharomyces*), and archaea, although these constitute a smaller proportion of the overall gut microbial community. An example of the archaea is the methane-producing *Methanobrevibacter smithii*, and in recent studies it has been implicated in irritable bowel syndrome (IBS) with constipation [11]. Bacteriophages have been shown to induce IBD through the reduction of bacterial diversity in the gut. Similarly, bacteriophages have been also used to treat antibiotic-resistant MRSA strains [12] and infections caused by *Mycobacterium abscessus* [13]. Recently, genetically modified bacteriophages were used for the treatment of pathogens in the gut, even intracellular pathogens.

2.2. Factors Influencing Gut Microbiota Composition

The composition and stability of the gut microbiota are shaped by a myriad of factors, both intrinsic and extrinsic to the host. Diet exerts a profound influence on gut microbiota composition, with dietary components serving as substrates for microbial metabolism and growth. For instance, high-fiber diets promote the growth of fiber-degrading bacteria, such as members of the Bacteroidetes phylum, whereas diets rich in saturated fats may favor the expansion of pro-inflammatory microbial taxa [1].

In addition to diet, host genetics play a role in shaping the gut microbiota composition. Twin studies have demonstrated that monozygotic twins exhibit more similar gut microbial profiles compared to dizygotic twins, indicating a genetic component to gut microbiota composition [14]. Furthermore, environmental factors, such as antibiotic exposure, stress,

and mode of birth (vaginal delivery vs. cesarean section), can significantly impact the assembly and development of the gut microbiota early in life [15]. Understanding the dynamic interplay between these factors is essential for unraveling the complex mechanisms governing gut microbiota composition and its implications for host health and disease.

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2.3. Functions of Gut Microbiota

The gut microbiota performs a myriad of functions that are essential for human health and well-being. These functions encompass nutrient metabolism, immune system modulation, synthesis of bioactive compounds, and communication with the central nervous system through the gut-brain axis.

2.3.1. Nutrient Metabolism

One of the primary functions of the gut microbiota is the fermentation of dietary fibers and complex carbohydrates that escape proximal digestion by host enzymes in the upper gastrointestinal tract and colonic organisms such as *Bacteroides*, *Roseburia*, *Bifidobacterium*, *Fecalibacterium*, and Enterobacteria. This process generates SCFAs, such as acetate, propionate, and butyrate, which serve as an energy source for colonic epithelial cells and play a crucial role in maintaining gut barrier integrity [16, 17]. Acetate, propionate, and butyrate usually found in 3:1:1 to 10:2:1 molar ratio; this ratio is consistent with the values reported in the intestine in early sudden deaths [17]. Acetate helps in the growth of other bacteria as an essential co-factor; for example, *Faecalibacterium prausnitzii* will not grow in pure culture in the absence of acetate. Propionate is also an essential energy source for the epithelial cells in the liver; it plays a vital role in gluconeogenesis. Butyrate is the essential SCFA for human health, as it is the primary source of energy for human colonocytes. Butyrate has the potential to act as an anti-carcinogen as it persuades apoptosis of colon cancer cells and regulates gene expression by inhibiting histone deacetylase. SCFAs also exhibit immunomodulatory effects, influencing the differentiation and function of immune cells in the gut [18]. Moreover, the gut microbiota contributes to the metabolism of other dietary components, such as proteins and fats, producing a variety of metabolites that can influence host physiology and metabolism. For example, microbial metabolism of dietary proteins can generate potentially toxic compounds, such as ammonia and phenolic compounds, which may have implications for gut health [19]. The gut microbiota has also been shown to impart a positive impact on lipid metabolism by suppressing the inhibition of lipoprotein lipase activity in adipocytes. They participate in the metabolism of dietary polyphenols, converting them into bioactive metabolites with potential health benefits. For example, microbial-derived metabolites of flavonoids, such as equol and urolithins, exhibit antioxidant, anti-inflammatory, and anti-cancer properties [20].

2.3.2. Immune System Modulation

The gut microbiota plays a pivotal role in shaping the development and function of the host immune system. It contributes to the maturation of gut-associated lymphoid tissue (GALT) and the differentiation of immune cells, such as regulatory T cells, which are critical for maintaining immune tolerance and preventing inappropriate immune responses. Through interactions with intestinal epithelial cells and immune cells, such as dendritic cells, macrophages, and T cells, the gut microbiota helps to maintain immune homeostasis and tolerance to commensal microbes while mounting appropriate immune responses against pathogens [21]. Furthermore, the gut microbiota helps to educate the immune system, distinguishing between harmful pathogens and beneficial commensal microorganisms. Dysregulation of this process can lead to immune-mediated diseases, such as inflammatory bowel disease (IBD) and allergies [22]. Specific bacterial species within the gut microbiota have been shown to promote the differentiation and activation of regulatory T cells (Tregs), which play a key role in dampening excessive immune responses and preventing autoimmune diseases [22]. Conversely, dysbiosis of the gut microbiota, characterized by alterations in microbial composition and function, has been implicated in the pathogenesis of immune-mediated disorders, such as inflammatory bowel disease (IBD) and allergies.

2.3.3. Synthesis of Vitamins and Bioactive Compounds

The gut microbiota is capable of synthesizing a range of vitamins and bioactive compounds that are important for host health. For example, certain bacterial species can produce vitamin K, which is essential for blood clotting, biotin and folate, which are essential for numerous cellular processes. Additionally, [23]. The normal gut microbiota has also been shown to impart a healthy metabolome in the serum by increasing the concentrations of pyruvic acid, citric acid, fumaric acid and malic acid, all of which are indicators of higher energy metabolism. [24]. Members of genus *Bacteroides* have been shown to synthesize conjugated lino-leic acid (CLA) that is known to be antidiabetic, antiatherogenic, antiobesogenic, hypolipidemic and have immunomodulatory properties.

2.3.4. Gut-Brain Axis Communication

Emerging evidence suggests that the gut microbiota can communicate with the central nervous system through the gut-brain axis, a bidirectional communication network between the gut and the brain. The gut–brain axis comprises the central nervous system, the neuroimmune and neuroendocrine systems, the parasympathetic and sympathetic sections of the autonomic nervous system and the gut microbiota [25]. The gastrointestinal tract is closely related to the central nervous system (CNS) which plays an important role in regulating gut function and homeostasis. In turn, the gut flora may affect the CNS and nerve cells, participate in the regulation of nervous system function, affect the pathogenesis and progression of nervous system-related diseases. There is an evidence from animal studies that gut bacteria affect brain chemistry and development and that enteric nervous system, which includes the sensory vagus nerve, appears to be able to distinguish between nonpathogenic and potentially pathogenic bacteria, and it may be crucial in mediating the effects of gut microorganisms on behavior [25]. [26] reported that microbial metabolites, such as neurotransmitters and neuroactive compounds produced in the gut, can influence brain function and behavior, potentially impacting mood, cognition, and stress responses.

3. Gut microbiota and disease

The gut microbiota plays a pivotal role in maintaining host health and homeostasis, and alterations in its composition and function have been implicated in the pathogenesis of various diseases.

3.1. Dysbiosis and Disease Pathogenesis

Dysbiosis, defined as an imbalance or maladaptation in the composition and function of the gut microbiota, has been implicated in the pathogenesis of numerous diseases. It has been associated with a wide range of conditions, including obesity, diabetes, IBD, allergies, and autoimmune disorders. [27]. Studies have demonstrated differences in the gut microbiota composition between lean and obese individuals, with obese individuals often exhibiting reduced microbial diversity and an altered abundance of specific bacterial taxa [28]. Furthermore, transplantation of gut microbiota from obese individuals into germ-free mice has been shown to induce weight gain and metabolic dysfunction, highlighting the potential causal role of gut dysbiosis in obesity [29]. One of the well-studied associations is between dysbiosis of the gut microbiota and IBD, which includes Crohn's disease and ulcerative colitis. Patients with IBD exhibit alterations in gut microbial composition, with reductions in microbial diversity and changes in the relative abundance of specific taxa, such as a decrease in Firmicutes and an increase in Proteobacteria. These microbial changes can contribute to intestinal inflammation and disrupt mucosal barrier function, further exacerbating disease progression [30]. Additionally, experimental studies using animal models of IBD have provided insights into the mechanistic interactions between the gut microbiota and host immune system in driving intestinal inflammation [30].

3.2. Gut Microbiota and Metabolic Diseases

The gut microbiota also plays a significant role in the development of metabolic diseases, such as obesity and type 2 diabetes. The alteration in microbiota composition in some instances may increase insulin resistance and thus induce an increase in insulin resistance type 2 diabetes. This is why recent studies have shown that the transplantation of the microbiota causes a change in susceptibility to metabolic disorders. Studies have shown that obese individuals harbor distinct gut microbial communities characterized by an increased capacity for energy harvest from the diet. This altered microbial profile can lead to metabolic endotoxemia, insulin resistance, and chronic low-grade inflammation, all of which are risk factors for metabolic disorders [31]. Furthermore, the gut microbiota can influence host metabolism through the production of bioactive metabolites, such as SCFAs, which can regulate adipocyte differentiation, glucose metabolism, and energy expenditure. Acetate, an SCFA, stimulates insulin secretion from the pancreas and thus when SCFA increases, the host will suffer from obesity. Dysbiosis of the gut microbiota, characterized by a reduction in SCFA-producing bacteria, has been associated with metabolic dysfunction and obesity [32].

3.3. Gut Microbiota and Immune-Mediated Disorders

In addition to IBD, dysbiosis of the gut microbiota has been implicated in the pathogenesis of other immune-mediated disorders, including allergies and autoimmune diseases. The gut microbiota plays a critical role in immune system development and regulation, particularly during early life. Perturbations in the gut microbiota composition, such as reduced microbial diversity and alterations in specific bacterial taxa, have been associated with an increased risk of allergic diseases, including asthma, atopic dermatitis, and food allergies [33]. These findings underscore the importance of early-life microbial colonization in shaping immune tolerance and susceptibility to allergic disorders. The hygiene hypothesis posits that alterations in the gut microbiota composition, resulting from improved sanitation and reduced exposure to diverse microbial communities, may contribute to the rising prevalence of allergic diseases [33]. Dysbiosis of the gut microbiota early in life has been associated with an increased risk of developing allergic conditions, such as

asthma and eczema [34]. Similarly, dysbiosis of the gut microbiota has been implicated in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis, type 1 diabetes, and multiple sclerosis, which are characterized by dysregulated immune responses against self-antigens. Alterations in gut microbial composition and function can lead to immune dysregulation and loss of tolerance to self-antigens, triggering autoimmune responses. Growing evidence suggests that dysbiosis of the gut microbiota may contribute to the pathogenesis of autoimmune disorders by modulating immune system function and promoting systemic inflammation [35]. Restoring gut microbial balance through interventions, such as probiotics or fecal microbiota transplantation, holds promise as a therapeutic strategy for immune-mediated disorders.

Dysbiosis of the gut microbiota has emerged as a common feature across various disease states, underscoring its importance as a potential diagnostic marker and therapeutic target for intervention. Further research is warranted to elucidate the mechanistic links between gut microbiota dysbiosis and disease pathogenesis and to develop novel microbiota-based interventions for disease prevention and treatment. Understanding the intricate interplay between the gut microbiota and disease pathogenesis is essential for developing targeted interventions to modulate the gut microbiota and mitigate disease risk.

3.4. Therapeutic Approaches Targeting Gut Microbiota

The dynamic and modifiable nature of the gut microbiota has spurred interest in developing therapeutic interventions aimed at restoring microbial balance (eubiosis) and ameliorating dysbiosis-associated diseases. These approaches include the use of probiotics, prebiotics, postbiotics, fecal microbiota transplantation (FMT), and dietary interventions, each offering unique mechanisms for manipulating the gut microbiota and improving host health.

3.4.1. Probiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Commonly used probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium*, which are known for their ability to modulate immune function, gut microbiota composition, improve gut barrier integrity, and inhibit the growth of pathogenic bacteria. Probiotic administration is suggested to restore microbial dysbiosis and maintain intestinal microbial balance by occupying host tissue and preventing colonization of pathogenic bacteria. *Lactobacillus* has been considered an option for preventing antibiotic-associated diarrhea in children [36]. For instance, [37] reported that *Lactobacillus casei* inhibits growth of *Helicobacter pylori*, and the co-colonization of *Lactobacillus rhamnosus* GG and *Bifdobacterium lactis* Bb12 promoted innate immune responses to human rotavirus. Probiotic supplementation has been shown in clinical studies, to be effective in preventing and treating various gastrointestinal disorders, such as antibiotic-associated diarrhea, irritable bowel syndrome (IBS), IBD, and allergies [36].

3.4.2. Prebiotics

Prebiotics are non-digestible dietary fibers that serve as substrates for beneficial gut bacteria, promoting their growth and activity. By selectively stimulating the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus* species, prebiotics can help restore microbial balance and enhance gut health [37]. They also serve as substrates for fermentation by these beneficial bacteria, prebiotics can enhance the production of SCFAs and other metabolites with health-promoting properties. The most well-known prebiotics are inulin, fructo-oligosaccharides (FOS), lactulose, and galacto-oligosaccharides (GOS). Studies have confirmed that taking prebiotics can stimulate the selective enrichment of probiotics in the intestinal tract, thereby regulating immune response and preventing pathogens [37]. Additionally, prebiotic supplementation has been shown to improve metabolic parameters, such as glucose metabolism and lipid profiles, in individuals with metabolic disorders.

3.4.3. Postbiotics

Postbiotics are bioactive compounds produced by probiotic bacteria during fermentation of prebiotics, and they exert beneficial effects on host health. These compounds include SCFAs, antimicrobial peptides, microbial cell components, and metabolic byproducts, which can modulate immune function, improve gut barrier integrity, and regulate inflammation [6]. Postbiotics offer a promising avenue for therapeutic intervention, as they provide a means to harness the health benefits of probiotics without the need for live microorganisms.

3.4.4. Fecal Microbiota Transplantation (FMT)

FMT involves the transfer of fecal material from a healthy donor to a recipient with the aim of restoring a healthy gut microbiota composition. FMT has emerged as a highly effective treatment for recurrent Clostridium difficile infection (CDI), a condition characterized by dysbiosis of the gut microbiota, with cure rates exceeding 90% in clinical trials [24].

Although these treatments showed promising results, they were investigated in preclinical models, or the sample sizes were too small. Emerging evidence suggests that FMT may also be beneficial in other conditions, such as IBD, IBS, and metabolic disorders, although further research is needed to establish its safety and efficacy in these contexts.

3.4.5. Dietary Interventions

Dietary interventions represent a non-invasive and accessible approach to modulating the gut microbiota. Certain dietary components, such as fiber-rich foods, polyphenols, and fermented foods, can promote the growth of beneficial bacteria and enhance microbial diversity in the gut [1]. For example, the *Bacteroides* genus is highly associated with the consumption of animal proteins, amino acids and saturated fats, which are typical components of Western diet, while the *Prevotella* genus is associated with the consumption of carbohydrates and simple sugars, which are typical of agrarian societies. People with a Bacteroides dominated gut microbiome will gain a *Prevotella*-dominated microbiome by switching from a Western diet to a carbohydrates-based diet for an extended period of time. Moreover, personalized dietary interventions based on individual gut microbiota profiles hold promise for optimizing gut health and preventing disease [1].

Therapeutic approaches targeting the gut microbiota offer exciting opportunities for the prevention and treatment of a wide range of diseases. Continued research into the mechanisms of action and long-term effects of these interventions will be crucial for advancing our understanding of the gut microbiota's role in human health and disease.

3.5. Future Directions and Challenges

The field of gut microbiota research is rapidly evolving, with ongoing advancements in technology and methodology enabling deeper insights into the complex interactions between the microbiota and host physiology. As we look to the future, several key areas of focus and challenges emerge, including the need for further mechanistic understanding, harnessing the therapeutic potential of the microbiota, and addressing ethical considerations. While considerable progress has been made in characterizing the composition and function of the gut microbiota, many questions remain regarding the underlying mechanisms driving microbiota-host interactions and their implications for health and disease. Elucidating the molecular pathways and signaling networks involved in microbiota-mediated effects on host physiology will be critical for developing targeted interventions and precision medicine approaches. Exploiting the therapeutic potential of the gut microbiota represents a promising avenue for preventing and treating a wide range of diseases. However, translating microbiota-based interventions from bench to bedside poses significant challenges, including standardization of protocols, identification of optimal microbial consortia, and ensuring safety and efficacy in diverse patient populations. Furthermore, personalized approaches that consider individual variations in gut microbiota composition and function will be essential for maximizing therapeutic outcomes. As the field of microbiota-based therapeutics continues to advance, ethical considerations surrounding the manipulation of the human microbiota warrant careful attention. Questions regarding informed consent, privacy rights, and the long-term consequences of microbial interventions on host health and ecosystem stability must be addressed to ensure responsible and equitable implementation of microbiota-based therapies. Additionally, efforts to promote diversity and inclusivity in microbiota research and therapy development are needed to mitigate potential disparities in access and outcomes. Advancements in high-throughput sequencing, metagenomics, metabolomics, and computational modeling have revolutionized our ability to study the gut microbiota and its functional dynamics. Continued investment in technological innovation will be crucial for overcoming existing limitations, such as the inability to culture the majority of gut microbes and the challenges associated with studying microbial-host interactions in complex ecosystems. Integration of multi-omics data and development of predictive modeling approaches will further enhance our understanding of microbiota-host dynamics and facilitate the design of personalized interventions.

The field of gut microbiota research holds great promise for revolutionizing our approach to human health and disease. By addressing key challenges and embracing interdisciplinary collaboration, we can harness the therapeutic potential of the microbiota to improve patient outcomes and advance the frontiers of biomedicine.

4. Conclusion

The gut microbiota plays a central role in human health, influencing a wide range of physiological processes and contributing to the development and progression of various diseases. Through its diverse metabolic activities, the gut microbiota impacts nutrient metabolism, immune system function, and the synthesis of bioactive compounds, highlighting its importance as a key mediator of host-microbe interactions. Through advances in high-throughput sequencing, metagenomics, and systems biology, our understanding of the gut microbiota has expanded dramatically in recent years, revealing its vast diversity and functional complexity. Dysbiosis of the gut microbiota, characterized by alterations in microbial composition and function, has been implicated in the pathogenesis of numerous diseases,

including obesity, inflammatory bowel disease, allergies, and autoimmune disorders. In response to these insights, there has been growing interest in developing therapeutic interventions targeting the gut microbiota, such as probiotics, prebiotics, postbiotics, fecal microbiota transplantation, and dietary modifications. These approaches offer promising avenues for modulating the gut microbiota and improving host health, although challenges remain in translating these findings into clinical practice. Looking ahead, future research efforts will focus on elucidating the mechanistic underpinnings of microbiota-host interactions, harnessing the therapeutic potential of the microbiota for personalized medicine, and addressing ethical considerations surrounding microbiota-based interventions. By embracing interdisciplinary collaboration and leveraging technological innovation, we can unlock the full potential of the gut microbiota as a novel target for disease prevention and treatment. By continuing to explore its complexities and harness its therapeutic potential, we can pave the way towards a future where microbiota-based interventions revolutionize healthcare and improve lives.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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