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Reviews in microbiome in health & disease

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Reviews in microbiome in health & disease

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Table of contents

05 Editorial: Reviews in microbiome in health & disease
Suhana Chattopadhyay and Leena Malayil

08 Insights into oral microbiome and colorectal cancer – on the way of searching new perspectives
Anna Kudra, Damian Muszyński, Bartosz Kamil Sobocki, Alessandro Atzeni, Ludovico Carbone, Karolina Kaźmierczak-Siedlecka, Karol Połom and Leszek Kalinowski

16 The role of microbiota in the pathophysiology of uterine fibroids – a systematic review
Lidia Korczynska, Natalia Zeber-Lubecka, Magdalena Zgliczynska, Elzbieta Zarychta, Kornelia Zareba, Cezary Wojtyla, Michalina Dabrowska and Michal Ciebiera

32 Alterations of gut microbiome following gastrointestinal surgical procedures and their potential complications
Christina Tsigalou, Afroditi Paraschaki, Nicola Luigi Bragazzi, K. Aftzoglou, Elisavet Stavropoulou, Z. Tsakris, S. Vradelis and Eugenia Bezirtzoglou

44 Erratum: Alterations of gut microbiome following gastrointestinal surgical procedures and their potential complications
Frontiers Production Office

45 Regulation of gut microbiota: a novel pretreatment for complications in patients who have undergone kidney transplantation
Jiajia Ye, Junxia Yao, Fangfang He, Jing Sun, Zheng Zhao and Yumei Wang

57 Psychobiotics and fecal microbial transplantation for autism and attention-deficit/hyperactivity disorder: microbiome modulation and therapeutic mechanisms
Min-jin Kwak, Seung Hyun Kim, Hoo Hugo Kim, Rahul Tanpure, Johanna Inhyang Kim, Byong-Hun Jeon and Hyun-Kyung Park

70 The gut-lung axis in the CFTR modulator era
Florian Lussac-Sorton, Éléna Charpentier, Sébastien Imbert, Maxime Lefranc, Stéphanie Bui, Michael Fayon, Patrick Berger, Raphaël Enaud and Laurence Delhaes

79 The oral-gut-circulatory axis: from homeostasis to colon cancer
Sofia C. Tortora, Maria Gonzalez Agurto and Laura A. Martello

94 Factors affecting the early establishment of neonatal intestinal flora and its intervention measures
Guangyu Ma, Yuguo Shi, Lulu Meng, Haolong Fan, Xiaomei Tang, Huijuan Luo, Dongju Wang, Juan Zhou and Xiaomin Xiao

106 An update: is there a relationship between *H. pylori* infection and nonalcoholic fatty liver disease? why is this subject of interest?
Xingcen Chen, Ruyi Peng, Dongzi Peng, Jia Xiao, Deliang Liu and Rong Li

117 Effects of plant natural products on metabolic-associated fatty liver disease and the underlying mechanisms: a narrative review with a focus on the modulation of the gut microbiota
Tianqi Cai, Xinhua Song, Xiaoxue Xu, Ling Dong, Shufei Liang, Meiling Xin, Yuhong Huang, Linghui Zhu, Tianxing Li, Xueke Wang, Yini Fang, Zhengbao Xu, Chao Wang, Meng Wang, Jingda Li, Yanfei Zheng, Wenlong Sun and Lingru Li

150 Research progress on the microbiota in bladder cancer tumors
Keyuan Lou, Junpeng Chi, Jitao Wu, Jian Ma, Shu Liu and Yuanshan Cui

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Editorial: Reviews in microbiome in health & disease

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Editorial on the Research Topic
Reviews in microbiome in health & disease

The gut microbiome, a complex and diverse community inhabiting the gastrointestinal tract, is vital for maintaining health and impacting susceptibility to numerous diseases. Its interactions with the host are diverse, from aiding digestion and regulating immunity to influencing metabolism and protecting against pathogens. These interactions are pivotal in the development of chronic diseases and autoimmune conditions. Firstly, the gut microbiome plays a significant role in digesting complex carbohydrates and fibers, producing short-chain fatty acids crucial for gut health. Therefore, any disruption to the normal gut microflora could contribute to the development of obesity and metabolic disorders (Liu et al., 2021). Secondly, the gut microbiome produces neurotransmitters and neuroactive compounds like serotonin and gamma-aminobutyric acid, which can impact the mood, behavior, and cognitive function of the host. Imbalances in the gut microbiome have been associated with mental health disorders like anxiety and depression (Xiong et al., 2023). Thirdly, the gut microbiome acts as a protective barrier against harmful pathogens by competing for resources and producing antimicrobial compounds. Dysbiosis, an imbalance in the gut microbiota, has been linked to various chronic diseases including inflammatory bowel diseases, type 2 diabetes, cardiovascular diseases, and autoimmune conditions (Tsai et al., 2021). Fourthly, evidence suggests that the gut microbiome plays a key role in the development of allergies and autoimmune diseases (Xu et al., 2019). Early exposure to diverse microbes is believed to contribute to proper immune system training, thereby reducing the risk of hypersensitivity reactions and autoimmune responses.

Overall, the gut microbiome is a dynamic and essential component of human health. Its role extends beyond digestion to influence immune function, metabolism, mental health, and protection against diseases. Understanding the intricate interactions between the host and its microbiome opens avenues for therapeutic interventions and preventive strategies, highlighting the significance of maintaining a balanced and diverse gut microbial community for overall well-being. This Research Topic emphasizes several reviews that underscore the critical role of the gut microbiome in maintaining a delicate balance between health and disease.

While it is known that dysbiosis in the gut microbiome is considered a key factor in the host's health and disease development through the microbiota-gut-brain (MGB) axis and the gut-lung axis, less is known about its impact extending to various conditions, including autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD), or cystic fibrosis. One such review discusses how scientific evidence suggests that imbalances

in the gut microbiome contribute to these disorders, highlighting the importance of the MGB axis in the treatment of ADHD and ASD (Kwak et al.). This review outlines the role of gut microbial colonization in early life and its connection to neurodevelopmental disorders like ASD/ADHD pathogenesis, emphasizing a promising therapeutic approach using psychobiotics and fecal microbial transplantation (FMT). Another review summarizes the impacts of the gut-lung axis on cystic fibrosis pathophysiology, highlighting the impact of cystic fibrosis transmembrane conductance regulator (CFTR) modulators in pulmonary and digestive microbiomes (Lussac-Sorton et al.). Albeit somewhat inconsistent, the review summarizes studies suggesting CFTR modulators that promote increased bacterial diversity with a reduction in conventional cystic fibrosis pathogens in the respiratory system and an increase in anti-inflammatory bacteria in the gut. This article showcases the use of CFTR modulators in the management of cystic fibrosis specifically for younger patients.

Furthermore, two other reviews highlight the importance of understanding the relationship between intestinal microbiome and surgical procedures. The first review by Ma et al. provides an overview of the pivotal role that early-life intestinal microflora provides in determining human health including the effects of multiple influencers like delivery mode, gestational age, and feeding method. The review highlights the importance of the maternal-infant symbiotic relationship in shaping an infant's gut microbiota, along with raising concerns about cesarean section's impact on the neonatal gut microbiome, emphasizing the need for long-term follow-up studies. Additionally, the review also explores natural and artificial reconstruction of intestinal flora in infants, intending to prevent and/or treat neonatal intestinal diseases. The second article by Tsigalou et al. reviews the association between peri-operative interventions and gut microbial community. While gastrointestinal surgeries have a significant impact on the epithelial barrier, the composition of the gut microbiome can influence surgical outcomes including complications like anastomotic leaks. The review highlights the current intervention studies with probiotics to address intestinal dysbiosis and reduce complications in surgical patients. A third review (Ye et al.) explores the connection between gut microbiome changes with kidney transplantation. Poor kidney function can disrupt the balance of intestinal microbiota, but administering prophylactic drugs to regulate the microbiota of patients may mitigate the occurrence and advancement of transplantation complications.

Two other review articles address chronic liver diseases in connection with gut microbiota with one exploring the use of plant natural products (Cai et al.) on metabolic-associated fatty liver disease (MAFLD) and the second one focusing on the relationship between *Helicobacter pylori* infection and nonalcoholic fatty liver disease (NAFLD) (Chen et al.). Both MAFLD and NAFLD, characterized by liver fat accumulation, lack approved drugs due to their complex nature. Recognizing the link between gut microbiota and MAFLD, there is growing interest in using plant natural products for its treatment. In Cai et al., the authors conduct a systematic review of the plant products that target the gut microbiota and show potential as safer and more effective treatments in the development of natural anti-MAFLD drugs. The second article focuses on the role of *H.pylori*, a gram-negative bacillus that has been suggested as an initiating factor for various diseases ranging from chronic gastritis, and Alzheimer's to NAFLD. Chen et al., conducted a meta-analysis on the extra gastric role of *H.pylori* in connection with the development of NAFLD including alterations in inflammatory cytokines, insulin resistance, and lipid metabolism. Furthermore, another systematic review (Korczynska et al.) focuses on pathogenetic pathways that are shared by the intestinal microbiome and the uterus, highlighting the importance of studying microbiome modulations for the treatment of uterine fibroids.

Furthermore, there are two reviews on the oral-gut-circulatory axis highlight the bidirectional influence of immune cells, inflammatory factors, circulating bacteria, and microbial metabolites on the homeostasis of oral and gut microbiota (Tortora et al. and Kudra et al.). Both of these comprehensive reviews focus on recent studies on associations between oral microbiota to cancer susceptibility, particularly colon cancer. While targeting systemic inflammation may have therapeutic potential, the development of non-invasive screening tools like personalized medicine, probiotics, and lifestyle modifications may offer preventative approaches. Finally, delving into the research progress on intratumoral microbiota associated with bladder cancer, Lou et al., summarize the role of different bacterial phyla in cancer onset, progression, and prognosis. They highlight the challenges that continue to persist, including ethical and methodological issues, necessitating future investigation for clinical insights.

The compilation of the above review articles in this Research Topic comprehensively delves into a broad spectrum of gut microbiome research. It unveils fresh perspectives, assesses novel tools, and facilitates discussion on multiple dimensions of gut microbiome homeostasis. Furthermore, it explores diverse facets of its intricate association with various ailments and diseases, providing an examination of this dynamic field.

Author contributions

SC: Writing – review & editing, Writing – original draft. LM: Writing – review & editing, Writing – original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Insights into oral microbiome and colorectal cancer – on the way of searching new perspectives

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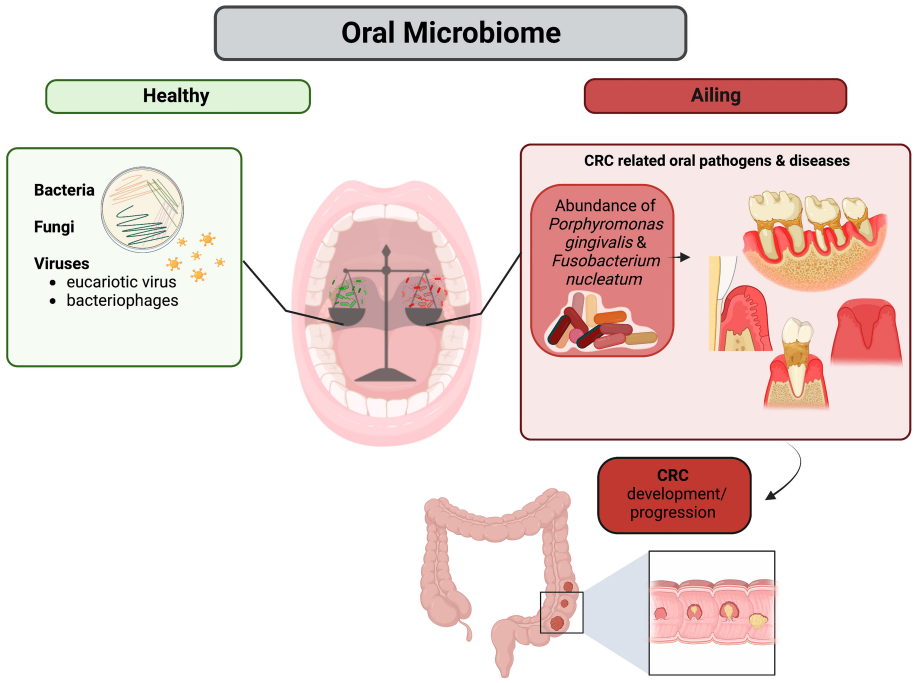
Microbiome is a keystone polymicrobial community that coexist with human body in a beneficial relationship. These microorganisms enable the human body to maintain homeostasis and take part in mechanisms of defense against infection and in the absorption of nutrients. Even though microbiome is involved in physiologic processes that are beneficial to host health, it may also cause serious detrimental issues. Additionally, it has been proven that bacteria can migrate to other human body compartments and colonize them even although significant structural differences with the area of origin exist. Such migrations have been clearly observed when the causes of genesis and progression of colorectal cancer (CRC) have been investigated. It has been demonstrated that the oral microbiome is capable of penetrating into the large intestine and cause impairments leading to dysbiosis and stimulation of cancerogenic processes. The main actors of such events seem to be oral pathogenic bacteria belonging to the red and orange complex (regarding classification of bacteria in the context of periodontal diseases), such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* respectively, which are characterized by significant amount of cancerogenic virulence factors. Further examination of oral microbiome and its impact on CRC may be crucial on early detection of this disease and would allow its use as a precise non-invasive biomarker.

KEYWORDS

colorectal cancer, oral microbiome, bacterial virulence factors, biofilm, periodontitis



GRAPHICAL ABSTRACT

1 Introduction

Nowadays, colorectal cancer (CRC) still represents one of the most commonly diagnosed types of cancer worldwide. According to the GLOBOCAN – Global Cancer Statistics 2020, colorectal cancer was assessed as the third (10%) most commonly diagnosed cancer globally and the second cause of death (9.4%) (Sung et al., 2021). Moreover, research work "Global colorectal cancer burden in 2020 and projections to 2040" reported that the number of new CRC cases will increase by 2040 approximately up to 3.2 million cases, causing a huge impact on global healthcare system (Yue and Pengfei, 2021). The highest rates are in the most developed countries, suggesting that diet (i.e., low intake of dietary fiber and diet with high content of saturated fatty acids, consumption of red meat), sedentary lifestyle and obesity, as well as environmental risk factors play a major role in cancer development (Geicho et al., 2015; Mármol et al., 2017; Kwong et al., 2018; Thanikachalam and Khan, 2019; Kaźmierczak-Siedlecka et al., 2020a; Sninsky et al., 2022). Moreover, alterations in "healthy" microbiome and microbial-derived metabolites (known as part of metabolome) might, among others, simulate local inflammatory response and increase the risk for CRC. Interestingly, in 2021 Wang et al. described age at diagnosis, male gender, poor oral hygiene, as well as altered salivary abundance of *Desulfovibrio desulfuricans* (anaerobic bacteria) as predict risk factors of CRC and incorporated them in a clinical nomogram (Wang Y et al., 2021). Over the past decade, dysbiotic changes in oral microbiome allowed better understanding of the pathogenesis of oral cancers and others distal organs disorders. For instance, the *Porphyromonas gingivalis* (Gram-negative oral anaerobe) is, on the one hand, a major periodontopathic pathogen involved locally in development of oral squamous cell carcinoma; on the other hand, it participates also in other localization, for instance in pancreatic carcinogenesis (Mysak et al., 2014; Öğrendik, 2017; Olsen and Yilmaz, 2019; Zhou and Luo, 2019). Another example is *Candida albicans*, whose infection induces several cancerous hallmarks, such as activation of proto-oncogenes, induction of DNA damage and overexpression of inflammatory signaling pathways, contributing to both oral cancer progression and gastric carcinogenesis (Engku Nasrullah Satiman et al., 2020; Zhong et al., 2021).

Considering this and other evidence, oral microbiome may distally affect the development/progression of CRC. Therefore, in this review the link between oral microbiome and CRC occurrence has been discussed. We presented the virulence factors of oral pathogens associated with CRC and the significance of creating biofilm by microorganisms. Moreover, we described the usage of oral microbiome as biomarker to detect CRC.

2 Oral microbiome in healthy individuals

Oral cavity represents the second mostly inhibited by microbes area of the human body (Verma et al., 2018). Approximately 772 prokaryotic species are comprised in the extended Human Oral Microbiome Database (eHOMD) (Verma et al., 2018). Six extensive phyla have been distinguished using 16S rDNA profiling, Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria,

Bacteroidetes and Spirochaetes (Verma et al., 2018). Different species of bacteria populate main seven regions of human mouth, i.e., gingival sulcus, cheek, attached gingiva, teeth, tongue, lip, hard palate, and soft palate (Dewhirst et al., 2010). Microbiome inhabiting those areas is not hazardous for our health as long as it does not colonies other areas of the human body. Although these bacteria are present in the oral cavity as part of its microbiome, more recent attention has focused on the correlation with several diseases, such as periodontitis, gingivitis, and dental caries, which have a definite connection to modifications in the oral microbiota (Willis and Gabaldón, 2020). These studies were carried out on so called "WEIRD" populations. The acronym referred to Western, Educated, Industrialized, wealthy, and Democratic countries, indicating a bias in psychology research toward these cultures, which at the time made up around 13% of the world's population (Willis and Gabaldón, 2020). Nevertheless, even within "WEIRD" populations oral microbiome may differ since there are numerous factors which could affect environmental conditions in oral cavity, such as overall hygiene, food and water intake, individual composition of saliva, lifestyle, and many others (Zaura et al., 2014).

The first study assessing the composition of oral mycobiota (fungal part of oral microbiota) in healthy individuals has been published in 2010 (Ghannoum et al., 2010). The authors reported that the most frequently identified genera of fungi were *Candida*, *Cladosporium*, *Aurebasidium*, *Saccharomycetales*, and *Aspergillus*. Moreover, *Fusarium* and *Cryptococcus* were also identified (Ghannoum et al., 2010). Interestingly, interactions between the bacterial and fungal fraction of the oral microbiome have been described. For instance, *C. albicans* initiates alterations of virulence factors of *Streptococcus mutans*, which is known as major cariogenic pathogen; notably, exoenzyme GtfB of *S. mutans* causes the formation of exopolysaccharide matrix and *C. albicans* intensifies that effect consequently contributing to growth of dental biofilm (Baker et al., 2017). The interactions between *C. albicans* and *F. nucleatum* as well as *C. albicans* and *Streptococcus oralis* were also observed (Baker et al., 2017). The effects of interactions between microbes contribute to the development of local and distal diseases. As it was mentioned above, *Candida* genus normally resides in an oral cavity with no observed pathological changes. However, in case of for instance: infections, immune-related disorders, diabetes, antibiotics taking, low production and secretion/flow of salivary, poor oral hygiene, and wearing dentures it caused oral candidiasis (Hu et al., 2019; Rodrigues et al., 2019; Martorano-Fernandes et al., 2020). Therefore, oral mycobiota seems to be significant in the context of CRC patients who are often treated with radiotherapy, chemotherapy and are at higher risk of *Candida*-associated diseases development. It should also be noted that dysbiotic alterations of intestinal fungal community is found in CRC patients. It is observed by, among others, reduced amount of *Saccharomyces cerevisiae* (Coker et al., 2019). Nevertheless, the data regarding the link between oral mycobiota and CRC is still strongly limited and that is associated with several reasons, for instance more complicated methodology than in case of bacteria analysis (Kaźmierczak-Siedlecka et al., 2020b).

Another important component of the oral cavity human microbiome is the oral virome, which, in healthy individuals, includes eukaryotic viruses as well as bacteriophages. Particularly, eukaryotic viruses are Herpesviriade, Papillomaviridae, and Anelloviridae, whereas the most common bacteriophages are Siphoviridae, Myoviridae, and Podoviridae. Oral virome is stable for a long period and, similarly as in case of bacterial oral microbiome, oral virome is different between individuals (Baker et al., 2017).

3 The impact of diet and other lifestyle-related factors on oral microbiome

Several studies focused on the impact of diet on the structure of human oral microbiome have recently emerged. A main risk factor, contributing to the growth of pathogenic bacteria as well as development of caries and biofilm expansion, seems to be an excessive intake of carbohydrates, especially sucrose (Sheiham, 2001; Moynihan and Petersen, 2004). Most common pathogenic bacteria use carbohydrates in processes of fermentation turning them into acidic products, which ultimately lead to the development of caries. Several lines of evidence suggest that richness of variety of oral bacteria can differ depending on sugar intake (Bostanci et al., 2021). In 2014 researchers attempted to evaluate whether long-term modifications in dietary habits have a significant impact on microbiota in saliva. Authors examined microbiome as well as metabolic profiles of 161 healthy patients who declared to follow a vegan, omnivore or ovo-lacto-vegetarian diet. After analysis of sequenced amplicons from the 16S rRNA gene's V1-V3 regions and the analysis of salivary metabolome through 1H-NMR and GC-MS/SPME, researchers came to the conclusion that long-term eating habits have negligible influence on salivary microbiome composition (De Filippis et al., 2014). Another interesting study results were presented by Zaura et al. where in order to better understand the ecobiological variety of the salivary ecosystem and the relationships between the salivary microbiome, salivary metabolome, and host-related biochemical salivary parameters, 268 healthy patients were evaluated after overnight fasting. This study revealed a correlation between daily protein intake and salivary pH, which indicates that diet may affect the environment in the oral cavity *via* altering salivary pH (Zaura et al., 2017).

Translational research projects, such as that conducted by Nagihan Bostanci in the 2021, have investigated smoking habits on oral microbiome composition. Patients were asked to answer a questionary describing them as "daily smoker", "occasional smoker", "former smoker", and "never smoker". Saliva samples were collected using SalivaGene Collector which enabled the quantification of DNA concentration using the Quant-iT Picogreen dsDNA test. Results demonstrated that smoking does not contribute significantly to the heterogeneity of oral microbiome, while a slight elevation of richness of oral bacteria species was observed in daily smokers in comparison with never smokers (Bostanci et al., 2021). According to research conducted by Le et al. consuming considerate amounts of alcohol may also influence oral microbiota. In this study researchers were looking for correlation between alcohol abuse and the diurnal variation of

salivary microbiome. 53 patients were tested for alterations in composition of oral microbiota and individual taxon abundance by 16S rRNA gene sequencing. Results stated that alcohol usage increased the richness of the salivary microbiome while decreasing its evenness. The oral microbiota composition altered dramatically in patients with alcohol abuse history. Furthermore, specific taxa, such as *Actinomyces, Leptotrichia, Sphaerochaeta*, and *Cyanobacteria*, which are known for their negative impact on homeostasis in oral cavity, were enriched in the research group (Li X. et al., 2022). The following study focused on the potential impact of sleep on the oral microbiota. A 16S rRNA gene sequencing analysis was performed to determine alterations. Material for this study was obtained with an Isohelix swab from several locations in the mouth cavity before and after sleep. As comparison to the pre-sleep schedule, the Chao1 index for samples from the buccal mucosa and gingival mucosa, as well as the Shannon index for buccal mucosa samples, were both significantly higher. Variations between before and after sleep may be due to changes in numerous variables influencing oral microbiome during sleep. For instance, during sleep, IgA concentrations rise, saliva pH lowers, and the temperature inside the mouth cavity decreases (Sotozono et al., 2021).

4 The alterations of oral microbiota in CRC

4.1 Dental biofilm and its significance

Human digestive system homeostasis is possible thanks to the symbiotic relationship between the bacteria inhabiting the gastrointestinal tract and the host. Despite being in different anatomical locations, the colon and the mouth cavity are both heavily populated by different microbiota (Koliarakis et al., 2019). Occasionally, symbiotic relationship occurring between bacteria and hosts oral cavity can be disrupted due to various factors such as neglect of hygiene, decreased production of saliva, change in diet, or weakened immune system. Such events may lead to extensive build-up of dental biofilm inside oral cavity on different surfaces, but mostly on uneven areas of teeth like pits and fissures, in interdental gaps and near gingiva (Chenicheri et al., 2017). Dental biofilm causes an increase of microbes' drugs resistance and host immune system functioning impairment. A complex multi-microbial community known as a dental biofilm is encased in a polymeric matrix that helps community expansion, bypasses the host's defenses and encourages colonization of mucosal surfaces through adhesion mediated by different glycoproteins (Chenicheri et al., 2017). Dental biofilms in diseases inside the oral cavity mostly develop in three stages (Kolenbrander et al., 2010). First stage begins right after cleaning when the surface of teeth is exposed to salivary component consisting of alpha-amylase, proline-rich proteins, mucins, and other proteins, with a glycoprotein covering layer leading to create pellicle. Between various glycoproteins, salivary components, and the tooth surface, a variety of interactions such as hydrogen bonds, acid-base interactions, calcium bridges, hydrophobic interactions van der Waals, and electrostatic interactions take place, causing conformational changes in the proteins forming the pellicle. Through strong interactions, bacteria may stay connected not only to the target surface but also to each other, promoting primary colonizers such as *Lactobacillus acidophilus*, *Veillonella*, *Neisseria*, *Actinomyces* spp. and *Streptococcus* spp. to occupy new areas. Mostly Gram-positive species colonize the supragingival surface of teeth while Gram-negative species colonize subgingival surface. As a byproduct of their carbohydrate fermentation process, oral streptococci create lactic acid, resulting in a fast reduction of environmental pH. *C. albicans* grows as yeast at acidic pH levels and as hyphal at alkaline pH levels, hence extracellular pH can affect hyphal development and consequently dental biofilm formation. For the dental biofilm to grow, communication among the interspecies in the plaque is required. Later, due intensive usage of substrates by primary colonizers, bacteria of the genus *Veillonella* can no longer obtain glucose, and they begin to break down lactic acid into acetic and propionic acid, gaining energy for growth and development.

4.2 Virulence factors of oral pathogens related to CRC development/progression

Herb Brody defined colorectal cancer as "a disease of modernity", highlighting the existing relationship between several environmental determinants and the incidence of colorectal cancer (Brody, 2015). Most pathogenic bacteria colonizing oral cavity may contribute to development or progression of CRC *via* its cancerogenic metabolites as well as other virulence factors. Many studies tried to examinate how different species of bacteria participate in carcinogenic processes which could take place inside the colon. Yiping W. Han identified the protein Fad2, exclusively encoded by *F. nucleatum* and *F. periodonticum*. Fad2, being not only adhesin but also an invasin, is essential for bacteria to first bind and then invade both host healthy and cancerous cells (Han, 2015). Other biomolecules presented on the surface of *F. nucleatum*, crucial in promoting development of CRC, are lipopolysaccharides (LPS), adhesin A (FadA) and fusobacterium autotransporter protein 2 (Fap2) (Coppenhagen-Glazer et al., 2015; Han, 2015). Thanks to modern molecular methods of detection such as qPCR, fluorescence quantitative (FQ)-PCR, droplet digital PCR (ddPCR) and fluorescence *in situ* hybridization (FISH), it was possible to establish that *F. nucleatum* was present in CRC neoplastic tissues (Marano et al., 2019). According to the studies conducted by Kensuke Yamamura, *F. nucleatum* occurred in 20% (4/20), 10% (2/20), and 45% (9/20) of esophageal, gastric, and CRC tissues, respectively (Yamamura et al., 2017). Different theories argue that *F. nucleatum* can be transmitted *via* hematogenous transmission, from its typical habitat in oral mouth to any part of human body, even that would be suitable for these bacteria to colonize, i.e., colon or even the pregnant uterus (Han, 2011). Another potentially cancerogenic oral pathogen is *P. gingivalis*, implicated in the pathogenesis of periodontitis, an inflammatory disease-causing atrophy of alveolar bone and gingiva inflammation. These hazardous bacteria can be responsible for promoting growth of other species of bacteria in its surrounding *via* various virulence factors, causing expansion of microbiome and leading to dysbiosis in that area (Xu et al., 2020). Furthermore, one

of the most troublesome virulence factors is lipopolysaccharide (LPS), which is an endotoxin present as component of bacteria cell wall. It seems that LPS could be responsible for end-organ damage as well as sepsis, both representing outcomes of systemic inflammation caused by increased release of cytokines and shoutable factors, released as a hosts' immunity response for the presence of LPS (Lien et al., 2000). Another important arguing issue is that *P. gingivalis* has two different types of fimbriae, minor fimbriae and major fimbriae, which allow bacteria to bond with hosts' cells and invade them causing inflammatory reaction (Enersen et al., 2013). *P. gingivalis* is also able to produce the gingipains, a family of cysteine proteinase which cleave polypeptides at the C-terminal after lysine residue and hydrolyze peptide bonds. Gingipains may be able to disintegrate different extracellular matrix components, such as complement factors, immunoglobulins, cytokines as well as collagens, and thus enable *P. gingivalis* to avoid immunological reactions and clearance by the host, leading to the expansion of pathogenic microbiome and the induction to expansion of pathogenic microbiome and induction of its cancerogenic input (Curtis et al., 2001). Gingipains (and also others virulence factors related to *P. gingivalis*) affect the host immune system and consequently cause both local and systemic disorders (Bregaint et al., 2022). Recent study revealed that gingipains are essential virulence factors for the stimulation of the MAPK/ERK signaling pathway and thus encouraging the CRC cells proliferation (Mu et al., 2020). In another study it was also shown that gingipains induce COX-2 expression and production of PGE_2 through their influence of human monocytes (i.e. activation of both MEK/ERK/AP-1 as well as IκB kinase/NF-κB p65 cascades) (Nakayama et al., 2022). It was revealed in many studies that *P. gingivalis* presence is enriched in colorectal mucosa as well as in the stool of CRC cancer patients (Koliarakis et al., 2019; Mu et al., 2020). Moreover, some studies proved that is a direct link between the abundance of bacteria in the oral cavity and in the gastrointestinal tumors microenvironment (Sobocki et al., 2021; Sobocki et al., 2022). It was also shown that *P. gingivalis in vitro* can adhere to CRC cells and then invade them only a few hours after administration. Therefore, *P.gingivalis* might promote CRC cells proliferation (Mu et al., 2020). Although the experimental data are still limited, there are indications that the role of *P.gingivalis* in CRC cancer prognosis is essential. In addition, oral hygiene and oral bacteria composition seem to influence the gut bacteria composition, cause dysbiosis, and in the end cancer promotion.

The summary of virulence factors of *F. nucleatum* and *P. gingivalis* is presented in Figure 1.

5 Oral microbiome as a biomarker for CRC

Oral microbiome may be used as a biomarker for the detection of oral cancer. The results of Hayes et al. study revealed the link between oral microbiome and head and neck squamous cell cancer (HNSCC) (Hayes et al., 2018). It was observed that abundance of oral *Corynebacterium* and *Kingella* was related to the decrease of the risk of HNSCC. However, oral microbiome may be established as biomarker not only for local oral cancer, but also CRC. Promising insights into finding a non-invasive oral microbial biomarker for early detection of colon cancer detection have recently emerged.

In a Zhang et al. study, oral microbiome (analyzed from oral swab) was analyzed using 16S rRNA sequencing (Zhang et al., 2020). Patients with CRC (n=161), colorectal adenoma (n=34), and healthy volunteers (n=58) were enrolled. Interestingly, the composition of oral microbiome and its diversity were significantly different between the three groups of participants. The highest diversity was observed in patients with colorectal adenoma (Zhang et al., 2020). Furthermore, Flemer et al., analyzing microbiota profiling from samples of oral swaps as well as from stool and colonic mucosae from patients suffering from CRC (n=99), colorectal polyps (n=32), and healthy individuals (n=103) highlighted that CRC heterogeneity may be linked to microbiota types that either predispose to or provide resistance to the disease (Flemer et al., 2018). Therefore, profiling the oral microbiome could provide a non-invasive biomarker for CRC. For instance, the results showed that *Prevotella* spp. and *Streptococcus* spp. may differ in abundance depending on occurrence of CRC (Flemer et al., 2018).

Another promising research, conducted by Yang et al., showed that oral pathogens, especially *Prevotella intermedia* and *Treponema denticola*, were positively associated to increased risk of developing colorectal cancer (Yang et al., 2019). From the examination of mouth rinse samples, which were sequenced using the 16S rRNA gene, a correlation was found between abundance of specific taxa of oral microbiota and possibility of CRC occurrence. Wang et al. investigated whether *P. gingivalis*, an oral bacterium belonging to red complex bacteria, is capable to promote the development of colorectal carcinoma. Overall, 77 fecal samples were collected (22 without colorectal diseases, 32 with colorectal adenoma, 23 with colorectal cancer), and *P. gingivalis* had greater abundance between patients affected by CRC rather than adenoma as well as healthy people.

In conclusion, these findings indicate that high number of these pathogenic bacteria may not only be associated with higher risk of having CRC locally advanced but may also be a crucial biomarker that helps increasing the probability of early detection of this disease (Wang X et al., 2021).

6 Oral microbiota and treatment efficiency

Although the treatment of CRC is fairly well established, and the surgical resection remains the cornerstone of curative intent approach (which is sometimes combined with downstaging preoperative radiotherapy and systemic therapy) interest in tailored medicine has grown in recent years (Dekker et al., 2019). New therapeutic non-invasive methods, focusing on patient-specific and disease-specific predictive biomarkers, could support the preparation of patients for the surgical treatment, reduce the side effects of anti-cancer therapy and inhibit the progression of disease.

Currently, a considerable amount of literature on the influence of gut microbiota on different treatment modalities in CRC has been

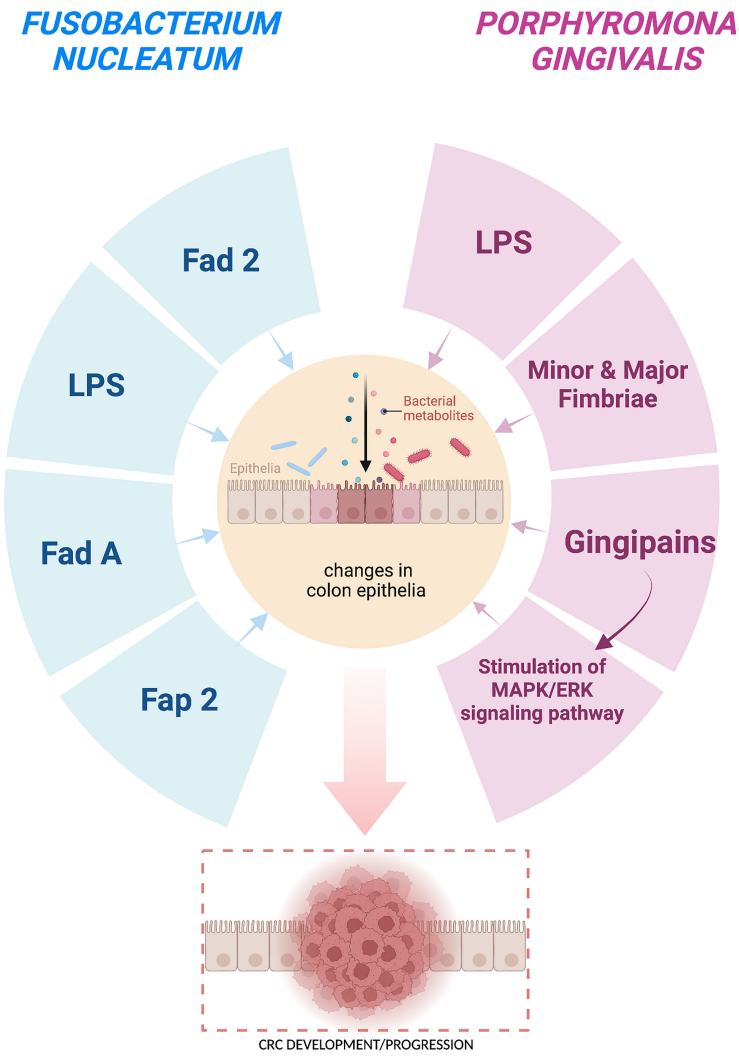


FIGURE 1
Carcinogenic effect of the virulence factors of *Fusobacterium nucleatum* and *Porphyromonas gingivalis* on the colon cells leading to CRC development/progression. Fad 2 – adhesin and invasion, LPS – lipopolysaccharides, Fad A – adhesin, Fap 2 – fusobacterium autotransporter protein 2, Gingipains – cysteine proteinases. This figure was created using *Biorender.com*.

published. However, although the oral microbiota appears to play a similar role, the number of evidence and studies showing this is limited. A study from Dong et al. gave an insight into the mechanism of action (Dong et al., 2021). Studying mouse models of CRC, they revealed that buccal *F. nucleatum* may migrate to CRC locus impairing the efficiency of radiotherapy. The administration of metronidazole reduced this effect (Dong et al., 2021). In addition, it seems that oral microbiota may influence the composition of gut microbiota, affecting specifically the tumor microbiota, but not the microbes in adjacent tumor tissues. With this mechanism oral microbiota may play a role in radiation-induced intestinal injury (Dong et al., 2021). Interestingly, Gao et al. study showed that *F. nucleatum* may stimulate response to tumor and the efficiency of PD-L1 blockade in mice. This bacterium induced PD-L1 expression *via* activation of STING signaling, increasing of interferon gamma and the level of CD8+ tumor infiltrating lymphocytes (Gao et al., 2021). These results may indicate the need to adjust the applied treatment to oral and gut microbiota composition.

Another study conducted by Yoshihara et al. confirmed that the management of periodontal disease might cause the decrease of *F. nucleatum* levels in stool of patients who underwent successful treatment. On the contrary, this was not observed in the group of patients with the treatment failure (Yoshihara et al., 2021). This therapeutic strategy seems to be promising and its impact on clinical outcomes in CRC patients should be further investigated.

Definitive randomized control trials should deeply characterize the impact of oral microbiota on different treatment modalities efficiency in CRC, taking into consideration the important conclusions drawn by the studies above mentioned. The missing links (i.e., secreted metabolites) between oral microbiota and therapy efficiency should be revealed in order to discover potential targets for treatment.

7 Conclusions

Upon presented information, we established that human oral microbiome is, on one hand, vastly important to keep balance between host and coexisting bacteria, on the other hand, it may cause serious damage to general health even beyond area of oral cavity. The structure of oral microbiome may be influenced by various factors, such as age, sex, oral hygiene, and general state of health. Oral mouth is heavily occupied by many different bacterial species that inhabit it. Notably, some of those microbes are extremely pathogenic and because of their numerous virulence factors they may turn out to be cancerogenic.

The metabolites produced by pathogenic bacteria, i.e. *P. gingivalis* and *F. nucleatum* increase the likelihood of CRC development and may even stimulate its growth. Some data suggest that these bacteria may be used, in the future, as non-invasive predictive biomarkers, to detect cancer at different stages (putting the emphasis on earl stage), predict its development and ultimately take appropriate preventive measures before CRC occur. However, this method is strongly limited, among others, due to the fact that periodontal diseases (caused by these bacteria) are more common than CRC. Additionally, the establishment of microbial biomarkers is complicated process, which should regard multiple factors, such as type of taken material (for instance dental plaque, unstimulated saliva), methods of analysis, stage of CRC, age, condition of dentition, the level of oral hygiene, etc. The analysis of oral microbiome and metabolites produced by oral microorganisms in the context of CRC may be a future perspective for oncology. Therefore, it is recommended to introduce more effective cooperation between dentists, oncologists, and oncological surgeons. This interdisciplinary cooperation may open/strength searching for new options for CRC patients.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The role of microbiota in the pathophysiology of uterine fibroids – a systematic review

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For a long time, the uterus had been considered a sterile organ, meaning that under physiological conditions the uterus would not be colonized by bacteria. Based on available data, it may be concluded that the gut and uterine microbiome are related, and that the role of this microbiome is greater than expected. Despite being the most common pelvic neoplasms in women of reproductive age, uterine fibroids (UFs) are still poorly understood tumors whose etiology has not been fully determined. This systematic review presents the relationship between intestinal and uterine dysbiosis and uterine fibroids. A systematic review of three medical databases was carried out: the MEDLINE/PubMed, Scopus and Cochrane. In this study, 195 titles and abstracts were reviewed, including only original articles and clinical trials of uterine microbiome criteria. Finally, 16 studies were included to the analysis. In recent years, researchers dealing with reproduction in a broad sense have focused on the microbiome in various locations to study its role in the pathogenesis and, consequently, the prevention and treatment of diseases of the genital organ. Conventional microbial detection methods are not suitable for identifying bacteria, which are difficult to culture. Next-generation sequencing (NGS) provides an easier and faster and more informative analysis of bacterial populations. It seems that gut microbiota dysbiosis has the potential to be a risk factor for uterine fibroids or affect the disease process. Some changes were shown in many types of bacteria, such as *Firmicutes, Proteobacteria, Actinobacteria* and *Verrucomicrobia* detected in fecal samples in patients with uterine fibroids. In view of the few results on the link between the microbiome and uterine fibroids, further intensive studies in humans and animal models are necessary, including the possible use of different microbiome modulations in the prevention or treatment of uterine fibroids.

KEYWORDS

microbiome, microbiota, 16S rRNA, NGS, bacteria, uterine fibroid, leiomyoma, pathophysiology

Introduction

It had been believed until recently that the uterine cavity and the upper parts of the female genital organ are a sterile environment. However, subsequent studies provided strong evidence that this view was no longer valid (Chen et al., 2017; Simon, 2018). It is not surprising, especially when considering the fact that bacterial cells in the human body account for 1-3% of its total weight and are equal in number to human cells (Moreno et al., 2016).

The use of next-generation sequencing (NGS) in the analysis of hypervariable fragments of the 16S rRNA bacterial gene showed the presence of a variety of microorganisms within the uterine cavity which were usually found in the vagina and in the colon (Pereira et al., 2016; Baker et al., 2018). According to available data, the difference between the vagina, cervix, and the uterine cavity is that the colonization of the uterine cavity is relatively low (Ichiyama et al., 2021).

Fertility disorders are the driving force behind numerous studies in gynecology and reproductive medicine. For a long time, researchers have been wondering whether there are proper microbiomes for specific parts of the reproductive organ, or whether alteration in those microbiomes might have negative consequences (Brandão and Gonçalves-Henriques, 2020; Vitale et al., 2022). The effect of female genital microflora on the ability to conceive is still unclear due to the scarcity and inconsistency of published data. Nevertheless, it seems that the flora dominated by *Lactobacillus* spp. plays a key role in determining fertility, and the presence of pathogens in the genitals may disturb issues associated with it (Vitale et al., 2022). Toson et al. have recently proposed that the physiological endometrial microbiota should be considered as a group of microorganisms that allows embryo implantation and live birth, regardless of the minimal presence of pathogenic bacteria (Toson et al., 2022).

Previous studies determined the normal uterine microbiome in women of childbearing age, where *Lactobacillus* spp. played a particular role (Mitchell et al., 2015). Other types of bacteria commonly detected in endometrial/uterine swabs included *Bifidobacterium*, *Gardnerella*, *Prevotella* and *Streptococcus* (Moreno et al., 2016). Further research showed that the abundance of *Lactobacillus* might be negatively correlated with the genus *Gardnerella*, *Bifidobacterium* and *Atopobium* and positively correlated with commensal bacteria, which are very important for the immune system, e.g., *Clostridium* and *Streptomyces* (Moreno et al., 2016).

Hormonal changes affect not only the uterine muscle and the uterine mucosa itself, but they also seem to affect the microbiome found in those areas. Current data suggest that the microbiome plays an important role in female reproductive endocrine system throughout the life by interacting with estrogens, androgens, insulin, and other hormones. An imbalance in the composition of the intestinal microflora may modify the course of numerous diseases and conditions, such as pregnancy complications, polycystic ovary syndrome, endometriosis, or reproductive organ tumors (He et al., 2021; Qi et al., 2021).

Available data indicated that exogenous progesterone might significantly alter the microflora of the endometrium (Brooks et al., 2017). Interestingly, according to Brooks et al., the use of oral hormonal contraceptives may positively influence the proper functioning of the reproductive system by increasing the quantity of *Lactobacillus* spp. and reducing bacterial taxa associated with bacterial vaginosis (BV) (Brooks et al., 2017). Progesterone may increase the α-diversity of both the vaginal and endometrial microbiome. Moreover, the quantity of bacteria that may interfere with proper functioning may increase after hormonal treatment (Toson et al., 2022). Notably, naturally occurring hormonal fluctuations during the menstrual cycle correlate with the instability of the microbial population, and a regular replacement of bacterial species occurs in the vagina during the cycle (Gajer et al., 2012). Significant changes also occur in the endometrial microbiome. The increased abundance of *Prevotella* spp. and *Sneathia* spp. may constitute the features of the proliferative and secretory phases, respectively (Gajer et al., 2012).

Despite being the most common pelvic neoplasms in women of reproductive age, uterine fibroids (UFs) are still poorly understood tumors whose etiology has not been fully determined (Ciebiera et al., 2020; Yang et al., 2022). They mainly affect women of childbearing age and are diagnosed in about 70% of with European-American women and in over 80% of African women throughout their lifetime (Giuliani et al., 2020). Uterine fibroids are heterogeneous in number, composition, and size. The risk factors for those tumors seem to include age, obesity, low vitamin D levels, and endogenous and exogenous hormonal factors (Yang et al., 2022). Heavy menstrual bleeding (HMB) is the most commonly mentioned abnormal symptom associated with uterine fibroids. Other symptoms may include anemia due to increased blood loss, pressure in the pelvic cavity and on organs adjacent to the uterus, urinary and digestive complaints, as well as reproductive disorders (Stewart et al., 2016). Uterine fibroids may require surgical treatment and are a major source of gynecological and reproductive dysfunction (Navarro et al., 2021).

Given that little is known about fibroids, it is obvious that a lot is still unknown about the interaction between the endometrial and myometrial microbiome and the immune response modulating inflammatory processes within the uterus. In view of the confirmed anti-inflammatory role of *Lactobacillus* spp. in the vaginal microenvironment, it is believed that the above may also contribute to disorders of uterine homeostasis by inducing the secretion of anti-inflammatory cytokines and the production of antimicrobial peptides. Therefore, the association between uterine and endometrial dysbiosis and immune dysregulation is highly possible (Toson et al., 2022).

The intestinal flora and host's body show a variety of reciprocal benefits. Numerous gut microbes affect the physiological functions of the host and affect the synthesis and secretion of hormones, trace elements, growth factors and immune system functions. In turn, the intestinal flora may be modified by hormonal interactions both *in vitro* and *in vivo*, thereby affecting the biological balance of the body (Rowland et al., 2018). The glucuronidase of intestinal bacteria improves estrogen reabsorption, and some intestinal bacteria can metabolize estrogen and are referred to as estrobolome (Sui et al., 2021). The high activity of estrobolome bacterial enzymes raises the

TABLE 1 Detailed search strategy in individual databases.

Pubmed	**64**	**("Leiomyoma"[Mesh] OR myom* OR leiomyom* OR fibromyom* OR (uterine AND fibroid*) OR (uterine AND fibrom*)) AND ("Microbiota"[Mesh] OR microbiot* OR microbiom* OR microfilm* OR flora OR microflora OR flora OR >microorganism*)**
Scopus	187	TITLE-ABS-KEY ((myom* OR leiomyom* OR fibromyom* OR (uterine AND fibroid*) OR (uterine AND fibrom*)) AND (microbiot* OR microbiom* OR microfilm* OR flora OR microflora OR flora OR microorganism*))
Cochrane	12	#1 "Leiomyoma"[Mesh] 2 myom* OR leiomyom* OR fibromyom* OR (uterine AND fibroid*) OR (uterine AND fibrom*) 3 Microbiota"[Mesh] 4 microbiot* OR microbiom* OR microfilm* OR flora OR microflora OR flora OR microorganism* 5 (#1 OR #2) AND (#3 OR #4)

The asterisk (*) represents any group of characters, including no character in PubMed.

level of free estrogen in the enterohepatic circulation by promoting an endogenous hormonal environment, leading to an increase in hormone levels, which can have a direct and indirect impact on the risk of developing uterine fibroids (Wang et al., 2020c). The abundance of intestinal microflora is closely correlated with the estrogenic metabolism. Numerous bacteria control estrogen content at the family and species level, with *Clostridium* and *Pneumococcus* exerting the most significant effect on estrogen metabolism (Wang et al., 2020a). Therefore, decreased estrogen levels are associated with impaired specific gut microbiota diversity (Fuhrman et al., 2014).

Pilot studies concerning the composition of the uterine cavity microbiome of women who developed fibroids showed a greater diversity of bacteria compared to control group women (Baker et al., 2018; Toson et al., 2022). It seems that fibroids may be associated with minimally altered vaginal and uterine microflora (Chen et al., 2017). For example, the endometrial microflora of patients undergoing hysterectomy for fibroids was dominated by

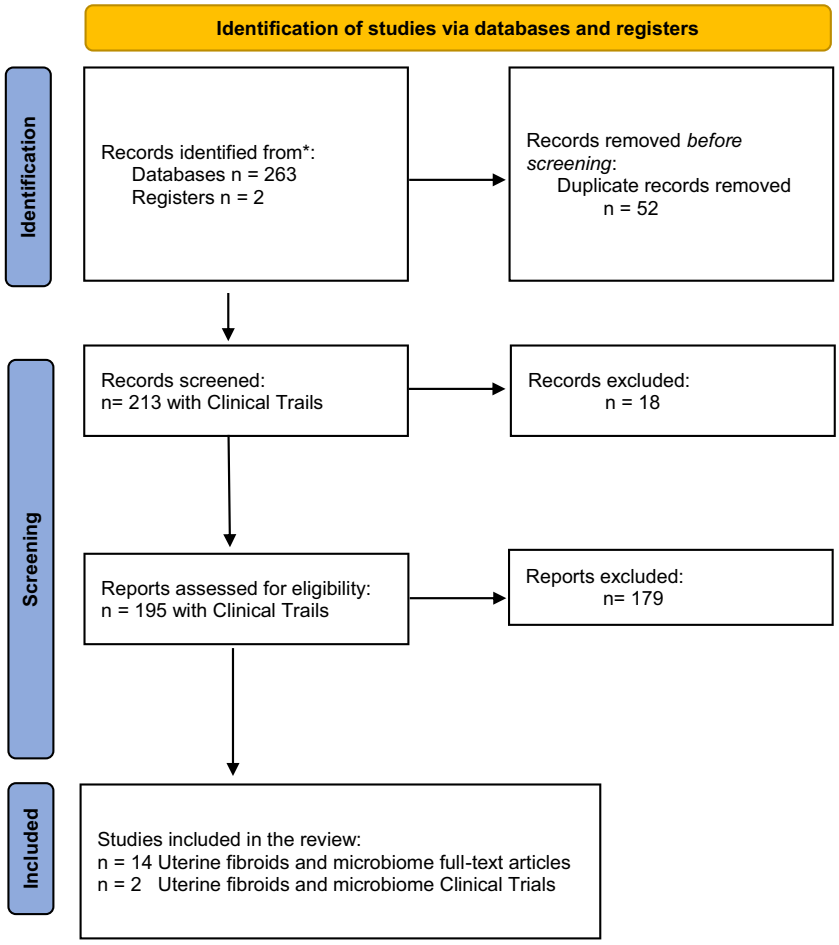

FIGURE 1
PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only.

TABLE 2 Data extracted from studies using culture-based technology to investigate the uterine, vaginal, endocervical, endometrial and gut microbiome and uterine leiomyoma (uterine myoma, uterine fibroid).

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
Teisala; 1987; Finland (Teisala, 1987)	To evaluate microbiological and histopathological findings from different levels of the endometrial cavity	10 menstruating women for hysterectomy indication 8 – uterine fibroids 2 – chronic pelvic pain		Range 36-49	Microbiological techniques	Tissue specimens from fundal, middle, and cervical area of the endometrium after removing the uterus.	Negative cultures of aerobic, anaerobic, and facultative bacteria			**Negative culture results of *C. trachomatis*, *N. gonorrhoeae*, *M. hominis*, *U. urealyticum*. Endometrial cavity of a nonpregnant uterus is sterile.**
Bertazzoni Minelli et al.; 1990; Italy (Minelli et al., 1990)	To assess the composition of the fecal flora in patients with breast cancer and uterine leiomyoma in comparison with a group of healthy women	18 patients with breast cancer 18 with uterine leiomyoma	30 healthy women	Range 25-52	Microbiological techniques	Stool sample, the first admission day		Anaerobic lactobacilli, streptococci (*Enterococcus faecium)* in groups of women with breast cancer and uterine leiomyoma	*Peptoniphilus asaccharolyticus* and *P. saccharolyticus* in groups of women with breast cancer and uterine leiomyoma	**Fecal bacteria reduce estrone to estradiol. The presence or absence of some bacterial species is important in modulating estrogen metabolism. The microflora may influence the metabolism of sex steroid hormones.**
Mikamo et al.; 1993; Japan (Mikamo et al., 1993a)	To identify the intrauterine bacterial flora in diabetic patients with various postoperative complications	Diabetic patients with abdominal hysterectomy because of uterine myoma 10 diabetic patients	Non-diabetic control patients 20 controls	Range 35-45	Quantitative bacteriological assay Anaerobic bacteria – RapID ANA II identification system (Innovative Diagnostic System, Inc., Atlanta, GA) combined with gas-liquid chromatography (GLC)	Swab from the endometrial cavity	Bacteria detected in the uterine endometrial cavity; 10 of ten diabetic patients with uterine myoma and 3 non-diabetic controls	*Enterobacteriaceae* (*Escherichia coli, Proteus* spp., *Enterobacter cloacae*, and *Klebsiella pneumoniae*		**Antimicrobial *Enterobacteriaceae* prevention of postoperative infections in gynecologic procedures in diabetic patients.**
Mikamo et al.; 1993; Japan (Mikamo et al., 1993b)	To identify the intrauterine bacterial flora in patients with uterine endometrial cancer	Patients with uterine endometrial cancer 20 – uterine endometrial cancer	Patients without complications other than uterine myoma 20 controls	Range 44-69	Quantitative bacteriological assay Anaerobic bacteria – RapID ANA II identification system (Innovative Diagnostic System, Inc., Atlanta, GA)	Endometrial cavity with a polyester fiber swab	*Enterobacteriaceae*, *Streptococcus agalactiae* and anaerobic bacteria detected in all patients with uterine endometrial cancer Patients without	*Enterobacteriaceae, Streptococcus agalactiae* and anaerobic bacteria		Products ofaerobicand anaerobic bacteria considered to contribute to endometrial carcinogenesis. Uterine endometrial

(Continued)

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TABLE 2 Continued

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
					combined with gas-liquid chromatography (GLC)		complications other than uterine myoma – no detection of bacteria			cancer provides favorable conditions for bacterial growth.
Møller et al.;1995; Denmark (Møller et al., 1995)	To evaluate whether the uterine cavity is non-sterile, in contradiction to previous suggestions	99 women admitted for hysterectomy 34 patients with uterine fibromyoma 29 patients with persistent irregular vaginal bleeding 10 patients with malignancy of the cervix (carcinoma *in situ* cervicis uteri)		Range 29-84	Microbiological techniques Histological examination	Cervical specimens Endometrial specimens	25% of all the patients harbored one or more microorganisms in the uterus	*Gardnerella vaginalis*, *Enterobacter* and *Streptococcus agalactiae*		Uterine cavity colonized with potentially pathogenic organisms. Inflammation of the uterine cavity should be evaluated *via* hysteroscopic examination before hysterectomy.

10.3389/fcimb.2023.1177366

TABLE 3 Data extracted from studies using sequencing-based technology to investigate the uterine, vaginal, endocervical, endometrial and gut microbiome and uterine leiomyoma (uterine fibroid).

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
Khan et al.; 2016; Japan (Khan et al., 2016)	To investigate microbial colonization in the intrauterine environment and cystic fluid of women with and without endometriosis	32 women with endometriosis	32 patients with uterine myoma	Range 21-52	16S rDNA sequence Illumina Miseq	Endometrial swabs Cystic fluid	Multiple bacteria detected in the endometrial swabs and cystic fluid collected from women with and without endometriosis	*Lactobacillaceae* in patients with uterine myoma	*Streptococcaceae, Staphylococcaceae, Enterobacteriaceae* in patients with uterine myoma	Sub-clinical infection in the intrauterine environment and in the cystic fluid of ovarian endometrioma.
Walther-Antoínio et al.; 2016; USA (Walther-António et al., 2016)	To investigate the uterine microbiome and its putative role in endometrial cancer	17 patients with endometrial cancer 4 patients with endometrial hyperplasia 10 patients with benign uterine conditions		18 years of age or older	16S rDNA V3-V5 region Illumina MiSeq	Vaginal swab, cervical swab, biopsies: fallopian, ovarian, peritoneal Urine samples	Structural microbiome shift in the cancer and hyperplasia cases, distinguishable from the benign cases	*Firmicutes (Anaerostipes, ph2, Dialister, Peptoniphilus, Ruminococcus*, and *Anaerotruncus), Spirochaetes (Treponema), Actinobacteria (Atopobium), Bacteroidetes (Bacteroides* and *Porphyromonas)*, and *Proteobacteria (Arthrospira)*		Suspected dominnation of *Stenotrophomonas* in UFs
Chen et al.; 2017; China (Chen et al., 2017)	To investigate potential bacterial markers for adenomyosis and endometriosis	110 women of reproductive age		Range 22-48	V5 to V4 Region Ion Torrent Personal Genome Machine Real-time qPCR Conventional bacterial culturing	Nylon flocked swabs from 6 locations (the vagina, cervical mucus, cervical canal, endometrium, fallopian tubes, fluid from the pouch of Douglas)	Over- represented microbial taxa, correlated with potential functions of the menstrual cycle in patients with adenomyosis or infertility due to endometriosis	Vaginal and cervical samples: *L. iners* in patients with hysteromyoma	Vaginal and cervical samples: *Lactobacillus* sp. in patients with hysteromyoma	Vaginal or cervical microbiota useful in the detection of common diseases in the upper reproductive tract.
Wang et al.; 2020; China (Wang et al., 2020b)	To investigate the effect of transabdominal hysterectomy on the diversity of the intestinal flora in patients with uterine fibroids	15 preoperative patients with uterine fibroids	15 postoperative patients with uterine fibroids	Range 40–45	High-throughput sequencing of the 16S rRNA gene Illumina HiSeq	Stool	Decreased abundance and diversity of the intestinal flora after abdominal hysterectomy	*Proteobacteria* after abdominal hysterectomy		After abdominal hysterectomy: reduced diversity and abundance of the intestinal flora; lower level of estrogen in the body

(Continued)

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TABLE 3 Continued

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
										after abdominal hysterectomy.
Riganelli et al.; 2020; Italy (Riganelli et al., 2020)	To explore structural variations of vaginal and endometrial microbiota in embryo implantation failure	34 patients undergoing personalized hormonal stimulation 1 – with G1 uterine fibroid		Range 22–43	16s rRNA V4-V5 Illumina MiSeq	Vaginal fluid, and endometrial biopsy	Significant difference between vaginal and endometrial microbiota	*Lactobacillus* in pregnant women		Uterine microbiota structurally differed from the vaginal microbiota. Reduction of barriers resulting from translocation from the vagina to the endometrium. Predictive "microbiota dysbiosis" before assisted reproductive technology (ART) treatment.
Liu et al.; 2021; China (Liu et al., 2021)	To evaluate how gut microbiota affects host immune response and induces an imbalance in cytokine levels	41 miscarriage patients 4 – with uterine fibroids	19 controls	Mean age 31.3 ± 5.0	The V3-V4 variable regions of the 16S rRNA gene llumina MiSeq PE300 Fecal metabolic profiling using liquid chromatography/ mass spectrometry (LC/MS) Cytokine quantification by flow cytometry	Stool	Microbial diversity reduced in the miscarriage patient group Microbe-associated metabolites (imidazolepropionic acid) Positively associated with changes in the levels of Th1/Th17 cytokines in the miscarriage group		*Prevotella_1, Prevotellaceae_UCG_003* and *Selenomonas* in the miscarriage group	The role of gut microbiota, stool metabolites and Th1/Th17-mediated immune response in miscarriage patients.
Kim et al.; South Kora;	To evaluate the clinical relationship between the	40 patients of reproductive age with RV	100 healthy women	Range 20- 55	16S ribosomal RNA gene	Flocked swab	Bacterial abundance significantly lower in patients with RV Species evenness and		*Lactobacillus* spp. patients with RV	Changes in vaginal microbial community strongly associated with RV.

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TABLE 3 Continued

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
2022 (Kim et al., 2022)	vaginal microbiome and the pathophysiology of recurrent vaginitis (RV)						diversity significantly higher in patients with RV Beta diversity significantly different between patients with RV and healthy individuals Higher species richness and diversity in patients with underlying uterine diseases (uterine leiomyoma, adenomyosis, and endometrial polyps)			Vaginal microbiome is valuable for detecting and treating gynecological diseases in the future.
Hua et al.; 2022; China (Hua et al., 2022)	To analyze alterations of the cervical canal microbiota in intrauterine adhesion (IUA) patients	23 patients with mild-to-severe IUA 8 women with infertility 3 women with submucous myomas 8 women with endometrial polyps		18 years of age or older	16S rDNA high-throughput sequencing	Cervical mucus	Lower diversity of bacteria in the group with moderate or severe IUA	*Firmicutes* in IUA patients *Firmicutes*/*Acinetobacteria* or genus *Lactobacillus*/*Gardnerella* in the severity of IUA		The severity of IUA associated with a higher bacterial load but lower diversity.
Mao et al.; 2022; China (Mao et al., 2022)	To investigate possible differences in gut microbiome compositions between patients with uterine fibroids (UFs) and healthy control subjects	42 patients with uterine fibroids	43 control subjects	Range: patients 24–52 controls 23–54	16S rRNA quantitative arrays	Stool	Significantly lower diversity in patients with UFs The microbial composition of UF patients deviated from that in healthy controls.	*Pseudomonas stutzeri*, *Prevotella amnii* in patients with UFs	*Bifidobacteria scardovii*, *Ligilactobacillus saerimneri*, *Lactococcus raffinolactis* in patients with UFs	Gut microbiota dysbiosis has the potential as a risk factor. UFs associated with alterations of gut microbiome diversity. Host–gut microbiota – role in the

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TABLE 3 Continued

Author, date, country	Study aim	Sample		Age	Detection method	Material	Study findings	Abundance of microbiota		Conclusions of the study
		Studied group, Subjects, *n*	Control group, Subjects, *n*					Increased	Reduced	
										development and prevention in UF pathogenesis.

Acinetobacter, *Cloacibacterium*, *Comamonadaceae* and *Pseudomonas*, while the species *Lactobacillus* were rare in the uterus (Winters et al., 2019). The results suggest that the systemic distribution of intestinal bacteria extends to the patients' fibroids following dysbiosis or impaired intestinal barrier (Yang et al., 2022). However, the above data were obtained from few studies, and the data need to be systematized.

Aim

The purpose of this systematic review is to provide a comprehensive summary of the fibroids and uterine microbiome literature published to date.

Material and methods

A systematic review of three medical databases was carried out: the MEDLINE/PubMed, Scopus and Cochrane. The last search was conducted on January 26, 2023. The details of the search strategy are presented in Table 1. As a result, a total of 263 articles were obtained. Using EndNote X9 automatic duplicate search tool, 42 duplicates were identified, and then another 10 duplicates were found *via* manual search. Finally, 195 titles and abstracts were reviewed, including only original articles and clinical trials of 281 uterine microbiome criteria (Figure 1).

Results

Conventional microbial detection methods are not suitable for identifying bacteria, which are difficult to culture. In turn, advanced molecular biology techniques can resolve this problem, but even such methods have limitations. The restrictions are primarily related to the possibility of detecting DNA derived from lifeless bacteria or DNA fragments. In addition, sensitive molecular biology methods, together with the low number of bacteria present in the tissue, carry a risk of contamination, which may very easily falsify the result. Experimental difficulties in the interpretation, determination of the endometrial microbiota composition are the result of conducting studies in various populations, using diverse sampling methods and a large number of research approaches as well as abundance of human DNA also create technical challenges (Abah et al., 2021). Conversely, despite numerous limitations, NGS methods seem to provide the widest variety of insights. The application of advanced sequencing and genome analysis methods resulted in rapid publications enhancing the uterine microbiota field. Today, uterine microbiota is an important research direction in human reproductive studies.

As regards uterine fibroids microbiome research, both conventional culture and NGS studies remain rather rare (Tables 2, 3). Due to the limited ability to cultivate larger quantities of bacterial species in laboratory procedures, metagenomics established a popular and useful tool applied in the analysis of bacterial composition residing in the reproductive system. NGS provides an easier and

faster analysis of bacterial populations, without cloning DNA sequences into a vector. Today, metagenomics represents a powerful tool used in microbiology which, *via* combining various molecular biology techniques, enables the study of the biodiversity of microbial populations and provides a better understanding of the role of individual bacteria in a particular environment, as well as the discovery of new genes (Table 3; Figure 2).

Search Results on ClinicalTrials.gov website revealed 426 studies for: Myoma/Fibroid; Uterus terms (Table 4). The issue of the uterine microbiome was tackled in 7 of them, and only 2 without the exclusion criteria of fibroid, leiomyoma or myoma (Table 5).

Discussion

The presence of the microbiota in the body is known to be important for human health. In recent years, advances in molecular biology techniques have not only allowed the confirmation of the occurrence of microorganisms in the human digestive, respiratory and urinary systems, but also prompted the detection of the presence of microorganisms in organs previously considered sterile, such as the uterine cavity (Dekaboruah et al., 2020).

For a long time, the uterus had been considered a sterile organ, meaning that under physiological conditions the uterus would not be colonized by bacteria. In 1950s, research based on bacterial culture methods revealed the first reports suggesting that the uterus was an organ with its own microbiota. Further development of diagnostic techniques and molecular biology methods in early 21st century allowed the reanalysis of the composition of the uterine microbiota, including bacteria which are not easily cultured under laboratory conditions (Franasiak et al., 2021).

Until the late 1980s, the results of most studies related to the microbiome of the myomal uterine cavity had indicated the sterility of the uterus (Teisala, 1987; Mikamo et al., 1993b) (Table 2). Several years later, a quantitative bacteriologic assay of swabs from the endometrial cavity after myoma uteri hysterectomy demonstrated an increased amount of *Enterobacteriaceae* family, including *Escherichia coli, Proteus* spp., *Enterobacter cloacae, and Klebsiella pneumoniae* (Mikamo et al., 1993a). In contrast, the authors expressed a different view of the microbiome of the uterine cavity affected by cancer. The results showed the distribution of *Enterobacteriaceae, Streptococcus agalactiae* and anaerobic bacteria detected in all patients with uterine endometrial cancer (Mikamo et al., 1993b). In conclusion, the products of aerobic and anaerobic bacteria contributing to endometrial carcinogenesis as well as uterine endometrial cancer provided preferential environment for bacterial growth. A turning point occurred when a study of nearly 100 women was conducted and revealed that myomal uterine cavity was colonized with potentially pathogenic organisms (Møller et al., 1995). In samples from the uterine cavity, a quarter of the women cultured one or more bacteria using microbiological techniques. *Gardnerella vaginalis, Enterobacter* and *Streptococcus agalactiae* pathogenic bacteria were identified. The deviations of obtained results were due to non-cultivation of bacteria under laboratory conditions or the presence of only lifeless bacteria in the material.

The modulating effect of the gut microbiome on the uterine cavity was highlighted in the 1990s. Using culture techniques, the composition of the stool microbiome was examined in patients with breast cancer and uterine leiomyoma (Minelli et al., 1990). The study showed fecal bacteria reduction of estrone to estradiol (Minelli et al., 1990). The findings confirmed the decreased amount of *Peptoniphilus asaccharolyticus* and *P. saccharolyticus* and an increased number of anaerobic lactobacilli and *Enterococcus faecium* in the gut microbiome of breast cancer and uterine leiomyoma women. The presence or absence of some bacterial species is important in modulating estrogen metabolism. Thus, the microbiome influenced the metabolism of sex steroid hormones (Hussain et al., 2021).

It has been known for a long time that the genus *Lactobacillus* represents the dominant component of the vaginal microbiota and plays a protective role for the vaginal microenvironment. The presence of Lactobacilli was also confirmed in the upper female reproductive tract (FRT) (Verstraelen et al., 2016; Chen et al., 2017; Benner et al., 2018). Chen et al. demonstrated the predominance of *Lactobacillus* bacteria (> 97.56%) in the cervix. In the uterus, *Lactobacillus* (30.6%), *Pseudomonas* (9.09%), *Acinetobacter* (9.07%), *Vagococcus* (7.29%), and *Sphingobium* (5.0%) were identified, while the fallopian tubes revealed the presence of *Acinetobacter* (18.27%), *Comamonas* (11.49%), *Pseudomonas* (9.9%), *Pseudomonadaceae* (9.1%), and *Dysgonomonas* (5.11%) (Chen et al., 2017). Further studies published in 2018 and 2019 confirmed the occurrence of bacteria from the *Lactobacillaceae* family not only in the vagina, but also in other segments of the female reproductive tract (Moreno et al., 2016; Koedooder et al., 2019). The protective role of *Lactobacillus* in the vagina was recognized. However, the precise mechanism of the effect and a possible impact on fertility have not been fully elucidated and require further research.

Today, we know that a gigantic number of human microbes influences the physiological functions of the human body at all times and has a profound effect on the synthesis and secretion of hormones and various growth factors. As a result, changes in the composition of the human gut flora may affect the body in various aspects, including the immune or hormonal system (Thursby and Juge, 2017). It is well known how important hormones (estrogens and progesterone) are in the pathophysiology of fibroids (Bulun, 2013). The associated growth factors are equally important, with transforming beta growth factor probably being the crucial one (Ciebiera et al., 2017).

Leaving aside the above words of introduction concerning new generation research and general disorders related to female reproductive tract colonization, our analyses of publications mostly indicate that almost no data are available on the microbiota and uterine fibroids. It seems almost unlikely assuming that uterine fibroids are the most common problem with which women present to gynecologists (Stewart et al., 2016). This problem has a large clinical impact because most papers that may be reviewed are not analyzed in terms of fibroids. The analyses concentrated on the fact that fibroids were not an exclusion criterion for other studies. Therefore, our conclusions will certainly not be strong.

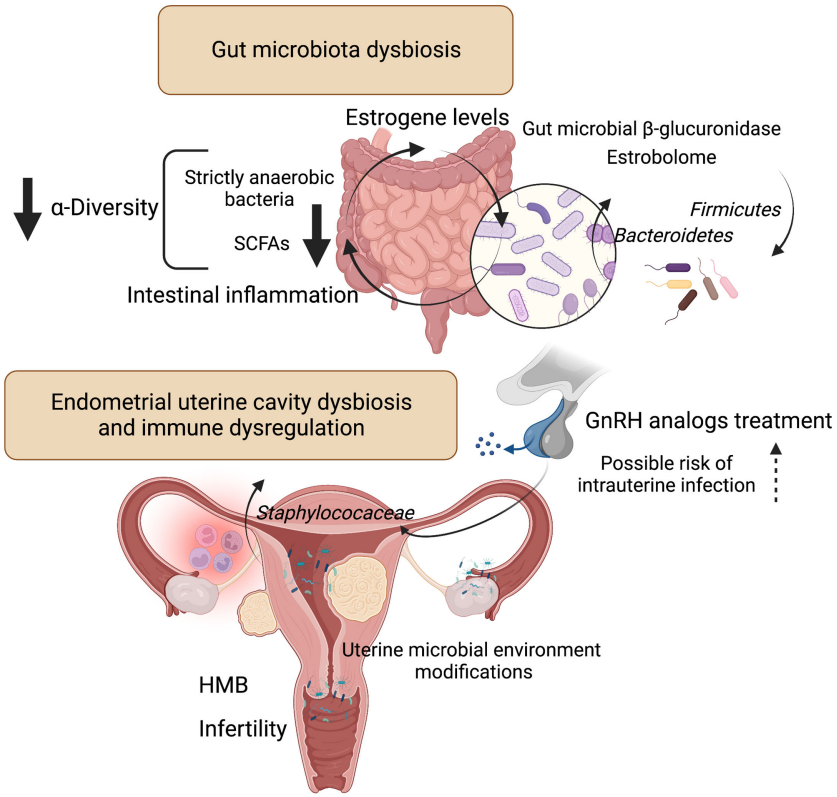

FIGURE 2
Influence of microbiota on estrogen metabolism and endometrial uterine cavity dysbiosis in patients with uterine fibroids and possible role of GnRH analogs treatment. Liver-conjugated estrogens excreted in the bile into the gastrointestinal tract could be deconjugated by intestinal bacteria that produce ß-glucosidase enzymes involved in estrogen deconjugation. This leads to their absorption into the bloodstream and allows them to bind to estrogen receptors. However, the relationship is a bidirectional - the gut microflora can also be affected by estrogen. The experimental evidence for the key role of gut bacteria in estrogen metabolism was observed decades ago. The estrobolome is defined as the gene *repertoire* of intestinal microflora with products capable to metabolize estrogens. By influencing and modulating the intestinal and hepatic circulation of estrogens, the estrobolome affects the excretion and circulation of estrogens. Numerous evidence indicates that dysbiosis of intestinal bacteria increases the chance of intestinal inflammation. This is particularly related to a decrease in strictly anaerobic bacteria and a simultaneous increase in facultative anaerobes such as Escherichia coli and Klebsiella. And SCFAs, produced mainly by strict anaerobes, have anti-inflammatory effects. According to one study (Khan et al., 2016), intrauterine higher colonization by *Staphylococcaceae* was revealed in a group of control patients who used GnRH analogs. Patients with fibroids after GnRH treatment had the highest staphylococcal levels compared to other groups. Figure 2 created with BioRender.com. Heavy menstrual bleeding (HMB); gonadotropin-releasing hormone (GnRH); short-chain fatty acids (SCFAs).

We identified 4 studies that have attracted our greatest attention in the analysis and that seem to be more significant in terms of the pathophysiology of uterine fibroids and possible clinical situations associated with them. We would like to focus on those publications below.

The first study on the uterine cavity microbiota was published in 2016 by Khan et al. The Japanese authors conducted a molecular analysis of intrauterine colonization with microorganisms of women with endometriosis (Khan et al., 2016). The study included a total of 64 women half of whom had endometriosis and the other half was not affected by the condition. In each group, 16 patients additionally received GnRH analogs for 4-6 months. The results were highly interesting. A high percentage of bacteria was detected in the swabs collected from the endometrium and from the fluid obtained from cysts. Metagenomic tests showed that the proportion of *Lactobacillaceae* was significantly reduced, while the proportion of *Streptococcaceae, Staphylococaceae, and Enterobacteriaceae* was significantly increased in the GnRH analog-treated groups of women with endometriosis compared to women who did not receive those drugs. Ordinary tests did not allow for the assessment of cystic fluid for microbial colonization. The results of the 16S metagenomic test were different. A much higher percentage of *Streptococcaceae* ($p<0.01$) and *Staphylococaceae* ($p<0.05$) was detected in the fluid collected from cysts of women with ovarian endometriosis compared to fluid collected from cysts without an endometriotic etiology. It is difficult to determine what effect those data have on uterine fibroids. However, a significantly higher colonization by *Staphylococaceae* ($p<0.05$) and an insignificant statistical difference for colonization by *Enterobacteriaceae* was revealed in a group of control patients who used GnRH analogs (due to uterine fibroids) compared to samples obtained from control group women who were not pharmacologically treated. Interestingly, according to the data obtained from the study, the group of patients with fibroids after GnRH treatment had the lowest overall percentage of streptococcal colonization and the highest Staphylococcal levels compared to other groups. It is not known what these data mean from the viewpoint of uterine fibroids and what the clinical

TABLE 4 Myoma/Fibroid; Uterus terms of 426 studies (*ClinicalTrials.gov* website, February 2023).

Terms	Search Results (studies)*	Entire Database (studies)**
Synonyms		
Uterine Fibroid	426	426
Leiomyoma	407	407
myofibroma	320	320
Fibroid	313	313
Uterine myoma	42	42
uterus myoma	3	3
Fibromyoma	3	3
Uterine Fibromas	2	2
Fibroid	426	426
Leiomyoma	407	407
myofibroma	320	320
Uterine myoma	42	42
uterus myoma	3	3
Fibromyoma	3	3
Uterine Fibromas	2	2

–No studies found; *Number of studies in the search results containing the term or a synonym; **Number of studies in the entire database containing the term or a synonym.

application may be. However, the role of drugs blocking the pituitary was shown not only to consist in regulating the growth of fibroids, but also to change the entire uterine environment, which may affect other important features such as fertility or a possible relationship with pregnancy. It certainly requires a lot of well-designed research, in which not endometriosis, but uterine fibroids will constitute the focus (Linder et al., 2021; Maxim et al., 2022). The authors concluded that the results indicated the presence of a subclinical infection in the intrauterine environment and, importantly, in the fluid collected from the lesions in the ovary. Furthermore, the authors emphasized the side effect of treatment with GnRH analogs associated with promoting an intrauterine and/or ovarian infection (Khan et al., 2016)

The second of the studies analyzed in the context of uterine fibroids concerned the effect of transabdominal hysterectomy on the intestinal flora in patients with uterine fibroids using high-throughput sequencing (Wang et al., 2020b). According to the authors, as could be predicted, estrogen levels decreased after transabdominal hysterectomy. However, post-hysterectomy high-throughput sequencing showed that the quantity and diversity of intestinal flora decreased.

It is known that total hysterectomy is one of more commonly proposed procedures in women with uterine fibroids who do not have fertility requirements. A group of doctors obviously struggle with such an approach and try to convince their colleagues to switch to more conservative surgical methods, use non-operative methods such as thermoablation (Lozinski et al., 2021) or switch to pharmacological treatment (Niaz et al., 2022). Complete hysterectomy affects the level of sex hormones. According to available data, serum AMH levels might be decreased after hysterectomy, with a greater reduction when total hysterectomy is performed in comparison with supracervical hysterectomy (Yuan et al., 2015). It is due to the fact that the anatomical structure of the uterus and ovary is closely correlated. A significant portion of the blood supplied to the ovary comes from the ascending branch of the uterine artery. Simultaneously, regardless of anatomical changes, thermal damage is common during procedures, which can impair the blood supply to the ovaries after complete hysterectomy, thus leading to a reduction in the secretion of sex hormones (Wang et al., 2020b).

So what is the significance of the results of the study by Wang et al.? Previous research demonstrated that a decrease in estrogen levels leads to a decrease in the diversity of the intestinal flora and a reduction in the abundance of thick-walled bacteria, such as *Clostridium* (Breban, 2016). Decreased estrogen levels in the study described leads to other situations, because of an increase in the quantity of *Firmicutes*, a decrease in the diversity of *Bacteroidetes* and an increase in the species diversity of *Proteobacteria*. The authors concluded that transabdominal hysterectomy could reduce the level of estrogen in the body and reduce the diversity and abundance of intestinal bacterial flora before and after surgery with the growth of *Proteobacteria* being the main difference in that case (Wang et al., 2020b). Obviously, it probably cannot be said that the mechanism underlying this regulation has already been determined. Only a deeper analysis can help understand whether the intestinal microflora may affect the risk of uterine fibroids by affecting endogenous estrogens (Yang et al., 2022).

Another analyzed study tackled the issue of interactions between the intestinal microflora and cytokine disorders in women with unexplained causes of miscarriages. Five patients

with fibroids were included in this study, according to the data provided. Regrettably, this group did not undergo any additional individual analyses (Liu et al., 2021). Multiple authors suggested that dysregulation within the cytokine and growth factor network is involved in the pathogenesis of unexplained pregnancy loss (Calleja-Agius and Brincat, 2008). As already known from the previous paragraphs, the microbiota affects the host's immune response. However, the question of how this dysbiosis impairs he cellular immune function in the event of miscarriage remains ambiguous. It is also known that factors related to the Th1 response, such as tumor necrosis factor alpha (TNF-α), increase in the serum of patients affected by miscarriage (Mallmann et al., 1991). TNF-α was also reported to inhibit trophoblast invasion and elevated TNF-α levels were identified in women who experienced miscarriage (Azizieh and Raghupathy, 2015). According to a large amount of data, TNF-α also seems to be an extremely important cytokine in the biology of uterine fibroids, associated symptoms and conditions (Ciebiera et al., 2018b). It was shown that its concentration was increased in women with symptomatic uterine fibroids (Ciebiera et al., 2018a; Kali and Cagiran, 2022). Currently available data suggest the occurrence of an inflammation-like condition in women with uterine fibroids, with TNF-α being a strong inducer of the state (Ciebiera et al., 2018b).

Liu et al. (Liu et al., 2021) demonstrated that the microbiological diversity and a relative abundance of *Prevotella_1*, *Prevotellaceae_UCG_003* and *Selenomonas_1* were significantly reduced in cases of miscarriage. Further analyses indicated that some microbial-related metabolites were positively associated with changes in Th1/Th17 cytokine levels in the miscarriage group. The authors concluded that a network between the intestinal microbiota, fecal metabolites and Th1/Th17 might mediate miscarriage recurrences. Moreover, the intestinal microflora in patients with a history of miscarriage showed a much higher concentration compared to controls. Given that only a few patients with uterine fibroids participated in this study, it is not possible to draw unambiguous conclusions about this particular condition. However, a number of factors and pathways, e.g., hormonal-immune ones, fit quite well into the same ones that affect uterine fibroids. Seemingly, it may be a very promising topic of research, e.g., to study why some fibroids may increase the risk of miscarriages or improper implantation.

Finally, the last study was designed for a group of patients with uterine fibroids. The authors used 16 rRNA high-throughput microarrays to compare gut microbiological differences between healthy women and women with uterine fibroids. Moreover, the authors aimed to determine the correlations and interactions between the intestines, microbiome and uterine fibroids (Mao et al., 2022). The study included 42 patients with uterine fibroids and 43 patients without a history of fibroids. The authors demonstrated very interesting correlations. Firstly, microbiome diversity in patients with uterine fibroids was significantly lower than in healthy controls. The microbiological composition of samples collected from patients with uterine fibroids differed from that of healthy women. Some changes were shown in many types of bacteria, such as *Firmicutes, Proteobacteria*, *Actinobacteria* and *Verrucomicrobia* detected in fecal samples in patients with uterine fibroids.

Further analysis of bacterial abundance showed that some species were present to a smaller extent (e.g., *Bifidobacteria scardovii, Ligilactobacillus saerimneri* and *Lactococcus raffinolactis*) and some were more abundant (e.g., *Pseudomonas stutzeri* and *Prevotella amnii*). Moreover, microbial interactions and specific networks in uterine fibroids exhibited lower connectivity and complexity as well as higher clustering properties compared to the second group. The results indicated it was highly possible that intestinal dysbiosis might be a significant risk factor for the development or growth of uterine fibroids. The data confirmed that uterine fibroids might be strongly associated with changes in the diversity of the gut microbiome. This provides a new direction for further analyses of the intestinal microflora and its links to the development and growth of uterine fibroids. The results will certainly provide an incentive to develop new research and further analyses. Furthermore, as the authors noted, they may also constitute pioneering reports in terms of new therapies for uterine fibroids, such as the development or use of probiotics to protect against uterine fibroids or support therapy (Mao et al., 2022).

Apart from the scarcity of publications on the microbiota and uterine fibroids, it is also quite surprising that there are practically no registered studies that could expand such knowledge. In preparing this systematic review, the authors also reviewed the clinicaltrials.gov database and, to their surprise, it turned out that only 7 out of 426 studies related to the microbiome were related to the uterine microbiome (only 2 were directly linked, and only 2 indirectly addressed the issue of uterine fibroids). As regards those two clinical studies, the first one concerns chronic endometritis and its effects on fertility and pregnancy. This study is particularly important because chronic endometritis is commonly diagnosed in the context of fertility problems, and the patient is often treated blindly with broad-spectrum antibiotics in this case. Uterine fibroids are not simply an exclusion criterion here and such patients can be recruited for the study (Table 5). The topic of the second study is different, i.e., it addresses implantation failures. The study focuses on the characteristics of the uterine microbiome in women with repeated implantation failures and in women with normal fertility. The absence of pregnancy after the transfer of a total of 5 high-quality embryos is the inclusion criterion in the study. As regards uterine fibroids, they are not an exclusion criterion in the study.

Conclusions

Metagenomics and traditional methods share some limitations, while also complement each other. Thus, this creates new diagnostic potential for research. The microbiota in the uterine cavity has been much less characterized. The upper female genital tract, consisting of the uterus, fallopian tubes and ovaries, was once considered a sterile environment. Although this approach has fundamentally changed over the years, there is still no current consensus on the basic female genital tract microbiota occurring in healthy women, nor its exact role in the formation of uterine fibroids. However, an increasing amount of strong evidence is available to support this changing concept.

TABLE 5 Clinical trials of the uterine microbiome, without the exclusion criteria of fibroid, leiomyoma, myoma (*ClinicalTrials.gov* website, February 2023).

Identifier	Sponsor	Condition	Intervention/ treatment	Study Type	Estimated enrollment	Aim	Material	Criteria		Outcome Measures
								Inclusion	Exclusion	
NCT05337072	Universitair Ziekenhuis Brussel	Endometritis; Chronic subfertility	Diagnostic Test: Microbiome study	Observational	1000 participants	To gain insight into the microorganisms that are present in the female reproductive tract based on various techniques	Endometrial biopsy and a vaginal swab	•Women planned for IVF/ICSI treatment •Women who undergo a diagnostic hysteroscopy in preparation for their treatment	•interventional hysteroscopy planned •previous history of chronic endometritis •use of antibiotics in the last 3 months	•Microbiome evaluated through metagenomics sequencing and high-throughput culturomics
NCT03405883	University Hospital, Ghent	Repeated implantation failure; Normal fertile	Vaginal swab and endometrial biopsy	Observational	40 participants	To characterize the uterine microbiome in women with repeated implantation failure as well as in normal fertile women	Obtained vaginal swab and endometrial biopsy in the midluteal phase of the cycle	•18-40 years old (max 39 years and 364 days at the day of signing the informed consent) •Negative serological tests for HIV, HBV, HCV, RPR for syphilis	•Hormonal contraception •Intra-uterine device use •Antibiotic treatment in the current cycle	Midluteal vaginal and endometrial microbiome profile Microbiome analysis using 16S ribosomal RNA sequencing

10.3389/fcimb.2023.1177366

This systematic review presents the relationship between intestinal and uterine dysbiosis and uterine fibroids. In recent years, researchers dealing with reproduction in a broad sense have focused on the microbiome in various locations to study its role in the pathogenesis and, consequently, the prevention and treatment of diseases of the genital organ. Recent studies of microbiomes of different locations have identified patterns of the bacterial composition of these sites depending on such factors as the population. The next step involves increasingly advanced research on health changes related to specific conditions, especially with regard to the impact of steroid hormone axes, and the interaction of estrogens with the gut microbiome.

Based on available data, it may be concluded that the gut and uterine microbiome are related and that the role of this microbiome is greater than expected. Numerous pathogenetic pathways are shared by the microbiome and fibroids, which allows a conclusion that the presence of fibroids or some symptoms may be dependent on changes in the microbiome. In view of the few results on the link between the microbiome and uterine fibroids, further intensive studies in humans and animal models are necessary, including the possible use of different microbiome modulations in the prevention or treatment of uterine fibroids.

Author contributions

Conceptualization, MC, NZ-L; Methodology, MZ; Search for references, MZ, NZ-L, LK; Writing—original draft preparation, NZ-L, MC, LK; Writing—review and editing, NZ-L, LK, MZ, EZ, KZ, CW, MD; Visualization, NZ-L, MZ; Supervision, MC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Alterations of gut microbiome following gastrointestinal surgical procedures and their potential complications

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Intestinal microorganisms play a crucial role in shaping the host immunity and maintaining homeostasis. Nevertheless, alterations in gut bacterial composition may occur and these alterations have been linked with the pathogenesis of several diseases. In surgical practice, studies revealed that the microbiome of patients undergoing surgery changes and several post-operative complications seem to be associated with the gut microbiota composition. In this review, we aim to provide an overview of gut microbiota (GM) in surgical disease. We refer to several studies which describe alterations of GM in patients undergoing different types of surgery, we focus on the impacts of peri-operative interventions on GM and the role of GM in development of post-operative complications, such as anastomotic leak. The review aims to enhance comprehension regarding the correlation between GM and surgical procedures based in the current knowledge. However, preoperative and postoperative synthesis of GM needs to be further examined in future studies, so that GM-targeted measures could be assessed and the different surgery complications could be reduced.

KEYWORDS

gut microbiota, microbiome, surgery complications, surgical disease, alterations in microbiota, peri-operative interventions

Abbreviations: GI, gastrointestinal; GM, Gut Microbiota; IBD, Inflammatory bowel disease CD Crohn's disease; NGS, Next-generation sequencing; UC, Ulcerative colitis; NOD2, Nucleotide oligomerization domain 2; CRC, Colorectal cancer; T2D, Type 2 diabetes; ICR, ileocolonic resection; LPS, Lipopolysaccharides; MSCNS, Methicillin-Susceptible Coagulase-Negative Staphylococci; RYGB, Roux-en-Y gastric bypass; SG, Sleeve gastrectomy; MBP, Mechanical bowel preparation; PPIs, Proton pump inhibitors; PN, Parenteral nutrition; SSIs, Surgical site infections; AL, Anastomotic leakage; POI, Postoperative ileus.

1 Introduction

Microbiome is a complex and dynamic ecosystem composed of a dense microbial population, in which hundreds of microbial species coexist (Wang et al., 2019). The average human body harbors 100 trillion of microorganisms, both inside and out, with the vast majority located in gastrointestinal (GI) tract (NIH HMP Working Group et al., 2009; Ursell et al., 2012). In fact, GI tract harbors 10 times more bacterial cells than human cells and carries 150 times more genes (microbiome) than the entire human genome (Thursby and Juge, 2017; Tsigalou et al., 2021). Over centuries, humans have ignored the importance of those microbial organisms which are proven to be essential for their wellbeing. Research has shown that humans live in close relationship with boundless communities of microorganisms that live on and within human bodies and play a major role in human health and disease (Tsigalou et al., 2021). In relation to that, in 2007 the Human Microbiome Project, consisting of multiple projects worldwide, started a research project using sequencing methods to characterize and describe the human microbiome and analyze how it can affect human health and disease (Turnbaugh et al., 2007; Tsigalou et al., 2021). Subsequently, there are several recent technologies of genomics sequencing, proteomics and metabolomics for the identification and analysis of GM (Alverdy et al., 2017).

In recent years, there has been a growing interest in the field of human gut microbiota (GM). Under normal conditions, GM prevents pathogens from crossing the intestinal barrier. Besides, it has been shown to contribute to prevention of nosocomial infections. On the other hand, a disruption to the microbiota homeostasis seems to be involved both in disease onset and development of complications after surgical procedures. Various studies have shown that there is an association between GM and certain diseases, such as inflammatory bowel disease (IBD) including Crohn's disease (CD) (Shimizu et al., 2011; Zaborin et al., 2014; Stavrou and Kotzampassi, 2017; Schmitt et al., 2019). Surgery turns out to have a significant impact on the GM with a great number of medical and surgical problems to be linked to perturbations of the microbiome (Morowitz et al., 2011). For instance, Schmitt et al. (Schmitt et al., 2019) shows that pancreatic surgery affects the GM, while Fang et al. (Fang et al., 2021) describes another types of surgery which affect th GM as well. Therefore, despite the improvement in operation techniques and the quality of general surgical care, postoperative complications remain a notable problem and a considerable number of patients experience postoperative morbidity. Patient age, medical comorbidities, longer procedural times, even the type of surgery are some of the well-recognized risk factors. Nevertheless, postoperative complications may occur even if a low number of risk factors exists. In recent years, the use of Next-generation sequencing (NGS) helped researchers to identify the intestinal microbial composition. NGS allows high-throughput sequencing of DNA samples, so that large numbers of bacterial genomes can be sequenced rapidly in a single experiment. Hence, by using these NGS techniques, researchers can better understand the different bacterial populations and see how microbial imbalances can lead to various health diseases. (Slatko et al., 2018; Gupta and Verma, 2019; Galloway-Peña and Hanson, 2020). Consequently, GM has been shown to play a crucial role in occurrence of postoperative complications (Schmitt et al., 2019). Thus, ongoing research has the potential to lead to new strategies which may enhance the outcomes of surgical procedures.

The key words surgery, microbiota and microbiome' were used to search for relevant studies published in Pubmed database between 2011 and 2022. After evaluation of the full text of the articles, the articles were selected according to the main topics of this review, which are gut microbial dysbiosis linked with human diseases, alterations of GM following surgery, impact of peri-operative interventions on GM and post-operative complications related to GM.

2 Gut microbial dysbiosis and surgical disease

The human gastrointestinal tract harbors several species of microorganisms, including bacteria, fungi, and viruses. The main microbial phyla present in GI tract are Firmicutes (e.g. *Clostridium, Lactobacillus*) and Bacteroidetes (e.g. *Bacteroides, Prevotella*) representing around 60% of gut microbiota, followed by Actinobacteria, Proteobacteria and Fusobacteria (Arumugam et al., 2011; Thursby and Juge, 2017; Rinninella et al., 2019). Each individual can be described with a unique GM profile while a balanced gut microbiota composition confers benefits to the host. The microbiota collaborates with the host's defenses and immune system to protect against pathogen invasion. Furthermore, it exerts profound influence on host metabolism by taking part in digestion of food ingredients leading to essential nutrients and vitamins production (Vernocchi et al., 2020). Contrariwise, imbalance of gut's microbial community, a condition called dysbiosis, alters the physiological functions of the host and, as a result, is associated with unhealthy outcomes and leads to pathogenesis of common human diseases. Dysbiosis contributes to the development of various disorders, including IBD, metabolic syndrome, diabetes, and cancer among many others (Carding et al., 2015; Belizário and Faintuch, 2018; Martinez et al., 2021). Hence, a broad range of surgical problems have been linked to disturbance of the GM composition.

2.1 IBD

IBD, which includes CD and ulcerative colitis (UC), is a chronic, progressive immune-mediated disease affecting the gastrointestinal tract and has become a global emergence disease (M'Koma, 2013). It is estimated that 0.3% of the European population has been diagnosed with IBD, which means that, approximately, a total of 2.5-3 million people is affected. In North America its prevalence is estimated to already exceed 0.5% of the population (Burisch et al., 2013; Coward et al., 2019; Hammer and Langholz, 2020).

Several factors, environmental and immunologic, can lead genetically susceptible hosts to inflammation. More recently, studies have associated alterations in GM with occurrence of IBD. Advances in cultivation-independent technologies showed decreased biodiversity of the gut microflora in those patients and intestinal dysbiosis has been well described (Ott et al., 2004; Manichanh et al., 2006; Frank et al., 2007; Dalal and Chang, 2014). In IBD, Enterobacteriaceae are enriched in the microbial flora and adherent-invasive *Escherichia coli* is commonly isolated from biopsy samples of those patients with CD (Darfeuille-Michaud et al., 2004). Neut et al (Neut et al., 2002) demonstrated that patients undergoing ileocecectomy are more likely to develop postoperative recurrence of CD when high counts of *E.coli* and *Bacteroides* were present. Thus, microbiome seems to play a significant role in development and progression of IBD (Manichanh et al., 2006; Dalal and Chang, 2014; Skowron et al., 2018; Glassner et al., 2020).

Several mutations in genes related to immune system are involved in microbiome-immune interactions and, therefore, in pathogenesis of IBD. It is clearly shown that there is a connection between intestinal flora and intestinal immune cells. Nucleotide oligomerization domain 2 (NOD2), for instance, plays an important role in immune function by recognizing bacterial cell wall proteins and contributing to commensal microbes' control in gut. Mutations in NOD2 gene is a strong genetic risk factor in the pathogenesis of IBD. NOD2- deficient mice have an altered microbiome and increased susceptibility to colitis, sensitizing the colonic mucosa to injury (Kobayashi et al., 2005; Petnicki-Ocwieja et al., 2009; Couturier-Maillard et al., 2013; Skowron et al., 2018).

Moreover, other risk factors related to the microbiome predispose the host to the development of IBD. Use of antibiotics can alter the composition of GM and a study in Denmark (Hviid et al., 2011) showed that early exposure to antibiotics in childhood can lead to IBD and CD. Dietary habits also influence the intestinal flora and play a significant role in shaping its composition. High-fat diets lead to dysbiosis, while plant-based diets affect the GM positively (Tsigalou et al., 2021). Hou et al (Hou et al., 2011) found that diets based in high intake of fats and protein are associated with increased risk of IBD, while high fiber, fruit and vegetable consumption were associated with decreased CD and UC risk. Diet-induced shifts in GM can explain those findings. In addition, breast-feeding seems to be protective against development of IBD. The microbial diversity in breast milk promotes immune tolerance and prevents infections (Xu et al., 2017). In general, a microbial-centered etiology is proposed to explain the development of IBD.

2.2 Colorectal cancer

Dysbiosis of gut microbiota is closely related to colorectal cancer (CRC). CRC is one of the most common types of cancer worldwide. It ranks third in terms of incidence and is the second most common cause of cancer death. Nearly 2 million new cases were diagnosed in 2020 and almost 1 million deaths occur per year (*Colorectal Cancer Awareness Month 2022 – IARC*, no date).

Recent reports have demonstrated that GM plays a crucial role in progression of CRC. Studies have shown alterations in the intestinal microbiota synthesis of patients with reduced bacterial diversity compared with healthy individuals (Chen et al., 2012). Also, several bacterial species have been associated with CRC. *Streptococcus bovis*, enterotoxigenic *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Enterococcus faecalis* and biofilms with species of *E.coli* are some of them (Chen et al., 2012; Wang et al., 2012; Kostic et al., 2013; Sears et al., 2014; Denizot et al., 2015; Veziant et al., 2016; Cheng et al., 2020).

Chronic inflammation is accepted as a risk factor for CRC and, therefore, patients with IBD are in higher risk of CRC development. Intestinal microbiota interacts, as mentioned, with the host immune system and, subsequently, immune responses to bacteria can lead to low-grade inflammation which can lead to tumorigenesis (Arthur et al., 2012). Furthermore, damaged host protective barriers, like intestinal epithelium in colitis, allow translocation of bacteria and exposure to bacterial products. The host though may respond by producing pro-inflammatory cytokines, such as IL-17, -23, TNF-a, which have the characteristic to be also pro-tumorigenic (Garrett, 2015; Skowron et al., 2018). Dejea et al. (Dejea et al., 2014) suggested that colon mucosal biofilm formation enhanced bacterial translocation across the gut barrier due to greater epithelial permeability, which promotes inflammation and may predict increased risk for CRC.

2.3 Obesity

Obesity is a complex metabolic disorder and a result of both genetic and environmental factors. Moreover, studies revealed that obesity is closely related to GM (Liu et al., 2021). It has been shown that GM differs in obese individuals. The microbial composition seems to show low diversity and variety in obese people, with an overgrowth of Gram-negative pathogens which promote lipopolysaccharides (LPS) diffusion and causing low grade chronic inflammation and increased intestinal permeability leading to obesity (Vallianou et al., 2019; Tsigalou et al., 2021; Zsálig et al., 2023). Many studies of the gut microbiome of obese individuals revealed significant alterations of intestinal bacterial phyla with increase in Firmicutes and decrease in the abundance of Bacteroidetes leading to an elevated Firmicutes/Bacteroidetes ratio (Ley et al., 2005). Furthermore, reduced abundance of *Bifidobacterium* is associated with obesity, whilst Streptococcaceae are associated with those individuals with higher BMI (Waldram et al., 2009) (Garcia-Mantrana et al., 2018). Other studies summarized the effect of *Lactobacillus* on body weight and found that *Lactobacillus paracasei* was reduced in overweight subjects, while *Lactobacillus reuteri* and *Lactobacillus gasseri* were increased (Million et al., 2012; Crovesy et al., 2017). Million et al. (Million et al., 2012) showed, also, reduced levels of *Methanobacteriales smithii* in obesity.

Therefore, dysbiosis of GM has been shown to be linked to obesity. However, further research in that field is needed to understand the interaction between GM and obesity.

2.4 Type 2 diabetes

Type 2 diabetes (T2D) is a metabolic disorder which has become a global health problem. While there are various risk factors associated with the development of T2D, emerging evidence suggests that gut microbiota may play a significant role in the development of this disease. Disturbances of GM may increase gut permeability and lead to signaling pathways, related to the insulin resistance in T2D patients. Therefore, research has shown a reduction in beneficial bacteria such as *Bifidobacteria* and an increase in Firmicutes (Sharma and Tripathi, 2019; Zhou et al., 2022)

Furthermore, GM can produce various metabolites that can impact glucose and lipid metabolism. For instance, short-chain fatty acids (SCFAs) produced by gut bacteria can affect insulin signaling and glucose metabolism. Nevertheless, dysbiosis has been associated with altered SCFA production in individuals with type 2 diabetes (Portincasa et al., 2022; Zhou et al., 2022).

Overall, further research is needed to fully understand the relationship between gut microbiota and type 2 diabetes so that potential therapeutic interventions that target the gut microbiota may improve glucose metabolism and insulin sensitivity.

3 Alterations in the Gastrointestinal Microbiome after surgery

Surgery has a large effect in the microbiome and can profoundly alter the synthesis of gut microbiota (Morowitz et al., 2011). Great number of studies have shown that surgical operations are associated with changes in microbiota composition.

Among studies that looked at IBD, Fang et al. (Fang et al., 2021) reported that intestinal surgeries reduce the diversity of GM in IBD patients. A total of 332 stool samples from 129 subjects suffering from UC or CD were collected. Both species and metabolite diversity differed among groups with a significant decrease in phylogenetic diversity after ileocolonic resection and colectomy. In addition, there was a significant increase in the expansion of *E.coli* in individuals who underwent surgery and, in particular, samples from IBD patients who underwent colectomy had the highest abundance of *E.coli*. Wright et al. (Wright et al., 2017) also indicated that there is a significant difference in bacterial composition 6 months and 18 months after surgery. A total of 141 mucosal biopsy samples from 34 CD patients were collected at surgical resection and at colonoscopy after surgery. In addition, 28 control samples were obtained. At 6 months, endoscopic recurrence was associated with elevated *Proteus* genera and at 18 months with reduced *Faecalibacterium*, *Desulfovibrio* and *Bilophila* abundance. At 18 months severe endoscopic recurrence was associated with increase in Proteobacteria. In patients with subsequent remission significant increases in the Firmicutes phylum, the Bacteroidaceae and Pasteurellaceae families and *Bacteroides* genus were observed when compared to those with recurrence. Mondot et al. (Mondot et al., 2016) showed that ileocolonic resection (ICR) in CD patients had a dramatic impact on gut microbial ecosystem. Twenty patients were included in the study. Six months after surgery, ten patients developed recurrence of CD lesions. Samples collected at time of surgery were enriched in Proteobacteria and harbored high levels of unusual, bacteria such as *Streptococcus mitis*, *Undibacterium oligocarboniphilum*, *Sphingomonas melonis* and *Gemella haemolysans*, while 6 months after surgery samples had elevated proportions of anaerobic bacteria belonging to Lachnospiraceae (*Clostridium nexile*, *Blautia wexlereae*, *Dorea longicatena*). Remission was characterized by increased levels of bacteria belonging to *Bacteroides*, *Dorea*, *Ruminococcus* and *Dialister* genera, whereas recurrence was associated with increased levels of *Gemmiger formicilis*, *Enterococcus durans* and *Ruminococcus lactaris*.

Furthermore, other studies demonstrate changes in the balance within the intestinal microbiota after colorectal surgery. Deng et al. (Deng et al., 2018) reported reduction of Bacteroidetes and Firmicutes and increase of Proteobacteria in patients undergoing surgery for CRC. Sze et al. (Sze et al., 2017) showed differences to the bacterial community after surgery in patients treated for adenomas or carcinomas. Microbial communities from patients with carcinomas changed more notable than those with adenomas after surgical treatment and were more similar to those of healthy people after surgery. Komatsu et al. (Komatsu et al., 2016) reported a significant reduction in the total number of bacteria and the number of dominant obligate anaerobes (such as *Clostridium coccoides* group, *Clostridium leptum* subgroup, *B. fragilis* group, *Bifidobacterium*, *Prevotella*, and *Lactobacillus* species) and increase in the abundance of Enterobacteriaceae, *Staphylococcus* (Methicillin-Susceptible Coagulase-Negative Staphylococci MSCNS), *Pseudomonas*, and *Clostridioides difficile* after colorectal surgery. Feng et al. (Feng et al., 2015) collected both faecal and mucosal samples before and six months after colorectal surgery. The abundance of bifidobacterial and lactobacilli in feces and the number of bifidobacterial in mucosa increased after surgery, while Firmicutes and Bacterioidetes decreased after surgery.

Schmitt et al. (Schmitt et al., 2019) analyzed 116 stool samples from 32 patients undergoing pancreatic surgery and examined changes in GM following pancreatic surgery. The samples were classified into three different microbial communities (A, B, C). Community B showed increase in *Akkermansia*, *Aeromonas*, Enterobacteriaceae and Bacteroidales and decrease in Lachnospiraceae, *Prevotella* and *Bacteroides*. Schmitt et al. also observed that the majority of patients experiencing complications showed microbial community B during the period after surgery.

Other studies assessed the remodeling of gut microbiota after bariatric surgery. Steinert et al. (Steinert et al., 2020) found that Roux-en-Y gastric bypass (RYGB) resulted in clear alteration in composition of gut fungal and bacterial microbiota. Before surgery, patients had higher levels of Firmicutes and Actinobacteria and lower levels of Verrucomicrobia in comparison with healthy individuals, which changed after surgery as the levels of those bacterial groups were not different anymore. Also, *Faecalibacterium* and *Bifidobacterium* showed a decrease after surgery and aerotolerant *Streptococcus* was increased postoperatively. The relative abundance of Proteobacteria was increased after surgery. Changes in fungal microbiota composition included decreases in *Candida* and *Saccharomyces* and increases in *Pichia*. Assal et al. (Al Assal et al., 2020) reported that gut microbial richness increased after RYGB and

the Firmicutes to Bacteroidetes ratio decreased. Shen et al. (Shen et al., 2019) showed that the levels of Verrucomicrobia and Proteobacteria increased after the surgery procedure and despite previous studies no changes were noticed in Firmicutes and Bacteroidetes. Paganelli et al. (Paganelli et al., 2019) determined alterations in microbiota composition in 45 obese patients who underwent crash diet followed by RYGB or sleeve gastrectomy (SG). Bifidobacteriaceae abundance decreased, whereas Streptococcaceae and Enterobacteriaceae increased after surgery and no significant differences appeared between both types of surgery in contrast to other studies (Liou et al., 2013). Murphy et al. (Murphy et al., 2017) examined gut microbiota changes after RYGB and SG surgery in obese patients with type 2 diabetes. RYGB led to increased Firmicutes and Actinobacteria phyla but decreased Bacteroidetes phylum. SG resulted in significantly increased Bacteroidetes phylum.

Overall, the results of those studies indicate that there is accumulating evidence that the microbiota can be significantly altered in patients undergoing different types of surgery. Changes following surgery procedures are noticed both in diversity and in the numbers of specific bacteria. Table 1 contains a summary of the articles mentioned in this review.

4 Impact of peri-operative interventions on microbiome

As more and more studies report changes in gut microbiota in patients undergoing surgery, it turns out that many routine techniques of surgical care can affect the host microbiome and, in consequence, the clinical outcomes. There is a variety of perioperative interventions such as mechanical cleansing of the bowel, use of antibiotics and other medication or type of nutrition, along with surgical injury itself and stress of surgery, that could impact the state of the microbiome (Stavrou and Kotzampassi, 2017).

Mechanical bowel preparation (MBP) is often used before abdominal surgeries and may lead to substantial change in GM as many studies show. It seems that MBP can alter the normal flora and, hence, it can provide the opportunity to several pathogens to thrive (Morowitz et al., 2011). It may take 14 days for the intestinal microbiota composition to recover to baseline (Nagata et al., 2019). Yang et al. (Yang et al., 2022) performed a study in which a total of 81 patients were enrolled and were divided into two groups, preparation and non-preparation group, whether they received MBP before the surgery or not. The findings concluded that, at phylum level, Bacteroidetes and Fusobacteria and, at family level, Pasteurellaceae and Neisseriaceae were obviously higher in the preparation group, whereas the abundance of Lactobacillaceae and Ruminococcaceae were higher in non-preparation group. Additionally, at genus level, *Bacteroides*, *Enterobacter*, *Fusobacterium*, *Veillonella*, *Haemophilus*, and *Neisseria* among others were obviously higher in the preparation group, while *Anaerotruncus*, *Coprobacillus*, *Lactobacillus*, and *Blautia* were higher in the group of patients who did not receive MBP. Another study also detected changes in the intestinal microbiota following bowel cleansing (Jalanka et al., 2015) and revealed that, immediately after the lavage, the intestinal microbiota was significantly different when compared with the baseline samples. Bacilli and *Clostridium* cluster IV genera decreased, while members of the Proteobacteria phylum and *Clostridium* cluster XIVa showed an increased abundance. Also, a twofold increase of Proteobacteria, including *Sutterella wadsworthia* and *Serratia*, was noticed after lavage. Similarly, Drago et al. (Drago et al., 2016) found a significant decrease in Firmicutes abundance immediately after colon cleansing and an increase in Proteobacteria. Reduction in Lactobacillaceae and increase in the levels of Enterobacteriaceae were observed as well, while Streptococcaceae showed a 4-fold increase after bowel lavage.

Furthermore, the use of perioperative antibiotics – both oral and intravenous administration- can impact the microbiota leading to a significant reduction of several bacterial counts after surgery. An increase of potentially pathogenic microorganisms is reported as well (Ohigashi et al., 2013; Lederer et al., 2017; Lederer et al., 2021). Also the duration of antimicrobial prophylaxis is associated with higher rates of surgical site infections, resulting for example in higher rates of *C. difficile* infections (Branch-Elliman et al., 2019). Hedge et al. (Hegde et al., 2018) studied on rats and found that broad spectrum antibiotics dramatically reduced the total microbial abundance and diversity. The relative abundance of Firmicutes was noticed to be decreased, whereas Bacteroidetes and Proteobacteria were enriched. Similar to other studies, Nalluri et al. (Nalluri et al., 2020) tried to evaluate the impact of perioperative antibiotic administration on gut microbiome in patients undergoing vertical sleeve gastrectomy and showed that routine antibiotics led to postsurgical changes in the intestinal microbiota. Other peri-operative medications, such as antacids and opioids, can also alter the microbiome composition. Antacids are a class of medicines that neutralize stomach acidity and patients treated with high doses of omeprazole tend to decrease the microbial diversity of colon (Kostrzewska et al., 2017). A significant lower abundance in gut commensals and decreased microbial diversity is also noticed in patients treated with proton pump inhibitors (PPIs). Furthermore, PPI use was associated with increases in Streptococcaceae (Jackson et al., 2016).

Moreover, opioids have been shown to disrupt gut homeostasis. Morphine is one of the most used opioid analgesic for severe pain. In a recent study with a morphine-murine model, the results revealed a significant shift in GM after morphine treatment with the pathogenic bacteria to be increased, even in short term. A significant reduction of beneficial microorganisms, such as *Lactobacillus* and *Bifidobacterium*, was also observed (Wang et al., 2018). Gicquelais et al. (Gicquelais et al., 2020) examined gut microbiota changes related to use of opioids among people and observed that individuals exposed to opioid agonists had alterations in GM with decreased diversity and richness. Therefore, use of morphine results in alterations in gut microbiome contributing to microbial dysbiosis (Herlihy and Roy, 2022).

Increasing evidence suggest that parenteral nutrition (PN) is associated with changes in the intestinal microbiota as well. The host diet affects nutrients availability and, in consequence, the gut microbiome. Parenteral type of nutrition though is required for patients when enteral feeding is not possible (David et al., 2014; Demehri et al., 2015). In a mouse model, PN leads to a relative loss of Firmicutes and to an expansion of Proteobacteria and Bacteroidetes (Miyasaka et al., 2013). In a neonatal pig model, a significant shift in

TABLE 1 Representative studies linking the type of surgery with alterations of GM.

Study Design	Type of surgery	Year	Alteration of Microbial Population	Outcome	References
Prospective longitudinal study	Surgery for IBD: Ileocolonic resection and colectomy	2020	Surgery lowered diversity and affected overall taxonomic, functional and metabolite profiles Elevated *E. coli* relative abundance in surgery samples	Long-term effect of intestinal surgeries on the gut microbiome of IBD patients	Fang, X. et al. (Fang et al., 2021)
Prospective, randomised, controlled trial	Surgery for IBD: resection in CD patients	2017	Microbial composition changed within CD patients. Resection samples were different to samples taken at colonoscopy at 6 and 18 months. Recurrence associated with elevated *Proteus* genera and reduced *Faecalibacterium*	Microbial factors and smoking independently influence postoperative CD recurrence. The genus Proteus may play a role in the development of CD	Wright, E. K. et al. (Wright et al., 2017)
Double-blind, randomised, placebo-controlled, 6-month clinical study	Surgery for IBD: ileocolonic resection in CD patients	2016	Bacterial profiles 6 months after ICR differed markedly from the profiles at time of surgery. Remission associated with increased *Bacteroides*, *Dorea*, *Ruminococcus* and *Dialister* genera Recurrence associated with increased facultative anaerobes like *Gemmiger formicilis*, *Enterococcus durans* and *Ruminococcus lactaris*.	ICR modifies th GM.	Mondot, S. et al. (Mondot et al., 2016)
Metagenomic study of the microbiota of CRC patients after surgery and chemotherapy	Surgery for colorectal cancer	2018	Reduction of Bacteroidetes and Firmicutes. Increase of Proteobacteria	Fecal microbiome-based approaches may provide additional methods for anti-cancer treatments	Deng, X. et al. (Deng et al., 2018)
67-person cohort study	Surgery for colorectal cancer	2017	Microbial communities from carcinoma group changed more notable	Biomarkers within the microbiota could be used to potentially evaluate the effect of treatment and to predict recurrence	Sze, M. A. et al. (Sze et al., 2017)
Single-center randomized, controlled trial	Laparoscopic colorectal surgery	2016	Reduction in dominant obligate anaerobes Increase in Enterobacteriaceae, *Staphylococcus* (MSCNS), *Pseudomonas*, and *Clostridioides difficile*	The microbial imbalance, in addition to the reduction in organic acids, could be improved by perioperative synbiotics treatment	Komatsu, S. et al. (Komatsu et al., 2016)
Feces and mucosal samples of five healthy volunteers and 17 patients with refractory constipation before and six months after subtotal colectomy were collected.	Subtotal colectomy	2015	Faecal samples: increase in *Bifidobacterium* spp and *Lactobacillus* spp/decrease in Firmicutes and Bacteroides Mucosal samples: increase in *Bifidobacterium*	Subtotal colectomy has been shown to normalize the number of intestinal flora and improving refractory constipation	Feng X. et al. (Feng et al., 2015)
Prospective, observational, clinical study	Pancreatic surgery	2019	Patients showing increase in *Akkermansia*, Enterobacteriaceae and *Bacteroidales* as well as decrease in Lachnospiraceae, *Prevotella* and *Bacteroides*, at least once during the observation period were found to have a higher risk for developing postoperative complications	Sequencing of the GM might represent a useful diagnostic tool in future clinical practice	Schmitt, F. et al. (Schmitt et al., 2019)
Pilot cohort study	RYGB	2020	Changes of gut bacterial and fungal microbiota Decrease in Firmicutes, Actinobacteria, *Candida* and *Saccharomyces* Increase in Proteobacteria and *Pichia*	Changes in intestinal fungal communities in RYGB patients that are distinct to changes in the bacterial microbiota	Steinert, R. E. et al. (Steinert et al., 2020)
Prospective cohort study	RYGB	2020	GM richness increased. Firmicutes/Bacteroidetes ratio decreased	Evaluating GM profile to predict T2D remission after RYGB and modulating by dietary interventions	Assal, K. et al. (Al Assal et al., 2020)
Non-Randomized interventional study	RYGB and SG surgery	2019	Verrucomicrobia and Proteobacteria increased after surgery. No changes in Firmicutes and Bacteroidetes	Microbiome partially mediates improvement of metabolism during the first year after bariatric surgery	Shen, N. et al. (Shen et al., 2019)
Observational study	RYGB and SG surgery	2019	Decreased Bifidobacteriaceae. Increased Streptococcaceae and Enterobacteriaceae No significant differences between the two types of surgery	Crash diet invoked temporary changes in GM, yet surgery was associated with changes in GM,	Paganelli, F. L. et al. (Paganelli et al., 2019)

(Continued)

TABLE 1 Continued

Study Design	Type of surgery	Year	Alteration of Microbial Population	Outcome	References
				that likely contribute to weight loss, independent of surgery type	
Longitudinal study	RYGB and SG surgery	2017	RYGB: Increased Firmicutes and Actinobacteria phyla/ decreased Bacteroidetes phyla SG: Increased Bacteroidetes phylum	RYGB showed greater and more predicted favourable changes in GM than SG	Murphy, R. et al. (Murphy et al., 2017)

GM was also observed and was characterized by reduction in bacterial concentration throughout the intestine and loss of microbial diversity. In addition, PN-dependent piglets were at higher risk of colonization by toxin-expressing *C. difficile* (Harvey et al., 2006). When complete intravenous nutrition is applied, a nutrient-deprived environment is created for bacteria in the gut and this hostile environment may favor Proteobacteria, which have been shown to survive in relative starvation states, in contrast to Firmicutes, as they dominate in nutrient-rich environment (Demehri et al., 2015).

Some studies suggest that anesthesia can also provoke unfavorable alterations in the composition and diversity of the gut microbiota. Lian et al (Lian et al., 2021) investigated the effects of surgery and anesthesia on the gut microbiota of mice. They found that exposure to anesthesia and surgery altered the abundance of certain bacterial species with *Escherichia–Shigella*, *Actinomyces*, *Ruminococcus_gnavus*_group, and Lachnospiraceae_FCS020_group to be enriched after anesthesia/surgery. Another study (Serbanescu et al., 2019)observed a decrease in bacterial diversity and depletion of commensal bacteria such as Clostridiales. It is notable that lower levels of Clostridiales have been associated with increased rates of infections (Becattini et al., 2017). Researchers also observed that the type of anesthetics that are used had a different impact on the changes in GM. Han et al. (Han et al., 2021)studied the effect of sevoflurane inhalation anesthesia. The intestinal microbiome of mice showed increased abundances of *Bacteroides*, *Akkermansia* and *Alloprevotella* and decreased abundancies of *Lactobacillus*.Furthermore, hypothermia during anesthesia and surgery is a relatively common occurrence in the surgical patient. Although limited research exists to link hypothermia with gut microbiota, some studies have suggested that hypothermia may contribute to changes in the composition of GM, leading to dysbiosis (Hart et al., 2011; Wang et al., 2021).

Last but not least, the operative stress can influence the composition of the microbiota and it may also be able to increase intestinal permeability through corticotropin-mediated mechanisms leading to translocation of microorganisms (Agnes et al., 2021). Generally, just the stress of surgery and injury itself, even with no use of antibiotics or other therapeutic interventions, may also decrease gut microbiota diversity (Ho et al., 2020).

5 Are post-operative complications related to the gut microbiota?

Postoperative complications are a serious problem, which lead to a higher morbidity and mortality rate and occur in up to 50% of patients undergoing major abdominal surgery (Trencheva et al., 2013; Young and Khadaroo, 2014; Lederer et al., 2021). Some of the most common surgical complications include surgical site infections (SSIs), anastomotic leakage (AL),postoperative ileus (POI) and malabsorption. As previously mentioned, recent studies highly suggest that surgery procedures have detrimental consequences for GM and, therefore, it is more than likely that there is an association between the patients' gut microbiota and surgical outcomes.

5.1 Anastomotic leak

Anastomotic leak is a devastating problem with serious long-lasting consequences. It is defined as a defect of the intestinal wall at anastomotic site resulting in a spillage of intestinal material outside the bowel which was sutured (Rahbari et al., 2010). The cause of AL appears to be multifactorial, with surgical technique generally being the primary contributing factor. After certain surgical procedures, a leak in the connection between two structures that where surgically joined can happen. However, the exact cause is often complex and involves several factors, except for the surgery technique and type of surgery, including the characteristics of patients, such as age, sex and pre-existing medical conditions (Sciuto et al., 2018). There is compelling evidence that gut microbiota is also a risk factor for leakage (Defazio et al., 2014; Sciuto et al., 2018).

Over 60 years ago, Cohn demonstrated a direct role of GM in AL occurrence. A dog model was developed in which decontamination led to complete healing of anastomosis and AL. On the other hand, animals that received saline alone developed major leakage (Cohn and Rives, 1955). Cohen et al. (Cohen et al., 1985) in 1985, also reported a protective effect of enteric antibiotics on colonic wound healing in rats and avoidance of AL. More recently, Schardey et al. (Schardey et al., 1994) created a rat model and suggested *Pseudomonas aeruginosa* as a causative species of AL. The study was focused on esophagoduodenal AL after total gastrectomy. Olivas et al. (Olivas et al., 2012) also showed that intestinal colonization with *P. aeruginosa* led to a significant high incidence of AL in a rat model.). Hence, studies suggest that the presence of specific disruptive species may result in the development of AL.

Other studies focused on the GM synthesis in patients experiencing AL. van Praagh et al. (van Praagh et al., 2016) investigated the composition of the microbiome at the anastomosis level from patients after rectal resection. This study

showed that patients with no AL had higher microbial diversity in contrast to patients who developed AL and showed less diversity with higher abundance of Lachnospiraceae.

In short, recent studied demonstrate an association between leakage and low microbial diversity, prevalence of Enterobacteriaceae and virulent microbiota (Gershuni and Friedman, 2019; Agnes et al., 2021).

5.2 Postoperative ileus

Postoperative ileus, a common postoperative complication, is defined as a prolonged absence of intestinal motility after surgical procedures and more often after abdominal surgery (Buchanan and Tuma, 2022).

Experimental studies and clinical observations revealed a potential link between intestinal microbiome and the pathogenesis of ileus. GM impairs intestinal peristalsis as a modulator of gut synapses or by activating dendritic cells, macrophages and monocytes. In particular, pathogenic iNOS (inducible nitric oxide synthase) produced by macrophages and monocytes, have been shown to induce POI by inhibiting smooth muscle cells. Furthermore, antibiotic administration leads to a considerable reduction of iNOS levels and, therefore, to reduced occurrence of POI, suggesting that macrophages and monocytes activation may depends on microbiota. Overall, further research is needed (Pohl et al., 2017; Bartolini et al., 2020; Agnes et al., 2021).

5.3 Postoperative infections

SSIs, which play a major role in postsurgical care as contributors to patient morbidity and mortality, are also highly suggested to be related to the gut microbiota. Overall, *Staphylococcus aureus* strains represent the most frequently found species in SSIs, followed by gut commensals such as *E. coli* and *E. faecalis*. In general, patients' microbial colonization seems to be the main source of infection as microorganisms causing infectious complications are often commensals of the human body, but further research has to focus on the association between GM and SSIs development (Young and Khadaroo, 2014; Lederer et al., 2017; Bassetti et al., 2020; Lederer et al., 2021).

5.4 Postsurgical complications on malabsorption and overall patient nutritional level

Malabsorption refers to the impaired ability of small intestine to absorb nutrients. Major intestinal reconstructive procedures, such as Roux-en-Y gastric bypass (RYGB), ileal pouch-anal anastomosis surgery (IPAA) or pancreatoduodenectomy may contribute to postoperative malabsorption, because of the surgical operation itself and changes in GM as well. The GM plays a crucial role in absorption of nutrients and alterations in its composition, which may follow surgical procedures, lead to changes in nutrient absorption (Shi et al., 2022; Zheng et al., 2023).

Most studies reveal an increase in *Bacteroides* and Proteobacteria and a decrease in Firmicutes (Luijten et al., 2019). After laparoscopic RYGB a higher abundance of aerotolerant bacteria such as *E. coli* and *Streptococcus* are noticed and, on the other hand, after sleeve gastrectomy(SG), anaerobes, especially *Clostridium*, are more abundant (Farin et al., 2020). Sanchez-Alcoholado et al. revealed changes in the microbiota population as well, with greater levels of *Akkermansia*, *Eubacterium*, *Haemophilus*, and *Blautia* after SG and higher levels of *Veillonella*, *Slackia*, *Granucatiella*, and *Acidaminococcus* after RYGB (Sánchez-Alcoholado et al., 2019). After RYGB patients seem to be at an increased risk of malabsorption as a result of trace element deficiency and osteopenia. Furet et al. (Furet et al., 2010) suggested that high levels of Gammaproteobacteria were related to diminished nutrient absorption after RYGB. In addition, the energy-reabsorbing potential of GM tends to be decreased following laparoscopic SG, indicated by the Bacteroidetes to Firmicutes ratio (Damms-Machado et al., 2015).

Additionally, intraoperative or postoperative antibiotic administration can affect the GM and reduce the microbial diversity, leading to malabsorption (Nalluri et al., 2020; Agnes et al., 2021).

6 Conclusion and discussion

In the past few decades, microbiome research has increased dramatically. The development of new molecular methods such as next-generation sequencing technology helped researchers to enhance their understanding of the complicated microbiota living within the human gut. It is now known that GM changes gradually with time, as people get older, and can also be affected by multiple factors leading to great differences in the composition between individuals (Cullen et al., 2020; Tang et al., 2020). Clearly, surgical operations and, mostly, gastrointestinal surgery can profoundly affect human microbiota. This can happen as a result of disruption of the epithelial barrier during surgery and translocation of bacteria or by other perioperative practices which can alter the microbiota, such as bowel preparation and antibiotic administration (Ferrie et al., 2021). On the other hand, GM composition can affect the surgical outcome and has been described to have a crucial role in surgery complications (Agnes et al., 2021).

Consequently, surgery can alter the population of microorganisms inhabiting the GI tract and may induce an imbalance of GM. Current research examines the factors that contribute to intestinal dysbiosis and focuses on trying to find a solution. Probiotics, which are live microorganisms found in food and supplements, have been shown to improve the intestinal microbial balance and restore the GM diversity. Besides, probiotic administration seems to reduce the total length of hospital stay, the days of intensive care and, in general, the infectious and other major complications (Zhang et al., 2012; Stavrou and Kotzampassi, 2017; Mustansir Dawoodbhoy et al., 2021). Therefore, modulation of the

GM with probiotics appears to be an effective method of reducing complications in patients undergoing surgery but the exact mechanisms remain unclear. Nevertheless, further studies in this field need to be done.

Overall, the results of studies indicate that GM seem to have a huge impact on surgical patients playing an important role in the development and progression of various surgical diseases. Therefore, it may be easier to predict the risk of developing of those complications and to prevent them by understanding the specific bacteria in a patient's gut. However, more studies in larger groups of humans need to be performed for a better understanding of the role of the microbiome in surgical disease and new microbiota-based approaches to surgical care need to be examined in order to lead to new treatments and better outcomes for the patients

Author contributions

Conceptualization, CT, SV, EB. Methodology, CT and NB. Investigation, resources AP, ZT, ES, KA. Writing—original draft preparation, AP, KA, ZT. Writing—review and editing, CT, NB, ES. Visualization, CT, SV. Supervision, EB. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Due to a production error, two author names were incorrectly spelled as "Eugenia Stavropoulou" and "Elisavet Bezirtzoglou". The correct spelling is "Eugenia Bezirtzoglou" and "Elisavet Stavropoulou". The publisher apologizes for this mistake.

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Regulation of gut microbiota: a novel pretreatment for complications in patients who have undergone kidney transplantation

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Kidney transplantation is an effective method to improve the condition of patients with end-stage renal disease. The gut microbiota significantly affects the immune system and can be used as an influencing factor to change the prognoses of patients who have undergone kidney transplantation. Recipients after kidney transplantation showed a lower abundance of Firmicutes and *Faecalibacterium prausnitzii* and a higher proportion of Bacteroidetes and Proteobacteria. After using prebiotics, synbiotics, and fecal microbiota transplantation to regulate the microbial community, the prognoses of patients who underwent kidney transplantation evidently improved. We aimed to determine the relationship between gut microbiota and various postoperative complications inpatients who have undergone kidney transplantation in recent years and to explore how gut microecology affects post-transplant complications. An in-depth understanding of the specific functions of gut microbiota and identification of the actual pathogenic flora during complications in patients undergoing kidney transplantation can help physicians develop strategies to restore the normal intestinal microbiome of transplant patients to maximize their survival and improve their quality of life.

KEYWORDS

kidney transplantation, gut microbiota, complication, diarrhea, rejection, probiotic

1 Introduction

Kidney transplantation is among the most effective treatments for end-stage kidney diseases compared with conventional therapies. Kidney transplantation was first introduced in the 1950s (Meena et al., 2021), and since then, it has drastically improved the survival rates of patients with renal complications (Hariharan et al., 2000).

Transplantation can cause various medical complications ranging from mild symptoms to life-threatening conditions (Adams, 2006). Herrington et al. (2019) revealed that cardiovascular disease (31% of deaths), infection (31%), and cancer (7%) were the leading causes of death following graft function in the first year after transplantation (Herrington et al., 2019). In the first year after kidney transplantation, the leading causes of death include cancer (29%), cardiovascular disease (23%), and infection (12%) (Hariharan et al., 2021). Furthermore, the high incidence of complications such as diarrhea, infection, and graft rejection after transplantation is a persistent challenge (Chong and Alegre, 2012; Mori et al., 2014). The administration of immunosuppressant drugs after transplantation, including tacrolimus and mycophenolate mofetil, is a primary strategy for preventing graft rejection (Tonshoff, 2020). It increases the likelihood of survival but concurrently results in a greater risk of complications after transplantation. Therefore, the development of new and feasible therapies that can attenuate the severity of complications after transplantation is necessary.

Contrary to expectations, substantial evidence has revealed that the adverse reactions observed after kidney transplantation are closely related to the gut microbiota of patients. The human body contains approximately 100 trillion microbial cells, including bacterial, archaeal, viral, and eukaryotic microbial communities, which directly inhabit the interface of the human body that is exposed to or connected to the external environment (Dominguez-Bello et al., 2019; Eng and Borenstein, 2019). Microorganisms play major roles in human health and can directly or indirectly affect human physiology in various ways (Dominguez-Bello et al., 2019). They can guide immune cells to rapidly and effectively respond to pathogenic invasion, simultaneously promote the completion of various metabolic functions, and affect most physiological functions *via* these basic functions (Gomaa, 2020).

Recently, it has been reported that gut microbiota dysregulation adversely affects the prognoses of patients undergoing kidney transplantation (Xu et al., 2017). Increasing evidence has indicated the presence of a bidirectional relationship between gut microbes and nephropathy (Anders et al., 2013; Rukavina et al., 2020). Gut microbes aid in rebuilding the host–microbiome symbiosis, which can help alleviate kidney disease to a certain extent (Al Khodor and Shatat, 2017). An increasing number of scholars and research teams are investigating the relationship between gut microbiota and complications after kidney transplantation, refining the research on this topic (Figure 1). However, current research and reviews have focused only on specific types of complications. In this article, we discuss the evidence regarding gut microbiota from a more comprehensive perspective to evaluate the relationship between gut microbiota and various kidney-related complications.

Abbreviations: KTRs, kidney transplantation recipients; UTI, urinary tract infection; AR, acute rejection; LPS, lipopolysaccharide; iFABP, intestinal fatty acid-binding protein.

2 The healthy profile of gut microbiota and kidney transplantation

The gut microbiome is an important but "invisible organ" in humans (Khanna and Tosh, 2014). Although this "organ" is not composed of actual organs, it is distributed throughout the body, with the intestine being the primary point of distribution (Gomaa, 2020). The total length of the gut is 175 m, leading to differences in the geographical distribution of gut microbiota in humans. The most abundant bacteria in the human gut are Firmicutes, *Escherichia*, and Bacteroidetes, which account for 95% of the total normal flora in healthy individuals; the remaining 5% include *Clostridium*, *Actinobacteria*, and other microbiota (Eckburg et al., 2005). Gut microbiota are among the most important components of the body and are related to various physiological conditions.

Gut microbiota, widely known as the mainstay of the digestive system in the body, were once considered to constitute an invisible organ with a metabolic capacity exceeding that of the liver (Nallu et al., 2017). In glycolipid metabolism, gut microbiota produce metabolites of short-chain fatty acids (colonic fiber fermentation), including acetate, propionate, and butyrate (Cummings et al., 1987), which positively contribute to the health and functioning of various systems. Along with mediating metabolic functions, the gut and microbiota, which collectively function as endogenous organs, play various physiological roles in the host, such as roles in liver damage, cardiovascular disease, sleep regulation, and circadian rhythms (Matenchuk et al., 2020), forming the microbe–gut–brain axis (Quigley, 2017), which maintains intestinal mucosal immunity (Shi et al., 2017). This suggests that gut microbiota dysbiosis is not only associated with digestive system diseases but also with other systemic diseases, such as obesity (Abenavoli et al., 2019), diabetes (Wu et al., 2020), and cardiovascular diseases (Witkowski et al., 2020). Winichakoon et al. (2022) comprehensively summarized and discussed major findings from *in vivo* and clinical data pertaining to the effects of gut microbiota on kidney transplantation (Winichakoon et al., 2022).

Kidney transplantation has been the gold standard renal replacement therapy for end-stage renal disease (ESRD) because it leads to superior survival and quality of life among patients compared with other replacement therapies (Wolfe et al., 1999). This has been consistently reported since the first successful kidney transplantation between identical twins in the 1950s (Force and Andreu, 2005). Transplantation and dialysis are the primary treatments for ESRD and these two treatments can significantly prolong a patient's lifespan and improve their quality of life. According to statistics, patients aged 20–39 years survived for 8 years on dialysis and 25 years after transplantation (Augustine, 2018). In the United States, the 5-year survival rates of patients who underwent primary kidney transplantations from deceased and living donors were 72% and 85% (Hariharan et al., 2021), respectively, which were slightly different from those in Australia (81% and 90%), Europe (79% and 87%), and Canada (81% and 91%) (Wang et al., 2016). Compared to dialysis, kidney transplantation not only increases life expectancy but also

improves the quality of life of several patients with ESRD. It also reduces the costs incurred due to other chronic treatments.

As noted earlier, after kidney transplantation, patients suffer from complications, such as cardiovascular disease (31% of deaths), infections (31% of deaths), and cancer (7% of deaths) (Hariharan et al., 2021). To reduce complications, patients undergoing kidney transplantation are chronically administered immunosuppressive agents to suppress the immune response after allogeneic kidney implantation. This inhibitory effect and the body's autoimmune response to constantly reject foreign bodies are key determinants of transplantation (Ranganathan et al., 2006). Although these complications are not life-threatening, they greatly reduce the patients' quality of life and affect their prognostic statuses. These complications differ from acute rejection, viral infection, cancer, and other uncontrollable risk factors and are closely related to the body's immunity. A delicate balance exists between the two, and if this balance is disrupted because of other factors, patients undergoing kidney transplantation experience postoperative complications including infection and transplant rejection. Studies have revealed that the homeostasis of the gut microbiota significantly influences the immune system and can be used to alter the prognoses of patients undergoing kidney transplantation (Gioco et al., 2020).

3 Gut microbiota profiles of patients after kidney transplantation

The effects of dysbiosis that activate human immune responses, allograft rejection, and the pharmacokinetics of immunosuppressive drugs remain largely unknown (Anders et al., 2013) and may result in certain complications after transplantation such as the risk of infection (urinary infections and infectious diarrhea), adverse immune phenomena (autoimmune hemolytic anemia), transplant rejection, and increased mortality. Human and mouse studies have revealed that the abundance of the gut microbiota is significantly inversely associated with transplant prognosis, the abundance of microbial species, the number of donors and recipients, the estimated glomerular filtration rate within 6 months of transplantation, and species-differentiated microbiota are associated with an increased frequency of infection-related complications after transplantation. Thus, the presence of certain species in the gut before kidney transplantation is significantly associated with subsequent rejection. Therefore, the microbiota can affect the quality of life of patients after kidney transplantation (Ranganathan et al., 2006). Winichakoon et al. (2022) reported that the microbiota of an individual could alter the immune response of organ transplantation hosts *via* specific signaling pathways, such as the Myd88 and TLR9 pathways (Winichakoon et al., 2022).

Kim et al. (2020) reported that differences in the donor–recipient microbial community before transplantation can affect the function of early allografts, and this relationship can affect the incidence of infection within 6 months of transplantation. In genetically unrelated donor transplantations, unrelated individuals exhibited a more pronounced correlation between graft function and microbial similarity (Kim et al., 2020). Kim et al. (2020) suggested that the effects of unknown environmental and traditional genetic factors on the microbial community should be considered during post-transplantation management. Therefore,

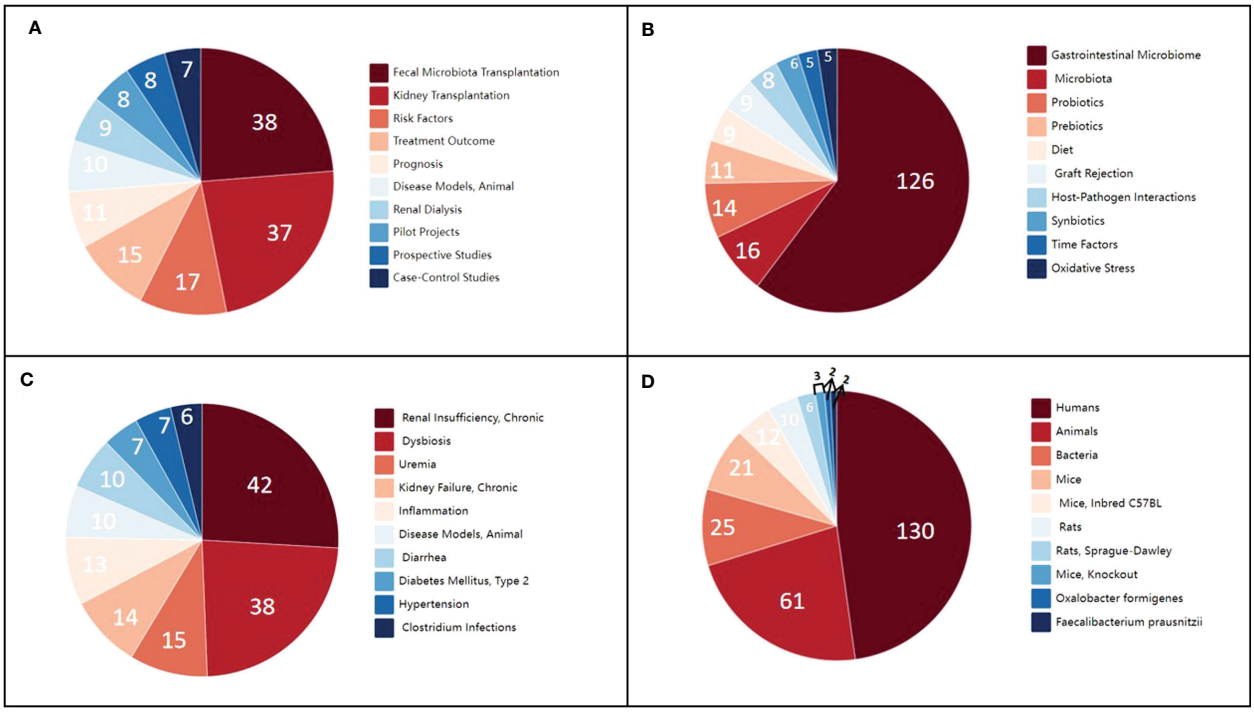

FIGURE 1
Clustering correlation between kidney transplantation and gut microbiota in published literature. **(A)** Analytical, diagnostic, and therapeutic techniques and equipment categories. **(B)** Phenomena and processes categories. **(C)** Diseases category. **(D)** Organisms category.

future studies investigating the relationship between gut microbiota and organ transplantation should consider sex, age, and ethnicity. Therefore, sex, age, and race should be considered when investigating the association between gut microbiota and organ transplantation.

Interestingly, we also found that various types of specific bacterial species were responsible for dysbiosis in patients undergoing kidney transplantation. Xiao et al. (2018) revealed that in densely populated areas in a multidimensional space composed of the same community (Xiao et al., 2018), the type and abundance of the human gut microbiota were similar. Arumugam et al. (2011) named this gut microbial composition "enterotype" (Arumugam et al., 2011; Costea et al., 2018) and found that its abundance was related to the age, sex, cultural background, and geographic location of individuals. In a study cohort, Costea et al. (2018) categorized enterotypes into three types and found that *Bacteroidetes* were dominant in Enterotype 1, *Prevotella* in Enterotype 2, and *Ruminococcus* in Enterotype 3. These intestinal types directly affect intestinal homeostasis and are closely associated with various acute and chronic diseases. In summary, based on this theory, personalized treatment should be administered in different populations; that is, different treatment methods should be selected for patients with different intestinal types.

Notably, a negative relationship exists between gut microbiota and the kidney. After drugs modulate the gut microbiota, kidney disease progression significantly slows down. When probiotics such as *Bacillus pasteuri* and *Lactobacillus sporogenes* were used to treat a rat model of chronic kidney disease (CKD), blood urea nitrogen (BUN) levels significantly reduced (Ranganathan et al., 2005), and the use of urease-positive *Pasteurella* reduced intestinal BUN levels. The concentration of urea, which also improves urinary protein excretion, reduces the glomerular sclerosis index (Ranganathan et al., 2006; Yoshifuji et al., 2016). Based on the findings of clinical studies summarized in Table 1, gut microbiota profiles differ considerably based on the pre- and post-transplantation status, as observed in 16S ribosomal ribonucleic acid polymerase chain reaction experiments.

4 Kidney transplantation and regulation of gut microbiota

As early as 2009, Jang et al. (2009) used mice to confirm the important role of the microecology of gut microbiota in kidney disease. They revealed that germ-free mice bred *via* technical means could be used to monitor *in vivo* abnormalities under the same

TABLE 1 Clinical studies on the profiles of gut microbiota in patients after kidney transplantation.

Reference	Population	Post-transplantation phyla	Outcomes
Kim et al. (2020)	67 KTRs/67 donors;	↓Prevotellaceae; ↓*Prevotella*	6-month allograft function
Lee et al. (2015)	19 KTRs/54.7 Compared with the baseline	↑*Firmicutes*	↑ *Faecalibacterium prausnitzii*
Lee et al. (2019a)	168 KTRs/ 54healthy	↓Faecalibacterium prausnitzii; Eubacterium dolichum; Coprococcuscomes; Eubacteriumrectale	↓ butyrate-producing gut microbiota
Swate et al. (2020)	139 KTRs/105 healthy	↑Actinobacteria,*Firmicutes*; *Proteobacteria*	↓ butyrate-producing bacteria
Fricke et al. (2014)	60 KTRs/58 Compared with the baseline	↑*Actinobacteria; Bacteroidetes; Firmicutes; Proteobacteria*	↑Cr; ↑Actinobacteria (*Bifidobacterales*) ↑ Firmicutes (*Peptostreptococcus*)
Zaza et al. (2017)	20 KTRs/62.3 Compared with the baseline	↑*Actinobacteria*; *Firmicutes*; *Proteobacteria*	EVE + MMF (↑ msrA); TAC + MMF(↑ fliNY, pilM)
Guirong et al. (2018)	16 KTRs/84 CKD/53 healthy	↑*Bacteroidetes; Proteobacteria*; ↓*Firmicutes*	↑ Metabolism of (carbohydrates/amino acids/xenobiotics) ↓Metabolism of (cofactors/vitamins/nucleotides/terpenoids)
Chan et al. (2021)	12 KTRs/ 51healthy; 12 donors/ 56healthy	↓*Firmicutes*_G; Acutalibacteracae familyRikenellaceae family; ↑*Firmicutes*_A; Roseburia intestinalis Faecalibacterium prausnitzii D	gut microbiota richness, diversity, composition, and functional parameters in kidney transplant recipients
Lecronier et al. (2020)	19 Controls/ 15 NODAT/ 16 Diabetic	↑*Lactobacillus sp.*;↓Faecalibacterium prausnitzii; A. muciniphila in patients with diabetes	Higher percentage of *Lactobacillus* sp. carriage, higher relative abundance of *Lactobacillus* sp., and lower relative abundance of *A. muciniphila*.

KTR, kidney transplantation recipient; CKD, chronic kidney disease; EVE, everolimus; MMF, mycophenolate mofetil; mrsA, macrolide transport system; fliNY, flagellar motor switch protein; pilM, pilus assembly protein; "↓", an upward trend in the abundance of gut microbiota and "↑", a downward trend in the abundance of gut microbiota.

exposure. A large population of killer T cells and extremely low concentrations of interleukin (IL)-4 were observed. However, after the gut microbiota were reintroduced to establish a stable gut microbiome, the conditions of the mice showed significant improvement (Figure 2). Accumulating evidence suggests that after gut microbiota dysbiosis affects the intestinal mucosa and causes intestinal inflammation, it eventually causes systemic inflammation by activating the nuclear factor (NF)-κB pathway (Anders et al., 2013). These findings provide a basis for the existence of the gut–immune axis *in vivo*. In view of the findings of these studies, a method to rebuild the intestinal microbiota and stabilize intestinal microecology can be developed, which can be innovatively used for the prevention and treatment of kidney diseases.

When gut microbiota are disturbed, inflammatory reactions can be activated *via* the NF-KB pathway (Anders et al., 2013), the degree of cresol and IL-4 (Jang et al., 2009)in intestinal mucosa can be reduced, and the degree of urea nitrogen can be increased (Ranganathan et al., 2005), which will lead to kidney damage.

The presence of multiple factors in patients before, during, and/or after kidney transplantation can result in changes in the type and quantity of the microbiota, thereby destabilizing it (Jang et al., 2009; Yoshifuji et al., 2016). Some scientists have revealed that the gut microbiota in patients undergoing kidney transplantation who have undergone fecal microbiota transplantation (FMT) are highly similar to the type composition of the donor's gut microbiota and will tend to gradually normalize (Ianiro et al., 2020). Wang et al. (2021a) revealed that in kidney transplantation recipients (RTRs), the dominant flora (Prevotella_9, relative abundance 35.9%) (Wang et al., 2021a) was *Bacteroides* (relative abundance 60.6%) after 2 months of FMT administration.

A plethora of evidence supports the idea that gut microbiota dysbiosis plays a vital role in the outcomes of organ transplantation, especially kidney transplantation. Modification of the gut microbiota in response to routine therapeutic approaches, such as the administration of immunosuppressive drugs and prophylactic antibiotics post transplantation (Kim and Song, 2020), may adversely affect graft outcomes, thus leading to graft rejection, infection, fibrosis, and alterations in drug metabolism. As summarized in Table 2, prebiotics, synbiotics, and FMT can transform a state of gut dysbiosis into colonization by healthy gut microbiota.

According to Guida et al. (2017), synbiotics can effectively lower plasma p-cresol levels in patients undergoing kidney transplantation. P-cresol generally accumulates when its production by the dysbiotic gut microbiome increases (Guida et al., 2017). In a study, 27 recipients of kidney transplantation with gastrointestinal symptoms received a prebiotic powder suspension with breakfast for 7 weeks (Chan et al., 2022). In this randomized placebo-controlled trial, prebiotics significantly suppressed gastrointestinal symptoms. In another study by Lin, three RTRs showed no further CDI recurrence or diarrheal symptoms during the follow-up period after receiving FMT treatment *via* colonoscopy (Lin et al., 2018) (Figure 3).

Furthermore, clinical studies have revealed that probiotic supplementation effectively improves kidney function. Ranganathan et al. (2010) and other researchers administered probiotic strains such as *Lactobacillus acidophilus*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* to 46 patients with CKD III or IV and found that their renal function and quality of life significantly improved after probiotic intake for 6 months (Ranganathan et al., 2010). Grosen et al. (2019) reported a case wherein a patient suffered from urinary tract infections (UTIs) caused by broad-spectrum lactamase-producing (ESBL+) *Klebsiella pneumoniae* but showed no symptoms of UTI after FMT treatment (Grosen et al., 2019). In 2021, Wang et al. (2021a) presented a case report where carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection was treated using FMT in China (Wang et al., 2021a). After FMT, not only did the CRKP test of urine and anal swab cultures yield negative results but a greater relative abundance of *Phascolarctobacterium* and *Lachnoclostridium*was noted along with the depletion of *Klebsiella*.

Kim reported that gut microbiota profiles considerably vary between recipients and donors (Kim et al., 2020). Recipients

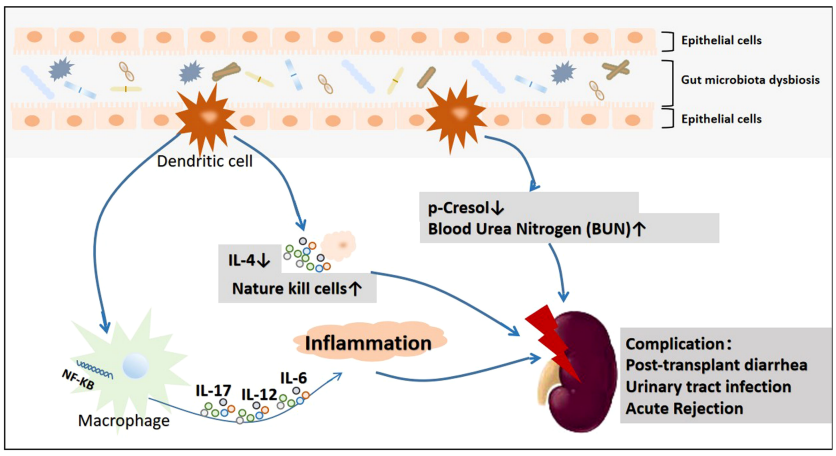

FIGURE 2
The primary mechanism underlying kidney damage after gut microbiota disorders.

TABLE 2 Effects of probiotics or synbiotics on kidney transplantation outcomes.

Reference	Population (mean ages)	Post-transplantationphyla	Outcomes
Wang et al. (2021a)	A 37-year-old woman with CRKP infection at 1 month after kidney transplantation	↑*Phascolarctobacterium* and *Lachnoclostridium*; ↓*Klebsiella*	Fecal microbiota transplantation (FMT) to treat an infection caused by CRKP for a patient undergoing kidney transplantation.
Biehl et al. (2018)	A 50-year-old woman undergoing immunosuppressive treatment after kidney transplantation 3 years ago	↓*Enterobacteriaceae*; ↑ classes of bacilli and clostridia	FMT for recurrent urinary tract infections.
Guida et al. (2017)	36 KTRs(mean age: 49.6 years) with transplantation vintage > 12 months	↓plasma p-Cresol	Treatment with synbiotics may be effective for lowering the plasma p-Cresol levels in KTRs.
Chan et al. (2022)	27peoplein the intervention group(mean age:52.9 years);29 peoplein the control group(mean age: 54.7 years)	Median GSRS score change −0.15/ −0.28/−0.07	Prebiotics significantly reduced gastro- intestinal symptoms.
Lin et al. (2018)	A 64-year-old woman, transplantation (2002); A 34-year-old woman, transplantation (2013); A 62-year-old woman, transplantation (2000)	With the resolution of diarrheal symptoms; no further CDI recurrence observed over 10 months of follow-up	Detailed FMT protocol and experience in treating a series of patients with SOT with rCDI.
Wei et al. (2019)	A 32-year-old Chinese woman, who developed DIHS-associated MODS, underwent FMT four times at a frequency of once every 6 days. The healthy control was a 23-year-old man who was a graduate student.	↑*Lachnospiraceae, Ruminococcaceae, Veillonellaceae* ↓ *Proteobacteria*, *Enterobacteriaceae*, and *Alcaligenaceae families*. At the genus level:↑ *Roseburia*, *Prevotella*, *Bacteroides*, *Oscillospira*, and *Faecalibacterium*	FMT is effective for treating intestinal failure associated with DIHS.

KTR, kidney transplantation recipient; CKD, chronic kidney disease; CRKP, carbapenem-resistant Klebsiellapneumoniae; FMT, fecal microbiota transplantation; CDI, Clostridium difficile infection; SOT, solid organ transplantation; GSRS, gastrointestinal symptom rating scale; DIHS, drug-induced hypersensitivity syndrome; "↓", an upward trend in the abundance of gut microbiota and "↑", a downward trend in the abundance of gut microbiota.

exhibited lower levels of *Prevotellaceae* and *Prevotella*, whereas the opposite was observed in donors. Lee et al. (2015) revealed that in patients after kidney transplantation, a lower diversity of Firmicuteswas observed (Lee et al., 2015). Lee et al. (2019a) also reported lower relative abundances of *Faecalibacterium prausnitzii*, *Holdemanella biformis*, *Eubacterium dolichum*, *Coprococcus comes*, *Subdoligranulum variabile*, and *Eubacterium rectale* (Lee et al., 2019a). Swarte et al. (2020) reported increased proportions of Firmicutes and Proteobacteria in 139 kidney transplant recipients and 105 healthy controls (Swarte et al., 2020). Fricke et al. (2014) reported that increased *Actinobacteria* and Firmicutes abundance is associated with an increased risk of graft dysfunction, as identified by an increase in chromium concentration after kidney transplantation (Fricke et al., 2014). Zaza et al. (2017) revealed increased abundances of Actinobacteria, Firmicutes, and Proteobacteria in patients who were administered different immunosuppressive drug regimens after kidney transplantation (Zaza et al., 2017). Contrary to expectations, Guirong et al. (2018) reported a decrease in Firmicutes abundance instead of an increase in recipients of kidney transplants (Guirong et al., 2018). Chan et al.

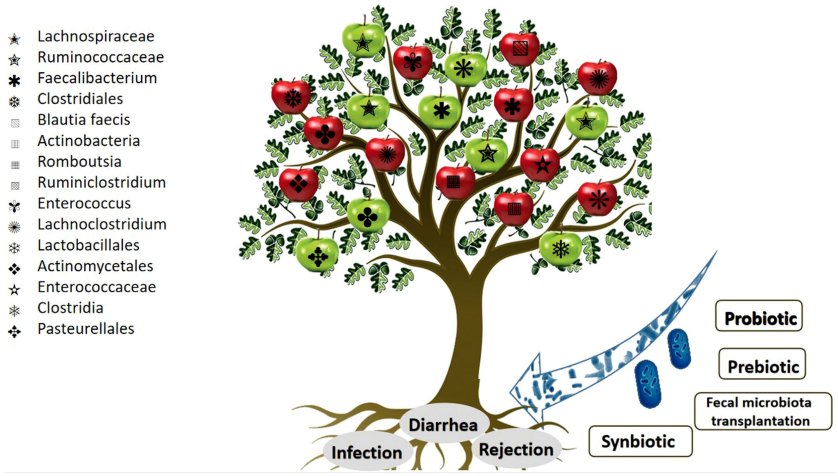

FIGURE 3
Schematic view of the link between gut microbiota and kidney transplantation complications. The red apples represent an upward trend in the abundance of gut microbiota. The green apples represent a downward trend in the abundance of gut microbiota.

(2021) classified Firmicutes into two types and reported a significantly greater abundance of Firmicutes A and a relatively lower abundance of Firmicutes G (Chan et al., 2021). This may explain the contradictory results reported by different studies.

5 Kidney transplantation outcomes and profiles of gut microbiota

5.1 Diarrhea after transplantation

Diarrhea after transplantation is a common complication observed after solid organ transplantation (Zhang et al., 2021), and a large registry analysis identified that diarrhea after transplantation negatively affects graft and patient survival (Aulagnon et al., 2014). Diarrhea is generally considered to exert a mild impact on patients' quality of life, but severe episodes have a significant impact on drug metabolism and the prognoses and survival of patients (Zhang et al., 2021), such as a drastic decline in the functionality of the transplanted kidney or a significant increase in patient mortality (Ekberg et al., 2007). According to Medicare claims registered with the United Network for Organ Sharing, diarrhea occurs in 20%–50% of solid organ transplant recipients in the first year after transplantation (Angarone and Snydman, 2019), with an incidence of diarrhea after kidney transplantation of 11.5% and the cumulative incidences of diarrhea at 2 and 3 years of 17.5% and 22.6% (Bunnapradist et al., 2008), respectively.

Along with certain dietary elements, antibiotic use, the induction of transplant-specific lymphatic failure (Li et al., 2013), and immune rejection also contribute to an imbalance in the gut microbiota. Some studies have revealed that only a few patients who harbored gastrointestinal pathogens during transplantation developed diarrhea after transplantation, and only a few patients exhibited the same pathogenic flora colonizing the gastrointestinal tract before and after transplantation (Westblade et al., 2019). Diarrhea can be induced by various factors. Bunnapradist et al. (2006) showed that tacrolimus and mycophenolate mofetil increase the risk of noninfectious diarrhea, graft loss, and patient death (Bunnapradist et al., 2006). Therefore, pathogens that cause diarrhea in the gastrointestinal tract are more likely to colonize and cause chronic infections in the body, forging a relationship with the progression of diarrhea after transplantation. Study findings have further indicated that gut microbiota can be isolated from diarrheal stool samples, with reduced microbial diversity and decreased abundance of commensal bacterial species. Most cases of diarrhea after transplantation are different from common infectious diarrhea, which is related to intestine-related dysbiosis. Patients undergoing kidney transplantation show a greater abundance of *Proteobacteria* than healthy individuals (Arslan et al., 2007). After kidney transplantation, patients are more susceptible to opportunistic pathogens than immunocompromised healthy individuals (Aulagnon et al., 2014).

Interestingly, Westblade et al. (2019) revealed that in the first 3 months after transplantation, 47 (33%) of 142 kidney transplant recipients developed diarrhea after transplantation, of which 24 recipients exhibited extensive microbial colonization (Westblade et al., 2019). Lee et al. (2014) reported differences in the diversity of the gut microbiota between healthy individuals and kidney transplant recipients. The latter exhibited an increase in *Enterococcus*, *Escherichia*, and *Lachnoclostridium*and a decrease in *Eubacterium*, *Anaerostipes*, *Coprococcus*, *Romboutsia*, *Ruminococcus*, *Dorea*, *Faecalibacterium*, *Fusicatenibacter*, *Oscillibacter*, *Ruminiclostridium*, *Blautia*, *Bifidobacterium*, and *Bacteroides*. Lee et al. (2014) also reported that an increase in *Faecalibacterium* abundance was positively correlated with future tacrolimus dosing at 1 month (Lee et al., 2014). Swarte et al. (2020) reported an increasing trend in the abundance of *Proteobacteria* and a lower abundance of *Faecalibacterium prausnitzii*, *Gemmiger formicilis*, *Eubacterium rectale*, *Coprococcus catus*, Co*prococcus comes*, and *Roseburia* (Swarte et al., 2020). This study also reported the depletion of butyrate-producing bacteria caused by antibiotics and immunosuppressive agents and this was considered to be associated with diarrhea after kidney transplant. Wei et al. (2019) reported increased abundance of *Lachnospiraceae*, *Ruminococcaceae*, and *Veillonellaceae*, and decreased abundance of Proteobacteria, *Enterobacteriaceae*, and *Alcaligenaceae* in a 32-year-old kidney transplant recipient. These findings reveal that butyrate-producing bacteria are lost in RTRs (Flint et al., 2015). Diarrhea after transplantation was not associated with common infectious diarrheal pathogens but with dysbiosis.

Norovirus has been attributed to approximately 25% of cases with diarrhea after transplantation. *Clostridium difficile* infection occurs in 2%–7% of kidney transplant recipients (Chan et al., 2022). Evidence from several studies has suggested that *C. difficile* also causes nosocomial infections that patients contract within the first few months after transplantation (Guida et al., 2017; Lin et al., 2018). Kujawa-Szewieczek et al. (2015) attempted to use *Lactobacillus plantarum* 299v as a preventive agent against this infection in patients receiving antibiotics and observed that probiotics could efficiently prevent *C. difficile* infection in RTRs (Kujawa-Szewieczek et al., 2015). Lee et al. (2019b) also reported that some patients continued to experience diarrhea despite repeated monitoring tests for *C. difficile* before FMT, which yielded negative results. The authors also revealed that the abundance of *Ruminococcus* significantly decreased in samples collected after the onset of diarrhea symptoms (Lee et al., 2019b) compared to samples collected before diarrhea. A summary of these findings is provided in Table 3.

Modulation of the gut microbiota profile *via* FMT or administration of probiotics has positively affected RTRs. Aulagnon et al. (2014) reported the clinical characteristics of a patient with chronic diarrhea after kidney transplantation (Aulagnon et al., 2014). After Masson's trichrome staining, the intracellular accumulation of calcium oxalate monohydrate was microscopically observed, and the deposition of massive crystals disrupted the tubular structure, thus inducing inflammation and fibrosis in the scarred area. Gu et al. (2018) showed that diarrhea symptoms in patients undergoing kidney transplantation improved

TABLE 3 Association between gut microbiota and diarrhea in kidney transplantation recipients: evidence from clinical studies.

Reference	Population (mean ages)	Key findings	Outcomes
Swarte et al. (2020)	Fecal samples were collected from 139 KTRs (50% men, mean age: 58.3 ± 12.8 years) and 105 healthy controls (57% men, mean age: 59.2 years).	↑***Proteobacteria***, ↓ *Actinobacteria* ↓ butyrate-producing bacteria	On comparing the gut microbiome of KTRs to healthy controls, KTRs were found to suffer from dysbiosis, a disruption in the balance in the gut microbiome.
Fricke et al. (2014)	Fecal samples were collected from 40 KTRs (63% men, mean age: 58 years).	↓*Proteobacteria, Escherichia*, *Porphyromonas* (urine), *Haemophilus*, *Neisseria*, *Pasteurella* (oral swabs)	Characteristics of the microbiota that can serve as diagnostic markers for transplant health and guide intervention strategies for improving transplantation outcomes.
Lee et al. (2014)	Fecal samples were collected from 26 KTRs (50% men, mean age: 56years).	↑*Faecalibacterium* in the dose escalation group	Distinct microbiota structures were observed in allograft recipients with post-transplant diarrhea, acute rejection, and *Enterococcus*UTI.
Lee et al. (2019b)	Fecal samples were collected from 71 KTRs: 25 from the diarrhea group (40% men, mean age: 56 years) and 46 from the no diarrhea group (59% men, mean age: 53 years).	↓*Eubacterium*, *Anaerostipes*, *Coprococcus*, *Romboutsia*, *Ruminococcus*, *Dorea*, *Faecalibacterium*,*Fusicatenibacter*, *Oscillibacter*, *Ruminiclostridium*, *Blautia*, *Bifidobacterium*, and *Bacteroides*; ↑*Enterococcus*, *Escherichia*, and *Lachnoclostridium*	Colonization with gastrointestinal pathogens, particularly *Clostridium difficile*, is commonly observed in patients after kidney transplantation but does not predict subsequent diarrhea.
Zhang et al. (2021)	Fecal samples were collected from 40 KTRs (42% men, mean age: 52 years) and 57 healthy controls (67% men, mean age: 56 years).	Suggesting fecal β-glucuronidase activity could be a novel biomarker for gastrointestinal tract-related MMF toxicity.	Fecal β-glucuronidase activity could be a novel biomarker for gastrointestinal tract-related MMF toxicity.

KTRs, kidney transplantation recipients; MMF, mycophenolate mofeti; "↓", an upward trend in the abundance of gut microbiota and "↑", a downward trend in the abundance of gut microbiota.

after FMT (Gu et al., 2018). Zhang et al. (2021) recently revealed that fecal β-glucuronidase activity could serve as a novel biomarker for gastrointestinal tract-related mycophenolate mofetil toxicity.

5.2 UTI

UTI is an infection primarily caused by bacteria invading urothelial cells, inducing an immune response in the body to resist infection (Fiorentino et al., 2019). The incidence of kidney transplantation is considerably higher in patients with UTIs than in the general population (Alangaden et al., 2006; Rice and Safdar, 2009; Karuthu and Blumberg, 2012). Asymptomatic bacteriuria constitutes 44% of UTI cases, whereas uncomplicated and complicated UTI account for 32% and 24% of the cases, respectively. Patients undergoing kidney transplantation account for approximately 40%–50% of all infectious complications (Meena et al., 2021), with bacteriuria detectable in approximately 40% of patients within the first year (Magruder et al., 2019). Furthermore, nearly 50% of UTI cases occur within 3 years of transplantation. UTIs not only affect patients' quality of life but also increase the risk of other complications in transplant recipients, which affect long-term graft survival and rejection (Castaneda et al., 2013; de Castro Rodrigues Ferreira et al., 2017).

Fiorante et al. (2010) reported that asymptomatic bacteriuria alone was associated with a seven-fold increased risk of pyelonephritis in a population of patients undergoing kidney transplantation (Fiorante et al., 2010), and each episode of UTI that occurred after the transplantation reduced graft function to some extent (Fiorentino et al., 2019). In a retrospective study, approximately 1%–3% of patients with UTIs developed bacteremia, and >20,000 kidney transplant recipients developed UTIs 6 months after transplantation (Lee et al., 2013), which significantly increased the risk of death and graft loss. Recent studies support the role of gut microbiota in UTI pathogenesis, with *Enterobacteriaceae* being the most common cause of UTI after transplantation (Lee et al., 2013). In a study of nontransplantation patients, children with *Escherichia coli* UTIs exhibited greater gut *E. coli* abundance than other children. In another experiment including kidney transplant recipients, the identification of urine strains revealed that the *E. coli* present in urine was genetically similar to the *E. coli* present in stool samples collected from the same participants, supporting the notion that gut microbiota dysbiosis is the primary source of UTIs (Magruder et al., 2020). Magruder et al. (2020) showed that RTRs with UTIs experienced the same shifts in microbial diversity, with an increased abundance of Firmicutes and Proteobacteria (Magruder et al., 2019; Magruder et al., 2020). A summary of these findings is provided in Table 4.

Interestingly, some patients with UTI after kidney transplantation show improvements in clinical symptoms and urinary sensation without antibiotics. A preliminary speculation may be related to the body's response to acute infection. This implies that unconventional methods can be used to stabilize the type and abundance of microbiota. Contrary to expectations, Biehl reported that FMT (via frozen capsulized microbiota) improved UTI symptoms in kidney transplant recipients (Biehl et al., 2018).

Gram-negative bacteria are responsible for the occurrence of 70% of UTIs in RTRs, with *E. coli* accounting for 30%–80% of kidney transplantation-related UTIs (Valera et al., 2006; Pelle et al., 2007). *Klebsiella*, *Pseudomonas aeruginosa*, other gram-negative bacteria, and *Proteus* are common pathogens that cause UTIs. When gut microbiota invade the body to induce UTI or bacteriuria, the bacteria continuously invade the urothelium by producing virulence factors and specific adhesins on the surface of the bacterial membrane to stimulate

TABLE 4 Association between gut microbiota and urinary tract infection in kidney transplantation recipients: evidence from clinical studies.

Reference	Population (mean ages)	Key findings	Outcomes
Lee et al. (2014)	Fecal samples of 26 KTRs (50% men, mean age: 56 ± 8 years). The median time after transplantation of KTRs was 3 months.	↑ *Enterococcus* ↑ *Enterococcus* UTI compared to 0% in the 23 patients without *Enterococcus* UTI	Distinct microbiota structures were observed in allograft recipients with post-transplantation *Enterococcus* UTI.
Magruder et al. (2020)	Fecal samples were collected from 168 KTRs:51 in the Enterobacteriaceae bacteriuria group (27% men, mean age: 57 years) and 117 in the no Enterobacteriaceae bacteriuria group (67% men, mean age: 53 years). Median time after the transplantation of KTRs was 6 months.	↑*Faecalibacterium* and *Romboutsia*; ↓*Lactobacillus* in the no *Enterobacteriaceae bacteriuria* group	Bacterial taxa associated with a decreased risk for *Enterobacteriaceae* UTI in KTRs, which will help support future studies reporting the modulation of gut microbiota as a novel treatment for preventing UTIs.
Magruder et al. (2019)	Fecal specimens from 168 KTRs. Median time after the transplantation of KTRs was 3 months.	↑*Escherichia* in the *Escherichia bacteriuria* group than in the no *Escherichia bacteriuria* group; ↑*Enterococcus* in the *Enterococcus* bacteriuria group	The results support a gut microbiota–UTI axis, suggesting that the modulation of the gut microbiota may be a novel strategy for preventing UTIs.

KTRs, kidney transplantation recipients; UTI, urinary tract infection; "↓", an upward trend in the abundance of gut microbiota and "↑", a downward trend in the abundance of gut microbiota.

urothelial cells, which produce pro-inflammatory factors, such as IL-8. These factors promote the migration of neutrophils to infected urothelial cells and facilitate inflammatory immune responses (Fiorentino et al., 2019). Modulation of gut microbiota may play a role in the treatment of UTI in RTRs.

5.3 Acute rejection

Thirty-nine percent of patients experience at least one acute rejection within 1 year of transplantation (Carron et al., 2019). Acute rejection can lead to graft failure within a short duration (Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, 2009).It can also lead to a greater three-fold risk of graft glomerular disease and loss of graft function in patients within 1 year of transplantation than in healthy controls (Gloor et al., 2007). Gut microbiota have been associated with the occurrence of acute rejection, and the relative abundances of *Lactobacillus*, *Enterococcus*, anaerobic bacteria, and *Clostridium* termites were found to be higher in patients with acute rejection, whereas those of *Clostridium*, *Bacteroides*, *Cyanobacteria*, and *Lachnospira*were lower (Lee et al., 2014). Wang revealed that 29 recipients of kidney transplantation (including 24 acute rejection recipients) reported a risk of graft rejection in response to altered proportions of Firmicutes and Bacteroidetes compared to those of *Proteobacteria*, *Actinobacteria*, *Lactobacillales*, *Clostridia*, and *Faecalibacterium*, with the abundances of the former decreasing and that of the latter increasing (Wang et al., 2021b). Carron et al. (2019) reported that the concentrations of circulating sCD14 and intestinal fatty acid-binding protein (iFABP) inversely correlated with a stronger prompt for acute rejection after transplantation in 788 recipients of kidney transplantation (Carron et al., 2019). A summary of these findings is provided in Table 5.

Some scholars have studied how the gut microbiota can cause acute rejection and proposed that if patients with ESRD maintain a stable total lipopolysaccharide (LPS) concentration for a long duration and a state of low LPS activity, the level of inflammatory biomarkers in patients undergoing kidney transplantation can be improved 1 year after transplantation. This could help protect patients who have undergone kidney transplantation from acute rejection (Carron et al., 2019). Authors have also proposed the observation of an intestinal epithelial cell damage marker, iFABP (Piton and Capellier, 2016), to assess the integrity of the intestinal epithelial barrier and the measurement of inflammatory biomarkers (sCD14 and cytokines) concurrently to explore the pathways and mechanisms underlying LPS elimination (Lee et al., 2014) and body-induced acute immune rejection.

6 Conclusion and perspectives

The gut microbiota exerts certain regulatory effects on various organ systems, and kidney-related diseases are no exception. An increasing number of individuals are now focusing on the bidirectional relationship between gut microbiota and kidney transplantation complications. Low kidney function can result in intestinal microbiota imbalance, and an imbalance in the microecological environment can further accelerate the progression of complications in kidney transplant recipients. However, prophylactic drugs can be administered to regulate the microbiota of patients undergoing kidney transplantation to reduce the occurrence and progression of subsequent complications. However, this question does not yet have a definitive answer.

The results of the human microbiome project are still being reported, and the roles of treatments with prebiotics and gut microbiota transplantation in human health and disease prevention remain unclear. However, it is conceivable that in the near future, we will use probiotics or other drugs that regulate gut microecology instead of traditional drugs that exert significant adverse effects. A

TABLE 5 Association between gut microbiota and acute rejection in kidney transplantation recipients: evidence from clinical studies.

Reference	Population (mean age)	Key findings	Outcomes
Lee et al. (2014)	Fecal samples of 26 KTRs (50% men, mean age: 56 ± 8 years). Median time after transplantation of KTRs was 3 months.	↓*Clostridiales*,*Bacteroidales*,*Ruminococcus*, *Bacteroides*,*Lachnospiraceae*,*Blautia*, *Eubacterium dolichum*; ↑*Lactobacillales*, *Enterococcus*, *Anaerofilum*, and *Clostridium tertium*.	Distinct microbiota structures were observed in allograft recipients with post-transplantation *Enterococcus* UTI.
Wang et al. (2021b)	Fecal samples were collected from 24 ARs (mean age: 35 years) and 29 KTRs (mean age: 38 years). The median time after the transplantation of KTRs was 3 months.	↓ Firmicutes, Bacteroidetes; ↑*Proteobacteria*, *Actinobacteria*, *Lacto- bacillales*, *Clostridia*, *Clostridiales*, and *Faecalibacterium*	Provide a foundation for further investigation on the role of gut microbiota in AR after kidney transplantation.
Carron et al. (2019)	Fecal samples of 934 individuals were collected: 146 study participants (mean age: 50 years) and 788 KTRs (mean age: 52.4 years). The median time after the transplantation of KTRs was 3 months.	The circulating sCD14 concentrations decreased significantly at 1 year after transplantation; significant decrease in biologically active LPS; circulating iFABP concentrations decreased significantly at 1 year after transplantation; circulating iFABP concentrations at 1 year after transplantation were inversely correlated with the GFR	Individuals with higher pre-transplantation sCD14 levels are less likely to develop AR after transplantation

"↓", an upward trend in the abundance of gut microbiota and "↑", a downward trend in the abundance of gut microbiota.

clinical team used probiotics for the postoperative treatment of patients undergoing kidney transplantation and reported a significant decrease in the incidence of diarrhea and intestinal complications. However, the number of test groups was small, and the results are not generally representative. More basic clinical studies are required to explore this topic.

It is necessary to evaluate whether this preventive treatment can accurately control the patient's condition and reduce the occurrence and progression of complications, and whether early microorganisms in the body interact with drugs including antibiotics and prebiotics. Conversely, *Clostridiales* has been reported as a potential mechanistic biomarker in different irritable bowel syndrome subtypes and represents a potential therapeutic target. We also identified a potential mechanistic biomarker that could be used as an early monitoring indicator of postoperative complications or disease development in patients undergoing kidney transplantation.

Author contributions

JXY, FFH, JS, ZZ, YMW and JJY drafted the manuscript, JXY, FFH, JS and ZZ wrote the manuscript. All authors read, edited and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Psychobiotics and fecal microbial transplantation for autism and attention-deficit/hyperactivity disorder: microbiome modulation and therapeutic mechanisms

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Dysbiosis of the gut microbiome is thought to be the developmental origins of the host's health and disease through the microbiota-gut-brain (MGB) axis: such as immune-mediated, metabolic, neurodegenerative, and neurodevelopmental diseases. Autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) are common neurodevelopmental disorders, and growing evidence indicates the contribution of the gut microbiome changes and imbalances to these conditions, pointing to the importance of considering the MGB axis in their treatment. This review summarizes the general knowledge of gut microbial colonization and development in early life and its role in the pathogenesis of ASD/ADHD, highlighting a promising therapeutic approach for ASD/ADHD through modulation of the gut microbiome using psychobiotics (probiotics that positively affect neurological function and can be applied for the treatment of psychiatric diseases) and fecal microbial transplantation (FMT).

KEYWORDS

autism spectrum disorder, attention-deficit/hyperactivity disorder, psychobiotics, fecal microbial transplantation, gut microbiome

1 Introduction

Two childhood-onset neurodevelopmental disorders that have been linked to gut microbial dysbiosis are autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) (Prosperi et al., 2022). ASD and ADHD are highly prevalent, commonly co-occur with each other, and share overlapping symptoms (Bundgaard-Nielsen et al., 2023). Both have been found to be related to environmental exposures in early life, like endocrine disrupting chemicals (EDC), and EDCs have been suggested to induce microbiota changes through the gut-brain-microbiota axis conferring susceptibility to neurodevelopmental disorders (Ramírez et al., 2022). In the early twenty-first century, many papers highlighted the connection between the brain and gut. The term "psychobiotics", defined as the probiotic bacteria-derived molecules exerting psychological potential to support mental health by targeting microbial interventions, was newly coined in 2013. The therapeutic potential of psychobiotics ranges from mood changes and anxiety to neurodegenerative diseases and neurodevelopmental disorders (Sharma et al., 2021).

ASD is characterized by impairments in behavioral domains such as social communication, restricted interests, and repetitive behavior. Although the high heritability of ASD suggests that genetics is a key factor in its pathogenesis (Tick et al., 2016). Gene-environment interactions have also been reported to be substantially involved, with estimates that more than 50% of neurobiology is driven by non-heritable factors (Cheroni et al., 2020). ADHD is defined as a neurodevelopmental disorder, and it is described by hyperactivity, inattention, and excessive impulsiveness. The pathogenesis of ADHD is complex, and representative factors have been investigated including genetic, environmental, and perinatal damage-associated factors (Kalenik et al., 2021). ASD and ADHD are highly comorbid, where 20-50% children with ADHD meet the criteria for ASD and 30-80% of ASD meet the criteria for ADHD (Rommelse et al., 2010). High co-occurrence rate challenges differential diagnosis and also worsens symptom severity and prognosis (Zhou et al., 2023). Based on this high comorbidity, extensive research has been conducted on the overlapping genetic factors and shared biological underpinnings of ASD and ADHD (Liu et al., 2020).

Here, we reviewed the literature on the influence of psychobiotics and fecal microbial transplantation (FMT) on the gut microbiome and behaviors/gastrointestinal (GI) symptoms related to ASD and ADHD, and the relationship between neurodevelopmental disorders and psychobiotics has received considerable attention in recent years. There is a bidirectional interaction in the microbiota-gut-brain (MGB) axis, and its modulation exerts beneficial effects on brain activity and behavior as potential treatments (Bundgaard-Nielsen et al., 2020). In light of these considerations, the gut-brain axis is an attractive target for developing novel therapeutics, such as the use of probiotics, for neurodevelopmental disorders (Sarkar et al., 2016). The main subject of our review is the commonly known gut microbiome in ASD and ADHD individuals, and its impact on the symptoms of these neurodevelopmental disorders. And we will also discuss the theoretical basis of the correlations and the therapeutic possibility of psychobiotics and FMT on neurodevelopmental disorders (Figure 1).

To the best of our knowledge, no systematic review of randomized controlled trials (RCTs) has been conducted so far, and there are relatively few clinical studies identifying the therapeutic effects of psychobiotics and FMT on ASD and

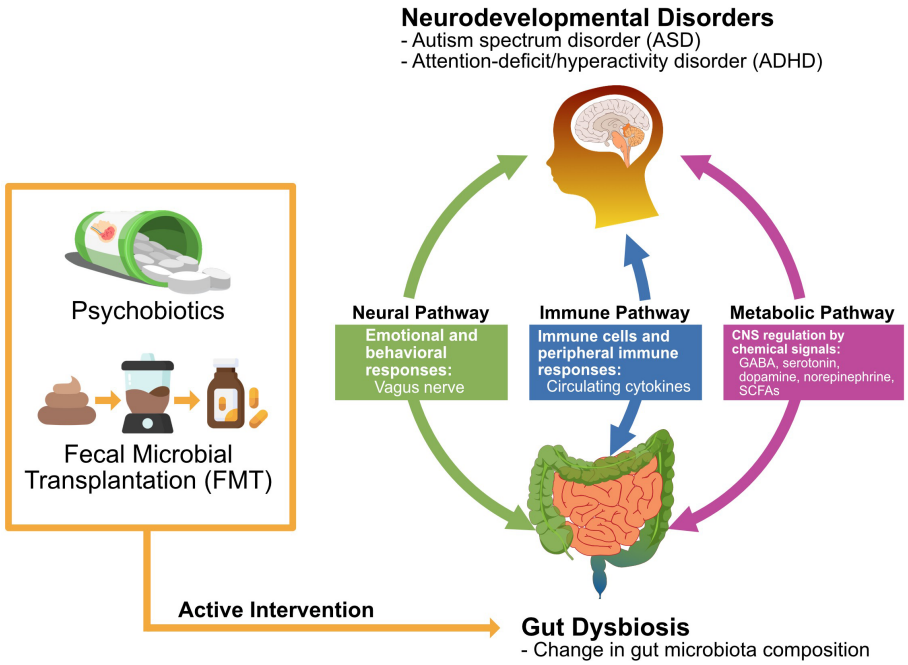

FIGURE 1
Action mechanisms by which psychobiotics and fecal microbial transplantation exert the potential therapeutic effect on ASD and ADHD. Abbreviations: γ-aminobutyric acid (GABA), short-chain fatty acids (SCFAs).

ADHD. Therefore, we thoroughly summarized the latest reports on potential therapeutic mechanisms and promising perspectives, in addition to observed changes in the gut microbiome composition and metabolites. Moreover, this review presents directions for future treatments that could be employed to directly manipulate gut microbiota during early life stages in humans to prevent the development of such diseases.

2 Role of gut microbiome axis

2.1 Early-life gut microbial colonization and development

Human microbial colonization begins in the fetus and continues to develop and modulate species abundance for approximately three years until the gut microbiome becomes adult-like (Figure 2A) (Arrieta et al., 2014; Senn et al., 2020). There is increasing evidence that the gut microbiota and its byproducts could play pivotal functions in the immune system maturation, development, and behavior of the host throughout the life cycle (Erkosar et al., 2013).

Microbiota development follows typical timely changes and the interplay between the gut microbiome and the rest of the human body that have been analyzed through metagenomics studies and recent strain-level profiling (Bäckhed et al., 2015; Selma-Royo et al., 2020). Especially microbial colonization of the newborn period is a critical process that affects long-term neurological outcomes and later-life health (Stiemsma and Michels, 2018; Niemarkt et al., 2019; Korpela et al., 2020).

2.2 Gut-brain axis and bidirectional communication

The past 15 years have seen the emergence of the microbiota as one of the critical regulators of gut-brain function through a complex network of signaling pathways, which has led to the appreciation of the importance of a distinct MGB axis (Cryan et al., 2019). There are various bidirectional communicating pathways between the gut microbiome and the brain, which include vagus nerve (VN), immunity with tryptophan metabolism, endocrine system, and enteric nervous system (ENS) with diverse bacterial byproducts, such as peptidoglycans, short-chain fatty acids (SCFAs), and branched-chain amino acids (Jena et al., 2020).

Bidirectional communication between the microbiota and the host through the gut-brain axis is an essential pathway for accessing the synergetic mechanism to modulate the host brain and behavior (Dinan and Cryan, 2017; Ronan et al., 2021). Studies to identify and examine the MGB axis have used different yet complementary microbiota interventions, including germ-free rodents, antibiotic-induced depletion, prebiotic/probiotic supplementation, gastrointestinal infection, and FMT (Cryan et al., 2019). Top-down signaling influences the motor, sensory, and secretory functions of the gastrointestinal tract *via* the efferent fibers of the VN. Bottom-up communication affects the function of the brain,

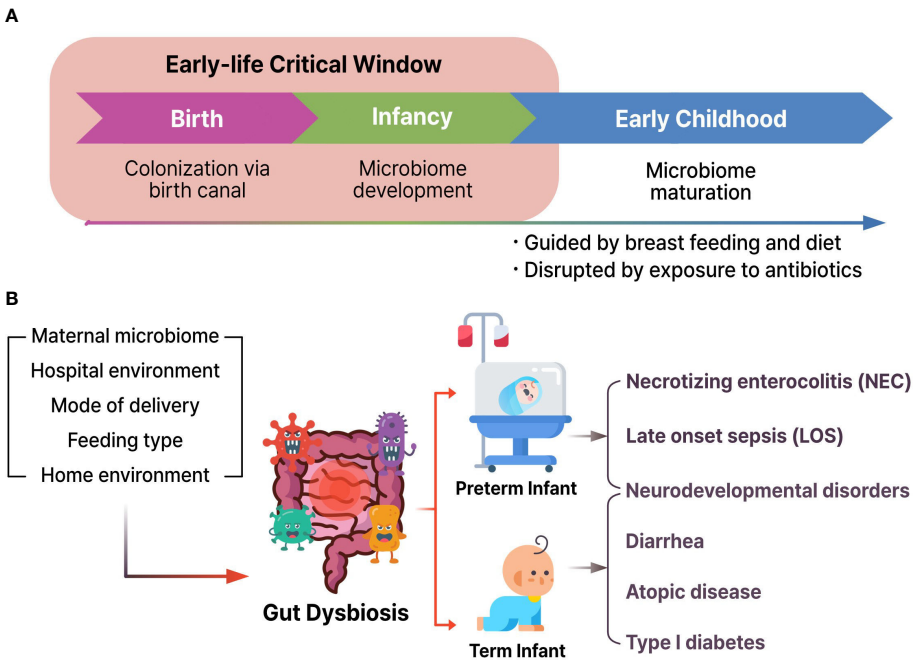

FIGURE 2
(A) Microbial colonization and development from birth to child. **(B)** Various environmental factors could induce gut dysbiosis and result in the pathogenesis of necrotizing enterocolitis, late onset sepsis, neurodevelopmental disorders, diarrhea, atopic disease, and type I diabetes.

especially the amygdala and hypothalamus, *via* the afferent vagal fibers.

3 Neurodevelopmental disorders and gut microbiome

3.1 Gut dysbiosis in neurodevelopmental disorders

Dysbiosis may play a role in the etiology and development of neurodevelopment disorders (Nagpal and Cryan, 2021). Earlier reports confirmed that the index of gut microbial α-diversity of 1-year-old children showed a close correlation with cognitive functions at 2-year-old (Carlson et al., 2018). Furthermore, additional human studies have suggested that the first year of life is the most authoritative period in human cognitive development (Figure 2B). These results indicate that the occurrence risk of diseases might be increased during fetal development and early life stages.

3.2 ASD and gut microbiome

In general, ASD patients frequently coincidently have various gastrointestinal disorders, and it might be due to the tight association between gut microbial disturbances and mental health. Moreover, these connections in ASD individuals have suggested increased gut permeability, called "leaky gut". If gut permeability arose, the gut barrier allows to cross the bacterial metabolites into animal's body, and it could negatively affect neurodevelopment during early childhood through the gut-brain axis. Fowlie and colleagues demonstrated that psychobiotics treatment in ASD patients has a potential to relieve ASD symptoms by modulation of the gut microbiome (Fowlie et al., 2018).

An important feature of ASD is its marked comorbidity with gastrointestinal symptoms. A high rate of ASD patients, ranging from 9% to 90%, report comorbid gastrointestinal symptoms such as constipation, abdominal pain, diarrhea, gas, and vomiting (Vuong and Hsiao, 2017). Moreover, the observed GI disturbances were strongly correlated with ASD severity. These GI problems suggest that the intestine plays an important role in ASD pathogenesis. Previous studies on microbiota composition in patients with ASD have shown highly heterogeneous results between studies, but the majority of them have found that the overall microbiota composition of ASD cases is different from that of the controls (Bundgaard-Nielsen et al., 2020). However, no specific bacteria are consistently associated with ASD diagnosis or severity in the literature.

Specifically for ASD, the specific composition of microbial taxa in the human gut, including *Firmicutes*/*Bacteroidetes* ratio, is reported to differ between the control and patient groups, with *Fusobacteria* and *Verrucomicrobia* abundances being lower in the patient group (De Angelis et al., 2013). Microbial taxa have specific roles in the production of substances, such as SCFAs, which are reported to have diverse neurobiological correlations in the context of the MGB axis, and 4-ethylphenylsulfate, which is a dietary tyrosine metabolite that is considered to induce ADS-like behavior among many others (Hsiao et al., 2013; Dalile et al., 2019). In an experiment with genetically engineered mice, maternal interleukin-17α secreted by Th17 cells was observed to induce behavioral and cortical issues in their offspring, suggesting a possible role for the cytokine receptor interleukin-17α in the modulation of ASD (Choi et al., 2016).

3.3 ADHD and gut microbiome

ADHD is a common childhood-onset neurodevelopmental disorder that persists into adulthood, with a worldwide prevalence of 5% (Polanczyk et al., 2007). ADHD is distinguished by symptom domains of inattention, hyperactivity, and/or impulsivity. Although some children might not reach the threshold of full diagnosis, ADHD traits are continuously distributed throughout the population (Brikell et al., 2021). ADHD is a complex genetic disorder with a high heritability rate of 76%; however, this estimate encompasses gene by environment interaction and such interactions may account for much of the etiology of ADHD (Faraone and Larsson, 2019). Diverse environmental factors, including perinatal factors (prematurity, low birth weight) and psychosocial determinants (adoption, child neglect), have been reported as reasonable factors to ADHD.

However, there are few reports on the role of the gut microbiome in ADHD patients. A recent systematic review found that all six included studies had distinct taxon findings between patients with ADHD and healthy controls. However, results varied between studies, and there was minimal consensus on which bacterial taxa correlated most with ADHD (Sukmajaya et al., 2021).

In the case of ADHD, there have been multiple attempts to find relationships between clinical features and differences of this disorder and healthy samples based on the gut-brain axis concept. A study that attempted to associate gut microbiota and plasma cytokine levels with ADHD showed a higher abundance of three genera (*Agathobacter*, *Anaerostipes*, and *Lachnospira*) and decreased levels of TNF-α in the ADHD group compared with that of the control group (Wang et al., 2022). However, the studies conducted so far have not been able to show clear relationships between microbial taxa and ADHD, compared to the relatively more established ASD studies (Bundgaard-Nielsen et al., 2020). Considering the relative paucity of scientific literature, more research efforts to clarify the possible relationship between ADHD and the gut-brain axis are required.

4 Psychobiotics: potential roles on MGB axis as treatment target

Psychobiotics, next-generation probiotics (NGPs) for the brain, are a special class of probiotics that positively affect neurological

function and can be applied for the treatment of psychiatric diseases (Cheng et al., 2019). They are different from typical probiotics in their ability to affect the gut-brain axis by modulating microbial composition, immune activation, VN signaling, and production of neuroactive metabolites, such as neurotransmitters, cytokines, SCFAs, and enteroendocrine hormones (Bermúdez-Humarán et al., 2019; Kwak et al., 2021; Morais et al., 2021). Considering this potential, psychobiotics have a wide-ranging application spectrum from stress alleviation to being an adjuvant in the treatment of diverse neuro-developmental and degenerative diseases (ADHD, ASD, Parkinson's disease, and Alzheimer's disease). Generally, conventional psychobiotic bacteria belong to the family *Lactobacilli*, and *Bifidobacteria* (Sharma et al., 2021). A summarized overview of clinical studies on the use of psychobiotics and FMT in individuals with ASD or ADHD is shown in Table 1.

TABLE 1 Treatment trials with psychobiotics and fecal microbial transportation in ASD and ADHD patients[1].

Types	Population	Method	Strain name	Dose	Main effects	Reference
ASD	85 patients (3–6 years)	Probiotics	*S. thermophilus, B. breve, B. lungum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii*	9.0×10^{11} CFU in 1st month 4.5×10^{11} CFU in following	ASD symptoms ↓ Inflammation, ↓ Oxidative stress ↓	(Santocchi et al., 2020)
ASD	131 patients (4–11 years)	Probiotics	*L. plantarum* PS128	3.0×10^{10} CFU BW < 30 kg 6.0×10^{10} CFU BW > 30 kg	Improve intestine ASD symptoms ↓	(Mensi et al., 2021)
ASD	35 patients (3–25 years)	Probiotics	*L. plantarum* PS128	6.0×10^{10} CFU	ASD symptoms ↓ *Veillonella*, ↑ *Streptococcus* ↑	(Kong et al., 2021)
ASD	80 patients (7–15 years)	Probiotics	*L. plantarum* PS128	3.0×10^{10} CFU	ASD symptoms ↓	(Liu et al., 2019)
ASD	22 patients (4–10 years)	Probiotics	*L. acidophilus*	5.0×10^{9} CFU	ASD symptoms ↓ Urinary arabinitol ↓	(Kałużna-Czaplińska and Błaszczyk, 2012)
ASD	30 patients (5–9 years)	Probiotics	*B. longum, L. rhamnosus, L. acidophilus*	5.0×10^{8} CFU	ASD symptoms ↓ Gut symptoms ↓ *Bifidobacterium* ↑ *Lactobacillus* ↑	(Shaaban et al., 2018)
ASD	1 patient (12 years)	Probiotics	*B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, L. delbrueckii, S. thermophilus, S. salivarius*	9.0×10^{10} CFU in *Bifido.* 8.0×10^{10} CFU in *Lacto.* 2.0×10^{11} CFU in *Strepto.*	ASD symptoms ↓ Gut symptoms ↓	(Grossi et al., 2016)
ASD	41 patients (4–11 years)	Prebiotics	Galactooligosaccharide	1.8 g for 6 months	Behavior improved *Bifidobacterium* ↑	(Grimaldi et al., 2018)
ASD	8 patients (2–11 years)	Synbiotics	*B. infantis* Bovine colostrum	2.0×10^{11} CFU 5.0– 10.0 g/day	Behavior improved Gut symptoms ↓	(Sanctuary et al., 2019)
ASD	13 patients (3–12 years)	Probiotics	*L. casei, L. plantarum, L. acidophilus, L. delbrueckii B. lungum, B. infantis, B. breve, S. thermophilus*	1.8×10^{6} – 3.2×10^{6} CFU	Behavior improved Gut symptoms ↓	(Arnold et al., 2019)
ASD	61 patients (2–16 years)	Probiotics	*L. fermentum, L. plantarum, L. salivarius* DSM 22	1.0×10^{10} CFU	Behavior improved	(Guidetti et al., 2022)

(Continued)

TABLE 1 Continued

Types	Population	Method	Strain name	Dose	Main effects	Reference
					Gut symptoms ↓	
ASD	18 children (7–17 years)	FMT	Standardized human gut microbiota (Hamilton, Weingarden et al., 2012)	2.5×10^{12} cells/day	ASD symptoms ↓	(Kang et al., 2017)
ASD	18 children (7–17 years)	FMT	Standardized human gut microbiota (Hamilton, Weingarden et al., 2012)	2.5×10^{12} cells/day	ASD symptoms ↓ Improved behavior	(Kang et al., 2019)
ASD	40 children (3–17 years)	FMT	Standardized human gut microbiota (Hamilton, Weingarden et al., 2012)	2.5×10^{12} cells/day	ASD symptoms ↓ Improved behavior	(Li et al., 2021)
ADHD	132 infants (2–13 years)	Probiotics	*L. rhamnosus* GG	1.0×10^{10} CFU	ADHD symptom ↓	(Pärtty et al., 2015)
ADHD	35 patients (4–17 years)	Probiotics	*L. rhamnosus* GG	1.0×10^{10} CFU	Improve QoL Improve cytokines	(Kumperscak et al., 2020)
ADHD	30 patients (4–16 years)	Probiotics	*B. bifidum* Bf-688	5.0×10^{9} CFU	ADHD symptom ↓ Weight gain ↑	(Wang et al., 2022)
ADHD	66 patients (5–55 years)	Synbiotics	*L. mesenteroides, L. paracasei, L. plantarum* B-glucan, inulin, pectin, starch	4.0×10^{11} CFU 2.5 g of prebiotics	ADHD symptom ↓	(Skott et al., 2020)
ADHD	38 patients (6–12 years)	Probiotics	*B. subtilis, B. bifidum, B. breve, B. infantis, B. longum, L. acidophilus, L. delbrueckii, L. casei, L. plantarum L. lactis, L. salivarius, S. thermophiles*	2.0×10^{9} CFU	ADHD symptom ↓	(Ghanaatgar et al., 2022)
ADHD	34 patients (8–12 years)	Probiotics	*L. reuteri, L. acidophilus, L. fermentum, B. bifidum*	8.0×10^{9} CFU	ADHD symptoms ↓ Inflammation ↓ Oxidative stress ↓	(Sepehrmanesh et al., 2021)
ADHD	1 patient (22 years)	FMT	Healthy doner's microbiota	Not applicable	ADHD symptom ↓ *F. prausnitzii* ↑ *B. longum* ↓	(Hooi et al., 2022)

[1]Abbreviations: autism spectrum disorders (ASD), attention deficit/hyperactivity disorder (ADHD), fecal microbial transplantation (FMT).

4.1 Therapeutic mechanisms/effects on ASD

Gut dysbiosis in ASD has been reported in numerous studies (Kho and Lal, 2018). Patients with ASD possess significantly altered gut microbiota, resulting in GI symptoms. When dysbiosis occurs in disorders such as irritable bowel disease and ASD, the psychobiotics would help the gut microbiota return to normal levels and have positive effects on psychiatric diseases. Consequently, various studies demonstrated that the use of psychobiotics for ASD individuals suffering from GI disorders would be a supplementary therapeutic method (Zheng Y et al., 2020).

4.1.1 Shift of gut dysbiosis toward eubiosis

4.1.1.1 Affecting the gut microbiome population

One study found reduced D-arabinitol levels, a metabolite of *Candida* species, in the urine of children with ASD after probiotic supplementation (Shaw et al., 1995). This result suggests that probiotics may prevent gastrointestinal colonization by *Candida* species. In another study, the levels of *Bifidobacterium* (known as beneficial bacteria, such as *Lactobacillus* species) were significantly lower in the stool of children with ASD (Shaaban et al., 2018). After probiotic supplementation, there was a significant increase in the colony counts of *Bifidobacterium* and *Lactobacillus* with significant improvement in the severity of ASD and gastrointestinal symptoms. A study in 2015 reported the effect of mixed probiotic

administration on gut microbiota composition in children with ASD (Tomova et al., 2015). The abundance of *Clostridia* and *Desulfovibrio* and *Bacteroidetes*/*Firmicutes* ratio were related to the severity of ASD and gastrointestinal symptoms. After probiotics treatment, the amount of *Firmicutes* significantly decreased, which resulted in an increase in the *Bacteroidetes*/*Firmicutes* ratio to a level similar to that observed in healthy children, *Bifidobacterium* increased, and *Desulfovibrio* decreased significantly. Moreover, a study of a mixture of *Lactobacillus* spp. and *Bifidobacterium* spp. in two different rodent ASD models indicated that a probiotic mixture could improve social behavioral symptoms by modulating the gut microbial population (Mintál et al., 2022).

4.1.2 Anti-inflammation and immunomodulation

4.1.2.1 Psychobiotics as immunomodulators

Psychobiotics have a potential to not only reconstruct the gut barrier function by resisting harmful bacteria, but also exert an immunomodulatory effect by reducing circulating hormones and pro-inflammatory cytokines in serum. The gut microbiota has been demonstrated to serve as a regulator of intestinal, systemic, and CNS resident immune cell function (Zheng D et al., 2020). Gut microbiota can communicate with the CNS by regulating intestinal and peripheral immune cells and peripheral immune responses *via* circulating cytokines (Arrieta and Finlay, 2012).

4.1.2.2 Psychobiotics reduce inflammation

Any peripheral inflammatory event induces VN to cause the suppression of the release of proinflammatory cytokines from intestinal macrophages (Daliri et al., 2016). Probiotics reduce gut inflammation through various mechanisms, such as reducing inflammatory cytokines and other immunomodulatory effects. For example, anti-inflammatory cytokines (IL-4 and IL-10) and proinflammatory cytokines (TNF-a, IL-1b, IL-2, IL-6, IL-8, IL-12, IL-17, and IL-18) are significantly changed by *Lactobacillus rhamnosus GG* (LGG) (Miyazawa et al., 2015; Cicenia et al., 2016; Fong et al., 2016; Wang et al., 2016; Aoki-Yoshida et al., 2017; Clarke, 2018; Cai et al., 2019).

4.1.2.3 Inflammatory cytokines change in ASD

In clinical studies, Tomova et al. found that TNF-α levels were strongly correlated with GI symptoms and showed a trend toward correlation with ASD severity (Tomova et al., 2015). Probiotic supplementation significantly decreased TNF-α levels in the feces of children with ASD. Similar to this study, Sanctuary demonstrated that psychobiotic supplementation could reduce the intracellular expression of certain cytokines in CD4+ T cells (Sanctuary et al., 2019). The frequency of CD4+/IL-13+ T cells was significantly lower after the treatment.

4.1.2.4 Animal studies on the anti-inflammatory effects of psychobiotics

Adıgüzel et al. demonstrated that dietary treatment with multispecies probiotics formulations (*Sptreptococcus thermophilus*, *Bifidobacterium breve*, *B. animalis*, *Lactobacillus helveticus*, *L. plantarum*, *L. acidophilus*, and *L. paracacei*) attenuated the inflammatory responses in a VPA-induced rodent ASD model. In particular, this study also showed that psychobiotic treatment decreased serum pro-inflammatory cytokine, IL-6 levels, and increased anti-inflammatory cytokine, IL-10 levels, with the improved status of diverse behavior tests including social interaction, anxiety, and repetitive behaviors (Adıgüzel et al., 2022). Alonazi also demonstrated that dietary psychobiotic supplementation could reduce levels of various serum inflammatory cytokines, such as IL-1β, IL-8, IL-10, and IFN-γ, in an ASD rat model induced by a neurotoxic dose of propionic acid (Alonazi et al., 2022).

4.1.3 Neural pathway and chemical signaling

4.1.3.1 Changing microbial signals (neuroendocrine signaling)

The gut microbiota influences the brain directly through neural pathways, including the VN and ENS. The VN connects the ENS and CNS and it could be activated by cytokines, which could be modulated by bacteria, and byproducts from bacteria including endotoxins and peptides. In particular, the neuropeptide could be sensed by receptors associated with dendritic cells in the gut, which could transfer the signals to the brain (Perez-Burgos et al., 2013). Psychobiotics modulate CNS-related behaviors through the VN pathway and the physiological response of various metabolites, including SCFAs, enteroendocrine hormones, cytokines, and neurotransmitters (Bravo et al., 2011; Dinan et al., 2014; Sgritta et al., 2019).

4.1.3.2 Hormones and metabolic changes

The levels of oxytocin and DHEA-S, which have been considered to be etiologies of ASD, were significantly lower in the plasma of children with ASD in a clinical study, and there was a trend towards a correlation between decreased DHEA-S levels and a lower *Bacteroidetes*/*Firmicutes* ratio which increased after probiotic implementation (Tomova et al., 2015). According to Grimaldi, increases in butyrate production, potentially positively affecting ASD, were detected in children with ASD following exclusion diets (Nankova et al., 2014; Grimaldi et al., 2018). Additionally, lower levels of amino acids (isoleucine, leucine, valine, alanine, and glutamine) and lactate were detected in the B-GOS group. The presence of amino acids in feces is associated with problems in gut barrier function (Marchesi et al., 2007).

4.1.3.3 Gut microbiome modulation and GABA metabolism

A study of psychobiotics in a rodent ASD model, which was induced by oral propionic acid ingestion, proposed that *Lactobacillus bulgaricus* and *Bifidobacterium infantis* could ameliorate glutamate excitotoxicity, a major autistic feature in this model. The therapeutic effect of these psychobiotics might be due to the reduction of oxidative stress, restoration of the depleted GABA signaling pathway, and upregulation of the GABA receptor's gene expression (Bin-Khattaf et al., 2022). Ingestion of *Lactobacillus rhamnosus* could connect bidirectional communication of the

gut-brain axis, and it could regulate emotional behaviors by controlling the GABA receptor expression in the VN (Bravo et al., 2011). In 2018, the specific bacterial species of ASD were identified in *Shank3* knock-out mice, and this study suggested that oral *Lactobacillus reuteri* ingestion could decrease repetitive behaviors by up-regulation of the γ-aminobutyric acid (GABA)-related metabolism (Tabouy et al., 2018).

4.2 Therapeutic mechanisms/effects on ADHD

The etiology of ADHD is multifactorial. However, emerging research has shown the involvement of modulation in the gut microbiome and its promising effect on the clinical course of ADHD (Kalenik et al., 2021). In general, the effect of probiotic supplementation could act in both direct and indirect ways on ADHD, and ADHD-suffering children have higher GI severity index grade than healthy children (Ming et al., 2018). These GI symptoms can be relieved by adjustment of the gut microbial community *via* probiotic administration, however, Rianda's randomized trial demonstrated that only one out of seven studies showed a positive effect of probiotics on cognitive function (Rianda et al., 2019).

4.2.1 Neurotransmitters and metabolites

4.2.1.1 Microbiome producing neurotransmitters

Bacteria can synthesize and respond to hormones and neurotransmitters. *Lactobacillus* species produce acetylcholine and GABA, *Bifidobacterium* species produce GABA, *Escherichia* produces norepinephrine, serotonin, and dopamine, *Streptococcus* and *Enterococcus* produce serotonin, and *Bacillus* species produce norepinephrine and dopamine (Galland, 2014). Other bacterial strains (*Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Escherichia coli*, *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei*) produce serotonin (O'Mahony et al., 2015).

4.2.1.2 Positive effects on dopaminergic and noradrenergic system

Various studies have suggested the dopamine hypothesis, which indicates that the enteric neurotransmitter release by nutrient intake could affect brain health and responses. And *Bacillus*, one of the representative bacteria to modulate the dopaminergic system, is known for its ability to produce dopamine and noradrenaline directly in the GI tract (Satti et al., 2023). Dysregulated dopaminergic and noradrenergic neurotransmission has been widely implicated in the pathophysiology of ADHD, and dopamine and norepinephrine play essential roles in behavioral, cognitive, and affective functions (Del Campo et al., 2011). In a study using patients with ADHD, *Bifidobacterium* was increased in patients with ADHD, which was linked with the enzyme involved in the dopamine precursor (phenylalanine) synthesis (Aarts et al., 2017)

4.2.1.3 Synthesis of serotonin beyond BBB

Serotonin also plays a role in ADHD pathogenesis, however, it affects brain function not directly, but *via* the nervous system (Banerjee and Nandagopal, 2015; Hou et al., 2018). On the other hand, gut microbiota directly act a biological role on the brain by modulation tryptophan's peripheral availability because tryptophan can cross the BBB and affect serotonin synthesis in CNS (Richard et al., 2009; Schwarcz and Stone, 2017).

4.2.1.4 GABAergic system and GABA production

A recent experimental study has demonstrated that *Lactobacillus rhamnosus* regulates, *via* the VN, emotional behavior and the central GABAergic system, which is also associated with neuropsychiatric disorders (Enticott et al., 2010). According to Pärtty, the early supplementation of *Lactobacillus rhamnosus* GG decreases the risk of developing ADHD, and Liang-Jen Wang suggested that oral probiotic *Bifidobacterium bifidum* (Bf-688) improves the clinical symptoms of ADHD. In addition, food supplement treatments containing *Lactobacillus acidophilus* and *Bifidobacterium* improve the self-control and attention of children with ADHD (Pärtty et al., 2015; Wang et al., 2022). These results are thought to be due to the role of *Lactobacillus* and *Bifidobacterium* as producers of GABA, which is known to decrease in patients with ADHD (Yunes et al., 2016).

4.2.2 Intermediate substances and metabolites

4.2.2.1 Vagus nerve

The VN is the longest cranial nerve in the body, and it delivers electronic signals from the body (lungs, liver, heart, GI tract) to the brain through sensory fibers. And this connection administers the GI tract's function *via* the metabolites from intestinal microorganisms. The VN is involved in functions such as mood control, immune response, and GI tract function *via* intestinal permeability and enteric reflex and influences the hypothalamic-pituitary-adrenal axis. Vagal afferent fibers sense microbiota signals indirectly through the diffusion of bacterial compounds, metabolites, or other cells located in the epithelium that relay luminal signals (Del Toro-Barbosa et al., 2020). The gut microbiome has the capacity to modulate the host's emotional and behavioral responses by acting on vagal afferents.

4.2.2.2 Short-chain fatty acids

Various host physiological metabolism could be regulated by SCFAs, in particular, gut barrier integrity, immune defense system, and lipid metabolism could be the major target of the SCFA. (Dalile et al., 2019). Moreover, SCFAs might directly influence neural function by reinforcing BBB integrity, modulating neurotransmission, influencing the levels of neurotrophic factors, and promoting memory consolidation. Increased evidence suggests a potential key role for SCFAs in gut-brain axis signaling (Silva et al., 2020). The ADHD group showed significantly lower concentrations of fecal acetate and butyrate than the control group, and various bacterial strains (*Bifidobacteria, L. salivarius, L. agilis, L. acidophilus, LGG, B. longum, B bifdum*, and *L. gasseri*) are known to increase SCFAs production (LeBlanc et al., 2017; Jung et al., 2022).

4.2.3 Immune pathway and anti-inflammation

4.2.3.1 Anti-inflammatory effects of probiotics

Lactobacillus rhamnosus GG is known to strengthen the gut permeability barrier by fortifying intestinal tight junctions, mucin layer thickness, and antigen-specific immunoglobulin A production (Asano et al., 2012). In particular, *L. rhamnosus GG* administrated participants showed a significant decrease in the serum levels of the pro-inflammatory cytokines (IL-6, IL-12 p70, and TNF-α). (Kumperscak et al., 2020).

5 FMT: rebuilding gut microecology

The definition of FMT is the transfer technique of a healthy donor's fecal specimen to the GI tract of a recipient patient to reestablish the normal gut microbiome. This technique has been focused in recent years because of the technical advances in metagenomics sequencing and the growing understanding of its function. FMT has been demonstrated to be able to reconstruct a normally functioning microbial community, making it an accepted therapy with biological plausibility. Considering the effect of FMT on the reorganization of gut microbiota, it is considered to have the potential for the treatment of neurodevelopmental diseases such as ASD through the interaction of the MGB axis. It is necessary to determine the optimal composition of the microbiome to be used for FMT by clarifying the structure or functional profile of the microbes associated with improved clinical outcomes (Zhuang et al., 2019). Bacterial diversity and health-associated functions, such as colonization resistance, can be restored using FMT. In addition to bioactive compounds, FMT is also a source of microbes, such as phages. These components come together in a symbiotic community, allowing better colonization of the GI tract (Goldenberg et al., 2018). In terms of the gut microbiota, FMT is considered an untargeted intervention.

5.1 Therapeutic mechanisms and their effects on ASD

5.1.1 Altering gut ecosystem

5.1.1.1 Bacterial diversity

FMT could serve as a protective treatment for reconstructing the gut microbiota at both the phylum and genus levels and has a therapeutic effect on ASD symptoms and gastrointestinal disorders (Li et al., 2021). A modified FMT protocol for children with ASD, termed microbiota transfer therapy, appears to be a promising approach to alter the gut microbiome and improve GI and behavioral symptoms of ASD (Kang et al., 2017; Kang et al., 2019). This protocol improved GI and ASD symptoms, and the microbiome persisted for two years after treatment, suggesting a long-term impact. Important changes in the gut microbiota at the end of treatment were observed during follow-ups, including significant increases in bacterial diversity and relative abundance of *Bifidobacteria* and *Prevotella*.

5.1.1.2 Engraftment of the donor microbiome

Li et al. showed that the gut microbial population of ASD children was altered by FMT with donor microbiota toward that of the healthy group. Especially, the FMT response significantly reduces the abundance of *Eubacterium coprostanoligenes* (Li et al., 2021). These data also indicated that decrement in the population of *Eubacterium coprostanoligenes* by FMT might be a curative technique for ASD symptoms and behaviors.

5.1.2 Modulating neurotransmitters

5.1.2.1 FMT alters the serum levels of neurotransmitters

Unlike probiotics, FMT refers to the transfer of the full spectrum of gut microbial communities containing more than 1,000 bacterial strains, and it might be more effective than psychobiotics in aspects of physiological regulation in the nervous system, endocrine system, and host behavior (Chen et al., 2022). In recent studies, FMT could exhibit a recovery effect on the serum levels of serotonin, GABA, and DA in the ASD cohort, which means that FMT might be an effective technique in regulating neurotransmitters *via* the MGB axis (Li et al., 2021). Moreover, FMT in the ASD cohort could decrease GABA and serotonin in serum, but the dopamine level was increased by FMT. It could be assumed that FMT may be an efficient approach to modulate neurotransmitter secretion for regulation of the central nerve *via* the MGB axis.

5.1.3 Regulating immune responses

5.1.3.1 Chemokines and microbiome

Alterations in the gut microbiota composition after FMT could significantly improve behavioral impairments and regulate immune responses in ASD. Chen and colleagues demonstrated that treatment using FMT with *in vitro* cultured healthy donor's intestinal microbiota had a positive effect on ASD symptoms in mouse ASD model (Chen et al., 2020). They observed amelioration of anxiety actions and repetitive performance with lower serum levels of metabolites, such as GRO-α and MIP-1α, and a conversely higher level of MCP-3, RANTES, and Eotaxin. Additionally, family or genus levels of S24-7, *Clostridiaceae*, *Prevotella*, and *Candidatus Arthromitus* were key microbial taxa in FMT treatment, and serum levels of chemokines were related to the relative abundance of these taxa.

5.1.3.2 Original donor vs *in vitro* cultured

In this study, both original donor microbiota transplantation and cultured microbiota transplantation improved behavioral abnormalities and chemokine disorders in an ASD mouse model and were effective in the modification of several key differential taxa in the gut microbial composition (Chen et al., 2020). These results of cultured microbiota transplantation suggest the possibility of using "donor-free FMT" and regulating the donor gut microbiota structure before transplantation during *in vitro* culture. The batch methods are fast, easy, and repeatable culturing techniques.

5.2 Therapeutic mechanisms/effects on ADHD

5.2.1 Neuroprotective effects of the transplanted microbiome

A case report provides preliminary evidence regarding the use of FMT in a patient with *C. difficile* infection and ADHD. The authors

suggested that gut microbiome modulation, particularly the gain or loss of specific microbial species and pathways involving the metabolism of SCFAs, tryptophan, and GABA, may merit further exploration as a potential therapeutic strategy for ADHD (Hooi et al., 2022). Among bacteria engrafted through FMT, *F. prausnitzii* may reduce neuroinflammation and alleviate ADHD symptoms. *F. prausnitzii* exhibits anti-inflammatory effects by increasing anti-inflammatory cytokines and decreasing inflammatory cytokines that promote neuroinflammation and development of ADHD. *L. ruminis* possesses genes contributing to the pentose phosphate pathway, which contributes to SCFA production. Engraftment of Lactobacillus genus may exert neuroprotection by producing anti-inflammatory SCFAs. (Basen and Kurrer, 2021).

6 Future perspectives and concluding remarks

Although the application of pre- and probiotics as psychobiotics remains promising, it is feasible that the effect of psychobiotics will be decreased over time due to the significant influence of environmental reasons occurred in child development (Grimaldi et al., 2018). Further studies should be needed to address the drug administration timing, the effect of different strain combinations, safety, and efficacy of probiotics. Furthermore, the novel therapeutic functions of the psychobiotics and commensal bacteria will be investigated in synthetic biology fields (Bin-Khattaf et al., 2022). For example, Korpela proposed that oral-fecal transplantation with diluted fecal samples from the maternal gut microbiome could restore normal gut microbiota in Cesarean-born infants (Korpela et al., 2020). Furthermore, a study demonstrated that using *E. coli* native to the target murine host to knock-in specific functions and apply them back to the host enabled the perpetual engraftment of transgenic bacteria in the intestine, which was demonstrated until the transformation stage of human borne *E. coli* (Asano et al., 2012). As such, various studies have focused on fortifying the modulating effects on the gut microbiome *via* adjustment at the molecular level.

The use of probiotics is feasible in children, and short-term supplementation has been shown to be safe. However, the long-term effects of repeated applications on the gut microbiome and the safety concerns of treatment are unknown. The actual efficacy of FMT has been proved by various studies using diverse animal models. However, Safety is the most important aspect in the FMT study because most ASD patients are children. In previous studies, the oral ingestion of human fecal suspensions was considered an unpleasant experience for patients and might cause side effects, including extra ingestion with acid inhibitors. As it has been known that a colon-release capsule coated with acid-resistant hydroxypropyl cellulose is the best formulation for patients (Kang et al., 2019).

This review summarizes the current knowledge on the positive effects and potential pathways of promising therapeutic interventions, including psychobiotic supplementation and modulation of the gut microbiome to improve the GI and behavioral symptoms of patients with ASD or ADHD. Development of the gut microbiome in early life plays an important role in the overall well-being of humans. Numerous studies have demonstrated that early alterations in the gut microbiome are closely related to neurodevelopmental disorders, such as ASD and ADHD. Nevertheless, the ambiguous and equivocal evidence of clinical studies makes it difficult to believe the therapeutic method targeting the MGB axis. To better understand the role of the gut microbiome in heterogeneous and complex ASD/ADHD pathogenesis, double-blind, randomized, controlled trials and treatments tailored to individual characteristics and the host microbiome are recommended. In particular, the process of intestinal microbiota colonization and establishment in the early stage of life is crucially affected by maternal conditions/diseases, mode of delivery, and exposure to antibiotics. Therefore, future studies are needed to determine more accurate therapeutic targets in immune, metabolic, endocrine, and neural pathways by mechanism validation through culturomics experiments of mainly modulated microbial populations and metabolomic analysis of the mother's skin, vagina, gut microbiota, and infant gut environments.

Author contributions

All authors researched the data for this article, made substantial contributions to discussions of the content, wrote the article, and reviewed and/or edited the manuscript prior to submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The gut-lung axis in the CFTR modulator era

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The advent of CFTR modulators represents a turning point in the history of cystic fibrosis (CF) management, changing profoundly the disease's clinical course by improving mucosal hydration. Assessing changes in airway and digestive tract microbiomes is of great interest to better understand the mechanisms and to predict disease evolution. Bacterial and fungal dysbiosis have been well documented in patients with CF; yet the impact of CFTR modulators on microbial communities has only been partially deciphered to date. In this review, we aim to summarize the current state of knowledge regarding the impact of CFTR modulators on both pulmonary and digestive microbiomes. Our analysis also covers the inter-organ connections between lung and gut communities, in order to highlight the gut-lung axis involvement in CF pathophysiology and its evolution in the era of novel modulators therapies.

KEYWORDS

gut-lung axis, microbiota, mycobiota, cystic fibrosis, CFTR modulators

1 Introduction

Cystic fibrosis (CF) is the most common severe life-limiting genetic disease in Caucasian populations. It affects more than 80,000 people worldwide, with a prevalence at birth of about 1 in 3,500 in Europe (Brown et al., 2017) but with a wide heterogeneity in its distribution. While it is diagnosed equally in men and women, its clinical expression appears to be more severe in women, which may be linked to estrogen involvement in the disease pathophysiology (Lam et al., 2021). CF is a monogenic disease with an autosomal recessive inheritance, caused by mutations in the gene coding the cystic fibrosis transmembrane conductance regulator (CFTR) protein, responsible for impaired chloride and bicarbonate secretions across epithelial cell apical membranes. More than 2,000 *cftr* gene mutations have been reported, which are classified into 6 groups according to the subsequent functional defect. These dysfunctions in the CFTR chloride channel cause an accumulation of viscous and dehydrated mucus in exocrine glands epithelia and

airway lumen. While pulmonary complications remain the main cause of morbidity-mortality in CF, the disease also affects other organs in particular the gastro-intestinal tract (Karb and Cummings, 2021). The mucus accumulation may lead to an obstruction of the intestinal lumen as well as pancreatic and bile ducts. People with CF (pwCF) also exhibit an increased risk of gastro-intestinal cancers compared to general population (Yamada et al., 2018). Hence, CF represents a multi-organ pathology responsible for a wide variety of symptoms across patients' lives (Figure 1), with respiratory and digestive manifestations playing key roles in disease progression.

In both the airways and gastro-intestinal tracts altered mucus composition leads to an imbalance in microbial communities. This dysbiosis also contributes to the establishment of chronic inflammation associated with functional lung and digestive decline (Enaud et al., 2019). Metagenomic approaches based on next generation sequencing have clearly facilitated the investigation of the CF microbiome (Françoise and Héry-Arnaud, 2020) which is of great importance to the understanding of the underlying mechanisms and to predict disease evolution. While assessment of both bacterial and fungal communities is well documented using metabarcoding approaches, the viral component of the microbiome requires shotgun metagenomics methods and remains largely unknown (Billard et al., 2017).

Pulmonary bacterial and to a lesser degree fungal microbiotas have been well documented in pwCF and healthy subjects (Figure 1) (Charlson et al., 2012; Delhaes et al., 2012; Zhao et al., 2012; Marsland and Gollwitzer, 2014; Willger et al., 2014; Nguyen et al., 2015; Dickson et al., 2016; Moran Losada et al., 2016; Dickson et al., 2017; Lamoureux et al., 2019; Enaud et al., 2020; Françoise and Héry-Arnaud, 2020; Avalos-Fernandez et al., 2022; Natalini et al., 2022; Hong et al., 2023). In the gut, microbial communities have also been well characterized (Figure 1) (Spor et al., 2011; Hoffmann et al., 2013; Hallen-Adams et al., 2015; Hoen et al., 2015; Nash et al., 2017; de Freitas et al., 2018; Enaud et al., 2018; Antosca et al., 2019; Coffey et al., 2019; Enaud et al., 2019); however it should be noted that fungal microbiota (also called mycobiota) has not been specifically addressed yet in pwCF digestive tract. Distant microbial florae display inter-organ connections involving a bidirectional crosstalk. As such, the gut-lung axis is an emerging concept describing how pulmonary and intestinal communities influence each other and are linked to clinical outcomes. In CF, this gut-lung axis is supported by studies results showing how gut bacterial microbiome is correlated with its pulmonary counterpart or the occurrence of respiratory complications (Enaud et al., 2020).

Before the 2010s, CF therapies were solely able to alleviate the disease symptoms. Over the last decade the development of CFTR modulators which directly address the underlying defects in the impaired protein has represented a turning point in the history of CF management (Davies et al., 2019). This has dramatically changed the disease's clinical course (Figure 1) leading to meaningful improvements in the daily lives of a large CF population and enabling to reach an average life expectancy of nearly 50 years (Karb and Cummings, 2021). In this review we will focus on CFTR modulators, their impact on pulmonary and intestinal microbiotas and discuss how these novel therapies may modify profoundly the gut-lung axis in CF and which long-term clinical benefit CF patients may expect.

2 CFTR modulators

Current advances in CFTR modulators have been reviewed recently (Meoli et al., 2021). Here, we will focus on CFTR modulators for which microbiota data have been published. Briefly, Ivacaftor (IVA) (Kalydeco®, Vertex pharmaceuticals,

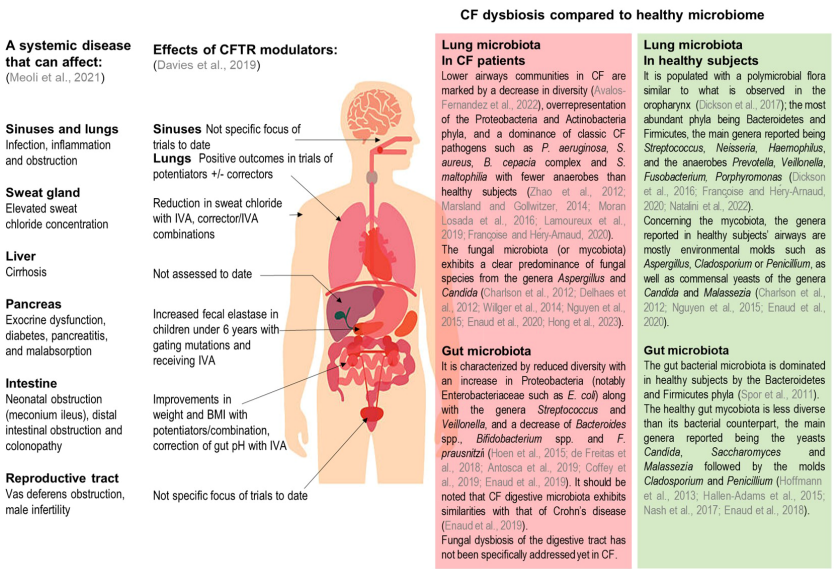

FIGURE 1
Summary of clinical manifestations, documented CFTR modulator effects, and of known lung and gut dysbiosis in pwCF. Adapted from Meoli et al., Pharmaceuticals 2021, and Davies et al., Kendig's Disorders of the Respiratory Tract in Children 2019. IVA, Ivacaftor; BMI, body mass index.

Boston, Massachusetts) was the first modulator to obtain approval by the FDA (2012); it belongs to the "potentiator" class, which increases CFTR gating at the apical surface of epithelial cells allowing for extended channel opening time. IVA was first approved in adult patients with at least one G551D gating mutation, representing only 2% of CF patients (McKone et al., 2003). Its use has been secondarily extended to other rare CFTR mutations with gating defects and nowadays from the age of 4 months upwards. Its clinical efficacy was demonstrated in children and adult cohorts, with an improvement in lung function (*i.e.*, increase of percent predicted forced expiratory volume in 1 second ($ppFEV_1$)), a decrease in the rate of pulmonary exacerbations, an improvement in nutritional status (*i.e.*, gain of weight and increase in body mass index (BMI)), an improvement in pancreatic function, and a strong reduction of the sweat chloride concentration (Ramsey et al., 2011; Davies et al., 2013). IVA displayed an acceptable safety profile (the most common adverse effects being headaches, oropharyngeal pain, upper respiratory tract infections and nasal congestion) (Ramsey et al., 2011; Davies et al., 2013). A risk of increased blood liver enzymes has been reported, justifying regular monitoring in all treated patients. No positive effects were observed when tested in patients with the more frequent F508del mutation (Flume et al., 2012), suggesting that a combination with a corrector is required to rectify the activity defect.

Several CFTR correctors have been developed to treat the protein misfolding and improve its trafficking to the apical surface, lumacaftor (LUM) being the first molecule of this class. Though LUM did not show any significant clinical effects as monotherapy (Clancy et al., 2012), it proved beneficial when associated with the potentiator IVA, and as such, has been FDA-approved as a combination since 2015 for patients aged 2 years and older, with homozygous F508del mutations (about 40% of pwCF (Wainwright et al., 2015)). The combination of lumacaftor-ivacaftor (LUM/IVA) (Orkambi®,Vertex pharmaceuticals) was evaluated in two randomized controlled trials, which showed modest but significant benefits on lung function at 24 weeks and on nutritional status (increase in BMI) (Wainwright et al., 2015). These benefits continued to be observed in the long term at week 96 (Konstan et al., 2017). LUM/IVA combination displayed an acceptable safety profile in the pivotal trials. However, clinical responses may also vary significantly amongst patients with the same genotype, and acute respiratory events (chest tightness, dyspnea and drop of $ppFEV_1$) were reported later on in real life studies, responsible for treatment discontinuation in approximately 20% of patients (Hubert et al., 2017; Jennings et al., 2017; Labaste et al., 2017; Burgel et al., 2020).

The second dual-combination of a CFTR corrector and potentiator was tezacaftor-ivacaftor (TEZ/IVA) (Symdeko® in the USA and Symkevi® in Europe, Vertex pharmaceuticals), FDA-approved in 2018 for patients aged 6 years and older, with homozygous F508del mutations or heterozygous in association with a residual-function mutation. TEZ/IVA combination showed significant clinical efficacy similar to that of LUM/IVA, but a better side-effect profile with less occurrence of acute respiratory events (Rowe et al., 2017; Taylor-Cousar et al., 2017; Lommatzsch and Taylor-Cousar, 2019). Thereafter, a triple-combination approach was developed by adding elexacaftor (ELX), a new CFTR corrector, to the former TEZ/IVA regimen. ELX and TEZ bind to different sites of the CFTR protein, displaying an additive effect to improve the protein processing. The triple-combination elexacaftor-tezacaftor-ivacaftor (ELX/TEZ/IVA) (Trikafta® in USA and Kaftrio® in Europe, Vertex pharmaceuticals) was approved in 2019 for patients aged 6 years and older having at least one F508del mutation (regardless of the other mutation on the second allele), representing nearly 90% of pwCF (Cystic Fibrosis Foundation, 2022; Vaincre la Mucoviscidose, 2022). ELX/TEZ/IVA has been assessed in several randomized clinical trials (Heijerman et al., 2019; Middleton et al., 2019; Zemanick et al., 2021) which demonstrated an unprecedented clinical improvement with an acceptable safety profile leading to a significant decrease in the number of lung transplantations (Burgel et al., 2021).

3 Pulmonary microbiota

CFTR modulators therapies lead to improved mucus hydration by correcting chloride channel activity, which improves mucociliary clearance and, in turn, induces changes in airway microbial communities. The impact of modulators on the pulmonary microbiome has been more extensively studied during IVA monotherapy (as the first developed molecule) in patients bearing at least one G551D mutation. Results of these studies are summarized in Table 1. Briefly, a first study assessing IVA effects reported no significant changes in bacterial alpha-diversity, but a trend towards decreased abundance of pathogens as well as a significant increase in the anaerobe *Prevotella* (Rowe et al., 2014). A subsequent study performed on 3 patients did not find any significant differences in total bacterial load and global community composition, but described a notable decrease in *Streptococcus mitis* abundance and increase in the anaerobe *Porphyromonas* (Bernarde et al., 2015). A third study reported a significant increase of bacterial diversity indices during the first year of treatment in patients chronically infected with *P. aeruginosa*, as well as a decrease in the relative abundance of *P. aeruginosa* with reciprocal increase of oropharyngeal bacteria such as *Streptococcus* and *Prevotella* (Hisert et al., 2017). Nonetheless, these changes were not sustained with a notable rebound observed during the second year of IVA monotherapy (Burgel et al., 2021). A more recent study reported increased richness and diversity of the pulmonary bacterial microbiome, correlated with lower levels of circulating inflammatory markers (Einarsson et al., 2021). By contrast, other studies did not find any significant changes in pulmonary bacterial microbiome related to IVA in patients with G551D or S1251N mutations (Peleg et al., 2018; Harris et al., 2020; Kristensen et al., 2021), in spite of limitations due to small sample sizes, short follow-up periods and differences in antibiotic exposures. Overall, these results suggest a trend towards increased bacterial diversity in the airways on IVA monotherapy, accompanied with a decrease but no eradication of *P. aeruginosa* and reciprocal increase in commensal anaerobes.

Regarding the impact of the LUM/IVA dual-combination on the airway microbiome, a first study reported similarly a moderate

TABLE 1 Impact of CFTR modulators on pulmonary and digestive microbiomes.

CFTR modulators assessed	Authors	Studied population	Follow-up period	Effects on bacterial microbiota	Effects on fungal microbiota
PULMONARY MICROBIOME					
IVA	Rowe et al., 2014	133 patients (with microbiome analysis limited to 14 patients), age 6 and older, at least one G551D mutation	6 months	Trend toward a decrease in relative abundance of CF pathogens. Significant increase in *Prevotella* relative abundance. Reduction of *P. aeruginosa* isolation from respiratory cultures in the whole 133 patient's cohort.	NA
	Bernarde et al., 2015	3 patients, age range 10-16, at least one G551D mutation	mean 10 months	No significant changes in global community composition. Decrease in *Streptococcus mitis* relative abundance and increase of *Porphyromonas*.	NA
	Hisert et al., 2017	12 patients (microbiome analysis in 8 patients), age range 22-57, at least one G551D mutation	≥ 2 years	Increase of alpha-diversity during the first year, with decrease in *P. aeruginosa* relative abundance and reciprocal increase of commensal bacteria such as *Streptococcus* and *Prevotella*. Reduction in *P. aeruginosa* loads (assessed by culture and qPCR) in the first year. Rebound during the second year.	NA
	Peleg et al., 2018	20 patients, age range 18-65, at least one G551D mutation	4 weeks	No significant changes in microbial composition. In subjects with stable antibiotic exposure, reduction in total bacterial load.	NA
	Harris et al., 2020	31 patients, age 10 and older, at least one G551D mutation	6 months	No significant changes in diversity and bacterial loads (total and *P. aeruginosa*).	NA
	Kristensen et al., 2021	16 patients, mean age 22.5, at least one S1251N mutation	2-12 months	Trend toward an increase in alpha-diversity. No significant changes in overall microbial composition.	NA
	Einarsson et al., 2021	14 patients, age range 13-39, at least one G551D mutation	mean 1 year	Increase in alpha-diversity, correlated with lower levels of circulating inflammatory markers.	NA
LUM/IVA	Graeber et al., 2021	14 patients, age range 12-41, F508del homozygous	8-16 weeks	Decrease in total bacterial load and increase in alpha-diversity (with reduced IL-1β concentration in sputa).	NA
	Neerincx et al., 2021	16 patients, median age 25, F508del homozygous	1 year	Temporary and moderate decrease in *P. aeruginosa* relative abundance (statistically unsignificant, not sustained after 1 year).	NA
	Hong et al., 2023	66 CF patients (18 treated with LUM/IVA and 6 with IVA), age 18 and older	no longitudinal follow-up	NA	Higher alpha-diversity compared to untreated patients
	Enaud et al., 2023	41 patients, age 12 and older, F508del homozygous	6 months	No significant changes in diversity and bacterial loads (total and *P. aeruginosa*). In a subgroup of patients uncolonized with *P. aeruginosa* at baseline, increase in alpha-diversity.	No significant changes in diversity and total fungal load.
ELX/TEZ/IVA	Pallenberg et al., 2022	31 patients, age range 12-44,	3-12 months	Increase in alpha-diversity (evenness) and beta-diversity. Decrease in total bacterial load and relative abundances of *P. aeruginosa* and *S. aureus*.	NA

(Continued)

TABLE 1 Continued

CFTR modulators assessed	Authors	Studied population	Follow-up period	Effects on bacterial microbiota	Effects on fungal microbiota
		at least one F508del mutation			
	Sosinski et al., 2022	24 patients, mean age 32, at least one F508del mutation	mean 6 months	Increase in alpha- and beta-diversity. Decrease of the abundance log-ratio of CF pathogens/anaerobes.	NA
	Schaupp et al., 2023	65 patients, age 12 and older, at least one F508del mutation	1 year	Increase in alpha-diversity, correlated with reduced inflammatory markers in sputa. Decrease in *P. aeruginosa* relative abundance at 3 months.	NA
DIGESTIVE MICROBIOME					
IVA	Ooi et al., 2018	16 patients, age range 5-50, at least one G551D or G178R mutation	median 6 months	Increase in *Akkermansia* relative abundance. Moderate decrease in *Enterobacteriaceae* abundance (statistically unsignificant), correlated with reduced fecal calprotectin.	NA
	Kristensen et al., 2021	16 patients, mean age 22.5, at least one S1251N mutation	2-12 months	Significant increase in alpha- and beta-diversity. No significant changes in specific genera abundances.	NA
	Pope et al., 2021	12 patients (with pancreatic sufficiency), age range 21-64, at least one R117H mutation	4.4 months	No significant changes in diversity and microbial composition.	NA
	Ronan et al., 2022	14 patients, age range 18-39, at least one G551D mutation	median 1 year	No significant changes in diversity and microbial composition.	NA
LUM/IVA	Pope et al., 2021	8 patients (with pancreatic insufficiency), age range 8-24, F508del homozygous	median 7 months	No significant changes in alpha-diversity. Trend toward microbial composition closer to patients with pancreatic sufficiency.	NA

IVA, Ivacaftor; LUM/IVA, Lumacaftor + Ivacaftor; ELX/TEZ/IVA, Elexacaftor + Tezacaftor + Ivacaftor; NA, not assessed.

decrease in *P. aeruginosa* relative abundance that did not reach statistical significance and was not sustained after 12 months of treatment (Neerincx et al., 2021). A second study described a significant decrease in total bacterial load and an increase in bacterial alpha-diversity calculated with the Shannon index, along with a reduced concentration of the proinflammatory cytokine IL-1β in pwCF sputa (Graeber et al., 2021). Regarding the mycobiome, one recent cross-sectional study showed that adult patients treated with modulators (the majority of whom were receiving LUM/IVA) had significantly higher fungal alpha-diversity compared to untreated patients (Hong et al., 2023). A last study did not find any significant differences in both airway bacterial and fungal microbiota and pulmonary inflammation assessed by calprotectin measurement (Enaud et al., 2023). However, when focusing on a subgroup of patients uncolonized with *P. aeruginosa* at LUM/IVA initiation, calprotectin levels were lower and a significant increase in bacterial alpha-diversity was observed after 6 months of therapy, suggesting that microbiome changes on LUM/IVA are dependent on *P. aeruginosa* colonization status.

Only three studies have addressed the effect of the most recent and promising ELX/TEZ/IVA triple-combination on pulmonary microbiome in pwCF wearing at least one F508del mutation. One study performed on 24 patients (Sosinski et al., 2022) demonstrated a significant increase in bacterial alpha- and beta-diversity in the sputa after treatment, as well as a trend towards a reduced bacterial load. Although the relative abundances of specific bacterial taxa were unchanged, the authors described a significant decrease in the CF pathogens to anaerobes log-ratio. These findings were partially confirmed by a subsequent study reporting a significant increase in the evenness of distribution of bacterial taxa, along with a decrease in total bacterial load and relative abundances of major CF pathogens *P. aeruginosa* and *Staphylococcus aureus* (Pallenberg et al., 2022). Similarly, the authors of a recent study described an increase in bacterial alpha-diversity after 1, 3 and 12 months on

ELX/TEZ/IVA (Schaupp et al., 2023) while the decrease in *P. aeruginosa* relative abundance was only significant at 3 months. In addition, the authors also reported a significant reduction in inflammatory markers (IL-1β, IL-8 and neutrophil elastase) in the sputa. All these findings appear to be consistent with those reported with previous generations of CFTR modulators.

4 Digestive microbiota

To date the impact of CFTR modulators on the intestinal microbiome has been less extensively studied compared to its pulmonary counterpart. Several studies suggest that IVA monotherapy may have an impact on the intestinal microbiome in pwCF with gating defects; such results are summarized in Table 1. A first study showed that IVA provokes a significant increase in the relative abundance of the bacterial genus *Akkermansia* (Ooi et al., 2018) which is known for its anti-inflammatory effects and as a biomarker of healthy gut mucosa (Derrien et al., 2017; Rodrigues et al., 2022). This finding was associated with a significant decrease in intestinal inflammation assessed by fecal calprotectin measurement, while bacterial alpha- and beta-diversities were unchanged. Conversely, another study reported a significant increase in both alpha- and beta-diversity indices after IVA treatment, whereas no significant changes were found in specific bacterial genera abundances (Kristensen et al., 2021). Two other studies did not show any significant effect related to IVA monotherapy on the gut microbiome (Pope et al., 2021; Ronan et al., 2022). Finally, trends toward a bacterial microbiota composition closer to subjects with normal exocrine pancreatic function were reported with LUM/IVA dual-combination (Pope et al., 2021). These limited findings need to be further validated by larger studies, especially with next-generation modulators like the ELX/TEZ/IVA triple-combination. Moreover, it must be noted that the intestinal mycobiota has not been assessed to date in patients treated with CFTR modulators. By extrapolation based on similarities regarding the bacterial component (Enaud et al., 2019) it may display similarities with patterns reported in inflammatory bowel diseases, which are characterized by a notable increase in the Basidiomycota/Ascomycota ratio during flares episodes and a decrease during remission (Sokol et al., 2017). In addition, although the intestinal mycobiome has been reported to influence airway outcomes (Zhang et al., 2017) no studies have currently examined this hypothesis or the role of the gut in the context of CF.

5 Discussion and perspectives: towards a gut-lung axis analysis in pwCF on CFTR modulators

In this review we have summarized the published data regarding the impact of CFTR modulators on pulmonary and digestive microbiomes respectively. However, these two microbial florae are not strictly independent as indicated by growing evidence describing inter-organ connections (Enaud et al., 2020; Françoise and Héry-Arnaud, 2020), and supporting the concept of a gut-lung axis. Due to the anatomical links the microbial communities of both tracts have direct interactions through gastroesophageal content inhalations and sputum swallowing (Enaud et al., 2020). Long-reaching interactions between the airways and intestine are also involved, mediated via the mesenteric lymphatic system through which microbial metabolites are exchanged (Bingula et al., 2017; Anand and Mande, 2018; Enaud et al., 2020). Among them, short-chain fatty acids synthesized by intestinal bacteria are of particular importance given their well-known immunomodulatory properties. Such fatty acids have been shown to mitigate the airway inflammatory response (Trompette et al., 2014; Halnes et al., 2017).

In pwCF, several studies suggest interactions between bacterial communities at the pulmonary and digestive levels. In infants with CF, a high degree of concordance was observed between bacterial genera evolution over time at both sites, with some genera colonizing the gut prior to their appearance in the respiratory tract (Madan et al., 2012). Furthermore, the gut microbiome is closely related to CF respiratory complications. Indeed, some gut microbiota alterations (such as a decrease of *Parabacteroides*) precede the onset of *P. aeruginosa* colonization, while its composition is associated with CF exacerbation in early life (Hoen et al., 2015). Regarding dietary exposures, breastfeeding was associated with microbial diversity of the respiratory tract as well as a prolonged time to first CF exacerbation (Madan et al., 2012; Hoen et al., 2015), the role of short-chain fatty acids being discussed but not demonstrated yet. Finally, the oral administration of probiotics (supposed to modulate the gut microbiota) showed a significant reduction in the number of pulmonary exacerbations, although the size of effect is unclear (Anderson et al., 2017).

The gut-lung axis involvement in pwCF receiving CFTR modulators has been far less extensively investigated. To date, only one study has assessed bacterial communities in both the airways and gut in 16 patients with the rare S1251N mutation treated with IVA monotherapy (Kristensen et al., 2021). The authors reported significant improvements in the gut microbiota diversity that were not observed in the respiratory samples (including sputum, nasopharynx and oropharynx samples). Nonetheless, these findings are limited by the small sample size (n=16, among which only 8 patients were followed during 12 months). In addition, the authors suggested a follow-up time in excess of 12 months to detect significant improvements in lung microbiota on CFTR modulators, since the development of respiratory tract microbiota appeared to be presaged by gut colonization (Madan et al., 2012).

Further studies are thus required to fully decipher the gut-lung axis evolution on modulator therapies, involving a joint analysis of pulmonary and fecal samples. Moreover, microbiome analyses have so far been almost exclusively limited to the bacterial kingdom, the fungal kingdom being neglected in the assessment of pwCF microbiotas on modulators, especially in the digestive tract analysis. In addition, the use of shotgun metagenomics approaches will allow to investigate the viral component of the microbiome, providing an exhaustive inter-kingdom assessment of CF microbial communities. The entire microbiome data should also be correlated with clinical

parameters and surrogate inflammatory biomarkers for a comprehensive evaluation of the CF disease landscape in a personalized medicine approach that next generation of CFTR modulators will enable (Yi et al., 2021; Enaud et al., 2023).

Regarding the populations studied, it should be noted that the patients included in the quoted studies were over 5 years of age, including a majority of adults with already established chronic colonization and infection (Table 1). The modulators' impact should also be assessed in future studies in large pediatric populations including children under 5 years of age, since CFTR modulator treatment will be initiated earlier, before the patients are chronically colonized and the gut-lung axis mucosa irreversibly damaged (Enaud et al., 2023). Moreover, other features such as pwCF gender should be considered, since differential outcomes between men and women treated with IVA have been described (Secunda et al., 2020). Finally, early published studies being focused on the assessment of patients treated with IVA monotherapy, future studies are needed to investigate the gut-lung axis in patients receiving combinations of modulators such as LUM/IVA, TEZ/IVA and ELX/TEZ/IVA.

In conclusion, the advent of CFTR modulators marks a turning point in the history of CF management and outcome, with unprecedented clinical improvements and favorable safety profiles, even if data about long-term adverse effects are not yet available. The impact of such molecules on the resident microbial communities of the respiratory and digestive tracts has only been partially deciphered, mostly with first-generation therapies such as IVA. While the results of available studies are not totally consistent, they suggest that CFTR modulators may induce an increase in bacterial diversity, associated with a decrease in conventional culture-based CF pathogens in the airways and an increase in anti-inflammatory bacteria in the gut. The impact of next-generation modulators on the gut-lung axis will have to be more extensively investigated in future studies, with additional data regarding the mycobiota and virobiota, especially in younger pwCF who will benefit even more from these innovative therapies.

Author contributions

FL-S: Conceptualization, Writing – original draft, Writing – review & editing. ÉC: Conceptualization, Writing – review & editing. SI: Conceptualization, Writing – review & editing. ML: Writing – review & editing. SB: Writing – review & editing. MF: Writing – review & editing. PB: Writing – review & editing. RE: Conceptualization, Writing – review & editing. LD: Conceptualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The oral-gut-circulatory axis: from homeostasis to colon cancer

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The human microbiota is widely recognized as providing crucial health benefits to its host, specifically by modulating immune homeostasis. Microbial imbalance, known as dysbiosis, is linked to several conditions in the body. The oral cavity and gut host the two largest microbial communities playing a major role in microbial-associated diseases. While the oral-gut axis has been previously explored, our review uniquely highlights the significance of incorporating the circulatory system into this axis. The interaction between immune cells, inflammatory factors, circulating bacteria, and microbial metabolites influences the homeostasis of both the oral and gut microbiota in a bidirectional manner. In this comprehensive review, we aim to describe the bacterial components of the oral-gut-circulatory axis in both health and disease, with a specific focus on colon cancer.

KEYWORDS

microbiota, immune response, dysbiosis, oral-gut axis, circulation, colon cancer

Introduction

Disturbance of the microbiota homeostasis has emerged as a significant factor correlated with a multitude of diseases within the human body. The study of the microbiome and its association with cancer has primarily focused on an organ-specific relationship, however, increasing evidence underscores the pivotal role played by the microbial and inflammatory milieu at distant anatomical sites as an important regulator in both healthy and pathological processes.

The oral cavity and gut harbor the two largest microbial habitats in the body and play a significant role in infectious diseases and in different types of cancer. The mouth is the entry point of the digestive tract and is continuously exposed to various exogenous element, including microorganisms, nutrients, and xenobiotics. Bacterial translocation can occur through different routes, such as swallowing, aspiration and through circulation or transported inside immune cells. Certain oral pathogens, such as *Porphyromonas gingivalis (P. gingivalis)* and *Fusobacterium nucleatum (F. nucleatum)*, have been found to invade and colonize the oral epithelial cells and periodontal tissues where the bacteria

can release virulence factors and toxins that disrupt the integrity of the oral mucosa. Additionally, chronic oral infections, such as periodontitis, can weaken the barrier function facilitating the translocation of oral pathogens into the bloodstream, ultimately reaching distant sites such as the liver, spleen, and gastrointestinal tract. In the liver, oral microbes can trigger a low-grade inflammatory reaction and impact various body sites through the release of cytokines. Once in the gut, these oral bacteria can interact with the existing gut microbiota, influencing its diversity and functionality. Changes in the microbial communities may trigger immune responses, inflammation, and increased intestinal permeability, allowing opportunistic bacteria to invade, and toxins and metabolites to leak into the bloodstream. Disturbances in either the oral or gut microbiota can cascade into the other, potentially affecting systemic health and underscoring their interconnectedness. This mutual relationship highlights the importance of considering both systems together when studying and addressing various health conditions.

In this review, we summarize the current knowledge of the oral-gut axis, including the role of the circulatory system in bacterial translocation and systemic inflammation while highlighting the importance of the interconnectedness of these systems. Both oral pathogens, *P. gingivalis* and *F. nucleatum* have been implicated in the pathogenesis of colon cancer by directly affecting the host cell signaling pathways involved in cell proliferation, apoptosis, and DNA damage repair. These microbial interactions can further exacerbate inflammation and promote the growth and survival of colorectal cancer cells. The presence of these oral pathogens in the gut underscores the connection between of the oral-gut axis in the context of carcinogenesis.

The immune response in oral and intestinal mucosa

The mucosal immune system constitutes a specialized and region-specific defense network safeguarding a substantial portion of the inner surface of human anatomy, encompassing the mucosal linings of the respiratory system, urogenital tract, oropharyngeal region, GI tract, and also the exocrine glands. Specialized functions in the oral cavity and GI tract lead to variations in immune responses. These include differences in mucosal surfaces, microbial composition, and immune cell populations. The main roles of the oral cavity, digestion and speech articulation, require different immune adaptations compared to the GI tract, which primarily handles nutrient absorption and hosts a larger and more diverse microbial community. These distinctions contribute to unique immune profiles and responses in each region (Moutsopoulos and Konkel, 2018).

The intricate mucosal surfaces of the oral cavity, including the gingiva, tongue, and buccal mucosa, constitute the first line of defense against pathogens. The oral mucosa components ensure the integrity of oral tissues and form a complex defense network that protect against potential threats while maintaining tolerance to harmless entities. Oral epithelia are multilayered barriers with highly diverse antigenic responses and different expression patterns of cytokeratin. Salivary glands secrete saliva that contains enzymes and proteins, like lysozyme and lactoferrin, which possess antimicrobial properties (Moutsopoulos and Konkel, 2018). The GI immune system has a unique characteristic known as oral tolerance allowing the immune system to be unresponsive or tolerant to ingested protein antigens. Although the immune system becomes more mature and less flexible as we age, the oral cavity and gut immune systems still actively maintain tolerance to dietary antigens in adulthood.

Both oral and intestinal mucosa have specialized lymphoid tissues known as mucosa-associated lymphoid tissue (MALT). In the oral cavity, MALT includes tonsils and adenoids, while in the gut, it encompasses Peyer's patches, isolated lymphoid follicles. These lymphoid tissues contain immune cells, including T cells, B cells, innate lymphoid cells (ILCs), and antigen-presenting cells (APC), which play a crucial role in immune responses (Moutsopoulos and Konkel, 2018; Suárez et al., 2021). In the gut, innate immune tactics involve employing a combination of defenses such as a protective mucus layer, antimicrobial peptides (AMPs), and the coordinated action of ILCs. These mechanisms work together to contain a significant portion of the microbial community within the interior space of the intestinal tract. ILCs are innate equivalents of T cells without antigen-specific receptors, playing pivotal roles in immune responses by producing effector cytokines, maintenance of mucosal barriers, such as the epithelial lining of the oral and intestinal mucosa, and regulating other immune cells including T cells, B cells, and dendritic cells. They are present in lymphoid and non-lymphoid organs, along with mucosal barriers exposed to allergens, commensal microbes, and pathogens whereas these cells are uncommon in the bloodstream. Different ILC subsets, including ILC1s, ILC2s, and ILC3s, each have their own specific functions and cytokine profiles. ILC3s are abundant in the intestinal mucosa and have been implicated in inflammatory bowel disease (IBD). They play a dual role: promoting tissue repair and maintaining mucosal homeostasis, while also contributing to inflammation when dysregulated. Additionally, ILCs have been suggested to have roles in tumor immune surveillance, contributing to the recognition and control of tumor cells. However, their specific functions and contributions to antitumor immunity are still being studied (Panda and Colonna, 2019).

Regulatory T cells (Tregs) and other regulatory immune cells play an essential role in suppressing immune responses and maintaining tolerance (Upadhyay et al., 2013). Microbial-associated molecular patterns (MAMPs) are conserved structural components of microorganisms that are recognized by the innate immune system. Biofilm and bacterial metabolic products like lipopolysaccharides (LPS) are the main MAMPs that can stimulate the expression and production of pro-inflammatory cytokines through activation of toll-like receptors (TLRs). TLRs serve as crucial mediators in the inflammatory pathways, significantly contributing to the orchestration of immune responses against a diverse range of ligands originating from pathogens. They play a vital link connecting adaptive immunity with innate immunity. TLRs recognize MAMPs and activate signaling pathways that lead to the production of pro-

inflammatory cytokines and chemokines, as well as the upregulation of co-stimulatory molecules and antigen-presenting molecules (Mackey and McFall, 2006).

Moreover, nucleotide-binding oligomerization domain-like receptors (NLRs) also communicate with several innate immune sensors and receptors in the oral and intestinal epithelial cells. NLRs binding and downstream signaling by cytokines, chemokines, and antimicrobial peptides production provoke an inflammatory reaction (Franchi et al., 2009; Cekici et al., 2014). One major function of NLR proteins is to regulate and modulate inflammatory signaling pathways, including NFκ-B and MAPK. Once activated, NFκ-B translocates to the nucleus, where it binds to target genes and induces the transcription of pro-inflammatory cytokines and other immune response genes. Similarly, the activation of MAPK signaling pathway leads to the production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) (Franchi et al., 2009). Therefore, NLR proteins play an important role in maintaining the balance between immune defense and inflammatory damage in the oral and gut microenvironment.

Neutrophils play a vital role in homeostatic immunity by serving as the front-line defenders against microbial threats. Rapidly responding to infection signals, neutrophils migrate to affected areas, where they engulf and neutralize pathogens through phagocytosis and releasing antimicrobial proteins and cytokines modulating the behavior of other immune cells. Additionally, neutrophils support the epithelial barrier's function by participating in the tissue repair process (Moutsopoulos and Konkel, 2018).

Dendritic cells (DCs) are another important antigen-presenting cell that play a critical role in linking innate and adaptive immunity. They derive from hematopoietic stem and progenitor cells (HSPCs) in the bone marrow and are found in various locations throughout the body. Being the most potent cells responsible for activating and directing naïve T cells, DCs in the oral region hold significant importance in orchestrating both immune responses and tolerance within the oral mucosa (Hovav, 2014). Unlike in murine models, the comprehensive examination of dendritic cells in human oral tissues remains limited, with the primary focus typically centered on Langerhans cells (LCs) (Hovav, 2014). Nonkeratinized mucosal regions, including the soft palate, ventral tongue, lip, and floor of the mouth, show the highest LC concentration, while the keratinized mucosa of the hard palate displays the lowest LC density (Daniels, 1984). LCs within the oral mucosa effectively collect oral fluids and bacteria, with their dendritic extensions reaching towards the surface, often constituting a diverse group. LCs become mobile and mature in response to inflammatory cytokines and pathogen-associated molecular patterns (PAMPs) released by oral mucosal pathogens. LCs are primarily responsible for presenting exogenous antigen-derived peptides through major histocompatibility complex (MHC) class II presentation to CD4+ helper T-cells and MHC class I-restricted cytotoxic (CD8+) T-cell responses. Furthermore, LCs stimulate Natural killer (NK) cells by producing cytokines, including IL-12 (Hovav, 2014).

Activated DCs migrate to the GALT, where they interact with naïve T cells, thereby initiating the adaptive immune response. This migration and subsequent T cell activation are crucial for immune surveillance and responses in diverse body regions (Hovav, 2014). A deeper exploration is necessary to comprehend how DC activation in oral epithelia and their migration across different tissues contribute to coordinating immune responses and maintaining immune tolerance throughout the body.

Th17 cells, a subset of CD4+ T cells, specialize in orchestrating immune responses at mucosal surfaces by producing IL-17 and other cytokines that recruit neutrophils and enhance antimicrobial defenses. The coordinated efforts of neutrophils and Th17 cells illustrate the intricate collaboration between innate and adaptive immunity in safeguarding mucosal homeostasis and combating infections. This dynamic interaction plays a crucial role in maintaining the delicate balance between protective immunity and immune tolerance within mucosal environments (Suárez et al., 2021).

The intestinal epithelium is acknowledged as the principal axis of mucosal immunity, given that approximately 70% of the total lymphocyte population resides within the gastrointestinal tract (Suárez et al., 2021). The GI mucosal immune system comprises three principal components: the epithelial layer, lamina propria, and the MALT, known as gut-associated lymphoid tissue (GALT). The epithelium and lamina propria serve as the frontline defenses, while the GALT functions as the central hub where adaptive immune responses are instigated, specifically within the context of the GI tract (Wu et al., 2014). The epithelium layer is primarily composed of intestinal epithelial cells (IECs), organized into distinct structures known as villi. Other specialized cell types coexist, including Goblet cells, tuft cells, enteroendocrine cells, and M cells. Goblet cells lubricate and shield the intestinal epithelial surface by secreting mucins. (Suárez et al., 2021). Control of paracellular permeability is crucial for preventing microbial invasion, and this is achieved through the regulation of various types of intercellular junctions (Suárez et al., 2021). The gut-associated lymphoid tissue consists of Peyer's patches and isolated lymphoid follicles (Wu et al., 2014). Goblet cells also play a role as luminal antigen-presenting cells to CD103+ DCs, facilitating the differentiation of Tregs. DCs extend their dendrites into the epithelium to capture antigens and subsequently migrate to the lamina propria, subsequently draining to secondary lymphoid tissues. Meanwhile, specialized M cells, present in the epithelium of Peyer's patches, facilitate the transfer of antigens to DCs, macrophages, and other APCs. In secondary lymphoid tissues, naïve T cells undergo activation upon interaction with APCs and subsequently migrate to the lamina propria (Wu et al., 2014).

Overall, the oral and gut immune responses exhibit both similarities and differences, sharing common features in terms of lymphoid tissues and immune cell populations. On the other hand, microbial compositions, exposure to antigens, immune tolerance mechanisms, and antibody production are different. It has been reported that immune cells present in the oral draining lymph nodes can transmigrate to various other lymphoid organs, including the gut (Morton et al., 2014). Consequently, oral inflammation could lead to the emergence of T cells reactive to oral pathobionts. These T cells have the potential to migrate from the oral mucosa to the intestine, where they could become activated by

specificmicrobes, potentially leading to the onset of intestinal inflammation.

Furthermore, the gut mucosa employs alternative immune mechanisms to regulate the production of microbiota-responsive effector T cells. Within the gut, a specific group of ILCs expressing MHC class II molecules hinders the expansion of T cells specific to the microbiota. It is plausible that the oral mucosa and gingiva do not possess these intricate tolerogenic mechanisms observed in the gut. Therefore, acute inflammation in the oral mucosa might lead to the development of microbiota-responsive T cells with potential pathogenic characteristics. Th17 cells normally present in the gut do not induce disease, unlike their significant pathogenic role in promoting inflammation driven by commensal bacteria in the oral cavity in both mice and humans. Upon migration to the gut mucosa, these cells may undergo functional conversion into Th17/Th1 mixed phenotype cells capable of producing interferon-γ (IFN-γ). This conversion might contribute to the development of colitis triggered by oral-origin T cells. In summary, migratory Th17 cells represent a double microbiota and immune mechanism linking the oral and gut environments (Kitamoto et al., 2020).

A comprehensive understanding of the immune dynamics operating in these sites is crucial for unraveling the complex interplay between the oral and gut immune responses. Such insights could pave the way for novel strategies aimed at modulating immune reactions and designing interventions for diseases that involve both oral and gut components.

Dysbiosis in the oral microbiota and host response

The oralome comprises the dynamic interactions coordinated between the ecological community of oral microorganisms and the host within the oral cavity (Radaic and Kapila, 2021). The mouth harbors over 700 species of bacteria, as well as fungi, viruses, and protozoa, making it the second-largest and diverse microbiota after the gut (Peterson et al., 2009). Interindividual differences also exist, but the principal function of the microbiome is the same in every person. Oral commensal microorganisms help maintain microbiota balance, inhibiting pathogen attachment and invasion. They support oral health by competing for resources, producing antimicrobials, and modulating the host immune response (Wade, 2013). The oral microbiome has been extensively characterized using both cultivation and culture-independent molecular techniques, such as 16S rRNA cloning. The six most abundant phyla identified in the oral microbiome are: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria, which together comprise 96% of the taxa (Dewhirst et al., 2010).

Biofilms are organized communities of similar or different aggregated bacterial cells encased in a self-produced polymeric matrix adhered to a surface (Donlan, 2002). Oral biofilm formation begins with the initial attachment of planktonic "early colonizer" bacteria, primarily saccharolytic aerobes and facultative anaerobes that utilize glycoproteins and salivary mucins as nutrients. Streptococcus species, making up around 80%, dominate this early colonization phase (Kreth et al., 2009). Following the attachment, surface-bound bacteria undergo shifts in their metabolic and gene expression profiles resulting in the generation of extracellular polymeric substances, including polysaccharides, proteins, lipids, and extracellular DNA (Radaic and Kapila, 2021). *F. nucleatum* plays a key role as a "bridge" bacterium within the biofilm by adhering to a diverse array of co-aggregated species through surface adhesins (Coppenhagen-Glazer et al., 2015).

The oral biofilm presents varying levels of oxygenation across its structural composition, providing the necessary environmental conditions for the attachment and proliferation of proteolytic obligate anaerobes, commonly referred to as "late colonizers" (Wade, 2013). Ahn et al. (Ahn et al., 2016) examined how *F. nucleatum* and *P. gingivalis* infections affect reactive oxygen species (ROS) production and bacterial complex formation in human oral cells. Despite both being anaerobic bacteria, *F. nucleatum* and *P. gingivalis* have different oxygen tolerances but are commonly found together at infection sites. ROS production primarily relies on NADPH oxidases (NOX) enzymes. Notably, *F. nucleatum* exhibits a 20-fold higher NOX activity compared to *P. gingivalis*, giving it a much greater ability to utilize oxygen molecules (Diaz et al., 2002). Similarly, the presence of *F. nucleatum* increases *P. gingivalis* attachment to human gingival fibroblasts by a factor of ten. Ultimately, the study (Ahn et al., 2016) revealed that *F. nucleatum* contributes to the co-aggregation of *P. gingivalis*, promoting the formation of bacterial complexes.

The most prevalent oral diseases that are dental caries and periodontitis are both associated with a disruption in the balance of oral microbiota. Dental caries is a biofilm disease caused by multiple microorganisms, influenced by dietary habits leading to the demineralization of tooth enamel through acid production by oral pathogens fueled by dietary sugars (Bowen et al., 2018). Periodontitis is a chronic inflammatory disease initiated by a dysbiotic biofilm in the gingival pocket. Its polymicrobial nature is driven by variations in subgingival microbiota composition and interactions, rather than new bacterial colonization (Aas et al., 2005; Bowen et al., 2018). The subgingival microbiota is typically dominated by *Firmicutes*, with fewer *Actinobacteria* and *Bacteroidetes*. In contrast, periodontitis is characterized by the presence of *P. gingivalis*, *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *F. nucleatum* (Paster et al., 2001; Donlan, 2002). Dominant species in the subgingival biofilm play a crucial role in driving dysbiosis and inflammatory response, in periodontitis development (Aas et al., 2005).

The aggregation of bacteria triggers local inflammation, increasing crevicular gingival fluid flow, leading to bleeding, and providing protein-rich nutrients. This promotes the growth of the Gram-negative anaerobes (Hoare et al., 2019). Within the periodontal pocket, the initial host response to the dysbiotic subgingival community involves NK cells, neutrophils, and granulocytes, initiating early inflammation. Subsequently, lymphocytes infiltrate, facilitating antigen presentation to dendritic cells. T cells, including CD8+ and CD4+ cells, generate a proinflammatory environment rich in cytokines like TNF-α, IL-1, IL-4, IL-10, IFN-γ, and transforming growth factor β (TGF-β)

(Pihlstrom et al., 2005). This inflammatory cascade leads to changes in the subgingival environment, contributing to shifts in the subgingival biofilm composition that drive the progression of periodontitis. Interestingly, certain periodontal pathogens directly play a role in promoting chronic inflammation by activating specific intracellular pathways (Hoare et al., 2019).

Several studies have linked periodontitis to systemic diseases, such as cardiovascular, diabetes mellitus, respiratory disease, adverse pregnancy outcomes (Geisinger et al., 2016), and increased risk of pancreatic and colon cancers (Pihlstrom et al., 2005; Momen-heravi et al., 2018; Kim et al., 2019; Koliarakis et al., 2019). Nevertheless, the available evidence about remains limited, necessitating further research to elucidate the nature of this connection and the underlying mechanisms. Potential mechanisms linking oral infections to systemic conditions encompass the spread of oral pathogens through transient bacteremia and intracellular infection in immune cells, the release of oral microbial toxins into the circulation at the site of injury, and interactions between oral pathogens and the host's immune response, resulting in systemic inflammation (Xiaojing et al., 2000). Further prospective investigations are imperative to gain a comprehensive understanding of the causal mechanisms linking oral dysbiosis and its potential impact on the development of colon cancer (Figure 1).

The circulation as a key component in the oral-gut axis

The mechanisms underlying bacterial leakage into the bloodstream remain unknown, with hypotheses ranging from dendritic cell processes, and oral pathogen-immune cells transport to dysfunctional epithelial junctions. Some studies reported pathogen *P. gingivalis* in the bloodstream of healthy individuals and those with periodontitis after activities like tooth brushing, flossing, or chewing food (Horliana et al., 2014). Tsukasaki et al. observed the formation of oral bacterial colonies in liver and spleen cells following prolonged ligature placement around a tooth in a murine model of periodontitis. The bacterial species were also present in the oral cavity but were notably absent in fecal samples. This suggests that the systemic spread of oral bacteria occurs when the oral barrier is compromised (Tsukasaki et al., 2018).

Severe periodontitis is associated with elevated levels of pro-inflammatory mediators and recruitment of immune cells to the affected site, including increased neutrophil numbers in the blood. The inflammatory response caused by this inflammatory chronic disease can disrupt the tight junctions between endothelial cells, leading to increased permeability of blood vessels. This increased permeability allows inflammatory mediators, bacterial components, and immune cells to enter the vessel walls and surrounding tissues, promoting systemic inflammation (Nakajima et al., 2010). Periodontal pockets surface area presents a large bacterial biofilm accumulation, ranging from 50 cm^2 to 200 cm^2, allowing bacterial products such as lipopolysaccharides or proteases to diffuse into the blood stream (Hujoel et al., 2001).

Via transient bacteremia, bacterial infection is cleared by the immune system. However, some can evade the immune response and survive in the bloodstream. It is known that leukocytes are not effective in recognizing and engulfing bacteria in high-velocity liquids, although erythrocytes can attract bacteria by electrical charges on their surface and kill them by oxidative attack. Despite

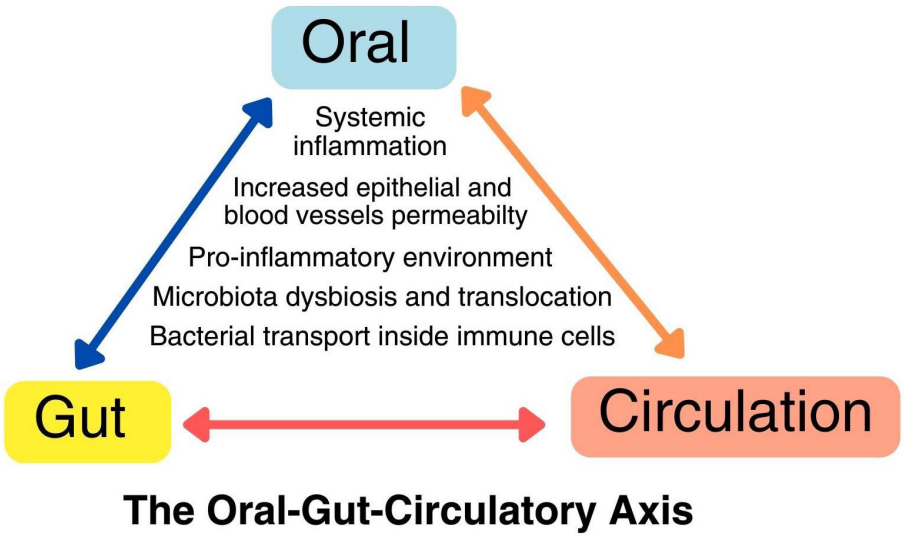

FIGURE 1
The oral-gut-circulatory axis. This axis involves a pro-inflammatory state and the recruitment of immune cells due to local dysbiosis, resulting in heightened permeability in both epithelial cells and endothelial cells of blood vessels. This increased permeability allows pathogens to spread through transient bacteremia and intracellular transport within immune cells like macrophages and neutrophils. This axis operates in a dynamic, bidirectional manner, leading to direct and indirect changes at distant body sites.

this, some bacteria can survive in the liver or spleen promoting low-grade inflammatory response and inducing cytokine secretion by other tissues/organs (Minasyan, 2014). Bacterial reservoir inside erythrocytes provides a long-term bacteria survival causing antibiotics ineffectiveness and immune reactions (Minasyan, 2017).

Individuals with periodontitis have elevated circulatory levels of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-alpha. Once in the circulation, these cytokines can then exert their effects on other tissues and organs, contributing to systemic inflammation and potentially leading to the development of comorbidities at distant sites (Nakajima et al., 2010). In addition, these cytokines are released in response to bacterial pathogens in the gums, acting as immune cells recruiters at the infection site.

Recent research suggests a potential link between immune cells activated in the oral cavity and the development of gut inflammation. It proposes that these immune cells can migrate from the oral cavity to the gut, where they can play a role in promoting inflammation. This is referred to as an indirect pathway, as the immune cells themselves do not directly trigger gut inflammation. Instead, they contribute to the inflammatory process by priming other cells or interacting with various components of the immune system. An alternative idea, called the Trojan Horse hypothesis, suggests that immune cells could transport pathogens throughout the body.

In periodontitis, *P. gingivalisis* found intracellularly in DCs, which affects the differentiation of these immune cells while using them as vehicles to enter the circulation and disseminate to distant organs (Carrion et al., 2012). On the other hand, recent research has shown that human neutrophils can carry and spread viable *F. nucleatum* after phagocytosis by using both an *in vitro* microfluidic device and a zebrafish model. The researchers previously found that *F. nucleatum* can suppress immune responses in neutrophils and survive within them, suggesting that intracellular bacterium can avoid the host's immune defenses and spread from the oral cavity (Ellett et al., 2023). Additionally, macrophages have been proposed as a communication network linking the oral cavity to distant sites. *P. gingivalis* has been shown to invade and persist within resident macrophages. Moreover, studies in animal models indicate that *P. gingivalis*-infected macrophages can produce cytotoxic extracellular vesicles, damaging distant organs. *P. gingivalis* has evolved intricate strategies to evade macrophage antimicrobial defenses. Understanding *P. gingivalis*-macrophage interactions may yield insights into disease mechanisms and potential therapies (Lin et al., 2022). Moreover, *F. nucleatum* can also survive within the cytoplasm of infected macrophages by the expression of indoleamine-2,3-dioxygenase, which can amplify the impairment of peripheral blood lymphocyte function. This dual effect allows *F. nucleatum*-infected macrophages to evade cellular death (Xue et al., 2018). These mechanisms suggest that common oral bacteria could connect oral and systemic diseases by utilizing the body's immune cells for transport. Further investigation should be done to study if this mechanism for immune cells-mediated bacterial dissemination could apply to other oral bacterial species and potentially contribute to the link between oral and systemic diseases.

From oral bacterial translocation to gut microbiota dysbiosis

The study of the oral and gut microbiomes has mostly been conducted in an organ-specific manner, which overlooks the fact that the mouth and gut are anatomically continuous regions; moreover, both regions are chemically connected through the passage of salivary fluids and digested food through the GI tract (Figure 2A). Saliva is estimated to contain about 10^6 bacteria per ml, which would result in people orally ingesting as many as 10^{12}-10^{13} bacteria day (Von Troil-Lindén et al., 1995). The oral cavity is the entry point for the digestive tract and is continuously exposed to various external factors, including microorganisms, nutrients, and xenobiotics. The Human Microbiome Project (Peterson et al., 2009) showed that more than half of the total bacteria in the human body are present in the GI tract (29%) and the oral cavity (26%). Interestingly, oral bacteria have also been found in distant sites such as the pancreas and gut, indicating direct crosstalk between microbiota at different locations (Chung et al., 2021).

The transmission and invasion of oral pathogens in the gut require the presence of at least two essential factors, one within the oral microbiota and the other within the gut microbiota. The first prerequisite is an elevated number of oral bacteria in the mouth caused by oral dysbiosis which enhances the likelihood of gut translocation. The second prerequisite requires the disruption of the intestinal mechanisms to resist colonization by opportunistic bacteria, which is typically conferred by gut dysbiosis. This disruption may be a necessary step to allow oral pathobionts, which have successfully traversed the gastric barrier, to establish colonization within the gut (Kitamoto and Kamada, 2022). Kageyama et al. (Kageyama et al., 2023) investigated both saliva and stool samples using 16S rRNA gene amplicon analysis and the amplicon sequence variant (ASV) approach to explore the translocation of oral bacteria to the gut. The study found that ASVs shared with an individual's salivary microbiota were present in the gut microbiota of 72.9% of subjects. Notably, the sharing and similarity of oral-gut microbes were more pronounced within an individual than across different individuals (Schmidt et al., 2019).

Overall, the acidic environment of the stomach plays an important role in protecting the host against potential infections from ingested microbes, as well as in regulating the composition of the gut microbiota. Research findings indicate that over 99% of oral microbes that are ingested are rendered inactive or eliminated as they traverse the stomach. The high acidity of the stomach environment makes it inhospitable for the survival of most bacteria. However, some microorganisms, such as *Helicobacter pylori (H. pylori)*, are adapted to survive in the acidic environment of the stomach and can colonize the gastric mucosa (Hunt et al., 2015). Long-term colonization by *H. pylori* can cause achlorhydria and decreased acid secretion, leading to changes in the gastric microbiota (Hunt et al., 2015). Additionally, gut colonization by oral-origin bacteria, Streptococcus and Veillonella, is observed in patients with gastric achlorhydria caused by long-term use of proton pump inhibitors (Schmidt et al., 2019). Furthermore,

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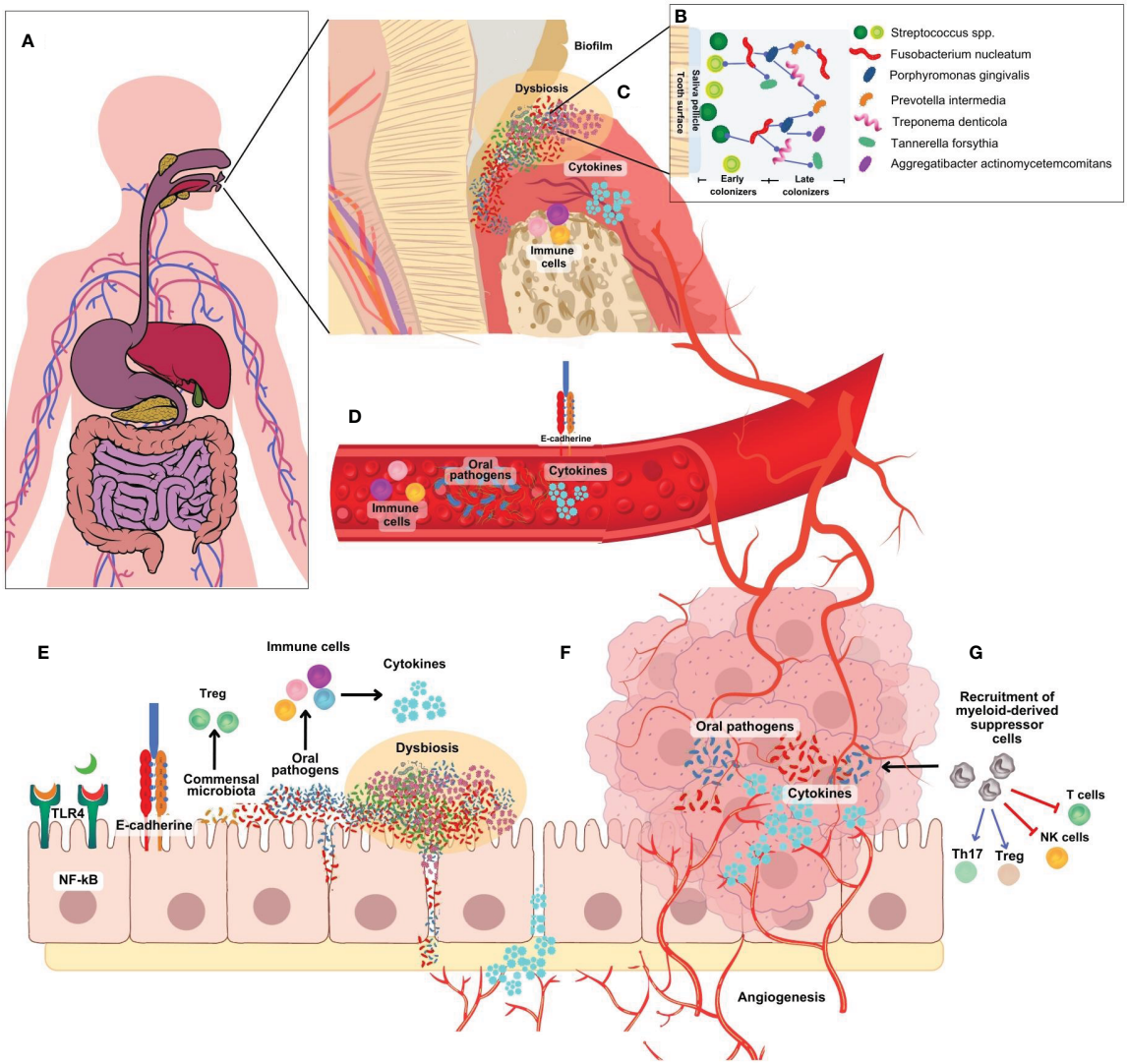

FIGURE 2
(A) Microbiome research has traditionally focused on specific organs, neglecting the continuous anatomical connection between the mouth and gut. **(B)** The development of an *F nucleatum-P. gingivalis* bacterial complex is central to the formation of oral biofilm. *F nucleatum* acts as a bridge attaching early colonizers like *Streptococcus* spp, *Actinomyces* spp, *P. gingivalis* and others. **(C)** In the periodontal pocket, initial host response is characterized by the infiltration of NK cells, neutrophils, granulocytes, and lymphocytes to present antigens to dendritic cells. T cells secrete cytokines such as TNF- α, IL-1, IL-4, IL-10, IFN- γ, TGF- β, RANK-L. **(D)** Bacteria, metabolites and inflammatory factors can travel from the oral cavity to other parts of the body into the blood vessels by increased endothelial permeability. **(E)** Oral bacteria, particularly *F nucleatum* and *P. gingivalis*, can infiltrate intestinal cells, provoke pro-inflammatory cytokine production, and induce a pro-inflammatory environment **(F)**. They activate various pathways linked to inflammation and cancer development and can disrupt the colon's epithelial barrier, increasing permeability to opportunistic bacteria and promoting chronic inflammation, angiogenesis, and cancer progression. **(G)** A diverse array of immune cells are recruited to the tumor site, including myeloid-derived suppressor cells (MDSCs). MDSC block T cells and NK cells activation, and recruitment and activation host suppressor cells such as FoxP3+ regulatory T cells.

aging displayed a significant correlation with an increased abundance of oral bacteria in the gut microbiota. This association could be attributed to the diminished barrier function of the gastrointestinal tract due to aging-related alterations (Kageyama et al., 2023).

The gut microbiota contains 1000-1150 bacterial species, some of the most abundant groups are *Bacteroidetes, Dorea/Eubacterium/ Ruminococcus, Bifidobacteria, Proteobacteria, and Streptococci/ Lactobacilli* (Qin et al., 2010). Maintaining a balanced relationship between the host and commensal microorganisms is crucial for proper functioning. The host provides a fit environment and nutrients for the microorganisms to thrive, while the commensal bacteria contribute to various physiological functions and offer protection against pathogens invasion. This intricate balance is achieved through complex interactions between the host and microbiota (Lee et al., 2013). Oral administration of *P. gingivalis* in C57BL/6 mice alters gut microbiota with an increase in *Bacteroides* and *Staphylococcus* and a decrease in commensal species such as *Firmicutes* and *Lactobacillus* when compared to sham-inoculated mice (Nakajima et al., 2015). *Lactobacillus* species are recognized as probiotics, known for their ability to promote digestive health and support immune function. Decreased levels of

probiotic bacteria can disrupt the gut balance, leading to dysbiosis and compromised intestinal barrier. This may increase intestinal permeability, allowing toxins and bacteria to enter the bloodstream and potentially trigger inflammation and health problems (Arimatsu et al., 2014; Gao et al., 2015; Kobayashi et al., 2020).

In mice administered with *P. gingivalis*, the expression of genes responsible for intestinal alkaline phosphatase (Akp3) and the tight junction protein (Tjp1) in the small intestine was both decreased. (Arimatsu et al., 2014). Additionally, Kobayashi et al. (Kobayashi et al., 2020) found that the effects on the gut microbiota were not solely due to *P. gingivalis*, but also due to the other oral bacteria such as *Streptococcus mitis, Streptococcus salivarius, Porphyromonas nigrescens*. Interestingly, the study found that dysbiosis led to a decrease in the abundance of Th17 cells, a type of immune cell involved in protecting against pathogens, and a decrease in the production of immunoglobulin A (IgA), an antibody important for mucosal immunity in the small intestine. IgA and Th17 cells play important roles in maintaining gut homeostasis and protecting against pathogen invasion. With the growing body of evidence highlighting the significance of interactions between gut microbiota and immune cells in maintaining the immune system balance, Morton et al, utilizing Kaede transgenic mice, observed a bidirectional immune cell trafficking between the gut and other organs (Morton et al., 2014). The migration of oral immune cells to the gut is a pivotal aspect of the mouth-gut axis in intestinal inflammatory conditions, such as colitis. During periodontal inflammation, Th17 cells are generated in the oral draining lymph nodes, specifically recognizing oral bacteria. Upon reaching the gut, these orally primed Th17 cells can be activated by translocated oral pathobionts, contributing to colitis development (Kitamoto and Kamada, 2022).

Interestingly, Nagao et al. (Nagao et al., 2022) hypothesized that oral pathogens in the oral cavity, known as pathobionts, could migrate to the intestinal tract and trigger an immune response that exacerbates periodontitis. They infected mice with *P. gingivalis* showing that the infection provoked pathobiont migration from the oral cavity to the intestine where they were recognized by DCs triggering the Th17-type immune response, leading to the production of IL-17 and other pro-inflammatory cytokines. Interestingly, the study revealed that Th17 cells can migrate from the intestine to the oral cavity upon oral infection. This suggests that the migration of Th17 cells may be one of the mechanisms by which the gut microbiota can affect periodontitis. These findings suggest that the oral-gut axis is bidirectional, and that the gut microbiota can affect the homeostasis of the mouth. It is crucial to further explore and understand the specific mechanisms involved in the communication between the oral and gut microbiota to gain insights into their collective impact on oral, gut, and systemic health. The examination of host-microbe interactions in murine models has played a crucial role in unraveling the gut-axis relationship. However, given the variations between the microbiotas of mice and humans, it is imperative to invest additional effort into comprehending how these findings apply to humans (Kitamoto and Kamada, 2022).

An imbalance in the oral microbiome can not only lead to oral disorders such as dental caries and periodontitis, but also systemic diseases such as irritable bowel syndrome, IBD (Tsuzuno et al., 2021; Kitamoto and Kamada, 2022), and colorectal cancer (CRC) (Flemer et al., 2018; Tortora et al., 2022). Metagenomic sequencing analysis revealed the presence of four species enriched in tumor samples in CRC patients with oral cavity origin: *Porphyromonas asaccharolytica, F. nucleatum, Prevotella intermedia*, and *Parvimonas micra*. Remarkably, these bacteria were found to form mutually beneficial networks within the microbial community where *F. nucleatum* occupied a central position within this network, suggesting a pivotal role for oral bacteria in shaping these interactions (Dai et al., 2018) similar to the role that *F. nucleatum* plays in oral biofilm as a "bridge" bacteria between early and late colonizers. Bacterial biofilms that invade the mucus layer can be observed on the colon mucosa in around 50% of CRC patients and roughly 13% of individuals without the disease (Tomkovich et al., 2019). These findings suggest that the composition and arrangement of the microbiota, rather than the overall health status of the human donor, are connected to the development of tumors. Additionally, given the association between bacterial biofilms and CRC, it is crucial to gain insight into the functional significance of bacterial organization within the colon mucosa in relation to CRC. The next phases of research should involve the retrieval of oral and colonic mucosal biofilms for comprehensive analyses, focusing on bacterial community composition and functional profiling to identify commonalities shared among these microbiota, providing valuable insights into the mechanisms underlying their role in disease development.

Oral pathogens in the carcinogenesis of colon cancer

The development and progression of colon cancer is associated with several factors including genetics, environmental, diet, lifestyle, and microbiota (Tortora et al., 2022). Several studies have highlighted the relationship between the gut microbiota and colon cancer (Cho et al., 2014; Fulbright et al., 2017; Koliarakis et al., 2019; Tortora et al., 2022). Oral diseases, such as periodontitis, involve chronic inflammation triggered by a multispecies bacterial community in the subgingival region in the mouth. While inflammation mainly occurs in the oral cavity, studies reveal that inflammatory agents, subgingival bacteria, and their components can disseminate, contributing to extraoral diseases like cancer (Hoare et al., 2019; Koliarakis et al., 2019). The association between periodontal disease and CRC was established in the Nurses' Health Study, in which women with moderate or severe periodontitis were at moderately increased risk of developing CRC (Momen-heravi et al., 2018; Nwizu et al., 2020).

As previously mentioned, the formation of an *F. nucleatum-P. gingivalis* bacterial complex is central to the pathogenesis of periodontitis (Ahn et al., 2016). *F. nucleatum* acts as a bridge attaching other early colonizers like *Streptococcus* spp, *Actinomyces* spp, *P. gingivalis* and others (Figure 2B). In the periodontal pocket (Figure 2C), the initial host response is marked by the infiltration of NK cells, neutrophils, and DCs,

followed by the subsequent influx of lymphocytes after antigen presentation. Recruited T cells subsequently contribute to the response by secreting cytokines like TNF-α, IL-1, IL-4, IL-10, IFN-γ, TGF-β, and receptor activator of nuclear factor kappa-B ligand (RANK-L) (Hoare et al., 2019). One of the complications of periodontal disease is the migration of bacteria from the oral cavity to other parts of the body. This occurs because there is an increase in the number of oral bacteria in the sub-gingival biofilm in intimate contact with the ulcerated gingiva creating an entry point for oral bacteria into the bloodstream, facilitating their dissemination to remote locations such as the colon (Figure 2D) (Nwizu et al., 2020). These bacteria are mostly multispecies with high numbers of *Clostridium, Peptostreptoccocus* and *Fusobacterium*.(Hoare et al., 2019) In the gut (Figures 2E-G), oral bacteria, can invade and adhere to intestinal epithelial cells, increase the production of pro-inflammatory cytokines, and contribute to a pro-inflammatory microenvironment (Bashir et al., 2016). These findings imply that *F. nucleatum* may exert a significant indirect influence on the development of periodontal diseases by promoting the proliferation of *P. gingivalis*, potentially contributing to the etiological factors involved. The ability of *F. nucleatum* to adhere to both host cells and other bacterial species enhances its capacity to form complex microbial communities and promote the colonization of harmful bacteria in the gut. These findings highlight the interconnected nature of the oral-gut axis and suggest that *F. nucleatum* and *P. gingivalis* may have significant influence not only in the oral cavity but also in gut (Figure 2F) (Castellarin et al., 2012). Interestingly, *F. nucleatum* and *P. gingivalis* are known to synergistically promote oral cancer progression (Binder Gallimidi et al., 2015). Similar studies should be proposed to study potential synergy between both oral pathogens and gut dysbiosis in colon cancer.

In the study by (Tsuzuno et al., 2021) *P. gingivalis* amplified gastrointestinal inflammation by directly engaging with the intestinal epithelial barrier in a susceptible host and showed a higher colitogenic potential compared with other periodontal pathogens such as *Prevotella intermedia* and *F. nucleatum* in mice. In summary, the potential mechanisms through which *P. gingivalis* reduces the protein level of ZO-1 *in vivo*, also known as tight junction protein-1, encompass several interconnected events: detachment of intestinal mucus bacterial invasion into intestinal epithelial cells, and cytosolic degradation of ZO-1(Tsuzuno et al., 2021). The study noted that following the intravenous administration of *P. gingivalis*, CD4+ T cells stimulation heightened the inflammatory response among colon and lamina propria lymphocytes, resulting in an elevated Th17/Treg ratio. These results suggest that the increase in Treg cells could potentially counterbalance the escalation of intestinal tissue inflammation induced by *P. gingivalis*. However, further research is imperative to gain a comprehensive understanding of this phenomenon (Li et al., 2022). There is growing evidence suggesting a potential link between chronic inflammation, such as colitis, and an increased risk of developing colon cancer. This connection underscores the intricate interplay between inflammation caused by dysbiosis and the development of colon cancer.

Numerous investigations have shown dysbiosis-related gut microbiota associated with *F. nucleatum* infection in the tumor tissues of CRC patients (Gao et al., 2015; Dai et al., 2018; Koliarakis et al., 2019; Huh and Roh, 2020; Yu et al., 2022). The argumentative aspect revolves around determining whether *F. nucleatum* contribution to CRC is correlational or causational. Compelling evidence substantiates both hypotheses. Numerous studies on the gut microbiota have consistently demonstrated a significant prevalence of *F. nucleatum* within the tumor tissue and fecal samples of individuals with CRC (Wang et al., 2021). Etiological investigations have further elucidated the role of *F. nucleatum* as a bacterium that promotes carcinogenesis at various stages of CRC development. Two different groups were the first to show an increased abundance of this oral pathogen in CRC tissues when compared to normal tissues (Castellarin et al., 2012; Kostic et al., 2013). A recent study highlighted that identical strains of *F. nucleatum* were identified in both the saliva and colorectal tumors of patients with CRC (Komiya et al., 2019), in addition to the findings that oral *F. nucleatum* translocate to the colon through the hematogenous route (Abed et al., 2020). This suggests that CRC-associated *F. nucleatum* likely originates from the oral cavity, underscoring a potential link between oral bacteria and the development of CRC.

Fusobacterium is numerous in human adenomas, proposing an early role in colon carcinogenesis (McCoy et al., 2013). Kostic et al. (Kostic et al., 2013) investigated the association of *F. nucleatum* in stool and colon samples from human colorectal adenomas and adenocarcinomas. It assessed the impact of *F. nucleatum* on cancer progression and tumor-related inflammation in $Apc^{Min/+}$ mouse model of intestinal tumorigenesis. The bacterium amplified tumor numbers and led to accelerated tumorigenesis in both the small intestine and colon, infiltration of distinct myeloid cell subsets into the tumors, and an NF-κB-driven proinflammatory profile, like F. nucleatum-positive colorectal carcinomas in humans. APC gene mutations typically manifest as early molecular alterations during the transition of epithelial cells into adenomas (Kostic et al., 2013). Hence, probably the early somatic mutations are responsible for tumor initiation, which occur before *F. nucleatum* accumulation in the tissue. This concept is further substantiated by the mechanisms described by which this bacterium contributes to CRC in the study by Rubinstein et al, where they showed that *F. nucleatum* through virulence factor FadA adhesin can adhere and invade tumor cells, and promote oncogenic and inflammatory responses to promote progression of CRC (Rubinstein et al., 2013).

Another study using the same $Apc^{Min/+}$ mouse model showed that *F. nucleatum* induced DNA damage and cell growth in CRC by activating the E-cadherin/β-catenin pathway (Guo et al., 2020). These genetic alterations play a role in disruption of the epithelial barrier and the mucous layer facilitating the infiltration of *F. nucleatum* and other opportunistic bacteria, allowing them to establish themselves within the tumor microenvironment. However, it is essential to acknowledge that the murine model utilized lacks full characterization, potentially limiting its ability to entirely recapitulate all aspects of *F. nucleatum*-associated CRC observed in humans. These collective findings suggest that *F. nucleatum* infection has been implicated as an added

environmental risk factor for CRC by promoting a proinflammatory microenvironment conducive to the progression of colorectal neoplasia. Moreover, the high abundance of *F. nucleatum* in CRC is associated with poorer survival(Flanagan et al., 2014; Mima et al., 2016; Komiya et al., 2019) and recurrence after chemotherapy (Yu et al., 2017).

There are three major virulence factors that contribute to the promotion of CRC: LPS, the adhesin FadA, and autotransporter protein Fap2 (Kostic et al., 2013; Abed et al., 2016; Lee et al., 2019). Fap2 operates as an inhibitor, dampening the tumor-killing effectiveness of T cells and NK cells. It achieves this by directly triggering the inhibitory receptor known as T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT), thereby contributing to a mechanism of tumor-immune evasion (Gur et al., 2015). Furthermore, Fap2 exhibits binding affinity towards a carbohydrate structure, specifically D-galactose-β(1–3)-N-acetyl-D-galactosamine (Gal-Gal/NAc), which is prominently present in CRC. Moreover, metabolic specialization may underlie the distinctive impact of *F. nucleatum* within the tumor milieu. As an asaccharolytic bacterium, it refrains from competing for glucose, a favored substrate in cancer metabolism, thereby conferring upon it a competitive advantage distinct from other microbes residing in the tumor microenvironment (Flynn et al., 2016).

Additionally, Fap2 triggers the release of proinflammatory cytokines, namely IL-8 and C-X-C motif chemokine ligand 1 (CXCL1), which enhances the migration of cancer cells (Casasanta et al., 2020). This effect contributes to the promotion of CRC cell invasiveness and potentially impacts disease progression. Synergically, LPS induces the secretion of a range of inflammatory cytokines, including IL-2, IL-6, IL-8, IL-10, IL-17, TNF- α, and upregulates NF-κB levels. On the other hand, FadA facilitates the attachment to epithelial and endothelial cells while also triggering inflammation. This interaction facilitates the attachment of *F. nucleatum* to CRC cells through the hematogenous route, contributing to the association between the oral pathogens and colon carcinogenesis (Abed et al., 2016). Furthermore, this adhesin activates pro-carcinogenic pathways directly within colon cancer cells, specifically by initiating E-cadherin-β-catenin signaling (Ito et al., 2015; Nosho et al., 2016; Rubinstein et al., 2019). In summary, *F. nucleatum* plays a role in promoting the initiation and progression of CRC through various mechanisms, including its localization within the colorectal environment, its ability to proliferate, promote immune suppression, facilitation of metastasis, and contribution to chemoresistance. These multifaceted effects collectively contribute to the complex relationship between *F. nucleatum* and CRC development.

F. nucleatum and *P. gingivalis* are involved in the direct activation of inflammation and carcinogenesis through multiple pathways. Although the precise role of these oral pathogens in the carcinogenesis of colon cancer is still unclear (Castellarin et al., 2012). Some studies suggest that *P. gingivalis* and *F. nucleatum* may directly interact and attach with intestinal cells and can disrupt the integrity of the epithelial barrier lining the colon. This disruption can lead to increased permeability of the barrier, allowing other opportunistic bacteria and their byproducts to enter the underlying tissues and alter the microbial communities in the gut (Figure 2F). This promotes chronic inflammation creating a favorable environment for cancer development and progression. These bacteria also stimulate angiogenesis, the formation of new blood vessels, which is essential for tumor growth as it supplies nutrients and oxygen to cancer cells, contributing to the growth and spread of cancer cells, bacterial metabolites, and inflammatory factors. Furthermore, they interact directly with colon cells, promoting signaling pathways that encourage cell survival, proliferation, and resistance to cell death – all hallmarks of cancer cells (Fulbright et al., 2017).

In response to the abnormal growth of colonic cells and the oral pathogens infection, a diverse array of immune cells are recruited to the tumor site (Figure 2G). Among them are tumor-infiltrating lymphocytes (TILs), including CD4+ helper T cells and CD8+ cytotoxic T cells, pivotal for recognizing and combating cancer cells. Macrophages, exhibiting both pro-inflammatory (M1) and anti-inflammatory (M2) functions, influence tumor progression and immune responses. DCs play a crucial role by capturing antigens and initiating immune reactions. NK cells directly target and eliminate cancer cells, restricting tumor growth. Conversely, myeloid-derived suppressor cells (MDSCs) can hinder immune responses by inhibiting T cell activity, allowing tumors to evade immunity. The intricate interplay and functions of these immune cells within the tumor context remain a focal point of research, offering potential avenues for innovative cancer treatments (Yin et al., 2020).

P. gingivalis is often recognized as highly proficient in evading and undermining the immune system, employing various tactics to elude and undermine immune defenses. While possessing an array of virulence factors, including gingipains, LPS, and fimbriae, *P. gingivalis* is known for invading epithelial, fibroblast, endothelial and specific immune cells. Leukocytes in circulation may function as a "Trojan horse" facilitating the oral bacterial dissemination through the bloodstream and spreading to the gut (Carrion et al., 2012). A recent review described the potential mechanisms of translocation of *P. gingivalis* from the oral mucosa by circulatory dissemination, like the Trojan Horse mechanism (de Jongh et al., 2023). A study showed that a *P. gingivalis* strain was able to significantly avoid phagocytosis and escape from macrophages (Werheim et al., 2020). Aside from macrophages, DCs are the other phagocytic cells that may play a role in blood dissemination of *P. gingivalis* (de Jongh et al., 2023). One mechanism is by engaging its fimbrial proteins with complement receptor 3 (CR3). CR3 is a major receptor for the phagocytosis of opsonized particles (Carrion et al., 2012). The clinical significance of this study (Carrion et al., 2012) lies in the remark that *P. gingivalis* strains expressing Mfa1+ infect DCs within oral mucosal tissues and the bloodstream of individuals with periodontitis. Subsequently, these infected DCs can spread to distant sites where angiogenesis occurs. El-Awady et al. investigated the mechanism of autophagy evasion by *P. gingivalis* promoting the survival within human monocyte-derived dendritic cells (MoDCs). This process is facilitated by its glycoprotein fimbriae, specifically Mfa-1, which interacts with the C-type lectin DC-SIGN found on DCs. The other primary fimbriae, known as

FimA, is a TLR2 agonist targets thereby inhibit the autophagic degradation of *P. gingivalis*, which may also facilitate its dissemination to distant sites within DCs (El-Awady et al., 2015). Furthermore, another investigation revealed that macrophages subjected to differentiation in the presence of IL-34, a predominant cytokine in the oral gingival environment, exhibit significantly diminished capacity to eliminate engulfed *P. gingivalis* (Almarghlani et al., 2022). In conclusion, *P. gingivalis* exhibits a remarkable capacity to evade immune defenses and establish persistence within diverse cell types, notably DCs and macrophages. Nevertheless, the specific role of *P. gingivalis* on intestinal inflammation has yet to be fully investigated. Additionally, the precise mechanisms of translocation from the oral mucosa to the gut remain unclear.

Despite a continuous influx of numerous oral bacteria into the gastrointestinal tract, the impact of periodontal pathogens on intestinal inflammation remains as an uncharted territory (Tsuzuno et al., 2021). In Figure 3 we depicted a comprehensive landscape of *P. gingivalis* and *F. nucleatum* virulence factors in the development and progression of CRC. Gingipain proteases produced by *P. gingivalis* can activate NF-κB and MMP-9, both significant for tumor invasion and metastasis. Furthermore, proteins associated fimbriae aid in the formation of biofilms, as well as the invasion and dissemination of the bacteria through blood DCs. Notably, Mfa1 fimbriae have been demonstrated to promote oncogenic signaling, recruiting tumor-infiltrating myeloid cells and promoting the expansion of immune-suppressive MDSC. Additionally, FimA can interact with *Streptococci, Actinomyces*, and *Treponema* spp and promote the release of the inflammatory cytokines TNF–α, IL–6, metalloproteinase-8 (MMP–8) and MMP –9 via the TLR4/NF–κB signaling pathway (Hoare et al., 2019). *P.* gingivalis binds to protease activated receptor and cleaves the MMP-9 active form, which subsequently facilitates tumor cell invasion and migration (Hoare et al., 2019; Yu et al., 2022). *P. gingivalis* displays antiapoptotic properties within epithelial cells through various mechanisms. These include the enhancement of both the PI3K/AKT and JAK/STAT3 signaling pathways, alongside the inhibition of caspase 3 and caspase 9 activity which are the final stage of the pathways of apoptosis. Additionally, *P. gingivalis*-triggered Akt–STAT3 signaling facilitates the expression of programmed cell death ligand 1 (PD-L1) while dampening CD8+ T-cell functionality, presenting another potential immune mechanism employed by the bacterium to further cancer progression (Yu et al., 2022). Also, *P. gingivalis* increases cell proliferation by modifying the activity of p53, cyclins and the WNT/β-catenin and MAPK/ERK pathways (Mu et al., 2020).

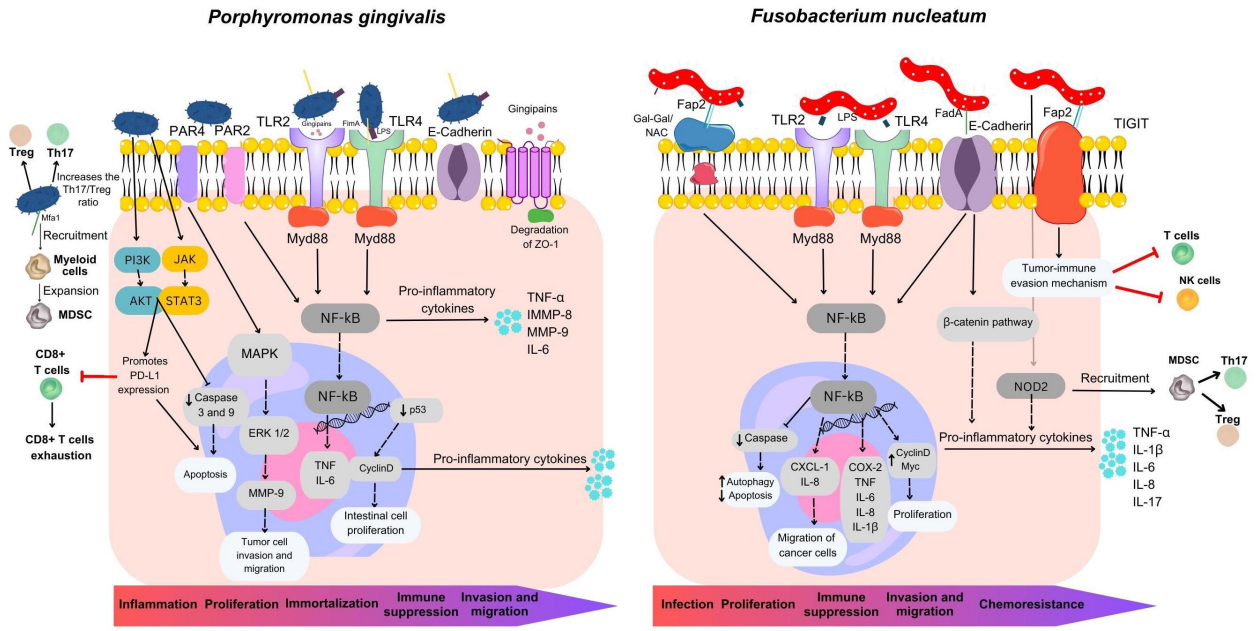

FIGURE 3
Overview of mechanisms utilized by *Fusobacterium nucleatum* and *Porphyromonas gingivalis* in colorectal carcinogenesis. *F. nucleatum* plays a multifaceted role in CRC development, contributing to its localization within the colorectal environment, proliferation, immune suppression, metastasis, and chemoresistance. It possesses three major virulence factors: Fap2, FadA, and LPS. Fap2 acts as an inhibitor, suppressing the anti-tumor activity of T and NK cells by engaging the inhibitory receptor TIGIT, facilitating tumor immune evasion. Fap2 also binds to the CRC-associated carbohydrate structure Gal-Gal/NAc, promoting attachment, and induces proinflammatory cytokine release, enhancing cancer cell migration. FadA invades and activates pro-carcinogenic pathways in colon cancer cells. *F. nucleatum*'s LPS stimulates inflammatory cytokine production, establishing a pro-inflammatory microenvironment driving CRC progression. It also targets caspase activation via NOD2, activating the IL-17F/NF-κB pathway, leading to intestinal damage, increased cytokine expression, and MDSC recruitment. *P. gingivalis* employs multiple processes to drive inflammation, cancer cell growth, invasion, metastasis, and immune suppression. It activates NF-κB and MMP-9 through gingipain proteases, promoting cancer cell invasion. Mfa1 fimbria induces oncogenic signaling and immune-suppressive MDSC expansion. Interactions with other oral bacteria lead to inflammatory cytokine release via the TLR4/NF-κB pathway. *P. gingivalis* enhances cell survival via PI3K/AKT and JAK/STAT3 while inhibiting caspase activity, modulating cell proliferation through various pathways, and manipulating immune responses by promoting PD-L1 expression and suppressing CD8+ T-cell activity via Akt-STAT3 signaling. These mechanisms collectively contribute to cancer progression.

Therefore, these results offer a valuable understanding of the multiple mechanisms employed by *P. gingivalis* to suppress the immune response. By elucidating the complex components and identify specific molecular targets of *P. gingivalis*-mediated immunosuppression, we can develop innovative strategies to augment the immune response that can be leveraged to enhance the effectiveness of immunotherapeutic approaches in colon cancer, ultimately benefiting patient outcomes.

Other oral bacteria, such as *T. denticola* and *T. forsythia* can also trigger the death of epithelial cells. Damaged cells release chemical signals in the form of chemokines, cytokines, and pro-inflammatory molecules that attract immune cells to the site, creating a specific environment for both innate and adaptive immune responses including MDSCs recruitment. If the cause of this process is not resolved, it can lead to chronic inflammation and persistent tissue damage (Jun et al., 2017). Both periodontitis and colon cancer have been associated with elevated MDSC levels, and increased levels of TNF-α, IL-1β, IL-6 and MMP-9 are also common factors in both conditions. In individuals without underlying health issues, hematopoietic stem cells undergo a developmental process, progressing into immature myeloid cells (IMCs). These IMCs can subsequently differentiate into granulocytes, monocytes, or mature into macrophages or DCs. In contrast, cancer patients experience a disruption in the maturation of IMCs, resulting in an elevated population of MDSCs. Importantly, both *F. nucleatum* and *P. gingivalis* have been demonstrated to induce the recruitment and proliferation of MDSCs (Kostic et al., 2013; Nosho et al., 2016; Sakamoto et al., 2021). These cells can suppress the activity of T cells, leading to T cell exhaustion and impaired anti-tumor immune responses (Cui et al., 2021). A previous report by Chen at al (Chen et al., 2020) found that the expression of NOD2, a gene coding for proteins associated with MDSCs, was upregulated in intestinal epithelial cells infected with *F. nucleatum*. *F. nucleatum* directs its actions towards caspase activation via NOD2, subsequently triggering the IL-17F/NF-κB pathway both *in vivo* and *in vitro* models. This cascade of events leads to damage to the intestinal epithelium and the upregulation of IL-1β, IL-6, IL-17F, and TNF-α (Chen et al., 2020). The activation of the TLR4/NF-κB signaling pathway by gingipain proteases and FimA, bacterial proteins produced by *P. gingivalis*, leads to an increased release of inflammatory cytokines which can have implications for the expansion and activation of MDSCs (Cai et al., 2019). Activated MDSCs within the tumor microenvironment can suppress both innate and adaptive immune responses in several ways. MDSCs inhibit the activity of T cells and NK cells and increase Treg numbers. MDSCs can also release reactive oxygen and nitrogen species, which can damage T cell receptors and interfere with T cell signaling (Figure 3) (Tang et al., 2021).

Based on the evidence, we propose that tumors exhibit elevated expression of genes related to MDSCs when co-infected with *F. nucleatum* and *P. gingivalis*. This co-infection synergistically enhances the presence of MDSCs within the tumor microenvironment, promoting the secretion of pro-inflammatory cytokines, producing sustained chronic inflammation, and leading to intestinal damage. These effects can lead to augmented gut permeability, enabling the infiltration of additional pathogens into the underlying tissues, thereby promoting the development of tumors.

MDSCs can also express PD-L1, which binds to PD1 on T cells, and causes secretion of IL-10 and TGF-β, which stimulate Treg activation and expansion (Tang et al., 2021). T cell exhaustion is a state of dysfunction characterized by the progressive loss of effector functions and sustained expression of inhibitory receptors, such as PD-1. Exhausted T cells are less capable of recognizing and eliminating cancer cells effectively. Targeting the recruitment and activity of MDSCs holds promise as a potential therapeutic strategy to alleviate T cell exhaustion and enhance anti-tumor immune responses. Through the suppression of MDSC recruitment or function, there exists a potential opportunity to recover T cell activity and support the role of the immune system in identifying and eradicating cancerous cells. This could involve targeting specific molecules or signaling pathways involved in the recruitment process. *F. nucleatum* and *P. gingivalis* have been shown to recruit MDSCs, leading to T cell exhaustion and impaired anti-tumor immune responses. Further research is needed to identify specific molecular targets and develop effective therapeutic interventions aimed at disrupting the immunosuppressive effects of MDSCs in the context of CRC and other cancers influenced by these bacteria.

Studying the link between oral bacteria and CRC also provides opportunities for prevention strategies. Promoting good oral hygiene practices and maintaining oral health may reduce the colonization and translocation of oral bacteria to the gut. Additionally, strategies that target the gut microbiota composition, such as dietary modifications or probiotic supplementation, can help create an environment that is less favorable for the growth and activity of these bacteria. As periodontitis is polymicrobial, mixed species potentially promote carcinogenesis both locally and in extraneous tissues, likely through complex microbial interactions. While mono-species effects on tumorigenesis pathways are elucidated, further research is required to understand the combined impact of oral species on carcinogenesis. Nevertheless, further research is needed to establish a clearer temporal relationship between *F. nucleatum* and CRC, which may be elucidated through prospective studies. By investigating the specific mechanisms by which *F. nucleatum* and *P. gingivalis* promote carcinogenesis, the field can not only develop sensitive and specific diagnostic tests that can aid in early detection and timely intervention but also identify potential therapies that specifically inhibit the activity of these bacteria or disrupt their interactions with host cells.

Conclusions and future perspectives

In conclusion, the oral-gut-circulation axis emerges as a dynamic model for host-microbial interactions, extending its influence well beyond the oral and gut systems including the circulatory system as an essential component. The oral cavity and gastrointestinal tract house the largest and most diverse microbial communities in the human

body. Recent research has revealed that these systems are not isolated but rather interact dynamically, shaping each other's composition and function. The circulatory system, as a conduit for pathogens, bacterial products, immune cell trafficking, and inflammatory factors, adds a layer of complexity to the oral-gut axis. Strategies aimed at mitigating systemic inflammation and circulatory factors may hold therapeutic promise in this context.

Moreover, there is an emerging link between oral microbiota and cancer susceptibility. Individuals diagnosed with periodontitis exhibit a significantly elevated colon cancer risk. Thus, maintaining oral health and treating oral infections such as periodontitis may hold the key to reducing the incidence of several conditions, including colon cancer. Based on the literature that shows the tumor-promoting potential of *F. nucleatum* in CRC, treatment targeting the reduction of *Fusobacterium* populations, particularly in the oral cavity where they are notably abundant and play an essential role in biofilm formation and structure, could potentially serve as a strategy to delay or prevent tumor progression in individuals at elevated CRC risk. Additionally, *P. gingivalis*, has emerged as another player in colon cancer pathogenesis. Together their ability to stimulate a pro-inflammatory milieu within the tumor microenvironment, modulate gut microbiota, and promote angiogenesis highlights their significance. Targeting these pathogens or their virulence factors could provide novel therapeutic avenues.

The development of non-invasive screening and diagnostic tools based on microbial signatures and inflammatory markers could significantly enhance current CRC screening practices. Traditional methods, such as colonoscopy or fecal occult blood tests, although effective, may face challenges related to patient compliance, invasiveness, and cost. Salivary and blood biomarkers, on the other hand, could provide a convenient, patient-friendly, and cost-effective alternative. Additional research is needed in prevention approaches such as personalized medicine based on microbiota profiles, probiotics for gut health restoration, and lifestyle modifications as non-invasive tools for the benefit of public health to decrease the incidence of conditions such as cancer.

Fostering multidisciplinary collaboration among experts from diverse fields will be instrumental in advancing our understanding of colon cancer and the development of immunotherapeutic interventions that target, for example, specific oral pathogens. In summary, the oral-gut-circulatory axis represents a promising frontier in colon cancer research, potentially reshaping our conception of this disease and opening innovative opportunities for prevention and treatment.

Author contributions

ST: Writing – original draft, Writing – review & editing, Conceptualization, Visualization. MA: Writing – review & editing. LM: Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Factors affecting the early establishment of neonatal intestinal flora and its intervention measures

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In recent years, it has become evident that early-life intestinal flora plays a pivotal role in determining human health. Consequently, it is imperative to explore the establishment of neonatal intestinal flora and its influencing factors. Early neonatal intestinal flora is influenced by a multitude of factors, including maternal and infant-related factors, as well as external environment. This review summarizes the colonization mechanism of intestinal flora in the early life of newborns and discussed their influence on the establishment of neonatal intestinal flora, taking into account factors such as delivery mode, gestational age and feeding mode. Additionally, this review delves into the natural or artificial reconstruction of intestinal flora colonization defects in infants born via cesarean section and premature infants, with the goal of establishing a theoretical foundation for preventing and treating issues related to neonatal intestinal flora colonization and associated diseases.

KEYWORDS

newborn, intestinal flora, caesarean section, premature delivery, breast milk

1 Introduction

The establishment of the human intestinal microbiota during early life plays a critical role in determining human, exerting profound effects on metabolic processes, growth, immune function, and even behavioral outcomes (Clemente et al., 2012). In recent years, there has been a substantial body of research focused on exploring the composition and functionality of the infant gut microbiota, revealing a distinct correlation between reduced microbial diversity and aberrant structural composition of the gut microbiota and the development of various diseases that may manifest in infancy or later in life, including conditions such as asthma, inflammatory bowel diseases, and metabolic disorders (Milani et al., 2017). Consequently, it is imperative to comprehensively investigate the governing

principles and contributing factors that shape the establishment of the neonatal gut microbiota during early life. With the rapid advancements in metagenomic sequencing technology, it is possible to explore the intricate internal patterns and interrelationships within the highly complex gut microbiota. In this review, we have undertaken a comprehensive review of the research progress pertaining to the colonization mechanisms of the early-life intestinal microbiota in newborns. We have delved into the extensive discussion of its profound influence on the establishment of the neonatal intestinal microbiota, considering critical factors such as delivery mode, gestational age, and feeding practices. Furthermore, we have conducted a rigorous analysis of both natural and artificial strategies for rectifying colonization deficiencies within the intestinal microbiota, specifically in the context of infants born via cesarean section and preterm birth. Our primary objective is to furnish a well-founded theoretical framework that can inform and guide proactive measures for the prevention and treatment of intestinal microbiota colonization anomalies and the resultant diseases affecting clinical infants.

2 Establishment of early intestinal flora

2.1 Discussions about the origin of intestinal flora colonization——utero or during childbirth

The traditional view suggests that the fetal environment *in utero* is sterile, and the colonization of the intestinal flora commences at birth. During childbirth, the neonate first encounters maternal intestinal, vaginal, and skin flora, as well as the surrounding environment. This initial contact facilitates the colonization and proliferation of the intestinal flora, which gradually evolves and establishes a mature microflora structure postnatally (Adlerberth, 2008). It is evident that maternal symbiotic bacteria play a crucial role in the early-life establishment of the neonatal intestinal flora. However, in line with pertinent research (Jiménez et al., 2005; DiGiulio et al., 2008; Aagaard et al., 2014; Collado et al., 2016; Chu et al., 2017b), bacteria were found in the placenta, umbilical cord and amniotic fluid in full-term pregnancy, which suggests that the fetus may not be entirely sterile *in utero* and that the initial colonization of the fetal intestinal flora may indeed commence within the maternal uterus. Jimenez E (Jiménez et al., 2005) conducted tests on umbilical cord blood flora from neonates delivered by cesarean section, revealing the presence of bacteria belonging to *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium*. Additionally, in animal experiments, the intestines of pregnant mice were inoculated with genetically labeled *enterococcus faecium*, and the labeled *enterococcus faecium* was detected in the feces of young mice delivered by cesarean section, suggesting the potential transmission of maternal microorganisms to the fetus *in utero* (Jiménez et al., 2008). The first meconium of a newborn can reflect the fetal situation *in utero*, and bacteria can be detected in the first meconium of a newborn (Kennedy et al., 2021; Turunen et al., 2021). These investigations provide compelling evidence supporting the concept of fetal intestinal flora colonization initiating *in utero*.

However, several studies have raised questions regarding whether fetal gut flora colonization truly initiates *in utero*. For instance, Kennedy KM (Kennedy et al., 2021) reported that they did not detect bacteria in the meconium collected from healthy newborns born via cesarean section. This suggests that, although microbial presence was observed in the fetal meconium for the first time, the majority of fetuses were delivered vaginally. The meconium collection typically occurs several hours to a few days after birth, and the microflora found in meconium may colonize during delivery or after birth. Therefore, it is plausible that the microflora found in meconium might colonize during delivery or in the postnatal period. Some studies have suggested an absence of flora in the amniotic fluid and placenta. RCR technology was conducted on the amniotic fluid of 344 women from 15 to 22 weeks of pregnancy, and the results showed that mycoplasma, bacteria and fungi in the amniotic fluid samples were negative (Rowlands et al., 2017). The amniotic fluid samples of 10 women who had cesarean section were tested, and the test results showed that the bacterial load in the amniotic fluid was no different from that in the blank control group (sterile Nacl), but the bacterial load in the amniotic fluid samples of 14 women with rupture of fetal membrane was 10 times higher than that of those who had cesarean section (Rehbinder et al., 2018). Amniotic fluid remains sterile due to the protective membrane. For infants with premature rupture of the membrane, intestinal flora colonization may initiate *in utero*, and fetal intestinal flora colonization may begin after the rupture of the membrane. Additionally, 16S rRNA sequencing technology was used to examine placenta-like flora in 29 women who did not give birth to full-term infants via cesarean section, and the results showed that there was no significant difference in the composition and structure of the bacterial spectrum between the placenta samples and the control group (Theis et al., 2019). Some scholars have raised concerns that bacterial flora determination technology primarily relies on bacterial 16S rRNA gene amplification, and the reagents and DNA extraction kits used may be susceptible to contamination from environmental bacteria. Such contamination could potentially affect the accuracy of bacterial flora determination and may not fully reflect the actual bacterial flora in the sample (Mühl et al., 2010; Salter et al., 2014). There is still a lot of controversy about whether fetal intestinal flora colonizes *in utero* or after delivery.

2.2 Origin of intestinal microflora in infants

To investigate the origin of intestinal microflora in infants and the contribution of various maternal bacterial flora from different body sites to the establishment of infant intestinal microflora, Ferretti P and colleagues (Ferretti et al., 2018) collected the bacterial samples from the mother's gut, vagina, skin and oral cavity. The results revealed that bacterial flora from these maternal sites contributed to the establishment of infant's intestinal

microflora. Maternal origins of neonatal gut microbiota are depicted in Figure 1. Notably, the maternal fecal microbiome accounted for the largest proportion of the contribution at 22.1%, followed by vagina (16.3%), mouth (7.2%) and skin (5%). Over time, the contribution of the maternal fecal microbiome increased, while the contributions from the vagina, mouth, and skin decreased. The colonization of the infant's intestinal tract by the mother's vaginal, oral, and skin flora was relatively transient. Interestingly, species from the maternal vaginal flora constituted approximately 3.5% of the total fecal microbial population in infants at 3 days after birth, but they were no longer detectable in the infant's feces by the first week. This phenomenon may be attributed to the differences between the vaginal and intestinal environments, as the maternal vaginal strains faced challenges in establishing a lasting presence in the infant's gut. The infant's intestinal environment. Given the transient nature of maternal vaginal colonization in the infant's gut, the feasibility and potential benefits of "vaginal seeding" warrant further discussion.

The microbial environment characterized by aerobic or facultative anaerobic bacteria, such as *Enterobacter*, *Enterococcus* and *Staphylococcus*, is established in the early stages of a newborn's intestine. These bacteria consume oxygen during their growth, thereby altering the intestinal microenvironment to favor the proliferation of anaerobic bacteria, including *Bifidobacterium*, *Clostridium*, and *Bacteroides* (Adlerberth, 2008). Within the first week of birth, the infant's intestinal flora undergoes changes influenced by the mother's vaginal and skin flora. The maternal intestinal flora becomes increasingly dominant in colonizing the newborn's intestinal tract. The alpha diversity of the initial flora decreases initially but gradually increases from the first to the third month of life. During this period, the infant's microflora is primarily composed of *Bifidobacterium* and *Bacteroides*. It's noteworthy that a stable gut microbiota resembling that of adults is typically established between the ages of 2 to 4 years (Stewart et al., 2018; O'Neill et al., 2020). Thus, neonates undergo a developmental process to establish their intestinal flora. In the initial stage, the intestinal flora is relatively simple and fragile, characterized by low species diversity, rendering it susceptible to external factors. Over time, however, the intestinal flora becomes relatively stable. This underscores the complexity of gut microbiota changes, with our current understanding representing only a fraction of the larger picture.

3 Influencing factors of neonatal intestinal flora establishment

The early neonatal intestinal flora is influenced by various factors, including maternal and infant-related factors and external environment (O'Neill et al., 2020). This article delineates the influence of factors such as delivery mode and gestational age on the establishment of the infant's intestinal microbiota (Figure 2). A schematic diagram illustrates how bacterial colonization in the infant gut microbiota changes under various influencing factors (Figure 3).

3.1 Mode of delivery

Intestinal flora plays a pivotal role in the early development of the neonatal immune system and metabolism. The initial colonization of the gut microbiota during the perinatal period represents a critical juncture in the human gut microbiome's ecological transformation. Fetuses born via cesarean section may

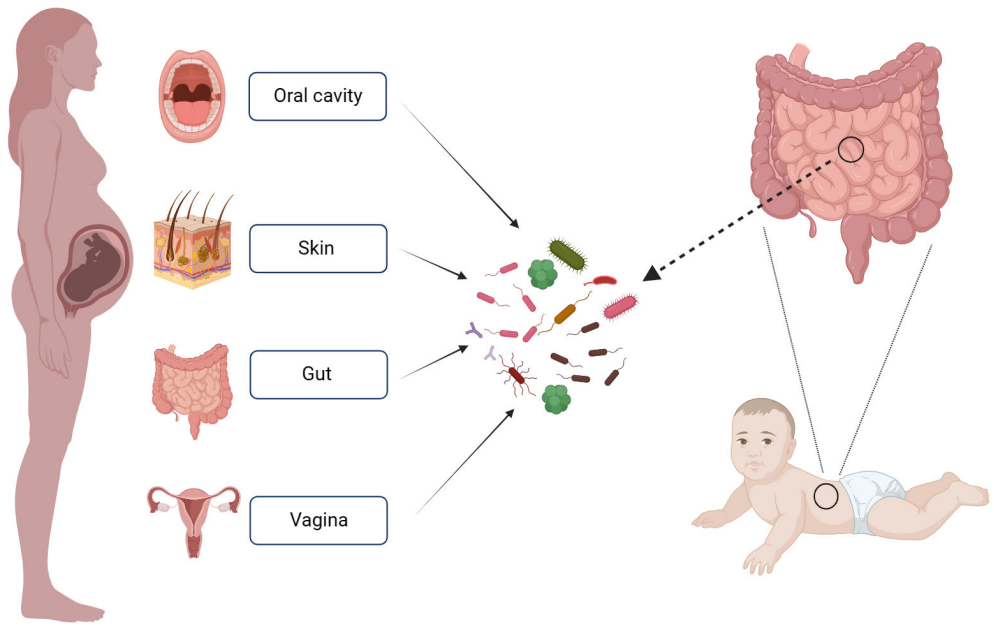

FIGURE 1
Maternal Sources of Neonatal Gut Microbiota. This figure was created with BioRender.com.

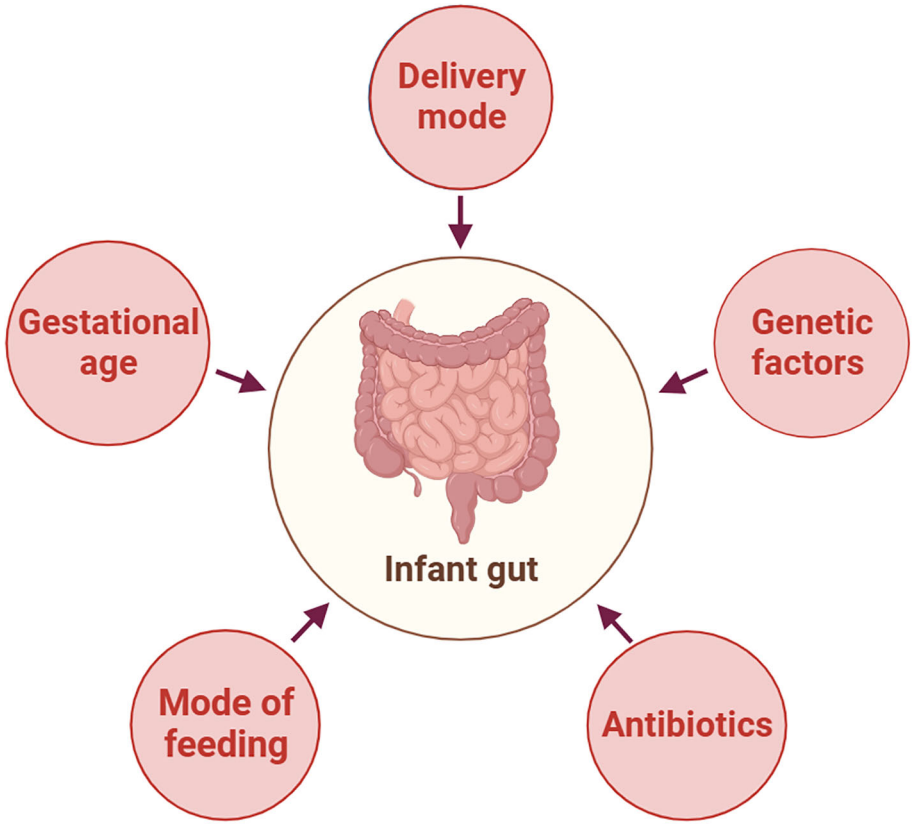

FIGURE 2
Factors Affecting the Composition of Neonatal Gut Microbiota. This figure was created with BioRender.com.

experience colonization defects in their intestinal flora, which can have enduring impacts on host's metabolism and immune development (Olszak et al., 2012). A comprehensive meta-analysis of 23 studies conducted in Northern Ireland has revealed that infants delivered via cesarean section face a 20% higher risk of developing asthma in both childhood and adulthood, in compared to those born through vaginal delivery (Thavagnanam et al., 2008). These findings have been meticulously adjusted to account for potential confounding factors such as maternal smoking, low birth weight, and the duration of breastfeeding. Furthermore,

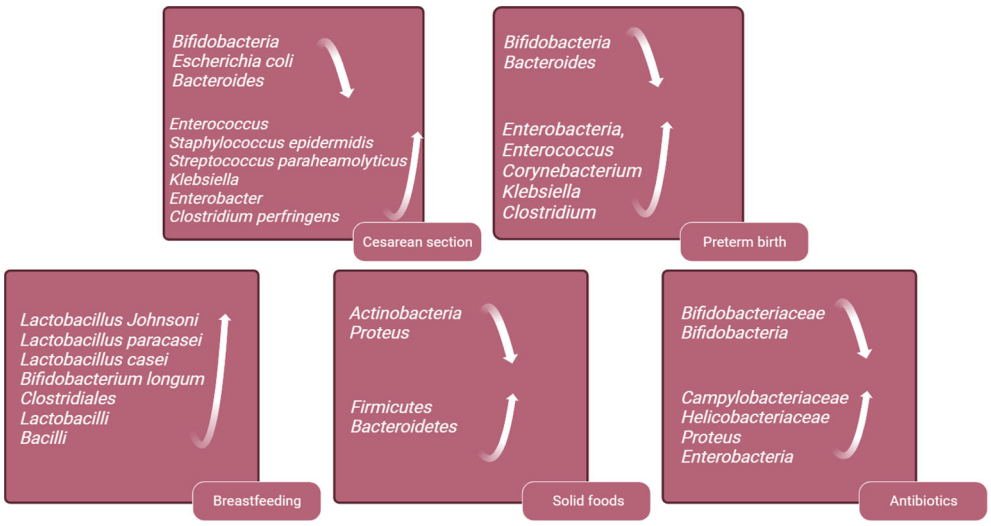

FIGURE 3
Schematic Diagram of Changes in Infant Gut Microbiota Bacteria Under Various Influencing Factors. This figure was created with BioRender.com.

additional studies have noted a heightened susceptibility to allergic rhinitis and atopic dermatitis in children born through cesarean section (Pistiner et al., 2008; Thavagnanam et al., 2008).

In addition, there is a growing focus on exploring alterations in the colonization patterns of gut microbiota and their potential links to long-term risks of obesity and metabolic syndrome. A prospective cohort study found that, even after accounting for influencing factors such as maternal weight and birth weight, children born via cesarean section exhibit a significantly higher incidence of obesity by the age of 3, compared to those delivered vaginally (Huh et al., 2012). In Brazil, another cohort study has reported an increased occurrence of central and peripheral obesity in adults associated with cesarean section (Mesquita et al., 2013). Moreover, infants born via cesarean section have a 23% higher risk of developing type 1 diabetes during childhood (Cho and Norman, 2013). Fetuses born via cesarean section are not exposed to the mother's vaginal flora but are initially exposed to flora from the mother's skin or the birthing environment. Early fecal samples from vaginally delivered infants are dominated by *Bifidobacteria*, *Escherichia coli* and *Bacteroides*, accounting for approximately 68.3% of the neonatal gut microbiome. In contrast, infants delivered by cesarean section exhibit depleted populations of these gut microbiome genera, which are replaced by *Enterococcus*, *Staphylococcus epidermidis*, *Streptococcus paraheamolyticus*, *Klebsiella*, *Enterobacter* and *Clostridium perfringens*. These microbiotas are typically found on the skin and in the hospital environment, with 83.7% of infants born via cesarean section carrying opportunistic pathogens during the neonatal period, compared to 49.4% of vaginally born infants. This difference may increase the risk of neonatal infection (Lax et al., 2017; Wampach et al., 2018; Shao et al., 2019).

Cesarean section interrupts the normal transmission route of maternal symbiotic bacteria to infants, potentially increasing the chance of opportunistic pathogen colonization in the intestines. The increasing prevalence of cesarean section in recent years not only increases the risk of pregnancy in the scar area of cesarean section in the second pregnancy, but may also lead to defects in the colonization of fetal intestinal flora. Chu et al. (Chu et al., 2017a) demonstrated that the effect of delivery mode on fetal intestinal flora only lasts for the first 6 months, with no significant difference in flora after this period. Shao et al.(Shao et al., 2019) suggest that although the impact of cesarean section on infants' intestinal flora diminishes over time, microbiome differences can still be detected up to the age of 1 year. Some studies have even found that differences in gut microbiota between cesarean section and vaginal delivery persist until the age of 7 (Penders et al., 2006; Neu and Rushing, 2011). The short-term or permanent effects of delivery mode on fetal microbiota still require long-term follow-up. Additionally, the routine use of antibiotics during cesarean section is a standard diagnosis and treatment process, so it remains uncertain whether antibiotics, as an interfering factor in comparing intestinal flora between vaginal and cesarean-born infants, impact the results.

The study conducted by Santos et al. presents a noteworthy finding that challenges the conventional understanding of the relationship between the maternal vaginal microbiome and the early development of the infant gut microbiome(Dos Santos et al., 2023). Traditionally, it has been believed that infants acquire their initial microbial communities during birth, particularly through exposure to maternal vaginal flora. However, the study's results suggest otherwise, indicating that the composition of the maternal vaginal microbiome may not have a significant impact on the early years of the infant gut microbiome. This finding sparks further inquiry into the multifaceted factors that shape early gut microbiota and their implications for child health and development.

3.2 Gestational age

Gestational age at delivery is another crucial factor that impacts the establishment of intestinal flora in infants. Gestational age at birth significantly influences the formation and development of the intestinal microbiota, particularly in preterm infants. Research indicates that gestational age at birth plays a crucial role in shaping the structure and diversity of the intestinal microbiota. Gregory et al. (Gregory et al., 2016) proposed that gestational age at birth determines the trajectory of microbiota development within the first three weeks after birth. Chernikova et al.(Chernikova et al., 2018) demonstrated that gestational age at birth has a notable impact on the composition of the intestinal microbiota, with extremely preterm infants having a Simpson index of 0.35, compared to 0.65 in preterm infants. Furthermore, Drell et al. (Drell et al., 2014) emphasized a positive correlation between corrected gestational age and microbiota diversity, highlighting the role of gestational age in the evolution of the microbiota during early childhood.

Many diseases in premature infants are closely associated with their intestinal flora, such as necrotizing enterocolitis (NEC) (Aguilar-Lopez et al., 2021). Impaired composition and functionality of the intestinal flora have a direct impact on the health and potential complications of preterm infants. A better understanding of the differences in intestinal flora between preterm and full-term infants can help address the intestinal flora deficiencies in preterm infants and reduce related diseases.

In comparison with full-term infants, preterm infants have lower intestinal flora diversity, delayed colonization of *Bifidobacteria* and *Bacteroides*, and an increase in opportunistic pathogens like *Enterobacteria*, *Enterococcus*, *Corynebacterium*, *Klebsiella* and *Clostridium* (Mai et al., 2013; Arboleya et al., 2015). In addition, studies have found that the levels of short-chain fatty acids, which are metabolites products of the flora, are lower in the gut of preterm infants compared to full-term infants (Bergström et al., 2014). Premature infants exhibit significant differences in their intestinal microbiota compared to full-term infants. These distinctions may render premature infants more susceptible to microbial imbalances, leading to alterations in metabolic byproducts and potential ramifications for immune system development, as well as the risk of related diseases. These disparities are often attributed to the influence of various medical factors that premature infants are frequently exposed to, including cesarean section delivery, lack of breastfeeding, mechanical ventilation, and the frequent use of antibiotics. These factors can

contribute to variations in the composition of the intestinal microbiota(Cuna et al., 2021). To improve the deficiency of intestinal flora in premature infants, this review will provide the following information.

3.3 Feeding mode

In recent years, the research and findings have primarily focused on the influence of maternal symbiotic flora on neonatal intestinal flora colonization but have often overlooked the impact of acquired breastfeeding. Breast milk is the best source of nutrition for infants, and the World Health Organization advocates exclusive breastfeeding until six months of age, with complementary foods gradually added, and breastfeeding continuing for at least two years (Binns et al., 2016).

When compared to formula-fed infants delivered vaginally, exclusively breastfed infants have a higher presence of probiotics in their gut, including *Lactobacillus Johnsoni*, *Lactobacillus paracasei*, *Lactobacillus casei* and *Bifidobacterium longum* (Bäckhed et al., 2015). The imbalance of intestinal flora in infants resulting from cesarean delivery can be corrected through subsequent breastfeeding. At six months postpartum, the composition and structure of the intestinal microbiome in infants delivered via cesarean section differ from those delivered vaginally, with fewer *Bacteroides* and *Shigella genera*, and more *Klebsiella*, *Veillonella* and *Enterococcus faecalis*. The differences in intestinal microbiome caused by delivery the mode of delivery can be mitigated by acquired exclusive breastfeeding (Liu et al., 2023). Breastfeeding can reduce the incidence of NEC and sepsis in preterm infants (Quigley et al., 2018). Breastfeeding is vital for the establishment of intestinal flora in preterm infants. Studies have shown that preterm infants who are breastfed have increased gut microbiome alpha diversity compared to formula feeding (Cong et al., 2016). There are more *Clostridiales*, *Lactobacilli* and *Bacilli* in the intestines of premature infants who are breastfed compared to formula feeding and donor milk feeding (Cong et al., 2017). Breast milk is beneficial to the intestinal microbiome development of premature infants and increases the microbial diversity in early life, and it is recommended to support breast-feeding for premature infants after birth.

The weaning process represents the final trajectory of infant gut microbiota formation. In this process, diet plays a crucial role in regulating the microbial community (Kumbhare et al., 2019). Before weaning, the gut microbiota's functional repertoire primarily involves gene expression related to the utilization of lactose (Tanaka and Nakayama, 2017). However, with the introduction of solid foods, the gene expression of the gut microbiota begins to increase in functions such as carbohydrate utilization, vitamin synthesis, and xenobiotic degradation. This transitional process is consistent with the rapid development of the immune system and changes in dietary intake.

The introduction of solid foods is another key factor in regulating the composition of the gut microbiota and marks the beginning of its maturation phase. This maturation of the microbial community is a gradually evolving process that ultimately leads to a more diverse and stable microbial profile (Davis et al., 2020). The introduction of solid foods triggers changes in the infant gut microbiota, and the supplementation of new nutrients increases the alpha -diversity of the gut microbiota, with *Firmicutes* and *Bacteroidetes* replacing *Actinobacteria* and *Proteus* as dominant phyla (Fallani et al., 2011; Koenig et al., 2011; Martin et al., 2016). Additionally, the total amount of short-chain fatty acids increases, with a significant rise in butyrate levels. The introduction of solid foods also increases the intake of plant-derived polysaccharides. During this stage, the composition of *Bifidobacterium* gradually shifts towards strains capable of metabolizing plant-derived polysaccharides, such as *Bifidobacterium longum* and *Bifidobacterium adolescentis* (O'Callaghan and van Sinderen, 2016). Simultaneously, *Bifidobacterium bifidum* redirects its metabolic capacity from human milk oligosaccharides (HMOs) towards mucin degradation.

Increased protein intake leads to an increase in the abundance of *Lachnospira* and a decrease in *Bifidobacterium* (Laursen et al., 2016). On the other hand, high fiber intake is associated with changes in the genus *Prevotella*. The addition of solid foods also marks the beginning of the transition of the infant gut microbiota structure towards an adult-like structure, with a microbial community becoming more complex to adapt to the plant-derived polysaccharides present in the adult diet, promoting mutualistic symbiosis between the host and microbiota (Koenig et al., 2011). This process continues until the age of two when the gut microbiota structure of infants reaches a stable state similar to that of adults.

3.4 Antibiotics

3.4.1 Effects of antibiotics on infant intestinal flora

Perinatal exposure to antibiotics has a significant impact on the early establishment of gut microbiota in offspring and increases the risk of childhood asthma, allergies, and obesity (Cox et al., 2014; Leclercq et al., 2017). Antibiotics affect an infant's gut microbiota in two ways: (1) Antibiotics can reach the fetal bloodstream through the umbilical cord. (2) Antibiotics alter the maternal vaginal and intestinal microbiome, leading to vertical transmission of infant intestinal flora abnormalities. A study on the effects of intrauterine antibiotic use on neonatal intestinal microbiota found that infants exposed to intrauterine antibiotic had decreased levels of Bifidobacteriaceae and an increased the proportion of potentially pathogenic microorganisms, including Campylobacteriaceae or Helicobacteriaceae (Nogacka et al., 2017). Another similar study found that infants treated with antibiotics during delivery had lower gut bacterial diversity, a decreased relative abundance of actinomycetes (especially Bifidobacteriaceae), and a higher relative abundance of Proteus in the gut microbiota (Zimmermann and Curtis, 2020). In full-term infants, the administration of antibiotics within a few hours after birth can reduce the level of Bifidobacteria in the gut and increase the level of Enterobacteria (Arboleya et al., 2015). The use of antibiotics during and after delivery can significantly affect the intestinal flora of infants, and it is

recommended to avoid antibiotics unless medically necessary to reduce infants' exposure to antibiotics.

Given that premature infants tend to have relatively fragile immune systems and are more susceptible to infections, antibiotics are commonly administered for therapeutic purposes (Groer et al., 2014; Yasmin et al., 2017). In fact, antibiotics rank among the most frequently prescribed medications in the Neonatal Intensive Care Unit (NICU). However, the use of antibiotics presents a double-edged sword. While antibiotics can reduce the mortality rate associated with infectious diseases in premature infants, they often disrupts and disturbs the composition of the intestinal microbiota, consequently increasing the risk of various diseases (Morowitz et al., 2022). Additionally, research indicates that premature infants, particularly those with extremely low birth weights, face an increased risk of developing conditions like sepsis and NEC as a result of prolonged exposure to antibiotics (Cantey et al., 2018; Vatne et al., 2023).

3.4.2 Influence of timing of antibiotic application in cesarean section

As the prophylactic application of antibiotics to pregnant women during cesarean section is a routine obstetrical diagnosis and treatment process, antibiotics that can cross the placenta may reach the fetus through the placental barrier. In order to prevent intrauterine exposure of newborns to antibiotics, antibiotics are administered after umbilical cord cutting in some countries to minimize the exposure of newborns to antibiotics (Stinson et al., 2018). It is worth to study whether the timing of antibiotics for cesarean section prophylaxis can affect the gut microbes of infants or not, thus revealing the influence of antibiotic exposure before umbilical rupture to fetal gut flora. Relevant studies have compared the use of antibiotics before skin incision and after the umbilical cord is clamped in the gut microbiome composition of full-term infants born via elective cesarean section. Surprisingly, there were no significant difference in the gut microbiome composition observed at 9 months of age, and the potential long-term effects of antibiotics cannot be ruled out based on this study (Kamal et al., 2019). Similar results were found in another study, which reported no difference in intestinal microbiota composition between 10 and 9 months after birth (Dierikx et al., 2022). Prenatal antibiotic exposure in infants born via cesarean section does not appear to affect early-life microbiome development, but long-term follow-up is needed.

3.5 Genetic factors and gender

There is growing evidence that genetic factors influence the gut microbiota of infants. The similarity of intestinal flora between identical twins is higher than that between fraternal twins, indicating that genetic factors have an impact on fecal microbial community (Stewart et al., 2005). A study has proved that there are differences in the gut microbiome in males and females, with male babies having a lower gut microbiome diversity index at birth, while female babies have a higher gut microbiome diversity index, and female babies have a higher abundance of *Clostridium difficile* and lower abundance of *Enterobacterium* than male babies (Cong et al., 2016). Another similar study demonstrated that the relative abundance of *Bacteroides* in the intestinal flora of 3-month-old male infants was lower than that of female infants (Kozyrskyj et al., 2016). Genetic factors may play a potential role in the establishment of infant intestinal microbiota, and the mechanism of sex difference on intestinal microbiota community is still unclear.

4 Interventions to compensate for the defects of neonatal intestinal flora colonization

4.1 "Vaginal seeding"

In recent years, there has been growing interest in a research topic called 'vaginal seeding' as a way to help newborns born by cesarean section establish a healthy gut microbiome. This involves swabbing newborns with gauze containing maternal vaginal fluid, starting from the lips and proceeding to different parts of the body to mimic the natural birth process. The idea is to introduce beneficial vaginal microbes to cesarean-born babies, with the aim of making their gut flora more similar to that of babies born through vaginal delivery. Studies have shown that this 'vaginal seeding' can indeed help cesarean-born babies establish a gut microbiome that resembles that of babies born vaginally.

However, it's important to note that current research has primarily focused on short-term effects, and we lack a clear understanding of the long-term consequences. There is also a concern about the potential risk of neonatal infection due to the introduction of vaginal bacteria during the 'vaginal seeding' process. Therefore, caution is needed, and more clinical studies are necessary to ensure the safety of this practice. If this practice is proven to be beneficial and safe, it's important to assess its acceptance among expectant mothers.

Furthermore, researchers have explored different methods of introducing maternal vaginal microbes to cesarean-born babies. For example, a study by Wilson et al. (Wilson et al., 2021) attempted to give cesarean section newborns an oral solution containing maternal vaginal microbial. Surprisingly, the results showed no significant difference in the composition and development of the babies' intestinal flora compared to those who received a standard oral saline solution. This suggests that the method of introducing mother's vaginal microbes may not be as effective in reconstructing the gut flora of cesarean-born babies as previously thought. More experiments are needed to verify the impact of various seeding methods on the reconstruction of the newborns' gut microbiome.

4.2 Maternal intestinal flora transplantation

It has been reported that a newborn's intestinal flora is primarily derived from the mother's intestinal flora, with seeding of the maternal vaginal flora playing a secondary role in the

colonization of the infant's fecal flora (Sakwinska et al., 2017; Turunen et al., 2021). Ferretti P et al. (Jiménez et al., 2005) demonstrated that maternal intestinal, vaginal, skin and oral flora all contributed to the establishment of infant's intestinal flora. Maternal fecal flora makes the most significant contribution to the infant's intestinal flora, accounting for 22.1% of the infant's intestinal microflora, followed by vaginal flora (16.3%). However, the contribution of vaginal flora species to infant feces diminishes over time. If vaginal microbiota isn't the primary source of intestinal microbiota in infants, then the effectiveness of "vaginal seeding" in restoring intestinal microbiota colonization defects in newborns born by cesarean section is questionable. It is suspected that fecal microbiota transplantation (FMT) may correct the imbalance of intestinal microbiota in infants born by cesarean section. In an experimental setting, within 10 hours of birth, the mother's fecal bacterial solution was mixed with breast milk and fed the cesarean-born infants, who did not experience adverse reactions. FMT treatment not only restored the intestinal microbiome of the cesarean-born infants but also led to the development of their gut microbiota being more similar to that of vaginally delivered infants. The microbiota of infants delivered by cesarean section with FMT in the early postpartum period was different from that of those delivered vaginally. However, from day 7 onwards, the gut microbiota of infants treated with FMT resembled that of infants delivered vaginally. Furthermore, FMT reduced the presence of intestinal opportunistic pathogens such as *Enterococcus*, *Enterobacter*, and *Klebsiella* in cesarean newborns (Korpela et al., 2020). Maternal intestinal flora transplantation has shown significant advantages in rectifying intestinal flora deficiencies in neonates born by cesarean section. Therefore, it may be feasible to consider the transplantation of maternal intestinal flora. However, the feasibility, safety, and clinical applicability of methods like 'vaginal seeding' and fecal bacteria transplantation still require further evaluation.

Further evaluation is needed to determine the feasibility and clinical applicability of 'vaginal seeding' and fecal bacteria transplantation as therapeutic methods to compensate for congenital defects in intestinal flora colonization.

4.3 Probiotics and prebiotics

Probiotics are live microorganisms that provide benefit to the host by colonizing the human body and changing the composition of the flora in a certain part of the host. The deficiency of newborn intestinal flora can be restored by probiotics. For premature infants, probiotics can enhance intestinal barrier function by regulating flora structure, reduce the colonization and migration of pathogenic bacteria, and promote the development and functional maturation of intestinal immune cells in newborns, thus reducing the incidence and mortality of NEC (Dermyshi et al., 2017; Gopalakrishna et al., 2019; Morgan et al., 2020). After oral administration of probiotics, preterm infants have a higher relative abundance of *Bifidobacterium* and *Enterobacterium*, and a lower relative abundance of *Escherichia coli*, *Enterococcus* and *Klebsiella* in the gut, and a lower incidence of NEC (Patole et al., 2014; van Best et al., 2020). Some studies have shown that early probiotic supplementation with *Lacticaseibacillus rhamnosus GG* (LGG) and *Bifidobacterium animalis* can affect the colonization of potential pathogens in infant intestines (Hui et al., 2021). Probiotics showed great benefit in restoring early intestinal flora colonization defects in preterm infants. It is recommended to apply probiotics in the early life of premature infants to prevent and treat intestinal flora defects and related diseases in premature infants.

Antibiotics can cause defects in infants' gut microbiota and are associated with health problems in later life (Hviid et al., 2011; Metsälä et al., 2013; Azad et al., 2014; Chu et al., 2015). Probiotic supplementation can reshape antibiotic-treated intestinal microbiota disturbances in mice (Grazul et al., 2016). In the study (Zhong et al., 2021), it was found that exposure to piperacillin-tazobactam reduced the richness of the gut microbiota in full-term infants and may interfere with *Bifidobacterium* and *Lactobacillus* reproduction. It was also found that simultaneous treatment with probiotics and antibiotics was beneficial to the gut microbiota and could lead to an increase in *Bifidobacterium*. When supplementation with probiotics was delayed, there appeared to be no effect and no benefit from intervening in the gut microbiota disrupted by antibiotics. This study emphasizes that probiotics should be taken at the same time as antibiotics in the treatment of infants with intestinal flora disorders caused by antibiotics, and its therapeutic effect will be greater.

The alpha diversity of intestinal flora was significantly lower in newborns delivered by cesarean section compared to those born vaginally. However, after probiotic supplementation, the alpha diversity and beta diversity of intestinal flora in infants delivered by cesarean section become similar to those delivered vaginally. Additionally, there was a significant increase in the abundance of *Lactobacillus* and *Bifidobacterium* in the gut (Yang et al., 2021). The effect of probiotics on intestinal microbiota of newborns delivered by cesarean section also depends on the feeding mode of infants. In comparison to formula milk powder, breastfeeding led to an increase in intestinal *Bifidobacterium* and a decrease in *Proteus* and *clostridium* in cesarean-born infants (Korpela et al., 2018). Therefore, it is recommended to supplement probiotics to newborns delivered by cesarean section, and the promotion of infant breastfeeding is encouraged.

Overall, when compared to non-supplemented formulas, these prebiotic-supplemented ones increase the softness of stools (Skórka et al., 2018). They might also be able to decrease the incidence of enteric infections and diarrhea, reduce eczema, and increase *Bifidobacteria* counts. In premature infants, researchers have extensively studied various combinations of non-human milk galacto-, fructo-, and acidic oligosaccharides. These prebiotic mixtures have been found to modify the fecal microbiome, lower fecal pH, enhance gastric motility, reduce feeding intolerance, and increase fecal sIgA (Westerbeek et al., 2013). However, despite these positive effects, there is currently no related research on whether prebiotics can restore the colonization defects in the gut microbiota of newborns delivered by cesarean section, and this requires further investigation.

5 Conclusions and perspectives

The question of whether fetal intestinal microbiota colonization occurs *in utero* or postnatally remains a subject of ongoing research with no definitive consensus. Traditional beliefs held that the fetal environment was sterile, with the infant's gut primarily being colonized following birth through exposure to maternal and environmental microorganisms. However, recent investigations have introduced the possibility of some degree of microbial exposure or colonization *in utero*. Despite this, the extent and clinical significance of such prenatal microbial colonization remain subjects of investigation. Several studies have reported the presence of microbial DNA in amniotic fluid, placenta, and meconium (the initial fecal material passed by a newborn), sparking inquiries into the potential for prenatal microbial colonization. Yet, questions persist about the origins and roles of these microorganisms in the developing fetus. To gain a more comprehensive understanding of the timing and mechanisms underlying the establishment of the infant gut microbiota and the potential occurrence of prenatal colonization, further clinical studies are essential.

In shaping the infant's gut microbiota, the review underscores the crucial role of the maternal-infant symbiotic relationship. Various microorganisms from different maternal body sites contribute to the development of the infant's intestinal microflora, with the maternal gut microbiota making the most significant contribution. The infant's gut microbiota undergoes multiple stages of change during the early period. It initially consists of aerobic or facultative anaerobic bacteria, gradually transitioning to anaerobic bacteria, such as *Bifidobacterium*, *Clostridium*, and *Bacteroides*. Understanding these transitions provides valuable insights into the formation of the infant's gut microbiota.

While the colonization of the infant's gut by maternal vaginal, oral, and skin flora is relatively short-lived, it has prompted discussions about the feasibility and potential benefits of the 'vaginal seeding' strategy. To determine when the infant's gut microbiota tends to stabilize, resembling that of adults, further in-depth research is needed. This understanding contributes to a better comprehension of the evolution of the intestinal microbiota.

Future studies should delve deeper into the origins and dynamic changes of the infant's gut microbiota to elucidate the potential impact of early microbial colonization on the health of children and adults. Additionally, there is a need for further research to explore the mechanisms and potential significance of prenatal microbial colonization.

Intestinal flora colonization is a complex process influenced by various factors, including delivery mode, gestational age, feeding mode, and antibiotic usage. The impact of delivery mode on newborn intestinal flora colonization is significant. Non-cesarean section indications should be advocated, and vaginal delivery should be selected as far as possible to avoid congenital colonization defects of infant intestinal flora. Understanding the impact of cesarean section on the neonatal gut microbiome and subsequent health outcomes is crucial. These findings shed light on the potential risks associated with cesarean section. Moreover, the need for long-term follow-up studies cannot be overstated. Such studies would allow us to gain a more comprehensive understanding of whether the effects observed persist over time or gradually diminish. This knowledge is crucial for healthcare professionals and parents alike. Exploring potential intervention measures to bridge the gap in gut microbiota between cesarean-born and vaginally born infants is another avenue for future research. This could include methods to promote the colonization of cesarean-born infants' gut with flora closer to that of vaginally born infants or strategies to maintain a healthy balance of gut microbiota after cesarean section.

Preterm infants are typically delivered by cesarean section, and most receive antibiotic treatment while lacking the breastfeeding. Prolonged exposure to the hospital environment can exacerbate colonization defects in the intestinal flora of preterm infants. Future research needs to delve deeper into the diversity of the gut microbiota in preterm infants, including differences compared to full-term infants, in order to comprehensively understand its formation and evolution. Additionally, there is a need to enhance investigations into the connections between gut microbiota and diseases relevant to preterm infants, such as necrotizing enterocolitis, to determine the specific impact of the microbiota on these conditions, thus providing a basis for future prevention and treatment. It's crucial not only to comprehend the composition of the gut microbiota but also to conduct in-depth research into their functions in metabolism, the immune system, and other physiological aspects to reveal more profound health associations. Long-term follow-up studies are indispensable to understand the extended development of the gut microbiota in preterm infants and their potential long-term health consequences, especially in adulthood, regarding potential risks related to the gut microbiota. Future research should focus on gaining a more comprehensive understanding of the formation and influence of the gut microbiota in preterm infants, with the goal of improving their health, reducing disease risks, offering more effective intervention measures, and enhancing their quality of life.

The use of antibiotics during the perinatal and postnatal periods can impact a baby's gut microbiota. It is standard practice to administer prophylactic antibiotics to pregnant women during cesarean section. In order to minimize early antibiotic exposure for newborns, some guidelines recommend using antibiotics to prevent infection after umbilical cord cutting. Current studies suggest that the timing of antibiotic administration does not appear to affect the establishment of the baby's gut microbiota. Breastfeeding is highly beneficial for establishing the intestinal flora in infants. It is recommended for newborns, and even in situations where breastfeeding is not feasible, it remains valuable. Breastfeeding contributes to the development of the intestinal microbiome of the intestinal microbiome in premature infants and enhances the microbial diversity in early life.

There are various perspectives on the source of neonatal intestinal flora colonization, including discussions about which components of the mother's flora play a more beneficial role in compensating for congenital neonatal intestinal flora colonization defects. Debates persist regarding the feasibility of practices such as 'vaginal seeding', maternal fecal flora transplantation, and the choice between single-flora or multi-flora transplantation

methods. More clinical studies are needed to validate these approaches. Breastfeeding, along with the use of probiotics and prebiotics, may have a beneficial effect in addressing colonization defects in the gut microbiota of preterm infants, newborns delivered by cesarean section, or infants affected by disorders related to gut microbiota establishment. Future research could consider gut microbes as a potential intervention to promote infant health.

Author contributions

GM: Writing – original draft. YS: Writing – original draft. LM: Writing – original draft. HF: Writing – original draft. XT: Writing – original draft. HL: Writing – original draft. DW: Writing – original draft. JZ: Writing – original draft. XX: Writing – review & editing.

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An update: is there a relationship between *H. pylori* infection and nonalcoholic fatty liver disease? why is this subject of interest?

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Helicobacter pylori (*H. pylori*) infection is thought to impact various extragastric diseases, including nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease. Meanwhile, the pathogenesis of NAFLD needs further research, and effective treatment for this disease remains elusive. In this mini-review, we enumerate and ponder on the evidence demonstrating an association between *H. pylori* infection and NAFLD. Primarily, we delve into high-quality meta-analyses and clinical randomized controlled trials focusing on the association studies between the two. We also discuss clinical studies that present opposite conclusions. In addition, we propose a mechanism through which *H. pylori* infection aggravates NAFLD: inflammatory cytokines and adipocytokines, insulin resistance, lipid metabolism, intestinal barrier and microbiota, *H. pylori* outer membrane vesicles and *H. pylori*-infected cell-extracellular vesicles. This mini-review aims to further explore NAFLD pathogenesis and extragastric disease mechanisms caused by *H. pylori* infection.

KEYWORDS

Helicobacter pylori, extragastric disease, nonalcoholic fatty liver disease, meta-analysis, pathogenesis

1 Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacillus that colonizes the human stomach. It is microaerophilic and has the ability to metabolize urea into ammonia and carbon dioxide. *H. pylori* is transmitted through the oral-oral and fecal-oral routes, and it infects approximately 4.4 billion people worldwide, with a prevalence rate of 44.3% (95% CI: 40.9-47.7) (Hooi et al., 2017; Sjomina et al., 2018; Zamani et al., 2018). An epidemiological survey based on family units revealed that the prevalence of *H. pylori* in China was approximately 40.66%, 43.45% in adults, and 20.55% in children and adolescents (Zhou et al., 2023). Multiple studies have substantiated that *H. pylori*

infection serves as the initiating factor in the progression of chronic gastritis to gastric cancer (Correa, 1992; Uemura et al., 2001; Kusters et al., 2006). In addition to peptic ulcers, gastritis, gastric cancer, and other gastric diseases, a variety of extragastric diseases, such as stroke, Alzheimer's disease, and nonalcoholic fatty liver disease (NAFLD), are closely related to *H. pylori* infection (Santos et al., 2020).

NAFLD refers to a spectrum of diseases, including simple hepatocellular steatosis, nonalcoholic steatohepatitis (NASH), NASH-associated cirrhosis, and hepatocellular carcinoma. With changes in diet and lifestyle, NAFLD is the the most common chronic liver disease (Chalasani et al., 2018; Younossi et al., 2020). Moreover, in the United States, NAFLD is the second leading cause of liver transplantation after alcoholic liver disease (Kim et al., 2018). The overall global prevalence of NAFLD was estimated to be approximately 29.1% (95% CI: 26.8-31.5), with the highest prevalence in Latin America (44.4%) and the lowest in Western Europe (24.6%). Moreover, the global prevalence of NAFLD is progressively increasing, rising from 24.4% in 1991-2006 to 36.0% in 2016-2020 (Henry et al., 2022). However, the pathogenesis of NAFLD remains unknown. The *multiple-hit* pathogenesis reviewed by Buzzetti et al. is widely accepted in academia and includes insulin resistance (IR), hormones secreted fromadipose tissue, nutritional factors, and gut microbiota (Buzzetti et al., 2016). No agents have been approved for the clinical treatment of NAFLD, and their treatment options rely mainly on weight loss through dietary modification and physical exercise (Wang and Malhi, 2018). Therefore, it is necessary to correctly discern the pathogenesis of NAFLD and propose targeted treatment options for NAFLD.

Since the initial report of *H. pylori* DNA being detected in the liver of NAFLD patients (Cindoruk et al., 2008), numerous studies have investigated the relationship between *H. pylori* infection and NAFLD (Kim et al., 2017; He et al., 2018; Yu et al., 2022). Based on the pandemic and the rate of *H. pylori* infection and NAFLD worldwide, a large proportion of patients have comorbid diseases. Despite the increasing severity of antibiotic-resistant forms of *H. pylori*, the eradication rate with multiple first-line treatment regimens remains more than 80% (Rokkas et al., 2021). Compared with NAFLD, for which no effective medical therapy is currently available, *H. pylori* can be eradicated in a large proportion of patients with comorbidities. In that case, it can delay or improve the progression of NAFLD and maximize the benefit of patients with comorbid conditions while greatly relieving the disease burden of NAFLD. However, there remains controversy about whether *H. pylori* infection is clinically associated with NAFLD, with some extensive multicenter clinical studies suggesting an association (Sumida et al., 2015; Kim et al., 2017; Doulberis et al., 2020) and others suggesting no association (Jamali et al., 2013; Okushin et al., 2015; Baeg et al., 2016). Even if they are relevant, mechanistic studies are needed to conduct research on how *H. pylori* infection impacts NAFLD. This mini-review intends to review the association between *H. pylori* and NAFLD and propose a hypothesis according to the summarized literature: *H. pylori* infection may exacerbate the development of NAFLD. Moreover, the authors explore the mechanism of the occurrence and development of NAFLD and the mechanism of extragastric diseases caused by *H. pylori*.

2 Preclinical studies

Basic research on *H. pylori* infection and NAFLD is rare, and no precise mechanism has been found. He et al. (He et al., 2018) established a mouse model of *H. pylori* infection, fed a high-fat diet (HFD) and a chow diet for six months, which showed that HFD plus *H. pylori*-infected mice had significantly increased abdominal circumference, fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C), and alanine aminotransferase (ALT) compared with HFD controls, and showed more severe hepatic steatosis, which was consistent with our hypothesis. Liver fibrosis is a progressive manifestation of NAFLD (Friedman et al., 2018). *H. pylori* infection has demonstrated to promote CCl_4-induced liver fibrosis in animal models (Goo et al., 2009), and that the proinflammatory signaling pathways may occur through transforming growth factor-beta1 (TGF-β1) (Ki et al., 2010).

The progress of experimental research on the association between *H. pylori* infection and NAFLD has been slow, which could be attributed to the following factors: (1) challenges in mimicking the complex hepatic physiological environment in cellular and molecular experiments; (2) *H. pylori* infection does not promote NAFLD through direct pathways; and (3) *H. pylori* infection is not associated with NAFLD. More experimental studies in line with human physiology, such as hepatic organoids, are needed to demonstrate whether *H. pylori* infection is associated with NAFLD.

3 Clinical studies

3.1 Meta-analyses revealed a positive correlation between *H. pylori* infection and NAFLD

Table 1 summarizes nine meta-analyses of clinical research on *H. pylori* infection with NAFLD (Wijarnpreecha et al., 2018; Liu et al., 2019; Mantovani et al., 2019; Ning et al., 2019; Zhou et al., 2019; Wei and Ding, 2021; Heydari et al., 2022; Ma et al., 2022; Xu et al., 2023). These studies included a minimum of 38,622 and a maximum of 218,573 participants. Most clinical studies included in these meta-analyses were conducted in the Asian region (China, Japan, and South Korea), and a few involved Europe, the United States, and Egypt. The results of the subgroup analysis suggested that *H. pylori* infection was stable and associated with NAFLD in Asian regions ($P < 0.01$), and there were no uniform results in other regions due to different inclusion studies in each meta-analysis (Mantovani et al., 2019; Ning et al., 2019; Zhou et al., 2019; Wei and Ding, 2021; Xu et al., 2023). This may be due to differences in the cytotoxin-associated gene A (Cag A) status of *H. pylori* and strain virulence between Asia and other regions (Yamaoka, 2009; Park et al., 2018). The heterogeneity of these meta-analyses was generally high, which may be related to the differences in the region, population, and methods of diagnosis included in the study. Only one meta-analysis had publication bias (Ning et al., 2019), which could be due to the small sample size of the studies. After removing

TABLE 1 Meta-analyses of the association between *H. pylori* infection and NAFLD.

Reference	Included studies (N)	Participants (N)	Main results	Odds ratio (OR)	Heterogeneity (I^2)	Publication bias
Wijarnpreecha K, 2018 (Wijarnpreecha et al., 2018)	6 (5 cross-sectional, 1 case-control)	38,622	A significantly increased risk of NAFLD among patients with *H. pylori* infection	1.21 (95% CI: 1.07–1.37)	49.00%	Indeterminant (evaluated only by Funnel plot)
Mantovani A, 2019 (Mantovani et al., 2019)	13 (2 cohort, 2 case-control, 9 cross-sectional)	81,162	*H. pylori* infection is associated with mildly increased risk of NAFLD	1.14 (95% CI: 1.05–1.23)	59.60%	No
Zhou BG, 2019 (Zhou et al., 2019)	15 (2 cohort, 2 case-control, 11 cross-sectional)	97,228	A positive association between *H. pylori* infection and the risk of NAFLD	1.19 (95% CI: 1.11–1.29)	66.00%	No
Ning L, 2019 (Ning et al., 2019)	12 (2 cohort, 2 case-control, 8 cross-sectional)	87,747	*H. pylori* infection was associated with the development of NAFLD	1.36 (95% CI: 1.22–1.53)	89.60%	Yes
Liu R, 2019 (Liu et al., 2019)	21 (2 cohort, 2 case-control, 17 cross-sectional)	145,091	*H. pylori* infection is one of the factors that promotes the progression of NAFLD	1.53 (95% CI: 1.34 - 1.75)	95.60%	No
Wei L, 2021 (Wei and Ding, 2021)	17 (2 cohort, 1 case-control, 14 cross-sectional)	91,958	A positive association between *H. pylori* infection and the risk of NAFLD	1.38 (95% CI: 1.23–1.55)	86.80%	No
Ma Z, 2022 (Ma et al., 2022)	25 (2 cohort, 6 case-control, 17 cross-sectional)	107,306	*H. pylori* infection was associated with an increased risk of NAFLD	1.30 (95% CI: 1.13–1.49) (Asian); 1.42 (95% CI: 1.04–1.94) (non-Asian)	94.30% (Asian);44.90% (non-Asian)	No
Heydari K, 2022 (Heydari et al., 2022)	22 (2 cohort, 2 case-control, 18 cross-sectional)	117,117	*H. pylori* infection increases the risk of developing NAFLD	1.22 (95% CI: 1.09-1.35)	NA	NA
Xu G, 2023 (Xu et al., 2023)	34 (4 cohort, 3 case-control, 27 cross-sectional)	218,573	*H. pylori* infection is associated with NAFLD	1.26 (95% CI: 1.17–1.36)	88.70%	No

NAFLD, non alcoholic fatty liver disease; CI, Confidence interval; NA, not available.

two studies with small sample sizes, publication bias was eliminated, and *H. pylori* infection remained significantly associated with NAFLD. All meta-analyses demonstrated a positive association between *H. pylori* infection and NAFLD. The odds ratio (OR) ranged from 1.14 to 1.53, which meant that the proportion of *H. pylori* infection in NAFLD patients was 1.14 - 1.53 times higher than that in their non-NAFLD counterparts. We hypothesize that *H. pylori* infection alone may have difficulty causing NAFLD, but *H. pylori* infection combined with a fast food diet and lifestyle disorders may exacerbate NAFLD levels.

The meta-analyses performed subgroup analyses according to race, region, diagnostic methods, and different study types to make the results more robust and credible and to reduce overall heterogeneity. However, most included studies were cross-sectional and could not illustrate the causal relationship between *H. pylori* and NAFLD. Second, correcting the effects of confounding factors such as hygiene level, dietary habits, physiological activity, and genetics from the clinical data collected by meta-analyses was difficult. With the specification of the NAFLD definition, the nomenclature of new fatty liver disease- metabolic dysfunction-associated steatotic liver disease (MASLD) (Rinella et al., 2023) will provide more accurate and high-quality clinical studies on the relationship between *H. pylori* infection and NAFLD/MASLD.

3.2 Dialectical discussion on studies negating the *H pylori*-NAFLD link

The above meta-analyses results suggest that *H. pylori* infection is positively associated with NAFLD. However, some clinical studies are controversial to this conclusion. We collected 13 clinical studies on *H. pylori* infection that had no bearing on NAFLD, including one bidirectional Mendelian randomization (MR) study (Liu et al., 2022), two clinical trials (Jamali et al., 2013; Polyzos et al., 2014), and ten cross-sectional studies (Okushin et al., 2015; Baeg et al., 2016; Cai et al., 2018; Fan et al., 2018; Kang et al., 2018; Lu et al., 2018; Han et al., 2021; Rahman et al., 2021; Wang et al., 2022a; Wernly et al., 2022), as shown in Table 2. Then, we provide a detailed dialectical discussion of clinical trials on studies needing revised or improved designs. A detailed discussion of the clinical randomized controlled trials (RCTs) by Jamali et al. (Jamali et al., 2013) and Polyzos et al. (Polyzos et al., 2014) is provided in Section 2.3.

TABLE 2 Clinical studies on *H. pylori* infection is not associated with NAFLD.

Reference	Region	Participants (N)	Research type	Main results	Odds ratio (OR)
Liu Y, 2022 (Liu et al., 2022)	Europe	NA	A bidirectional Mendelian randomization study	No evidence for a causal link between *H. pylori* infection and NAFLD	1.05 (95% CI: 0.78-1.41)
Jamali R, 2013 (Jamali et al., 2013)	Iran	100 (50:50)	Randomized open-label clinical trial	*H. pylori* eradication might not affect LFC, LFT, lipid profile, and insulin resistance in dyspeptic NAFLD patients	NA
Polyzos SA, 2014 (Polyzos et al., 2014)	Greece	13 (6:7)	prospective clinical research	*H. pylori* eradication had no long-term effect on hepatic steatosis.	NA
Okushin K, 2015 (Okushin et al., 2015)	Japan	1,802	cross-sectional study	*H. pylori* infection status did not show significant association with NAFLD	1.13 (95% CI: 0.99-1.28)
Baeg MK, 2016 (Baeg et al., 2016)	Republic of Korea	3,663	cross-sectional study	*H. pylori* infection is not a risk factor for NAFLD	1.13 (95% CI: 0.97-1.31)
Kang SJ, 2018 (Kang et al., 2018)	the United States	5,404	cross-sectional study	CagA positive *H. pylori* group did not demonstrate an association with NAFLD; CagA negative *H. pylori* group had a significant association with NAFLD	1.05 (95% CI: 0.86-1.18) (cagA positive) 1.30 (95% CI: 1.01-1.67) (cagA negative)
Lu LJ, 2018 (Lu et al., 2018)	China	1,867	cross-sectional study	participants with NAFLD had no statistically significant differences of *H. pylori* infection than those without NAFLD	1.13 (95% CI: 0.92-1.39)
Fan N, 2018 (Fan et al., 2018)	China	21,456	cross-sectional study	*H. pylori* infection is not independently associated with the risk of NAFLD	1.00 (95% CI: 0.70-1.30)
Cai O, 2018 (Cai et al., 2018)	China	2,051	cross-sectional study	*H. pylori* infection does not increase the NAFLD prevalence rate or to be associated with, or a risk factor for, NAFLD.	0.94 (95% CI: 0.70-1.27)
Han YM, 2021 (Han et al., 2021)	Republic of Korea	1,784	cross-sectional study	*H. pylori* seropositivity was not associated with CAP-defined NAFLD	0.96 (95% CI: 0.78-1.19)
Rahman MM, 2021 (Rahman et al., 2021)	Bangladesh	767	cross-sectional study	There was no relationship observed between *H. pylori* seroprevalence and NAFLD.	1.50 (95% CI: 0.94- 2.39)
Wang W, 2022 (Wang et al., 2022a)	China	71,633	cross-sectional study	*H. pylori* infection was not an independent risk factor for NAFLD.	1.02 (95% CI: 0.97-1.08)
Wernly S, 2022 (Wernly et al., 2022)	Austria	5,338	cross-sectional study	No independent association was found between *H. pylori* infection and NAFLD.	0.96 (95%CI: 0.82-1.13)

NAFLD, non alcoholic fatty liver disease; CI, Confidence interval; NA, not available; LFC, liver fat content; LFT, liver function tests; CAP, controlled attenuation parameter; Cag A, cytotoxin-associated gene A.

MR, which uses single nucleotide polymorphisms as instrumental variables to investigate the causal relationship between exposure factors and disease, have been widely used in epidemiological causal inferences in recent years (Davey Smith and Hemani, 2014). Liu et al. (Liu et al., 2022) showed no causal link between *H. pylori* infection and NAFLD. Moreover, *H. pylori* infection was not significantly associated with triglycerides (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C), or FBG. However, in this study, *H. pylori* infection diagnosis was based on serological testing and only involved European participants, which could have made the results potentially over-evaluated.

The cross-sectional study results were quite different. The lower 95% CI of the OR for some studies (Okushin et al., 2015; Baeg et al., 2016; Rahman et al., 2021; Wang et al., 2022a) was very close to 1, suggesting that the statistical results have questionable reliability in practice. This may be due to (1) different *H. pylori* strains circulating in various regions; (2) *H. pylori* infection not being diagnosed uniformly, such as ^{13}C- urea breath test and serum *H. pylori* antibody detection; (3) NAFLD being diagnosed differently, such as ultrasonography and hepatic steatosis index (HSI) and NAFLD liver fat score (NAFLD-LFS); and (4) differences in race and living routine between regions. Fan et al.(Fan et al., 2018) showed a significant association between *H. pylori* infection and NAFLD after adjusting for age and sex (OR = 1.1, 95% CI: 1.0 - 1.1, $P = 0.004$). Nevertheless, after adjusting for body mass index (BMI) and systolic and diastolic blood pressure, there was no significant

relationship between them (OR = 0.9, 95% CI: 0.9 - 1.0, $P = 0.097$). Finally, fasting plasma glucose, hemoglobin A1C (HbA1c), triglycerides, total cholesterol, HDL-C, LDL-C, and serum creatinine were adjusted (OR = 1.0, 95% CI: 0.7 - 1.3, $P = 0.753$). After adjusting for the metabolic index, *H. pylori* infection was not associated with NAFLD, which suggested that *H. pylori* infection may lead to metabolic disturbances rather than directly causing NAFLD. Our cross-sectional study of 16,942 participants also presents a significant link between *H. pylori* infection and metabolic index in humans (unpublished data), consistent with existing findings (Buzás, 2014).

3.3 Clinical RCTs on *H. pylori* eradication in *H. pylori*-positive NAFLD patients

Clinical RCTs are the gold standard for assessing the theoretical efficacy of clinical interventions. We collected recent RCTs on *H. pylori* eradication therapy in *H. pylori*-positive NAFLD patients (Jamali et al., 2013; Polyzos et al., 2014; Abdel-Razik et al., 2018; Maharshi et al., 2020; Yu et al., 2022). According to clinical RCTs, we speculated that eradicating *H. pylori* in *H. pylori* -positive NAFLD patients contributes to improving metabolic parameters.

The first randomized open-label clinical trial (Jamali et al., 2013) finally enrolled 100 patients with NAFLD randomly divided into a lifestyle modification group and a lifestyle modification plus *H. pylori* eradication group. After six months, it was found that liver fat content, liver function tests, lipid profile, and IR were improved in both groups compared with baseline levels. Nonetheless, there was no statistically significant difference between the two groups. Notably, the results of a recent randomized controlled trial of *H. pylori* eradication in NAFLD patients were the opposite. Yu et al. (Yu et al., 2022) enrolled 191 NAFLD patients with *H. pylori* infection and randomly divided them into untreated (health education and lifestyle guidance) and treated (health education and lifestyle guidance plus 14 days of *H. pylori* quadruple therapy) groups. One year later, the patient's metabolic index and FibroScan controlled attenuation parameter (CAP) values improved compared to those before treatment. The metabolic index [FBG, HbA1c (%), homeostatic model assessment of insulin resistance (HOMA-IR), TG, and BMI], CAP value, and inflammatory parameters [white blood cells, high-sensitivity C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α)] of the treated group were significantly improved compared with those of the untreated group. These two prospective studies came to a paradoxical conclusion, which may be mainly due to differences in inclusion criteria and follow-up times. Jamali et al. included NAFLD patients with ALT and AST greater than the upper limit of normal (ULN) who developed dyspeptic symptoms. Yu et al. included NAFLD patients with ALT and AST < 2 times the ULN and no gastrointestinal disease symptoms. In other words, Jamali et al. included patients with moderate and severe NAFLD; Yu et al. included patients with mild NAFLD. There was no difference in *H. pylori* eradication between the two groups in the former and a significant improvement between the two groups in the latter, illustrating the necessity of early eradication of *H. pylori* in NAFLD patients with *H. pylori* infection. Polyzos et al. (Polyzos et al., 2014) recruited 13 patients with biopsy-proven NAFLD who were divided into *H. pylori* (+) and *H. pylori* (−) groups according to whether they had *H. pylori* infection, both of whom received standard instructions for diet and exercise, and the *H. pylori* (+) group received *H. pylori* eradication therapy. Hepatic steatosis, HSENSI [Homocysteine, serum glutamic oxaloacetic transaminase, Erythrocyte sedimentation rate, and Nonalcoholic Steatohepatitis Index] were assessed twelve months later. The results indicated that *H. pylori* eradication had no long-term effect on magnetic resonance imaging -assessed hepatic steatosis. However, they suggested a trend toward improved NAFLD fibrosis score and HSENSI with *H. pylori* eradication. Interestingly, a retrospective study by the Polyzos team (Doulberis et al., 2020) suggested that active *H. pylori* infection was significantly associated with liver function, HOMA-IR, and liver fibrosis stage. The inclusion criteria for this study were patients with NAFLD demonstrated by liver biopsy and divided into *H. pylori* (+) and *H. pylori* (−) groups according to the presence or absence of *H. pylori* infection demonstrated by gastric biopsy. The findings of the same team seem paradoxical, probably because prospective studies included insufficient participants.

Abdel-Razik et al. (Abdel-Razik et al., 2018) followed 369 NAFLD patients (171 *H. pylori*-positive and 198 *H. pylori*-negative) for 24 months. They found that *H. pylori* eradication significantly reduced IR, lipid profile, HSI, and NAFLD-LFS and increased HDL. Maharshi et al. (Maharshi et al., 2020) followed 64 NAFLD patients (36 *H. pylori*-positive and 28 *H. pylori*-negative) for six months and found that *H. pylori* eradication improved hepatic steatosis and metabolic parameters. These findings also coincide with the results of another prospective RCT (Gen et al., 2010): beneficial impacts of *H. pylori* eradication therapy on IR, atherogenic lipid abnormalities, and low-grade inflammation.

4 The possible mechanism of *H. pylori* infection exacerbating NAFLD

Currently, there is no direct experimental mechanistic evidence that *H. pylori* infection impacts NAFLD, and the primary explanation focuses on IR, inflammatory cytokines or adipocytokines, lipid metabolism, and the intestinal barrier (Li et al., 2013; Cheng et al., 2017; Doulberis et al., 2021). We summarize the latest experimental evidence on the effects of *H. pylori* on the above factors and propose a new pathway: extracellular vesicles (EVs) or *H. pylori* outer membrane vesicles (*H. pylori*-OMVs), as shown in Figure 1. It should be noted that these possible mechanisms do not act independently on the development of NAFLD, such as multiple inflammatory cytokines that can also be involved in IR, lipid metabolism disorders, and intestinal barrier dysfunction. These factors are jointly engaged in multiple hits in NAFLD.

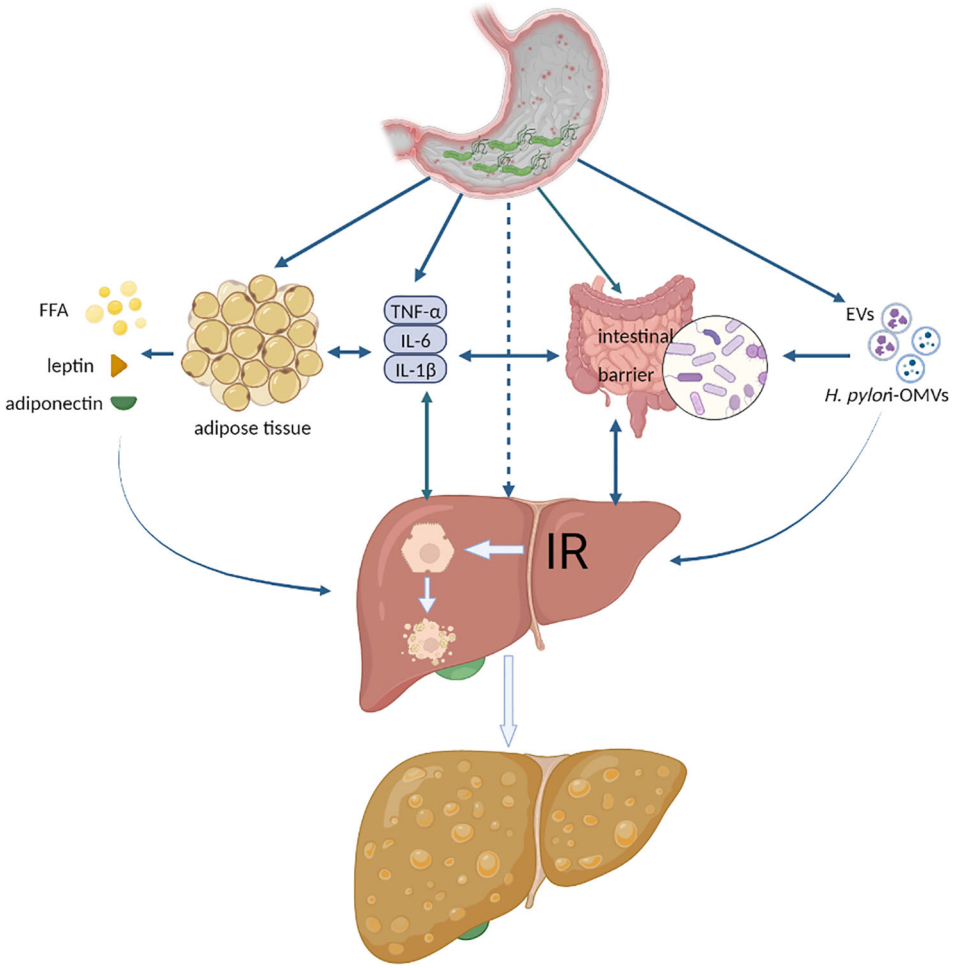

FIGURE 1
The possible mechanisms of *H. pylori* infection exacerbating NAFLD. FFA, free fatty acid; IR, insulin resistance; EVs, extracellular vesicles; *H. pylori*-OMVs, *H. pylori*- outer membrane vesicles. Authors hypothesize that systemic low-grade systemic inflammation caused by *H. pylori* infection aggravates NAFLD. Various inflammatory cytokines act directly or indirectly on adipose tissue, liver, and intestine to trigger or aggravate IR, disrupt the intestinal barrier, lead to hepatocyte steatosis, or activate liver fibrosis. Another possible mechanism is extracellular vesicles released by *H. pylori*-infected host cells or outer membrane vesicles secreted by *H. pylori*, which act directly with the liver and promote the development of NAFLD.

4.1 Inflammatory cytokines and adipocytokines

Inflammatory cytokines play a critical role in the pathology of *H. pylori* infection and NAFLD. In patients with persistent *H. pylori* infection, the body may lead to a chronic low-grade inflammation state and increased levels of the NOD-like receptor protein 3 (NLRP3) inflammasome (Pérez-Figueroa et al., 2016) and inflammatory cytokines, such as interleukin-1beta (IL-1β), IL-6, and TNF-α (Buzás, 2014). Inflammasomes and inflammatory cytokines are secreted by *H. pylori*-infected gastric epithelial cells and mucosal and circulating monocytes (Algood and Cover, 2006), reaching the liver via the circulatory system. NLRP3 and IL-1β participat in the whole process of liver inflammation, including IR and liver fibrosis (Tilg et al., 2016). IL-1β is directly involved in IL-1β/TNF-induced hepatocyte necrosis (Shen et al., 2020), promoting hepatic steatosis (Stienstra et al., 2010; Negrin et al., 2014), with positive feedback amplification of inflammation-induced IL-1β and TNF-α (Petrasek et al., 2012). In addition, researchers have found that IL-1β inhibits the fibroblast growth factor 21 (FGF 21) coreceptor beta-Klotho (KLB) to suppress FGF21 physiological effects (Zhao et al., 2016), which is crucial in regulating hepatic lipid metabolism and anti-inflammation (Xu et al., 2009). Mitsuyoshi et al. found that NLRP3, procaspase-1, IL-1β, and IL-18 mRNA levels were significantly increased in the livers of NAFLD patients compared to healthy controls (Mitsuyoshi et al., 2017). Accordingly, choline-deficient amino acid-defined diet-induced hepatomegaly, liver inflammation, and fibrosis were significantly ameliorated in Nlrp3 knockout mice compared with wild-type mice (Wree et al., 2014). Similar to IL-1β and NLRP3, TNF-α, and IL-6 play a direct or indirect role in the progression of NAFLD. TNF-α directly increases the expression of mast cell proteinase 1, Tgfb1, and tissue inhibitor of metalloproteinase 1 in hepatocytes, participating in hepatic lipid metabolism, inflammation, and liver fibrosis processes (Kakino et al., 2018). It is well-known that TNF-α also phosphorylates the serine of insulin receptor substrate-1 (IRS-1) to induce IR (Hotamisligil et al., 1996). Studies have demonstrated that IL-6 is involved in NASH progression through

the IL-6/signal transducer and activator of transcription 3 (STAT3) signaling pathway (Cai et al., 2016; Li et al., 2022).

Visceral white adipose tissue (WAT), when infiltrated by inflammatory cells, releases adipocytokines (including adiponectin, leptin, and resistin) and inflammatory factors (such as TNF-α and IL-6), which are involved in regulating IR and inflammation via endocrine or paracrine mechanisms (Kershaw and Flier, 2004; Stojsavljević et al., 2014). Adiponectin plays a role in alleviating IR and reducing intrahepatic triglyceride accumulation and is well known as a protective factor for NAFLD. On the one hand, adiponectin activates AMP-activated kinase (AMPK) by binding to adiponectin receptor 2 on the surface of hepatocytes, inhibits acetyl-CoA carboxylase, and decreases malonyl-CoA production, thereby increasing β-oxidation of fatty acids (FAs). On the other hand, adiponectin inhibits glycogenolysis and gluconeogenesis by inhibiting glucose-6-phosphatase and phosphoenolpyruvate carboxyl kinase mRNA expression (Combs and Marliss, 2014). Normally, hepatic leptin activates the phosphatidylinositol-3 kinase/Akt (protein kinase B)/mammalian target of rapamycin (mTOR) pathway mainly by binding to hepatocyte surface leptin receptor b, suppressing hepatic glucose production and improving insulin sensitivity. In addition, leptin can also activate the Janus kinase 2/STAT3 signaling pathway in the liver and regulate the function of suppressors of cytokine signaling 3 (SOSC3) function, a negative feedback regulatory molecule of the leptin receptor signaling pathway (Polyzos et al., 2015). However, the association of *H. pylori* infection with serum adipocytokines remains debated, as clinical observational studies have reached contradictory conclusions. Chen et al. found no significant difference in circulating leptin and adiponectin levels between *H. pylori*-positive and *H. pylori*-negative patients (Chen et al., 2015). Abdel-Razik et al. found a significant decrease in serum leptin and no significant change in adiponectin levels after *H. pylori* eradication therapy (Abdel-Razik et al., 2018), while Ando et al. found a significant increase in serum adiponectin levels after *H. pylori* eradication therapy (Ando et al., 2013). Future large-scale, prospective studies are needed to find specific links between *H. pylori* infection and adipocytokines.

4.2 Insulin resistance

IR, a central cause of NAFLD development, plays a significant role in hepatic triglyceride deposition, the inflammatory response, and hepatic fibrosis progression (Watt et al., 2019). Numerous studies have shown that *H. pylori* infection is an independent risk factor for IR. A cross-sectional study involving 1107 participants found that IR patients had a significantly higher prevalence rate of *H. pylori* infection than non-IR patients, even after adjusting for sex, age, BMI, waist circumference, visceral and subcutaneous adipose tissue, smoking status, alcohol consumption, dietary habits, and physical activity (Gunji et al., 2009). In *H. pylori*-infected patients, fasting glucose, fasting insulin, HbA1c, and HOMA-IR values decreased significantly after *H. pylori* eradication compared with those before treatment (Dogan et al., 2015). In addition, multiple meta-analyses have also indicated a link between *H. pylori* infection and IR (Polyzos et al., 2011; Azami et al., 2021). Animal studies have shown that HFD-fed mice with *H. pylori* infection developed more severe IR than HFD-fed mice alone, and mice fed a HFD for 12 weeks plus *H. pylori* infection were obese similar to mice fed a HFD for 24 weeks (He et al., 2016). Further studies revealed that *H. pylori* infection increases the expression of the inflammation-related transcription factor c-Jun. C-Jun can bind to the promoter region of the miR-203 gene to inhibit miR-203 expression, an inhibitor of the insulin negative feedback regulator SOCS3, and ultimately promote hepatic IR through the c-Jun/miR-203/SOCS3 pathway (Zhou et al., 2015).

4.3 Lipid metabolism

Hepatocyte steatosis is the primary pathological manifestation of NAFLD; its essence is lipid metabolism disorder in hepatocytes (Powell et al., 2021). Clinical studies have suggested that *H. pylori* infection affects lipid metabolism (Buzás, 2014; Watanabe et al., 2021). A large-cohort propensity score-matched analysis revealed that eradicating *H. pylori* could alleviate the deterioration of lipid metabolism but not return to uninfected levels. Specifically, HDL-C continued to decrease, LDL-C continued to rise in *H. pylori*-infected patients after *H. pylori* eradication therapy, and their lipid changes were significantly greater than those in participants with persistent *H. pylori*-negative status and significantly smaller than those in patients with persistent *H. pylori*-positive status (Wang et al., 2022b).

Increased triglyceride synthesis [fatty acid uptake, *de novo* lipogenesis (DNL)] and decreased consumption [fatty acid β-oxidation, very low-density lipoprotein (VLDL) transport] in the liver are the leading causes of triglyceride deposition (Kawano and Cohen, 2013). Donnelly et al. (Donnelly et al., 2005) demonstrated hepatic triglyceride deposition in NAFLD patients, 59% from plasma nonesterified fatty acids [also called free fatty acids (FFAs)], 26% from *de novo* lipogenesis, and 15% from the diet. FFAs in plasma are mainly derived from adipose tissue lipolysis. There is no direct linkage to the evidence that *H. pylori* infection increases adipose tissue lipolysis, whereas chronic systemic inflammation triggered by *H. pylori* infection induces WAT lipolysis (Xu et al., 2003). It has been found that fatty acid synthase and ATP-citrate lyase, critical enzymes of DNL in the gastric mucosa of *H. pylori*-infected patients, are upregulated, which means that DNL in the gastric mucosa of *H. pylori*-infected patients is increased (Chen et al., 2020), but whether *H. pylori* infection directly affects DNL in the liver needs further experimental research. Moreover, *H. pylori* infection affects the intestinal barrier and gut microbiota, affecting diet-derived lipid metabolism (see Section 4.4). VLDL is a carrier for transporting TGs synthesized by the liver to peripheral organs or circulation. When hepatic lipid production is excessive and VLDL transport capacity is not matched, hepatic steatosis and lipotoxicity produced

by hepatic triglyceride accumulation. It causes dyslipidemia, on the other hand (Heeren and Scheja, 2021). Circulating VLDL is significantly higher in *H. pylori*-infected patients than in non-infected patients (Işıktaş Sayılar et al., 2015), which may result indirectly from IR caused by *H. pylori* infection or endoplasmic reticulum stress in hepatocytes.

4.4 Intestinal barrier and microbiota

Substantial evidence has demonstrated that *H. pylori* infection impacts intestinal barrier function. Under pathological conditions such as hypoxia, inflammatory response, and intestinal microbiota disorders, intestinal bacteria, and their metabolites pass through the damaged intestinal barrier, enter the circulation and participate in the development of NAFLD (Sanduzzi Zamparelli et al., 2016).

A clinical study matching participants' sex, age, BMI, alcohol consumption, smoking, proton pump inhibitor usage, history of peptic ulcer disease, and dietary habits found that *H. pylori* infection was associated with alterations in the fecal microbiota and increased overall diversity of fecal microbes (Frost et al., 2019). Experimental studies have found that mice fed a HFD plus *H. pylori* infection have increased intestinal abundance of *Helicobacter* and decreased abundance of *Lactobacillus*, with a loss of diversity. Meanwhile, *H. pylori* infection also aggravated HFD-induced hyperglycemia, which could not be restored even with *H. pylori* eradication (Peng et al., 2021). Another study in mice fed a HFD plus *H. pylori* infection found that the expression of tight junction components, such as occludin, zonula occludens-1, and claudin-1, which are important components of the intestinal barrier, was significantly decreased, indicating that *H. pylori* infection directly affects intestinal barrier function (He et al., 2016). Further studies revealed that CagA -containing exosomes increased intestinal permeability by upregulating Claudin-2 expression through activation of CDX2 (Caudal-related homeodomain transcription 2) (Guo et al., 2022). Intestinal barrier dysfunction gives rise to (1) increased intestinal permeability, intestinal bacteria and their metabolites (such as dimethylamine, trimethylamine) and lipopolysaccharide (LPS) in the liver, triggering a liver inflammatory response, hepatocyte damage, and liver fibrosis (Cui et al., 2019); (2) intestinal epithelial cells release inflammatory cytokines to promote NAFLD (Wigg et al., 2001); and (3) intestinal nutrient absorption dysfunction and metabolic substances such as choline deficiency (Spencer et al., 2011).

4.5 EVs or *H. pylori*-OMVs

H. pylori-OMVs are bilayer membrane spherical vesicles released from *H. pylori* and contain various bacterial elements, such as LPS, outer membrane proteins (OMPs), and virulence proteins [such as CagA and Vacuolating cytotoxin A (Vac A)]. *H. pylori*-OMVs can influence bacterial survival, transmit toxins and virulence factors, and regulate the host immune response and genetic material transfer (Parker and Keenan, 2012). EVs are membranous vesicles secreted by *H. pylori*-infected host cells that maintain intercellular communication, promote inflammatory responses, and manipulate the lesion microenvironment (González et al., 2021). Extracellular vesicles are divided into two categories according to diameter: exosomes and microvesicles, the former of which have been most studied for their biological characteristics.

H. pylori-OMVs and *H. pylori*-infected host cell-derived EVs have been found to accelerate the development of various diseases (Chmiela et al., 2018; González et al., 2021; Qiang et al., 2022). CagA-containing exosomes have been demonstrated in the circulation of both CagA-positive *H. pylori*-infected patients and mice (Shimoda et al., 2016; Xia et al., 2020). Exosomes in the blood circulation reach the liver directly, impair endothelial function, activate hepatic Kupffer cells, and promote the hepatic inflammatory response and hepatocyte damage (Kazankov et al., 2019; Xia et al., 2020). Additionally, Zahmatkesh et al. (Zahmatkesh et al., 2022) found that exosomes derived from *H. pylori*-OMVs-infected hepatocytes activated hepatic stellate cells and upregulated the overexpression of liver fibrosis markers (vimentin, cadherin 1, and catenin beta 1). However, the role of *H. pylori*-OMVs and EVs in NAFLD needs further exploration.

5 Conclusion

H. pylori infection and NAFLD are chronic diseases, and their prolonged progression may lead to irreversible damage to the body. If *H. pylori* eradication therapy can delay or improve the physiological status of *H. pylori* positive NAFLD patients, it will significantly alleviate the disease burden of NAFLD. Numerous clinical and animal studies have suggested a link between *H. pylori* infection and NAFLD. We summarize here meta-analyses investigating the association between *H. pylori* infection and NAFLD, analyze some studies that oppose the association between them, and enumerate a number of high-quality evidence.

Understanding the relationship between *H. pylori* infection and NAFLD contributes to comprehending the mechanisms by which *H. pylori* leads to extragastric disease. This comprehension helps clinicians better understand and manage NAFLD and facilitates NAFLD patients with *H. pylori* infection to benefit from *H. pylori* eradication. Future multicenter prospective studies are needed to illustrate how *H. pylori* eradication will improve the physiological status of NAFLD patients or whether *H. pylori* eradication has additional benefits for NAFLD patients. In experimental studies, it is critical to determine whether *H. pylori* infection affects the progression of NAFLD in a direct (e.g., EVs or *H. pylori*-OMVs) or indirect (e.g., systemic chronic low-grade inflammation, IR) manner.

Author contributions

XC: Writing – original draft, Conceptualization, Data curation, Visualization. RP: Writing – original draft, Data curation, Visualization. DP: Writing – original draft, Data curation, Visualization. JX: Writing – original draft, Data curation, Visualization. DL: Writing – review & editing, Methodology, Supervision. RL: Writing – review & editing, Methodology, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of plant natural products on metabolic-associated fatty liver disease and the underlying mechanisms: a narrative review with a focus on the modulation of the gut microbiota

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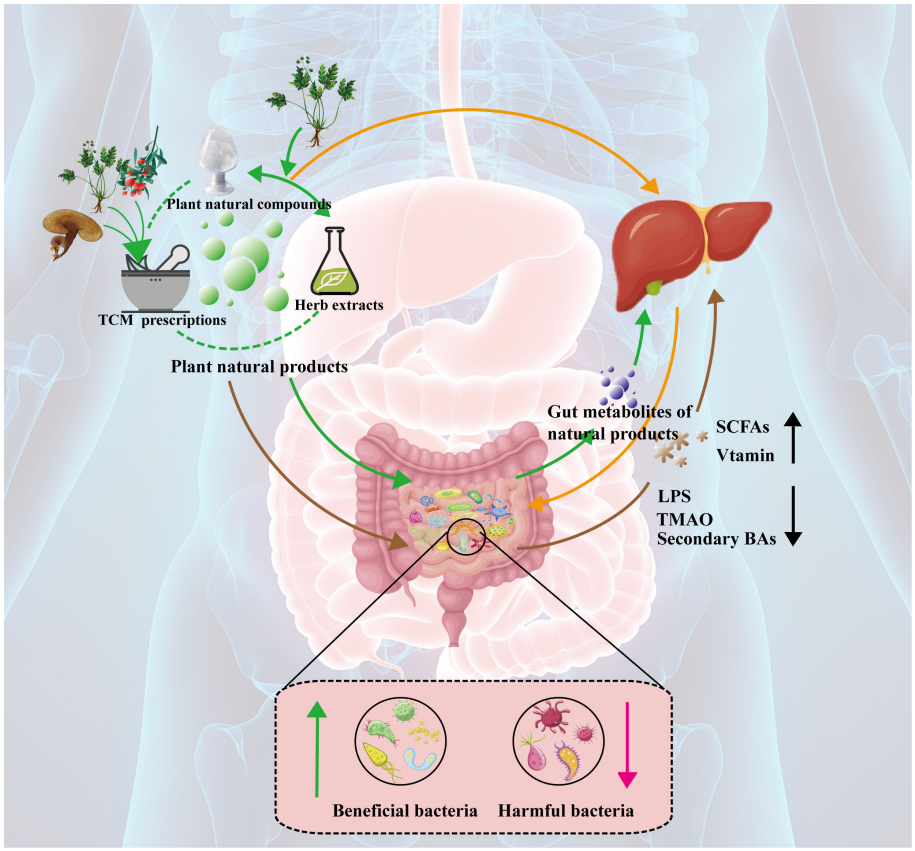
Metabolic-associated fatty liver disease (MAFLD) is a chronic liver disease characterized by the excessive accumulation of fat in hepatocytes. However, due to the complex pathogenesis of MAFLD, there are no officially approved drugs for treatment. Therefore, there is an urgent need to find safe and effective anti-MAFLD drugs. Recently, the relationship between the gut microbiota and MAFLD has been widely recognized, and treating MAFLD by regulating the gut microbiota may be a new therapeutic strategy. Natural products, especially plant natural products, have attracted much attention in the treatment of MAFLD due to their multiple targets and pathways and few side effects. Moreover, the structure and function of the gut microbiota can be influenced by exposure to plant natural products. However, the effects of plant natural products on MAFLD through targeting of the gut microbiota and the underlying mechanisms are poorly understood. Based on the above information and to address the potential therapeutic role of plant natural products in MAFLD, we systematically summarize the effects and mechanisms of action of plant natural products in the prevention and treatment of MAFLD through targeting of the gut microbiota. This narrative review provides feasible ideas for further exploration of safer and more effective natural drugs for the prevention and treatment of MAFLD.

KEYWORDS

MAFLD, gut microbiota, plant natural products, metabolite, gut-liver axis



GRAPHICAL ABSTRACT

Highlights

- The gut microbiota may be a new target for MAFLD.
- Plant natural compounds can prevent and treat MAFLD by targeting the gut microbiota.
- Herb extracts could prevent and treat MAFLD by targeting the gut microbiota.
- TCM prescriptions could prevent and treat MAFLD by targeting the gut microbiota.
- Changing the structure and metabolites of the gut microbiota is the mechanism of action of plant natural products.

1 Introduction

Metabolic-associated fatty liver disease (MAFLD) is a new term for nonalcoholic fatty liver disease (NAFLD) (Eslam et al., 2020) and is a standard positive diagnosis based on metabolic factors and independent of alcohol use. Currently, the diagnostic criteria for MAFLD are based on evidence of hepatic steatosis (demonstrated by biopsy, imaging or validated serum biomarkers), in addition to one of the following criteria: overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation defined by the presence of at least two metabolic risk factors, including high waist circumference, hypertension, hypertriglyceridemia, hypo-HDL cholesterolemia, prediabetes, insulin resistance, and elevated high-sensitivity C-reactive protein levels. Recently, the prevalence rate of MAFLD has increased consistently. The global prevalence of MAFLD is already approximately 25% (Younossi et al., 2016), reaching approximately 70% in the obese population (Quek et al., 2022). MAFLD has become the most common chronic liver disease in the world. MAFLD comprises a continuous spectrum of liver diseases, including not simple fatty liver (NAFL) but also dynamic disease that can progress to steatohepatitis (NASH), decompensated cirrhosis and even hepatocellular carcinoma (HCC), and has gradually become an important cause of liver failure and liver transplantation. Moreover, the development of MAFLD often occurs in combination with various metabolic disorders and contributes to the progression of serious diseases, such as gout (Kuo et al., 2010), type 2 diabetes mellitus (T2DM) (Tanase et al., 2020), hypertension (Ma et al., 2021), and cardiovascular disease (Targher et al., 2016). Therefore, it is particularly important to find appropriate and efficient ways to control and treat MAFLD.

Currently, traditional management strategies for MAFLD largely focus on weight reduction and lifestyle modification. Lifestyle interventions consist primarily of dietary interventions and exercise interventions. Most studies of dietary weight loss have

shown limited average weight loss (<5%) after 12 months of intervention, and there are some risks associated with long-term ketogenic diet interventions (Anekwe et al., 2020). For exercise interventions, it is recommend that the average adult complete at least 30 min of moderate-intensity aerobic exercise on 1 day and no less than 4 times per week for at least 16 weeks (Shojaee-Moradie et al., 2016). Both interventions (dietary and exercise interventions) are not only long-lasting and slow but also difficult to adhere to, leading to poor patient compliance. Pharmacological interventions are usually used in patients with MAFLD who fail to respond to conventional treatments. Pharmacological interventions prevent the progression of hepatitis and liver fibrosis by reducing liver fat accumulation and alleviating inflammatory damage. Studies have shown that drugs for type 2 diabetes can be used to treat patients with MAFLD (Mitrovic et al., 2022), but there are several toxic side effects. In fact, there are no Food and Drug Administration (FDA)-approved drugs for MAFLD treatment. Therefore, there is an urgent need to find safe and effective anti-MAFLD targets and drugs.

The gut microbiota plays an important role in regulating gut development, regulating host nutrient metabolism, preventing pathogenic bacterial colonization, and maintaining the gut barrier and immune function (Zhang et al., 2015). In recent years, numerous studies have shown that gut ecological dysbiosis is closely associated with the progression of obesity (Fei and Zhao, 2013; Cheng et al., 2022), type 2 diabetes (Ma et al., 2019) and cardiovascular disease (Xu H. et al., 2020). In particular, many studies have indicated that the gut microbiota is associated with the development of MAFLD (Le Roy et al., 2012). There are differences in the gut microbiota between healthy individuals and MAFLD patients, and MAFLD patients have poorer gut microbial ecological diversity and reduced bacterial abundance. Moreover, germ-free mice exhibited significantly reduced sensitivity to diet-induced hepatic steatosis (Bäckhed et al., 2004). Based on these findings, the gut microbiota has become a potential new therapeutic target for MAFLD.

A natural product is defined as "a product derived from a plant, animal or microbial source, also known as natural sources." (Paola et al., 2023) Normally, plant natural compounds, herb extracts and traditional Chinese medicine (TCM) prescriptions have been considered the main forms of plant natural products. Historically, plant natural products, especially TCMs, have been used since ancient times and in folk medicine for the treatment of many diseases and illnesses. Recently, plant natural products have attracted much attention for the treatment of MAFLD due to their multiple targets and pathways and few side effects. However, their low bioavailability has limited their further development. Many research studies have indicated that only some plant natural products are absorbed in the small intestine, while most of these products reach the colon, where the composition of the gut microbiota can be affected, facilitating the metabolism of the microbiota and alleviating MAFLD. Recent studies have suggested that plant natural products may exert their hypolipidemic and hepatoprotective effects by altering the structure of and metabolite production by the gut microbiota (Zhang N-N. et al., 2023). However, the effects of plant natural products on MAFLD and the underlying mechanisms are also poorly understood and need to be further clarified, especially given the data from the last five years.

Based on the above information and to address the potential therapeutic role of plant natural products in MAFLD, we systematically summarize the effects and mechanisms of action of plant natural compounds, herb extracts and TCM prescriptions in the prevention and treatment of MAFLD through targeting of the gut microbiota. This review summarizes studies from the past 5 years reported in databases, including PubMed, Web of Science, Google Scholar, and X-MOL, which were filtered using the keywords "gut microbiota" and/or "MAFLD". Those on human-modified substances, such as plant natural product-related nanoparticles or synthetic derivatives, are beyond the scope of this review. Overall, this narrative review provides feasible ideas for the further exploration of safer and more effective natural anti-MAFLD drugs.

2 A potential mechanism for the treatment of MAFLD by targeting the gut microbiota and its metabolites

The gut microbiome is a complex ecosystem composed of bacteria, archaea, fungi, protozoa and viruses that not only participates in nutrient digestion and immune regulation under host physiological and pathological conditions but also serves as a bridge between the gut and other extragut tissues (Kuziel and Rakoff-Nahoum, 2022). Thus, changes in the gut microbiome may affect the health of the host. Many studies have indicated that the gut microbiota is closely associated with the progression of MAFLD (Safari and Gérard, 2019; Hrncir et al., 2021; Shen et al., 2017; Boursier et al., 2016). For example, the ratio of Firmicutes to Bacteroides is greater in patients with MAFLD than in healthy people, and the abundances of Proteobacteria and Enterobacteriaceae increase while that of Ruminococcaceae decreases in these patients (Boursier et al., 2016). The abundance of *Prevotella* decreases significantly with increasing liver inflammation (Chen and Vitetta, 2020). However, the mechanism through which the gut microbiota affects MAFLD remains incompletely understood. With the development of metabolomics technology, changes in gut metabolites during the development of MAFLD have gradually been revealed (Chen and Vitetta, 2020). Among them, the roles of bile acid (BAs), lipopolysaccharides (LPSs), short-chain fatty acids (SCFAs), trimethylamine-N-oxide (TMAO) and vitamins have received widespread attention. These findings may lead to the identification of potential mechanisms of action for the treatment of MAFLD and to new feasible ideas for intervention therapy for MAFLD, as shown in Figure 1.

2.1 The gut microbiota may ameliorate MAFLD by regulating the BA pool composition

Bile acids (BAs) are cholesterol metabolites that play an important role in the balance of cholesterol and energy metabolism and the absorption of nutrients in the small intestine. The liver is the main site for BA synthesis. BAs are synthesized in the liver and subsequently secreted into the bile duct and stored in

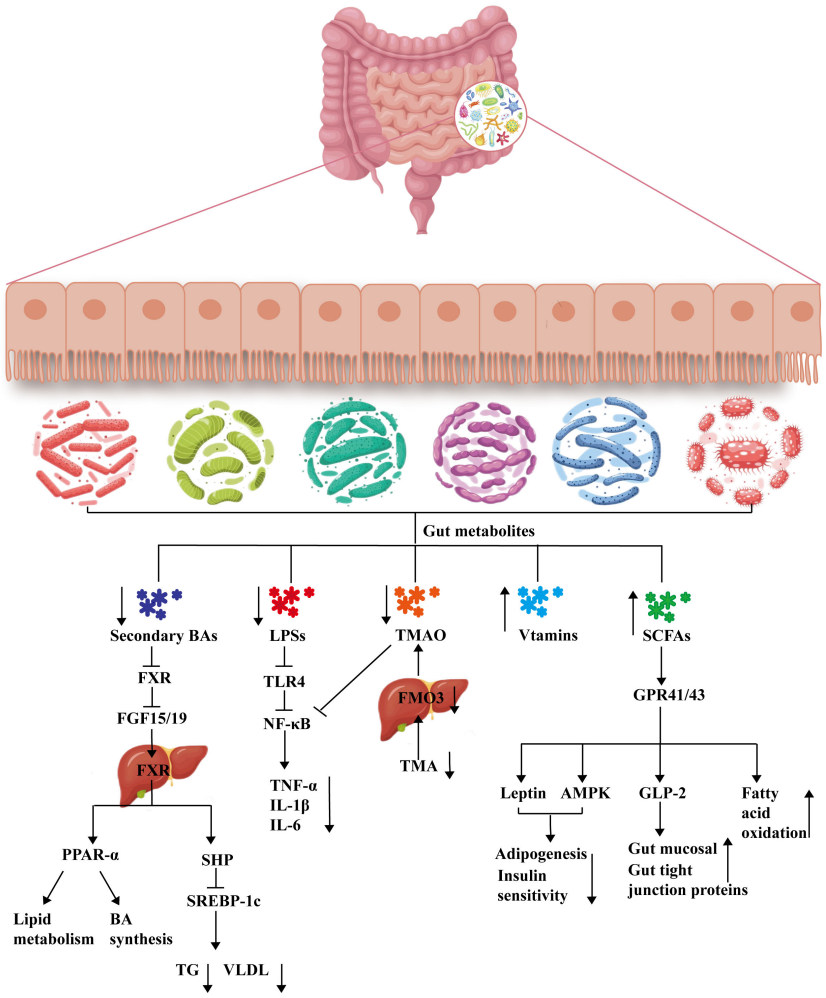


FIGURE 1
Mechanisms of gut metabolism involved in the treatment of MAFLD. BA, bile acid; FXR, farnesoid X receptor; FGF15/19, fibroblast growth factor 15/19; PPAR-α, peroxisome proliferator-activated receptor alpha; SHP, small heterodimer partner; SREBP-1c, sterol regulatory element binding protein-1c; TG, triglyceride; VLDL, very low-density lipoprotein; LPS, lipopolysaccharide; TLR4, Toll-like receptor-4; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1β; IL-6, interleukin-6; TMAO, trimethylamine-N-oxide; TMO, trimethylamine; FMO3, flavin monooxygenase; SCFA, short-chain fatty acid; GPR41/GPR43, mammalian G protein-coupled receptor 41/43; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; GLP-2, glucagon-like peptide-2 receptor.

the gallbladder. After eating, the gallbladder contracts, and the stored BAs are expelled into the small intestine. In the small intestine, 95% of the BAs are reabsorbed by the small intestine and returned to the liver via the hepatic portal vein, with another approximately 5% exiting the body in the feces or entering systemic circulation (Di Ciaula et al., 2017). BAs act as emulsifiers to promote the absorption and transport of lipids and other substances in the small intestine and as indispensable signaling molecules that bind to a variety of receptors, including the nuclear receptor farnesoid X receptor (FXR), vitamin D receptor (VDR), pregnane X receptor (PXR) and the membrane-bound G protein-coupled receptor Takeda G protein-coupled receptor 5 (TGR5), which exert essential effects to regulate the balance of BA metabolism, glycolipid metabolism and energy metabolism (Perino and Schoonjans, 2022). Recently, much attention has been given to the interaction between BAs and the gut microbiota (Long et al., 2017). Gut microbes can directly modify BAs, such as bacteria that produce bile salt hydrolases (BSHs), which catalyze the conversion of primary bile acids to secondary bile acids, resulting in decreased levels of coupled BAs that may activate intestinal FXR expression and promote hepatic steatosis (Yang and Wu, 2022). Most studies have suggested that BA levels are elevated and that the BA composition is substantially altered in the serum and liver of MAFLD patients (Ferslew et al., 2015; Kalhan et al., 2010). It has also been established in animals that changes in the expression of BA-metabolizing enzymes and transporters that occur with the progression of NASH-related liver fibrosis lead to an increase in the plasma total bile acid (TBA) concentration (Suga et al., 2019). Based on the above information, the gut BA pathway might participate in the treatment of MAFLD.

Changes in composition are the main way that the gut microbiota alters BAs, and these changes affect the efficiency of binding of BAs to their receptors. For example, the order in which BAs bind and activate FXR is chenodeoxycholic acid > lithocholic

acid > deoxycholic acid > cholic acid; the most efficacious BA ligands for TGR5 are in the order lithocholic acid > deoxycholic acid > chenodeoxycholic acid > cholic acid (Chiang, 2013). BAs regulate lipid and glucose metabolism mainly through the receptors FXR and TGR5 (Schaap et al., 2013). On the one hand, BAs can reduce triglyceride levels through the pathways of the FXR and the small heterodimer partner (SHP), as well as sterol regulatory element-binding protein 1c (SREBP-1c) (Watanabe et al., 2004). In addition, FXR activation promotes the expression of peroxisome proliferator-activated receptor alpha (PPAR-α), which facilitates the regulation of lipid metabolism, glucose homeostasis and anti-inflammatory activity (Carr and Reid, 2015; Pineda Torra et al., 2003). On the other hand, BAs not only activate TGR5 to improve insulin sensitivity and reduce obesity (Brighton et al., 2015; Thomas et al., 2009) but also inhibit the expression of cytochrome P450 7A1 (CYP7A1) by activating FXR and TGR5, thus inhibiting BA synthesis (Goodwin et al., 2000). Many studies have shown that TGR5 or FXR agonists can reduce lipogenesis, alleviate cholesterolemia, induce energy expenditure, and reduce liver inflammation (Pineda Torra et al., 2003; McMahan et al., 2013; Jadhav et al., 2018). BAs affect not only the gut-liver axis but also the gut-brain axis. BA-TGR5 signaling reduces the expression of Agouti-related protein (AgRP)/neuropeptide Y (NPY) and temporarily blocks the release of neuropeptides in AgRP/NPY neurons, which in turn inhibits feeding behavior, which may also be relevant to the treatment of MAFLD (Perino et al., 2021). Therefore, activation of FXR or TGR5 by regulating the gut microbiota to change the BA pool composition is expected to be the main therapeutic mechanism for the treatment of MAFLD.

2.2 Increased concentrations of SCFAs may ameliorate MAFLD in the gut microbiota

Under the action of anaerobic microorganisms in the mammalian colon, carbohydrates are degraded and fermented to produce large amounts of short-chain fatty acids (SCFAs), and acetic acid, propionic acid, and butyric acid make up approximately 90%-95% of the total SCFAs. SCFAs play an active role in the pathogenesis of MAFLD through portal vein entry into the liver and regulation of the inflammatory response, lipid metabolism and glucose metabolism (Zhou and Fan, 2019). SCFAs can also protect the gut barrier (Liu et al., 2020). Studies have shown that MAFLD patients have fewer SCFA-producing bacteria in the gut and decreased levels of SCFAs in the feces (Raman et al., 2013). In addition, numerous studies have demonstrated that SCFA or butyrate supplementation can repair the gut barrier and ameliorate NASH (Jin et al., 2015; Zhou et al., 2017; Gart et al., 2021; Ye et al., 2018). Therefore, the gut SCFA pathway might be a potential target for the treatment of MAFLD.

The specific receptors for SCFAs identified are mainly mammalian G protein-coupled receptor 41 (GPR41), mammalian G protein-coupled receptor 43 (GPR43), and G protein-coupled receptor 109 A (GPR109A). GPR41 and GPR43 are highly expressed in adipocytes, enteroendocrine cells and immune cells (polymorphonuclear cells and macrophages) (Maslowski et al., 2009), while GPR109A is expressed in adipocytes, hepatocytes and colon cells (Singh et al., 2014). These receptors usually mediate anti-inflammatory effects directly (Thorburn et al., 2014). Recent studies have shown that mammalian G protein-coupled receptors, especially GPR41, GPR43, and GPR109A, play important roles in metabolism, inflammation, and disease regulation and may be potential new drug targets for the treatment of certain metabolic diseases (Tan et al., 2014; Sekiguchi et al., 2015). The different SCFAs had the following order of activity against GPR43: propionic acid (C3) ≥ acetic acid (C2) = butyric acid (C4) > valeric acid (C5) > capric acid (C6) = formic acid (C1) (Brown et al., 2003; Nilsson et al., 2003). Activation of GPR43 directly inhibits lipid degradation (Hong et al., 2005; Ge et al., 2008). Thus, the gut microbiota may regulate lipid metabolism by affecting the SCFA composition and the expression of receptors in the gut. Moreover, SCFAs may inhibit adipogenesis through GPR41 or GPR43 by stimulating leptin secretion from white adipocytes in mice and suppressing appetite (Xiong et al., 2004; Zaibi et al., 2010). On the other hand, SCFAs also ameliorate gut inflammation and reduce gut mucosal injury. SCFAs may promote gut mucosal growth and development by inducing human glucagon-like peptide-2 activation; SCFAs may inhibit gut inflammation by activating GPR43 to protect the liver from portal vein-derived gut microbes while decreasing insulin sensitivity in adipose tissue and activating the hepatic adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway, which directly or indirectly plays a protective role in liver health (Zhu et al., 2014). In conclusion, SCFAs and their receptors, which are regulated by the gut microbiome, play essential roles in regulating lipid metabolism and the inflammatory response, thereby improving the pathological progression of MAFLD.

2.3 The gut microbiota may ameliorate MAFLD by reducing LPS levels

LPSs are glycolipids that can be found on the surface of bacteria and are produced mainly by gram-negative bacteria. LPSs are endotoxins that are transported through the serum circulation to target tissues and are recognized by immune cells. Most LPSs activate inflammatory signaling pathways to secrete proinflammatory factors, cause the body to enter a state of chronic low-grade inflammation, induce metabolic abnormalities, and exert few anti-inflammatory effects through immune cells (Bertani and Ruiz, 2018). The gut microbiota is the main source of LPSs in healthy individuals (Candelli et al., 2021). Differences in the composition of the gut microbiota determine whether LPSs are toxic. For example, LPS produced by *Bacteroides* is harmless (Poli and Orange, 2017), while LPS produced by *Escherichia coli* is highly toxic and increases the level of fecal calprotectin, which is a marker of gut inflammation (Orivuori et al., 2015). Moreover, the serum LPS concentration was found to be positively correlated with the abundance of the aerobic bacteria *Escherichia coli* and *Enterococcus* and negatively correlated with the abundance of the anaerobic bacteria *Lactobacillus*, *Bifidobacterium* and *Bacteroides*, which is consistent with the results of microbiota changes in MAFLD patients (Xue et al., 2017).

Toll-like receptor 4 (TLR4) is an important member of the TLR family. Studies have shown that TLR4 is a key pattern recognition

receptor for LPSs and plays an important role in the connection between the innate immune system and metabolic syndrome (Csak et al., 2011; Maddie et al., 2022; Sharifnia et al., 2015). During the progression of MAFLD, serum LPS levels and hepatic TLR4 expression were elevated in patients, while the gut microbiota diversity and biological colonization resistance of gut microorganisms were decreased (Thuy et al., 2008; Dapito et al., 2012). In addition, the correlation between hepatic TLR4 and the gut microbiota also showed the above trend. These findings suggest that LPSs and TLR4 are key molecules in the pathogenesis of MAFLD and that liver injury mediated by the LPS-TLR4 signaling pathway may be involved in the progression of MAFLD. Many studies have indicated that gut LPS and TLR4 activation is associated with diet-induced MAFLD onset, and the use of LPSs can serve as noninvasive tools for the diagnosis and grading of MAFLD severity in overweight and obese patients (Hegazy et al., 2020). Therefore, activation of TLR4 by regulating the gut microbiota to reduce LPS levels could regulate lipid metabolism and the inflammatory response and is expected to be the main therapeutic mechanism for the treatment of MAFLD.

2.4 The gut microbiota may affect MAFLD by regulating TMAO

Trimethylamine-N-oxide (TMAO) is an important product of the enterohepatic axis that is produced by the metabolism of trimethylamine (TMA) by the gut microbiota and enters the liver via the enterohepatic axis, where it is then oxidized by enzymes such as flavin-containing monooxygenase 3. Clinical studies have shown a significant increase in the serum TMA, TMAO and choline levels in patients with MAFLD compared to those in healthy individuals and that there is an association between high circulating TMAO concentrations and MAFLD as well as NASH (Theofilis et al., 2022; Flores-Guerrero et al., 2021; León-Mimila et al., 2020; Shi et al., 2022). TMAO may promote the progression of MAFLD through several mechanisms. For example, TMAO may directly enhance the development of MAFLD by affecting oxidative stress (Li X. et al., 2021); TMAO stimulation leads to increased expression of unfolded protein response-related proteins (glucose-regulated protein 78, X-box binding protein 1, and Derlin-1) (Shi et al., 2022), which may lead to hepatocyte lipid metabolism disorders and inflammation, causing the development and progression of MAFLD and ultimately to death (Lebeaupin et al., 2018; Song and Malhi, 2019). Furthermore, serum TMAO levels were found to be positively associated with total serum BA levels and hepatic CYP7A1 expression, suggesting that TMAO can increase BA synthesis and shift hepatic BA components toward FXR antagonistic activity, thereby exacerbating hepatic steatosis (Tan et al., 2019). However, it has also been shown that TMAO supplementation enhances the ability to repair tissue damage by increasing the number of endothelial cells in mice, thereby enhancing the integrity of the blood–brain barrier and protecting it from inflammatory damage, which may be beneficial in ameliorating NASH. For example, Zhou et al. reported that TMAO supplementation restored gut microbiota diversity, reduced liver fibrosis, and protected vascular function in mice, suggesting a possible protective effect of TMAO metabolic retrotransposition in the gut (Zhou et al., 2022). Therefore, reducing elevated TMAO levels by regulating the gut microbiota is expected to be the main therapeutic mechanism for the treatment of MAFLD.

2.5 The gut microbiota may affect MAFLD by regulating gut vitamins

Vitamins are essential nutrients for the normal growth of humans and animals, and deficiency and overdose can lead to metabolic disorders and reduced growth performance (Brennan, 2006). The human body cannot synthesize most vitamins and must obtain them from the diet or rely on symbiotic bacteria in the gastrointestinal tract for their synthesis (Hill, 1997). For example, *Lactobacillus* and *Bifidobacterium* species are capable of synthesizing most water-soluble B vitamins, and *E. coli* is capable of synthesizing vitamin K. The development of MAFLD has been shown to disrupt the gut microbiota; therefore, MAFLD may affect the synthesis of vitamins in the body through the gut microbiota. Clinical studies have indicated that vitamin D (VD) levels are markedly lower in patients with MAFLD than in those without this disease (Chung et al., 2016; Tian et al., 2020). The biological roles of VD include not only maintaining the balance of calcium and phosphorus metabolism in the body and participating in bone reconstruction but also reducing insulin resistance, modulating immunity, protecting cardiovascular health, and exerting antifibrosis and anti-inflammation effects (Chun et al., 2019; Whiting and Calvo, 2021). Numerous epidemiological investigations have shown that VD deficiency is closely associated with MAFLD, cirrhosis and obesity, and there is also evidence that VD has a protective effect against MAFLD (Tian et al., 2020; Du et al., 2023; Barchetta et al., 2011; Ciardullo et al., 2023; Barchetta et al., 2020; Barchetta et al., 2017; Pop et al., 2022). Furthermore, VD supplementation can alter the abundance and diversity of the gut microbiota. Zhang et al. reported that VD supplementation could mitigate MAFLD by increasing the relative abundances of *Prevotella* and Porphyromonadaceae and decreasing the relative abundances of *Mucispirillum*, *Acetatifactor*, *Desulfovibrio* and *Oscillospira* (Zhang X-L. et al., 2023). In addition to VD, numerous studies have shown that supplementation with vitamin B12 (Li L. et al., 2022), vitamin C (Lee et al., 2022), vitamin E (Perumpail et al., 2018), or a vitamin-rich diet could alleviate the progression of MAFLD. Therefore, regulating the gut microbiota by vitamin supplementation may be an additional modality for MAFLD treatment.

2.6 Other metabolites

In addition to the above metabolites, the gut microbiota secretes metabolites such as sphingolipids, amino acids, phenolic acids, and ethanol. However, the relationship between these metabolites and the progression of MAFLD requires further investigation. In summary, alterations in the gut microbiota and its metabolites may be potential therapeutic targets for MAFLD.

3 Plant natural products ameliorate MAFLD by regulating the gut microbiota

Plant natural products, including plant natural compounds, herb extracts and TCM prescriptions, have attracted much attention in the treatment of MAFLD due to their effects on multiple pathways and multiple targets and their few adverse reactions. Plant natural products can not only affect the gut microbial composition but also regulate gut microbial metabolism. This review systematically summarizes the effects and mechanisms of action of plant natural compounds, herb extracts and TCM prescriptions in the prevention and treatment of MAFLD through the targeting of the gut microbiota reported in studies published from 2017 to 2023 (Table 1).

3.1 Plant natural compounds ameliorate MAFLD by regulating the gut microbiota

Plant natural compounds mainly include active ingredients, such as polyphenols, lignans, alkaloids, saponins and polysaccharides. Polyphenols can be divided into flavonoid and nonflavonoid phenolics. Modern pharmacological studies have isolated the numerous active monomeric compounds mentioned above, as shown in Figure 2, and have shown that these active monomers can act as therapeutic agents for MAFLD by modulating the gut microbiota and its metabolites.

3.1.1 Polyphenols

3.1.1.1 Flavones

Baicalin is the main active component of the Chinese herb *Scutellaria baicalensis* Georgi. Recent studies have shown that baicalin is a typical multitarget and multipathway drug with hepatoprotective, antitumor, antibacterial, anti-inflammatory, antidepressant and antioxidant effects. Studies suggest that the effect of baicalin in treating MAFLD may be related to the gut microbiota. Baicalin inhibits liver fibrosis and inflammation by increasing the relative abundance of lactobacilli (Liu et al., 2022). Moreover, baicalin increases the abundance of SCFA-producing bacteria, especially butyric acid-producing bacteria, which is beneficial for improving gut homeostasis (Ju et al., 2019). On the other hand, baicalin may reduce hepatic cholestasis and alleviate cholestasis-induced liver fibrosis by increasing the activity of BA-metabolizing enzymes and key receptors such as FXR and TGR5 through targeting of the gut microbiota (Hu et al., 2021).

Luteolin (3',4',5,7-tetrahydroxy flavone) is a natural flavonoid that is widely found in vegetables, fruits, and natural herbs and has anti-inflammatory, antioxidant, antitumor and immunomodulatory effects. Recent studies have shown that luteolin intervention markedly regulates the gut microbial composition in MAFLD rats by decreasing the abundances of Desulfovibrionaceae and Coriobacteriaceae to reduce plasma LPS concentrations, inhibiting the TLR4/nuclear factor kappa-B (NF-κB) signaling pathway in the liver, and reducing the secretion of proinflammatory factors (Liu X. et al., 2021). Moreover, luteolin can increase the abundances of *Lactobacillus* and *Bifidobacterium*. Therefore, luteolin may alleviate MAFLD by restoring the microbiota imbalance.

Hyperoside (quercetin-3-O-galactoside) is a flavonol glycoside found in *Crataegus pinnatifida* Bunge., *Artemisia capillaris* Thunb., and *Hyperlcurn perforatum* L. that has anti-inflammatory, hepatoprotective, and antioxidant protective effects. Recent studies have shown that hyperoside can alter the high-fat diet (HFD)-induced gut microbiota composition. Hyperoside modulates the BA composition by inhibiting gut microbes involved in BSH activity, which increases hepatic FXR receptor activation, promotes free fatty acid β-oxidation, and inhibits *de novo* fatty acid synthesis (Wang S. et al., 2021). Therefore, the regulation of BA synthesis and transport by hyperoside may be the mechanism underlying the alleviation of MAFLD.

Myricetin (3,3,4,5,5,7-hexahydroxyflavone) is a flavonoid with antioxidant, anti-inflammatory, antibacterial, anticancer, antidiabetic and hepatoprotective effects (Song et al., 2020). Myricetin supplementation not only increases the abundance of SCFA-producing bacteria to reduce liver lipid synthesis and liver inflammation in HFD-induced MAFLD but also modulates insulin resistance by increasing the fecal butyric acid concentration, which may also benefit the treatment and prevention of MAFLD (Sun et al., 2021).

Quercetin (3,3,4',5,7-pentahydroxyflavone) is an abundant polyphenolic flavonoid with a variety of bioactivities including antioxidant, anti-inflammatory, antiapoptotic, immunoprotective and anticancer properties. Quercetin can improve gut microbiota imbalance (Wang T. et al., 2023) and modulate gut microbiota metabolites to ameliorate MAFLD. On the one hand, quercetin maintains lipid homeostasis and reduces hepatic steatosis by regulating gut BA metabolism and activating FXR and TGR5 in the liver (Yang et al., 2019; Chen et al., 2021). On the other hand, quercetin can reverse gut microbiota disorders and inhibit the endotoxemia-mediated TLR-4 pathway, which in turn inhibits inflammatory vesicle responses and reticulostriatal pathway activation, leading to blocked lipid metabolism abnormalities (Porras et al., 2016).

Dihydromyricetin, the most abundant flavonoid in *Ampelopsis grossedentata* (Hand.-Mazz.) W.T. Wang (vine tea), has been proven to have anti-inflammatory, antioxidant, antihypertensive, hepatoprotective, lipid-modulating and antitumor effects. Studies have indicated that dihydromyricetin can prevent and ameliorate MAFLD by regulating the gut microbiota and its metabolites. Dihydromyricetin not only affects BSH activity by reducing the abundance of *Lactobacillus* but also enhances BA binding and BA transport in the liver and inhibits FXR-related signaling pathway-mediated BA reabsorption in the ileum. In addition, dihydromyricetin can improve gut mucosal barrier function; increase the expression of gut tight junction proteins such as zonula occludens protein 1 (ZO-1), Occludin, and Claudin1; improve gut microvillus structure; and reduce peripheral serum LPS levels (Tong, 2018).

Silybin is isolated from the seeds of *Silybum marianum* (L.) Gaertn. (milk thistle) and is widely used as a hepatoprotective agent

TABLE 1 Mechanism of action of plant natural compounds and herb extracts in the treatment of MAFLD involves modulating the gut microbiota and its metabolites.

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
Flavones	Baicalin	Male C57BL/6 J mice	Fed with HFD; at the same time, baicalin, 200 mg·kg^{-1}·d^{-1} was administered for 15 weeks	*Lactobacilli*, Butyric acid-producing bacteria ↑	BA metabolism; FXR expression; TGR5 expression	(Liu et al., 2022; Ju et al., 2019; Hu et al., 2021)
	Luteolin	Male Wistar rats	HFD fed with luteolin, 25,50, or 100 mg·kg^{-1} ·d^{-1} for 8 weeks; HFD was fed for 12 weeks, and luteolin was administered continuously from the fifth week	Desulfovibrionacea, Coriobacteriaceae; ↓ *Lactobacillus*, *Bifidobacterium* ↑	LPS production; SCFA production; Gut barrier; TLR4/NF-κB signaling pathway; LXR-SREBP-1c signaling pathway	(Liu X. et al., 2021)
	Hyperoside	Male Wistar rats	HFD fed concurrently with hyperoside, 0.6 or 1.5 mg·kg^{-1}·d^{-1} for 20 days	Gut microbiota involved in BSH activity ↓	Hepatic FXR; BA metabolism and excretion; Cholesterol metabolism	(Wang S. et al., 2021)
	Myricetin	Male Wistar rats	Fed HFD containing 0.5% myricetin for 12 weeks; antibiotic treatment for one week, subsequently FMT with myricetin for 2 weeks and fed HFD for 12 weeks	SCFA-producing bacteria *Allobaculum*; Butyric acid-producing bacteria ↑	LPS/TLR4/NF-kB pathway; Gut barrier; Lipid synthesis	(Sun et al., 2021)
	Quercetin	Male C57BLKS/J background db/db and db/m mice	db/db mice were fed with a normal diet for 8 weeks as the model group; db/db mice were administered 100 mg·kg^{-1}·d^{-1} quercetin for 8 weeks	Increased beneficial bacteria, inhibited harmful bacteria, reduced the ratio of Firmicutes to Bacteroidetes	BA and cholesterol metabolism; FXR/TGR5 signaling pathway; LPS/TLR4 signaling pathway; TMAO metabolism	(Wang T. et al., 2023; Yang et al., 2019; Chen et al., 2021; Porras et al., 2016)
	Dihydromyricetin	Male C57BL/6 mice	HFD supplemented with dihydromyricetin, 50, 150, or 450 mg·kg^{-1}·d^{-1} for 16 weeks	*Alistipes*, *Mucispirillum*, *Oscillibacter* ↓	Metabolic endotoxin; BA metabolism; Gut barrier; FXR-related signaling pathway	(Tong, 2018)
	Silybin	Male C57BL/ 6J mice	HFD fed with silybin, 100,300 mg·kg^{-1} ·d^{-1} for 8 weeks; HFD feeding was performed for 17 weeks, and silybin was administered from the tenth week	SCFA-producing *Blautia*, *Bacteroides*, *Akkermansia*; ↑ *Alloprevotella*, *Lactobacillus* ↓	Gut barrier; Mitochondrial function; Apoptosis and oxidative stress	(Li X. et al., 2020)
	Tectorigenin	Male C57BL/6 N mice	HFD fed concurrently with tectorigenin, 25, 50 mg·kg^{-1}·d^{-1} for 6 weeks	*Akkermansia*; ↑ *Turicibacter*, *Dubosiella*, *Faecalibaculum* ↓	BA metabolism; LPS production; Gut barrier;LPS/TLR-4/NF-κB/TNF-α pathway; Gut FXR	(Duan et al., 2022)
	Puerarin	Male C57BL/6 mice	Fed with MCD; at the same time, puerarin, 0.2 g·kg^{-1}·d^{-1} was administered for 4 weeks	Butyrate producing bacteria *Roseburia*; *Akkermansia muciniphila*; ↑ LPS producing bacteria *Helicobacter* ↓	Gut barrier; Liver oxidation; Pyrimidine metabolism; One-carbon metabolism; Amino acid metabolism;Glycolysis, tricarboxylic acid cycle; Synthesis and degradation of ketone bodies	(Gong et al., 2021; Wang L. et al., 2019)

(Continued)

10.3389/fcimb.2024.1323261

TABLE 1 Continued

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
	Nobiletin	Male C57BL/6 mice	Fed with HFHS; at the same time, nobiletin, 100 mg·kg-1·d-1 was administered for 12 weeks	*Allobaculum stercoricanis*, *Lactobacillus casei* ↑	Myristoleic acid metabolism; Primary bile acid biosynthesis and PPAR signaling pathways	(Li et al., 2023)
	Naringin	Male C57BL/6 mice	Fed with HFD; at the same time, 0.07% naringin was administered for 8 weeks	*Allobaculum*, *Alloprevotella*, *Butyricicoccus*, *Parasutterella*, *Lachnospiraceae NK4A136 group*; ↑ Campylobacter, Coriobacteriaceae UCG-002, Faecalibaculum, Fusobacterium ↓	Lipid synthesis; LPS production	(Mu et al., 2020)
Nonflavonoid phenols	Curcumin	Male C57BL/6 mice	HFD supplemented with curcumin, 125 mg·kg^{-1}·d^{-1} for 10 weeks	*Desulfovibrio*; ↓ *Akkermansia*, SCFA-producing bacteria *Bacteroides*, *Parabacteroides*, *Alistipes*, *Alloprevotella* ↑	BA metabolism; Nrf2/FXR/LXR-α signaling pathway; SCFA production;AMPK-mTOR signaling pathway; Gut barrier integrity	(Li S. et al., 2021; Yan et al., 2018; Li R. et al., 2020)
	Resveratrol	Male C57BL/6 J mice	HFD with resveratrol, 300 mg·kg^{-1}·d^{-1} for 16 weeks	*Desulfovibrio*, *Lachnospiraceae NK4A316 group*, *Alistipes*; ↓ SCFA-producing bacteria *Allobaculum*, *Blautia* ↑	Gut barrier; Gut metabolites 4-hydroxyphenylacetic acid and 3-hydroxyphenylpropionic acid	(Wang et al., 2020a; Wang P. et al., 2019; Wang et al., 2020b; Yin et al., 2020)
Lignin	Schisantherin A	Male C57BL/6J mice	Fed with HFD; at the same time, schisantherin A was administered for 6 weeks	Firmicutes; ↓ Bacteroidetes ↑	Gut barrier; Gut inflammation; LPS production;LPS-TLR4 signaling pathway	(Yu S. et al., 2022)
Alkaloids	Berberine	WT and FXR$^{int-/-}$ mice with a C57BL/6J background Male C57BL/6J mice and FXR$^{-/-}$ mice on a C57BL/6J background	HFD with berberine, 150 mg·kg^{-1} ·d^{-1} for 8 weeks; FXR$^{int-/-}$ mice were fed with HFD and subsequently with berberine, 150 mg·kg^{-1} ·d^{-1} for 8 weeks HFD-DSS feeding with berberine, 150 mg·kg^{-1} ·d^{-1} for 4 weeks, HFD-DSS feeding for 16 weeks, berberine was administered from the thirteenth week	BSH-producing bacteria ↓ *Clostridiales*, Lactobacillaceae, *Bacteroidales* ↑	Gut FXR pathway; CD36 expression; BA metabolism; Lipid synthesis and metabolism; BSH activity Gut FXR/FGF15/NF-κB pathway; BA metabolism	(Sun et al., 2016) (Shu et al., 2021)
	Nuciferine	Male Sprague–Dawley rats	HFD feeding with nuciferine, 10 or 25 mg·kg^{-1} ·d^{-1} for 8 weeks	Mucus-associated microbes *Akkermansia muciniphila*, Ruminococcaceae; SCFA-producing bacteria; ↑ LPS-producing microbiota Desulfovibrionacea ↓	Ileal and liver FXR signaling pathway; BA metabolism; Gut barrier; SCFA production; LPS/TLR4/MyD88/NF-κB signaling pathway	(Sun et al., 2022; Fan et al., 2022; Wang Y. et al., 2020)

(Continued)

10.3389/fcimb.2024.1323261

TABLE 1 Continued

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
	Capsaicin	Male Sprague–Dawley rats	Fed with HFD; at the same time, 0.05% and 0.1% capsaicin were administered for 4 weeks	*Bacteroidales S24-7*, *Akkermansia*, *Allobaculum*; ↑ `*Desulfovibrio*, *Lactobacillus* ↓	BA metabolism; SCFA production; Liver FXR/FGF15 signaling pathway; Inflammatory signaling pathway; Lipid synthesis and metabolism	(Gong et al., 2022)
	Betaine	Female Kunming mice	HFD and 1% betaine for 23 weeks	*Akkermansia muciniphila*, *Lactobacillus*, *Bifidobacterium* ↑	SCFA production; TLR4/MyD88 signaling pathway; TMAO metabolism; Gut barrier	(Du et al., 2021; Wang F. et al., 2019)
	Sinapine	Male C57BL/6J (B6) mice	HFD fed concurrently with sinapine (500 mg·kg^{-1}·d^{-1} rapeseed oil, ≥98% purity) in rapeseed oil for 12 weeks	Lactobacillaceae, Akkermansiaceae, *Blautia*; ↑ Desulfovibrio ↓	SCFA production; Endotoxin production; SCFA-GPR43 inflammatory pathways	(Li Y. et al., 2019)
Saponins	Astragaloside IV	Male C57BL/6 N mice	HFD with 12.5, 25, or 50 mg·kg^{-1} ·d^{-1} astragaloside IV for 12 weeks	BSH-expressing bacteria ↓	BSH activity; Liver FXR/SHP signaling pathway; Ileal FXR/FGF15 signaling pathway; BA metabolism	(Zhai et al., 2022)
	Ilexsaponin A_1	Male C57BL/6 mice	HFD with ilexsaponin A_1, 120 mg·kg^{-1}·d^{-1} for 8 weeks	BSH-expressing bacteria ↑	BSH activity; Gut FXR pathway; BA metabolism; LPS production; Inflammatory signaling pathway	(Zhao et al., 2021)
	Ganoderic acid A	Male specific pathogen-free Kunming mice	HFD with ganoderic acid A, 15 or 75 mg·kg^{-1}·d^{-1} for 8 weeks	*Eisenbergiella tayi*, *Alistipes, senegalensis*, *Oscillibacter valericigenes*, *Bacteroides acidifaciens*, *Mucispirillum schaedleri*, *Bacteroides eggerthii*; ↑ *Parabacteroides goldesteinii*, *Anaerotruncus colihominis*, *Barnesiella intestinihominis*, *Lactobacillus murinus* ↓	BA synthesis and excretion; SCFA production; Lipid oxidation and liver inflammation; Liver FXR expression	(Guo et al., 2020)
	Ursolic acid	Male hamsters	HCD with 0.2% or 0.4% ursolic acid for 6 weeks	SCFA-producing bacteria *Bifidobacterium* ↑	LPS production; SCFA production; Cholesterol metabolism	(Hao et al., 2020)
	Soybean saponin A_2	Male C57BL/6J (B6) mice	MCD with soybean saponin A_2 1, 50 or 100 mg·kg^{-1}·d^{-1} for 16 weeks	Erysipelotrichaceae, *Faecalibaculum*; ↓ *Desulfovibrionaceae (Desulfovibrio)* ↑	BSH activity; BA metabolism; Ileum FXR/FGF15 signaling; Gut inflammation;"gut-liver axis"	(Xiong et al., 2021)

(Continued)

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TABLE 1 Continued

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
Polysaccharides	α-D-1,3-glucan	Male C57BL/6J mice	Fed HFD supplemented with LPS at 200 μg/kg per day in autoclaved water (LPS was added for 4 weeks and stopped for 2 weeks) from week 4 to week 16; at the same time, 50 or 100 mg·kg-1·d-1α-D-1,3-glucan was administered for 16 weeks	*Lactobacillus*, *Phocea*, *Ruthenibacterium*, *Flavonifractor*, *Oscillabacter*, *Flintibacter*, *Butyricicoccus* ↑	SCFA production; LPS production; Gut barrier; Gut inflammation	(Li Q. et al., 2022)
	MDG-1	Male C57BL/6J mice	HFD with 2‰, 4‰, or 8‰ MDG-1 for 8 weeks, HFD was fed for 16 weeks, and MDG-1 was administered from the ninth week	SCFA-producing bacteria; *Akkermansia muciniphila*; ↑ Endotoxin-producing pathogens ↓	Endotoxin metabolism; SCFA production; Inflammatory response; Liver lipid metabolism	(Wang X. et al., 2019; Zhang L. et al., 2021)
Plant extracts	Gypenosides	Male Sprague–Dawley rats Male C57BL/6 specific pathogen-free (SPF) mice	HFD with gypenosides. 50,100, or 150 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for 8 weeks HFD with gypenosides, 100 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for 4 weeks; HFD was fed for 14 weeks, and gypenosides were administered from the eleventh week	SCFA-producing bacteria *Akkermansia*, *Bacteroides*, *Parabacteroides*; ↑ *Desulfovibrio*, *Escherichia-Shigella*, *Helicobacter* ↓	SCFA production; LPS production; Gut inflammation BA metabolism; Liver FXR/SHP signaling; TLR4/NF-κB signaling; TMAO metabolism	(Zhong F-W. et al., 2022) (Li H. et al., 2020)
	Panax notoginseng total saponins	Male C57BL/6J mice	ob/ob and HFD-fed mice were fed for 4 weeks; *Panax notoginseng* total saponins at 800 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ were administered from 5 weeks to 12 weeks	*Akkermansia muciniphila*, *Parabacteroides distasonis* ↑	TLR4 inflammatory pathway; Gut barrier; SCFA production; SCFA/GPR41 signaling pathway; Leptin-AMPK/STAT3 signaling pathway	(Xu Y. et al., 2020; Xu et al., 2021)
	Astragalus Polysaccharide	Male C57BL/6J mice	HFD mixture with 4% *Astragalus* polysaccharide for 12 weeks	SCFA-producing bacteria, *Desulfovibrio vulgaris*; *Proteobacteria*, *Epsilonbacteria* ↑	Gut barrier; TLR4/NF-κB signaling pathway; SCFA/GPR41/GPR43 signaling	(Hong et al., 2021; Zhong M. et al., 2022)
	Lycium barbarum polysaccharide	Male Sprague–Dawley rats	HFD with *Lycium barbarum* polysaccharide, 50 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for 8 weeks; HFD was fed for 18 weeks, and *Lycium barbarum* polysaccharide was administered from the eleventh week	SCFA-producing bacteria *Marvinbryantia*, *Lachnospiraceae NK4A136 group*, *Butyricicoccus* ↑ Enterococcaceae ↓	Gut barrier; SCFA production; LPS production; LPS/TLR4/NF-κB signaling pathway; AMPK inflammatory pathway; Lipid metabolism	(Gao et al., 2021; Yang M. et al., 2021; Yang Y. et al., 2021)
	Platycodon grandiflorum neutral polysaccharides	Male C57BL/6 mice	Fed a HFD with *Platycodon grandiflorum* neutral polysaccharides, 300 mg/kg/day for 14 weeks	Firmicutes/Bacteroides ratio; *Desulfobacterota* ↓	LPS inflammatory pathway; Gut barrier	(Song et al., 2023)
	Ganoderma lucidum polysaccharide	Male C57BL/6J mice	Fed an HFD with *G. lucidum* polysaccharide, 100 or 300 mg/kg/day for 12 weeks	SCFA-producing bacteria *Allobaculum*, *Bifidobacterium Christensenellaceae R-7 group* ↑	SCFA production; SCFA/GPR43 signaling pathway; LPS production; TLR4/NF-κB signaling pathway; Gut barrier	(Sang et al., 2021)

(Continued)

10.3389/fcimb.2024.1323261

TABLE 1 Continued

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
	Eucommia leaf extract	Male C57BL/6 J mice	HFD with eucommia leaf extract, 320 mg/kg/day for 12 weeks; HFD-(HFD+eucommia leaf extract) FMT after 3 days of antibiotic treatment for 8 weeks	Erysipelotrichaceae; ↓ Ruminococcaceae ↑	SCFA production; SCFA/GPR41/GPR43 signaling pathway; Gut barrier; Lipid metabolism	(Wang et al., 2023a; Wang et al., 2023b)
	Penthorum chinense Pursh. extract	Male C57BL/6J mice	HFD with 2, 4, 8 g/kg/day *Penthorum chinense* Pursh. extract for 8 weeks, HFD was fed for 16 weeks, and *Penthorum chinense Pursh.* extract was administered from the ninth week	*Clostridium IV, Clostridium XIVb, Lactobacillus* ↓	BSH activity; BA and cholesterol metabolism; Gut FXR/FGF15 pathway; Liver FXR	(Li X. et al., 2022)
	ZhiHeShouWu ethanol extract	Male Kunming mice	HFD with ZhiHeShouWu ethanol extract, 0.34, 0,68, or 1.35 g/kg for 5 weeks, HFD was fed for 8 weeks, and ZhiHeShouWu ethanol extract was administered from the fourth week	SCFA-producing bacteria *Phascolarctobacterium*; ↑ *Desulfovibrio* ↓	Lipid oxidation; BA metabolism; Gut barrier; Gut FXR/FGFR4 pathway	(Dai et al., 2021)
	Ganoderma lucidum ethanol extract	Male Wistar rats	HFD with *G. lucidum* ethanol extract, 150 mg/kg/day for 8 weeks	SCFA-producing bacteria *Alistipes*, Desulfovibrionacea, Peptococcaceae, *Alloprevotella* ↑	SCFA production; SCFA-GPRs41/43 signaling pathway; Lipid and cholesterol metabolism	(Guo et al., 2018)
	Phyllanthus emblica L. aqueous extract	Male C57BL/6J mice	CDAHFD with *Phyllanthus emblica* L. aqueous extract, 0.9, 1.8, or 3.6 g of crude drug/kg for 6 weeks, CDAHFD was fed for 12 weeks, and *Phyllanthus emblica* L. aqueous extract was administered from the seventh week	*Bifidobacterium*, *Alistipes*; ↑ Muribaculaceae ↓	SCFA production; Gut inflammation BA metabolism; Amino acid metabolism	(Luo et al., 2022)
	Ginsenoside extract	Male C57BL/6J mice	HFD with ginsenoside extract, 100 or 200 mg·kg^{-1}·d^{-1} for 12 weeks	Muribaculaceae, *Akkermansia* ↑	SCFA production; LPS production; Lipid synthesis and metabolism; NF-κB/IκB signaling pathway	(Liang et al., 2021)
	Blueberry extract	Male C57BL/6J mice	Fed with HFD; at the same time, 0.5% (m/v) blueberry extract was added to water for 15 weeks	*Akkermansia*, *Lactobacillus*, *Bifidobacterium*; ↑ *Desulfovibrio* ↓	BA metabolism; BSH activity; Gut barrier; FXR/SHP/SREBP-1c signaling pathway;Lipid synthesis;	(Guo et al., 2019)
	Jamun fruit extract	Male C57BL/6J mice	Fed with HFD; at the same time, jamun fruit extract, 100 mg·kg-1·d-1 was orally gavaged for 8 weeks	*Bacteroides*, *Prevotella*, *Alloprevotella*; ↑ *Clostridium XlVb* ↓	SCFA production; Hepatic lipid synthesis; Insulin resistance	(Xu et al., 2019)
	Camu camu extract	Male C57BL/6J mice	Fed with HFHS; at the same time, 200 mg/kg of resuspended crude extract of camu camu was administered for 8 weeks	*Akkermansia muciniphila*, *Bifidobacterium*, *Barnesiella*; ↑ *Lactobacillus*; ↓	BA metabolism; LPS production; Gut inflammation; Energy consumption; Brown adipose tissue conversion	(Anhê et al., 2018)

(Continued)

10.3389/fcimb.2024.1323261

TABLE 1 Continued

Category	Compound name	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
	Guava polysaccharides	Male C57BL/6 mice	Fed with HFD; at the same time, guava polysaccharide, 100 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ was administered for 11 weeks	*Clostridium XlVa*, *Enterorhabdus*, *Parvibacter* genera; ↑ *Mucispirillum* ↓	SCFA production; Insulin resistance; Hepatic lipid accumulation; Hepatic inflammation	(Li Y. et al., 2022)
	Noni fruit polysaccharide	Male Sprague-Dawley rats	HFD fed with noni fruit polysaccharide, 100 mg·kg-1 ·d-1 for 5 weeks; HFD was fed for 9 weeks, and noni fruit polysaccharide was administered from the fifth week	*Eubacterium coprostanoligenes*, *Lactobacillus*, *Ruminococcaceae UCG 014*, *Parasutterella*, *Ruminococcus 1*; ↑ *Prevotella 9*, *Collinsella, Bacteroides* ↓	SCFA production; LPS production; Hepatic oxidative stress and inflammation; Colonic epithelium permeability	(Yang X. et al., 2020)
	Polygala japonica Houtt. aqueous extract	Male C57BL/6J mice	Fed with MCD; at the same time, Polygala japonica Houtt. aqueous decoction equivalent to 20 g/kg, 40 g/kg, and 80 g/kg of the crude drug was administered for 7 weeks	*Dubosiella*, *Akkermansia*, *Turicibacter*; ↑ *Faecalibaculum*, *Lachnospiraceae NK4A136 group* ↓	Oxidative stress; Liver inflammation; Tryptophan metabolism; Histidine metabolism	(Liao et al., 2023)
	Quzhou Fructus Aurantii extract	Male C57BL/6J mice	Fed with HFD; at the same time, 300 mg·kg-1·d-1 Quzhou Fructus Aurantii extract was orally gavaged for 12 weeks	*Akkermansia*, *Alistipes*; ↑ *Dubosiella*, *Faecalibaculum*, *Lactobacillus* ↓	LPS production; TLR4/NF-κB signaling pathway; Gut inflammation; Gut barrier	(Bai et al., 2019)

MAFLD, metabolic-associated fatty liver disease; HFD, high-fat diet; HCD, high-cholesterol diet; MCD, methionine/choline-deficient diet; HFD-DSS, HFD supplemented with 1% dextran sulfate sodium in drinking water; CDAHFD, choline deficient, L-amino acid-defined, high-fat diet with 0.1% methionine; HFHS, high-fat high-sucrose diet; BA, bile acid; SCFA, short-chain fatty acid; LPS, lipopolysaccharide; TMAO, trimethylamine-N-oxide; BSH, bile salt hydrolase; CD36, platelet glycoprotein 4; FXR, farnesoid X receptor; TLR4, Toll-like receptor-4; TGR5, Takeda G-protein-coupled receptor 5; GPR41/GPR43, mammalian G protein-coupled receptors 41/43; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor alpha; Nrf2, nuclear factor erythroid 2-related factor 2; LXR-α, liver X receptor alpha; SREBP-1c, sterol regulatory element binding protein-1c; AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; MyD88, myeloid differentiation primary response protein 88; FGF15, recombinant fibroblast growth factor 15; STAT3, signal transducer and activator of transcription 3; SHP, small heterodimer partner; mTOR, mammalian target of rapamycin; FGFR4, fibroblast growth factor receptor 4.
"↑" indicates an increase in the relative abundance of bacteria; "↓" indicates a decrease in the relative abundance of bacteria.

10.3389/fcimb.2024.1323261

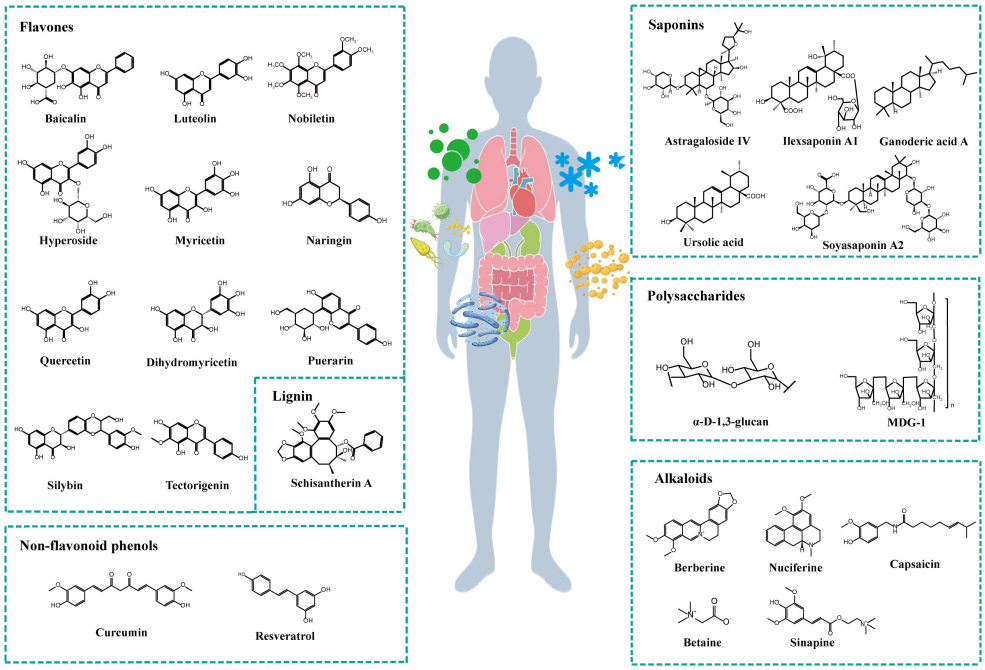

FIGURE 2
Chemical structures of plant natural compounds.

(Federico et al., 2017). Studies suggest that the effect of silybin in treating liver diseases may be achieved by modulating the gut microbiota and its metabolites (Li X. et al., 2020). Mechanistic studies revealed that silybin supplementation increased the relative abundances of the SCFA-producing *Blautia*, *Bacteroides* and *Akkermansia*, thereby increasing the gut levels of acetate, propionate and butyrate while suppressing the levels of formate, ultimately exhibiting protective effects against MAFLD and hepatitis. In addition, silybin improved gut mucosal barrier function by increasing ZO-1 and Occludin expression. However, silybin also inhibits some known beneficial bacteria, such as *Alloprevotella* and *Lactobacillus*; therefore, the relationship between silymarin-induced alterations in the gut microbiota and the alleviation of MAFLD needs to be investigated in greater depth.

Tectorigenin, a methoxy isoflavone with three hydroxyl groups, can be isolated from many medicinal plants, such as *Iris unguicularis*, *Belamcanda chinensis* (L.) DC., and *Pueraria lobata* (Willd.) Ohwi. Modern medical studies have shown that tectorigenin has anti-inflammatory, antioxidative, and antidiabetic effects on oxidative stress injury. Duan et al. (Duan et al., 2022) reported that tectorigenin could restore gut barrier function by promoting the growth of *Akkermansia* and inhibiting *Turicibacter*, *Dubosiella* and *Faecalibaculum*, which in turn reduced LPS levels and inhibited the LPS/TLR-4/NF-κB/tumor necrosis factor alpha (TNF-α) pathway to alleviate liver inflammation. Furthermore, tectorigenin can also activate the expression of hepatic and gut FXR, which is involved in the reduction of serum TBA levels and the excretion of fecal BAs, to reduce lipid accumulation and bacterial translocation. Therefore, tectorigenin may potentially mediate the gut-liver axis to attenuate MAFLD.

Puerarin (7,4-dihydroxy-isoralone-8-glucoside) is an active isoflavone glycoside extracted from the dry root of *P. lobata* that has vasodilatory, cardioprotective, antioxidant, anti-inflammatory, and anti-insulin resistance effects. It was found that puerarin treatment could inhibit the activity of the LPS-producing *Helicobacter* species, promote the activity of the butyrate-producing *Roseburia* species, and increase the abundance of *Akkermansia muciniphila* (Gong et al., 2021) to increase gut expression levels of Mucin 2 and Reg3g and protect gut barrier function by increasing ZO-1 and Occludin expression *in vivo* and *in vitro* (Wang L. et al., 2019). Thus, the anti-MAFLD activity of puerarin may be closely associated with the regulation of the gut microbiota.

Nobiletin is a polymethoxyflavonoid that is the main component of Citri Reticulatae Pericarpium and has been shown to have anti-obesity (Lee et al., 2012), antihyperglycemic (Lee et al., 2010), antihypercholesterolemic and anti-MAFLD activities (Kim et al., 2021). Recently, Li et al. (Li et al., 2023) reported that nobiletin treatment not only ameliorated high-fat high-sucrose feed-induced lipid accumulation but also reversed the dysbiosis of the gut microbiota in mice with MAFLD and increased the relative abundance of *Allobaculum stercoricanis* and *Lactobacillus casei*. Moreover, untargeted metabolomics revealed that nobiletin modulated the metabolism of the long-chain fatty acid myristoleic acid and experimentally demonstrated the protective effects of fecal transplantation as well as the administration of bacteria or metabolites to treat MAFLD. These results suggest that nobiletin may be a potential treatment for MAFLD, but further studies are needed.

Naringin, a naturally occurring flavonoid found predominantly in the rinds of grapefruit and citrus fruits, has been reported to have antihyperglycemic (Ahmed et al., 2017) and antihyperlipidemic properties (Yu X. et al., 2022). Mu et al. (Mu et al., 2020) reported that naringin treatment increased the relative abundance of *Allobaculum*, *Alloprevotella*, *Butyricicoccus* and *Parasutterella* and downregulated the expression of the sterol regulatory element binding protein 1, fatty acid synthase, acetyl-CoA carboxylase 1, and stearoyl-CoA desaturase 1 proteins, effectively attenuating hepatic *de novo* fatty acid synthesis. On the other hand, naringin treatment reduced the relative abundance of the deleterious *Campylobacte*r species and the proinflammatory *Faecalibaculum* and *Fusobacterium* species, which may be associated with the reduction in LPS levels in the serum and hepatic inflammatory cells of mice with MAFLD. Thus, naringin may attenuate NAFLD by preventing gut ecological dysregulation, but experimental evidence confirming that naringin attenuates MAFLD by directly modulating the gut microbiota is lacking.

3.1.1.2 Nonflavonoid phenols

Curcumin is a natural polyphenol compound isolated from *Curcuma longa* L. that has been proven to have antiobesity, anticancer, antioxidant, hepatoprotective and other biological activities. Studies suggest that curcumin may ameliorate MAFLD by regulating the gut microbiota through the enrichment of the SCFA-producing bacteria *Bacteroides*, *Parabacteroides*, *Alistipes* and *Alloprevotella* and a reduction in the abundance of the endotoxin-producing *Desulfovibrio* (Li S. et al., 2021). In addition, curcumin can inhibit hepatic lipogenesis and promote BA metabolism by regulating the nuclear factor erythroid 2-related factor 2 (Nrf2)/FXR/liver X receptor alpha (LXR-α) pathway (Yan et al., 2018). Furthermore, the results of a comparative study of curcumin and metformin showed that they had similar effects in reducing hepatic steatosis, improving gut barrier integrity and regulating the gut microbiota in rats with HFD-induced obesity and that curcumin may prove to be a novel adjuvant therapy for MAFLD (Li R. et al., 2020).

Resveratrol is a natural polyphenolic compound that is mainly isolated from *Vitis vinifera* L. (grape) and has anticancer, anti-inflammatory, antioxidant, and antiobesity effects. Resveratrol can attenuate HFD-induced steatosis through modulation of the gut microbiota composition (Wang et al., 2020a; Wang P. et al., 2019; Wang et al., 2020b; Yin et al., 2020). Resveratrol not only reduced the relative abundances of the harmful bacteria *Desulfovibrio*, *Lachnospiraceae NK4A316 group* and *Alistipes* but also increased the relative abundances of the SCFA-producing bacteria *Allobaculum* and *Blautia* (Wang et al., 2020b) and the gut metabolites 4-hydroxyphenylacetic acid and 3-hydroxyphenylpropionic acid, contributing to the improvement in lipid metabolism (Wang et al., 2020a). Furthermore, transplantation of the resveratrol-induced microbiota into HFD-fed mice also showed therapeutic effects (Wang et al., 2020a; Wang P. et al., 2019; Wang et al., 2020b; Yin et al., 2020). These results demonstrated that resveratrol has the potential to regulate the gut-liver axis to ameliorate MAFLD.

3.1.2 Lignin

Schisantherin A is an active substance isolated from the fruit of *Schisandra chinensis* (Turcz.) Baill., a perennial deciduous woody liana with antiparkinsonian, anti-inflammatory, hepatoprotective, ischemia–reperfusion injury prevention and osteoclast formation inhibition effects (Xiao et al., 2022). The hepatoprotective effects of schisantherin A may be achieved by improving gut inflammation and modulating the gut microbiota (Yu S. et al., 2022). On the one hand, schisantherin A treatment alleviated the HFD-induced imbalance in the gut microbiota by reducing the abundance of Firmicutes and increasing the abundance of Bacteroidetes. Furthermore, antibiotic treatment demonstrated the role of the gut microbiome in the schisantherin A-mediated improvement in liver inflammation. On the other hand, schisantherin A treatment reduced gut LPS production and release in HFD-fed mice and inhibited the LPS-TLR4 signaling pathway to ameliorate gut permeability impairment and inhibit the progression of MAFLD to NASH.

3.1.3 Alkaloids

Berberine, also known as safranin, is isolated from the root of *Coptis chinensis* Franch. and is traditionally used to treat diarrhea. Modern pharmacological studies have reported that berberine is beneficial for treating metabolic disorders, such as type 2 diabetes, dyslipidemia, and MAFLD/NASH. However, because the bioavailability of berberine is low, the gut microbiome and microbe-derived metabolites are thought to be key factors involved in the mechanisms of action of berberine (Cheng et al., 2021). Berberine may exert its lipid-lowering effects mainly through the regulation of BA turnover and thus through the gut FXR signaling pathway (Sun et al., 2016; Shu et al., 2021). Sun et al. reported that berberine increased taurine-coupled BA levels by reducing the relative abundance of BSH-producing bacteria, which activated the gut FXR pathway (Sun et al., 2016). Shu et al. reported that berberine alleviated NASH by modulating the gut microbiota and BA metabolism and upregulating gut FXR and recombinant fibroblast growth factor 15 (FGF15) expression and FGF15 secretion to further inhibit adipogenesis and NF-κB activation in the liver (Shu et al., 2021).

Nuciferine, the major functional aporphine alkaloid from the dried leaves of *Nelumbo nucifera* Gaertn., has been shown to be useful for reducing body weight, lowering serum and liver lipids, and alleviating hepatic steatosis and liver damage. Nuciferine supplementation not only reduced the abundances of BSH-producing and 7α-dehydroxylated bacteria, leading to the accumulation of coupled BAs as FXR antagonists to inhibit gut FXR signaling but also regulated the BA cycle *in vivo* by modulating the levels of the rate-limiting enzymes CYP7A1 and cytochrome P450 27A1 (CYP27A1) and the BA transporters bile salt export pump (BSEP) and Na^+-taurocholate cotransporting polypeptide (NTCP) in the liver (Sun et al., 2022). In addition, nuciferine elevated the relative abundances of mucus-associated microbes (*Akkermansia muciniphila* and Ruminococcaceae) and SCFA-producing bacteria to improve gut barrier integrity and reduce liver inflammation by decreasing the abundance of LPS-producing

microbes (Desulfovibrionaceae), reducing LPS production, and inhibiting the TLR4/myeloid differentiation primary response protein 88/NF-κB signaling pathway (Fan et al., 2022; Wang Y. et al., 2020).

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is one of the active ingredients of *Capsicum annuum* L. (pepper) and has anti-statogenic, antioxidant, anti-inflammatory and antifibrotic effects. Studies have indicated that capsaicin treatment increases the relative abundances of *Bacteroidales S24-7*, *Akkermansia* and *Allobaculum*, leading to the accumulation of SCFAs, which in turn enhances lipid accumulation and decreases TG and TC levels. Furthermore, capsaicin decreased BSH activity by inhibiting *Lactobacillus*, which increased the levels of conjugated BAs, especially tauro-β-muricholic acid (T-β-MCA), which in turn inhibited the enterohepatic FXR-FGF15 axis to regulate the composition of the BA pool (Gong et al., 2022). These results suggested that capsaicin can mitigate MAFLD by regulating the gut microbiota and the composition of BAs and SCFAs.

Betaine is a quaternary ammonium-type water-soluble alkaloid with antioxidant and anti-inflammatory effects. Betaine is an amino acid (trimethylglycine) that is a necessary intermediate in the catabolism of choline. Betaine supplementation can increase the abundances of *Akkermansia muciniphila*, *Lactobacillus* and *Bifidobacterium* and promote the production of SCFAs (Du et al., 2021). In mice lacking a gut microbiota, the ability of betaine to prevent HFD-induced obesity and metabolic syndrome was significantly reduced, which suggested that betaine can alleviate MAFLD by regulating the gut microbiota. In addition, Wang et al. (Wang F. et al., 2019) reported that betaine can reduce liver lipid accumulation by improving gut BA and TMA-related oxidative metabolism in blunt mouth bream.

Sinapine accounts for 70-80% of rapeseed polyphenols and may have the potential to ameliorate MAFLD. It was shown that sinapine intervention could regulate the HFD-induced gut microbiota, increase the relative abundance of the probiotic bacteria Lactobacillaceae, and inhibit endotoxin production by reducing the relative abundance of *Desulfovibrio*. Moreover, supplementation with sinapine could increase the abundance of the SCFA-producing bacteria Akkermansiaceae and *Blautia*, which increase SCFA levels and upregulate the GPR43 receptor to inhibit gut inflammatory factor expression (Li Y. et al., 2019). Therefore, sinapine has a therapeutic effect on MAFLD by regulating the gut microbiota.

3.1.4 Saponins

Astragaloside IV is one of the major saponin compounds extracted from the roots of *Astragalus membranaceus* (Fisch.) Bge. and has anti-inflammatory, anti-liver fibrosis, antioxidative stress, anti-asthma, antidiabetic, immunomodulatory and cardioprotective effects (Li et al., 2016). Astragaloside IV inhibits gut FXR expression by reducing the abundance of BSH-expressing bacteria and decreasing BSH activity, thus increasing the level of T-β-MCA, which is often accompanied by a decrease in FGF15 and subsequent activation of hepatic FXR, leading to the inhibition of hepatic steatosis (Zhai et al., 2022). Moreover, fecal transplantation experiments further demonstrated that the action of astragaloside IV is dependent on the gut microbiota. Therefore, alterations in the gut microbiota and BAs may be involved in the mechanism of action of astragaloside IV for treating MAFLD.

Ilexsaponin A_1 is one of the most abundant triterpenoid saponins and has antithrombotic and anticoagulant properties; it was isolated from *Ilex chinensis* Sims, a small evergreen tree with red fruit. Previous studies have shown that the combination of ilexhainanoside D and ilexsaponin A_1 modulates the gut microbiota, restores gut barrier function, and ameliorates gut inflammation (Zhao et al., 2019). A separate study of ilexsaponin A_1 revealed that ilexsaponin A_1 may exert cholesterol-lowering and MAFLD-inhibiting effects by altering the gut microbiota and BA metabolism (Zhao et al., 2021). Ilexsaponin A_1 intervention enhanced BSH activity by increasing the relative abundance of BSH-producing bacteria, which increased BA uncoupling and excretion in the ileum. Moreover, ilexsaponin A_1 intervention also increased FXR and BSEP expression and decreased NTCP expression, which increased hepatic BA efflux and reduced BA uptake. In addition, intake of ilexsaponin A_1 also decreased the serum LPS content and the expression of inflammatory cytokines.

Ganoderic acid A is one of the most abundant triterpenes isolated from the red fungus *Ganoderma lucidum* (Leyss. ex Fr.) Karst. and has antinociceptive, antioxidative and anticancer pharmacological effects. Ganoderic acid A intervention increased the relative abundances of *Eisenbergiella tayi*, *Alistipes senegalensis*, *Oscillibacter valericigenes*, *Bacteroides acidifaciens*, *Mucispirillum schaedleri* and *Bacteroides eggerthii* but significantly reduced the relative abundances of *Parabacteroides goldesteinii*, *Anaerotruncus colihominis*, *Barnesiella intestinihominis*, and *Lactobacillus murinus*, which increased the production of SCFAs in the gut. In addition, ganoderic acid A treatment can interfere with the regulation of BAs. Ganoderic acid A not only upregulated the expression of liver genes (FXR, CYP7A1 and NTCP) involved in BA homeostasis and reduced liver BA levels but also promoted the excretion of BAs through the feces (Guo et al., 2020). Therefore, the ability of ganoderic acid A to improve MAFLD may be mediated by regulation of the gut microbiota and metabolites.

Ursolic acid is a natural pentacyclic triterpene carboxylic acid that occurs naturally in various fruits and vegetables, such as apples, blueberries and cranberries, and has antioxidant, anti-inflammatory, anticancer and hepatoprotective effects. Recent studies have indicated that the addition of ursolic acid is effective at promoting the growth of *Bifidobacterium*, which is recognized as a genus of SCFA-producing bacteria. *Bifidobacterium* species, as recognized probiotics, have been shown to be effective at limiting the production of the endotoxins LPSs. In addition, ursolic acid can promote the production of SCFAs, including acetic acid, propionic acid and butyric acid, in feces (Hao et al., 2020). Therefore, ursolic acid holds promise as a potential therapeutic agent for ameliorating MAFLD, but further studies are needed.

Soyasaponins are phytochemicals found in *Glycine max* (L.) Merr. (soya bean) and that have antioxidant, anti-inflammatory, hypoglycemic, cholesterol-lowering, and hepatoprotective effects. Soyasaponin A_2 is a monomer of soyasaponins, and Xiong et al. (Xiong et al., 2021) reported that the hepatoprotective effect of soyasaponin A_2 against NASH may be achieved by modulating the

gut microbiota metabolite BAs. On the one hand, soyasaponins A_2 may alleviate methionine–choline-deficient (MCD) diet-induced steatohepatitis by reducing the abundance of Erysipelotrichaceae and *Faecalibaculum* species. On the other hand, it may not only effectively block gut-liver circulation and the reabsorption of BAs in the terminal ileum by forming mixed micelles with BAs but also directly bind to BAs and promote their excretion via feces.

3.1.5 Polysaccharides

Radix Puerariae thomsonii, the root of the botanical family *Fabaceae* species *Pueraria montana* var. *thomsonii* (Benth.) MR Almeida, can be used as food or medicine. Recently, an α-D-1,3-glucan isolated and purified from *Radix Puerariae thomsonii* was shown to not only reduce HFD-induced liver injury, inflammation, glucose metabolism, and steatosis but also regulate the gut microbiota and its metabolites (Li Q. et al., 2022). α-D-1,3-glucan treatment not only increased the relative abundance of *Lactobacillus*, *Phocea*, *Ruthenibacterium*, *Flavonifractor*, *Oscillabacter*, *Flavinibacter*, and *Butyricoccus* to increase butyric acid, propionic acid and acetate levels but also promoted the integrity of the gut barrier by increasing the expression of tight junction proteins (ZO-1, Occludin, and Claudin-4) and mucin (Mucin 2). In addition, α-D-1,3-glucan administration significantly reduced the serum levels of the proinflammatory factor TNF-α and LPSs and inhibited HFD-induced inflammation. In conclusion, α-D-1,3-glucan is expected to be a potential drug for alleviating MAFLD.

MDG-1, a β-D-fructose polysaccharide isolated and purified from the root of *Ophiopogon japonicus* (L.f.) Ker-GawL., prevents HFD-induced obesity and hyperlipidemia in mice. MDG-1 modulates the structure of the gut microbiota by promoting the growth of beneficial SCFA-producing bacteria and reducing the abundance of endotoxin-producing pathogens to inhibit the inflammatory response and liver lipid metabolism (Wang X. et al., 2019). In addition, MDG-1 intervention markedly promoted the growth of *Akkermansia muciniphila*, the abundance of which was inversely proportional to MAFLD (Zhang L. et al., 2021). Therefore, MDG-1 may be a potential agent for preventing and treating MAFLD by targeting the gut microbiota.

3.2 Herb extracts ameliorate MAFLD by regulating the gut microbiota

Plant natural products also include extracts with diverse functions, including total polyphenols, total saponins, and total polysaccharides. Plant natural products may act through a single compound in the total extract, but this phenomenon has not been studied at the monomer level because of certain limitations, such as those of isolation techniques. On the other hand, the complex compounds of the extracts may interact with each other and work together to exert therapeutic effects. Studies have shown that some herbal extracts can alter the gut microbiota and its metabolites to exert beneficial effects against MAFLD, as shown in Figure 3.

Gypenosides are the main components of *Gynostemma pentaphyllum* (Thunb.) Makino, a herb known as “southern ginseng”. These compounds are widely used as hepatoprotective agents in Asia, and their mechanism of action may be related to improvement of the gut microbiota. Gypenosides not only promoted the growth of SCFA-producing bacteria (*Akkermansia*, *Bacteroides* and *Parabacteroides*) and increased the content of SCFAs (acetate, propionate and butyrate) but also decreased the relative abundances of harmful bacteria (*Desulfovibrio*, *Escherichia-Shigella* and *Helicobacter*) to inhibit LPS production, thereby suppressing inflammation (Zhong F-W. et al., 2022). In addition, gypenosides also inhibited hepatic lipid synthesis by modulating the gut BA composition and increasing hepatic FXR and SHP levels (Li H. et al., 2020).

Panax notoginseng saponins are the main bioactive components of *Panax notoginseng* (Burk.) F.H. Chen, also known as “the king of ginseng”, and have been found to reduce serum lipid and liver lipid accumulation. *Panax notoginseng* saponins promoted the growth of *Akkermansia muciniphila* and *Parabacteroides distasonis*, which activated the leptin-AMPK/signal transducer and activator of transcription 3 signaling pathway to promote energy expenditure and reduce lipid accumulation (Xu Y. et al., 2020). In addition, *Panax notoginseng* saponin supplementation can increase the levels of long-chain fatty acids (oleic acid and 2-palmitoyl glycerol) and SCFAs (acetic acid, butyric acid, propionic acid, etc.) in feces, and then large amounts of SCFAs enter the liver to activate GPR41, further ameliorating steatosis and inflammation (Xu et al., 2021). Therefore, *Panax notoginseng* saponins may alleviate MAFLD by regulating the structure and function of the gut microbiota.

Astragalus polysaccharide is an active ingredient isolated from *A. membranaceus* that has antioxidant, anti-inflammatory, hypoglycemic, antiviral, hypolipidemic and immunomodulatory effects. Mechanistic studies suggest that *Astragalus* polysaccharide may ameliorate MAFLD by altering the gut microbiota composition. Hong et al. (Hong et al., 2021) reported that *Astragalus* polysaccharide can specifically enrich SCFA-producing bacteria, such as *Desulfovibrio vulgaris*, which can effectively produce acetic acid and inhibit the expression of liver fatty acid synthase and platelet glycoprotein 4 protein to exert anti-MAFLD effects. Zhong et al. (Zhong M. et al., 2022) reported that Mongolian *Astragalus* polysaccharide intervention could decrease the ratio of Firmicutes to Bacteroides and increase the abundances of *Proteobacteria* and *Epsilonproteobacteria*. In addition, Mongolian *Astragalus* polysaccharide supplementation may alleviate liver inflammation and lipid accumulation in MAFLD treatment through the gut microbiota by modulating TLR4-mediated inflammatory pathways and the SCFA-GPR signaling pathway.

Lycium barbarum polysaccharide is the main bioactive component of *Lycium barbarum* L. (goji berry) and has many pharmacological properties, such as serum glucose-lowering, serum lipid-lowering, anti-inflammatory, antioxidant and antitumor effects. Numerous studies have demonstrated that *Lycium barbarum* polysaccharide can alleviate MAFLD by altering the gut microbiota composition and SCFA metabolism (Gao et al., 2021; Yang M. et al., 2021; Yang Y. et al., 2021). *Lycium barbarum* polysaccharide intervention increased the relative abundances of the SCFA-producing bacteria *Marvinbryantia*, *Lachnospiraceae NK4A136 group* and *Butyricicoccus*, which increased the content of fecal SCFAs (Yang M. et al., 2021; Yang Y. et al., 2021). Moreover,

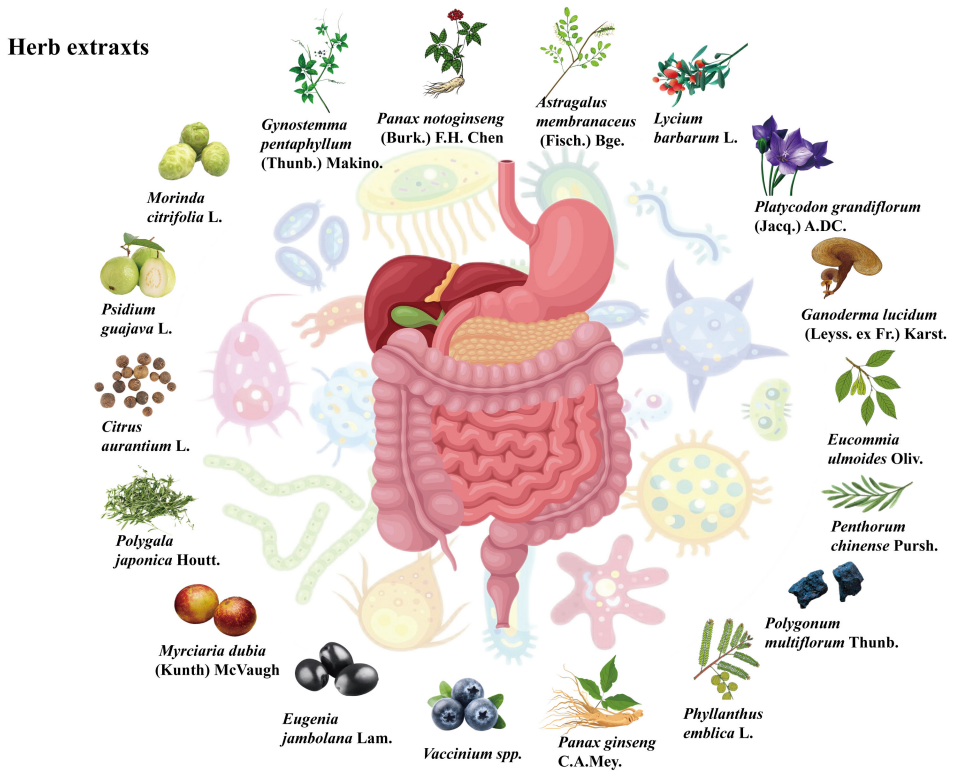

FIGURE 3
Sources of the herb extracts.

it also inhibited the LPS/TLR4/NF-κB signaling pathway by inhibiting the increase in the harmful bacteria Enterococcaceae and their metabolite LPSs (Gao et al., 2021), thereby alleviating MAFLD. Therefore, *Lycium barbarum* polysaccharide is expected to be a promising treatment for MAFLD.

Platycodon grandiflorum neutral polysaccharides are active components isolated and purified from *Platycodon grandiflorum* (Jacq.) A.DC. (balloon flower) that have antioxidative stress and immunomodulatory activities. Song et al. (Song et al., 2023) studied mice with HFD-induced obesity and reported that *Platycodon grandiflorum* neutral polysaccharide treatment could reduce the Firmicutes/Bacteroides ratio and inhibit the release of LPSs into the gut by reducing the abundance of *Desulfobacterota*, preventing an inflammatory response and disrupting gut energy metabolism. In addition, *Platycodon grandiflorum* neutral polysaccharides can increase gut tight junction protein expression and ameliorate gut leakage. Although the lipid-lowering effect of *Platycodon grandiflorum* neutral polysaccharides has been confirmed, whether they can prevent MAFLD through the gut microbiota requires fecal microbiota transplantation (FMT) experiments.

G. lucidum polysaccharides are the main active components isolated from *G. lucidum* and have immunomodulatory, antioxidant, anti-inflammatory, antitumor, antiobesity and antidiabetic effects. Several studies have reported that *G. lucidum* polysaccharides can prevent obesity and MAFLD by regulating the gut microbiota (Sang et al., 2021). *G. lucidum* polysaccharides increased the relative abundances of SCFA-producing bacteria and potential probiotics, such as *Allobaculum*, *Bifidobacterium* and *Christensenellaceae R-7 group*, which activated the GPR43 receptor in adipose tissue to regulate metabolism and inhibit obesity. In addition, *G. lucidum* polysaccharides inhibited the HFD-induced inflammatory response by reducing the serum LPS concentration and inhibiting the TLR4/NF-κB signaling pathway. Therefore, *G. lucidum* polysaccharide may be a potential agent for targeting the gut microbiota to prevent obesity, hyperlipidemia and MAFLD.

Eucommia, isolated from the dry roots of *Eucommia ulmoides* Oliv., is a Chinese medicine and food homolog that has antiosteoporotic, anti-inflammatory, hypoglycemic and hypolipidemic effects. Recent studies have indicated that Eucommia leaves have similar active ingredients and antihyperlipidemic effects as Eucommia bark and can modulate the gut microbiota to alleviate lipid metabolism disorders (Wang et al., 2023a; Wang et al., 2023b). Eucommia leaf extract supplementation increased the relative abundance of Ruminococcaceae to promote the production of butyric acid, which upregulated the expression of GPR43 to reduce the area of adipocytes and lipid accumulation. Furthermore, it reduced the relative abundance of the harmful Erysipelotrichaceae species. Therefore, Eucommia leaf extract has the potential to alleviate MAFLD by targeting the gut microbiota.

Penthorum chinense Pursh. has been widely used as a traditional Chinese functional food to prevent and treat liver diseases. Numerous studies have demonstrated the therapeutic effects of *P.*

chinense extract on MAFLD through alterations in the gut microbiota and its metabolite BAs (Li X. et al., 2022). *P. chinense* extract reduced the relative abundances of *Clostridium IV*, *Clostridium XIVb*, and *Lactobacillus* to inhibit BSH activity, inhibited BA uncoupling and dehydroxylation, and increased taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid (TUDCA) levels. TCDCA and TUDCA, which are naturally occurring FXR antagonists, can lower cholesterol levels by inhibiting gut FXR activity, downregulating FGF15 expression, activating hepatic BA synthase activity, and promoting cholesterol conversion. In addition, *P. chinense* extract increased chenodeoxycholic acid production, which activated hepatic FXR expression and increased hepatic FXR-induced BA excretion levels, thus further promoting the conversion of cholesterol to BAs.

ZhiHeShouWu is obtained by processing *Polygonum multiflorum* Thunb. in black bean juice and is often used clinically to regulate lipid metabolism. Studies have shown that supplementation with ZhiHeShouWu ethanol extract could increase the relative abundances of the SCFA-producing *Phascolarctobacterium* species and reduce the relative abundance of *Desulfovibrio* (Dai et al., 2021). In addition, the ZhiHeShouWu ethanol extract affected BA metabolism by remodeling the gut microbiota, thus inhibiting the expression of gut FXR genes, accelerating cholesterol metabolism *in vivo*, and maintaining cholesterol homeostasis. Therefore, ZhiHeShouWu ethanol extract may improve MAFLD by regulating the gut microbiota composition and maintaining gut barrier function.

G. lucidum, used in TCM for thousands of years, has a variety of activities, such as hepatoprotective, antitumor and antiaging activities. As described above for *G. lucidum*, its hepatoprotective effect is associated with the regulation of the gut microbiota. The *G. lucidum* ethanol extract was also able to improve HFD-induced gut microbiota disorders due to its abundance of triterpenoids (Guo et al., 2018). The *G. lucidum* ethanol extract significantly elevated the relative abundances of *Alistipes*, Desulfovibrionaceae, Peptococcaceae, and *Alloprevotella* to increase gut propionic acid and butyric acid levels, which have been associated with positive effects on gut health, such as anti-inflammatory effects and improvement in glucose homeostasis and other metabolic symptoms. Therefore, the mechanism of action of the *G. lucidum* ethanol extract in improving MAFLD may involve regulation of the gut microbiota and SCFAs.

Phyllanthus emblica L. is an edible medicinal fruit used to treat hepatobiliary disorders, viral hepatitis, alcoholic hepatitis, MAFLD, and hepatocellular carcinoma. It has been shown that *P. emblica* aqueous extract can improve MAFLD by remodeling the gut microbiota (Luo et al., 2022). On the one hand, *P. emblica* aqueous extract elevated the relative abundances of *Bifidobacterium* and *Alistipes* and reduced the relative abundance of Muribaculaceae. Remodeling of the gut microbiota helped to increase SCFA levels, suppress gut inflammation and maintain gut homeostasis. On the other hand, *P. emblica* aqueous extracts affected choline-deficient, L-amino acid-defined, high-fat diet-induced metabolic disorders by modulating primary BA biosynthesis and taurine and hypotaurine metabolism.

Ginsenosides are the main bioactive components isolated from *Panax ginseng* C.A.Mey. (ginseng) and have many biological effects, such as antiobesity, antihyperglycemia and anti-MAFLD effects. Studies have shown that various isoforms of ginsenosides, such as Rg1 (Hou et al., 2020), Rg2, Rh1 (Wang F. et al., 2021), CK (Zhang J. et al., 2021), Rb2 (Huang et al., 2017), Rc (Yang Z. et al., 2020), and Rf (Chen et al., 2022), have the potential to alleviate MAFLD. Liang et al. (Liang et al., 2021) reported that ginsenoside extract treatment could modulate HFD-induced gut microbiota imbalance and attenuate ecological dysbiosis-mediated gut leakage and metabolic endotoxemia, which may be a potential mechanism of action for improving MAFLD. Ginsenoside extract supplementation elevated the relative abundances of Muribaculaceae and *Akkermansia*, which promoted the production of SCFAs in the gut, thereby providing energy to colonic epithelial cells and improving lipid accumulation and liver inflammation. In addition, ginsenoside extract treatment also reduced the serum LPS concentration and attenuated metabolic endotoxemia in HFD-fed mice.

Blueberries (*Vaccinium* spp.) are rich in polyphenols and are known for their antioxidant and cardiovascular protective properties (Emily et al., 2023). Studies have shown that blueberry extract may ameliorate HFD-induced obesity by modulating the composition of the gut microbiota (Guo et al., 2019). On the one hand, blueberry extract significantly increased the relative abundance of *Akkermansia*, *Lactobacillus*, and *Bifidobacterium*, which increased BSH activity, resulting in lower plasma and liver TG concentrations in mice. In addition, blueberry extract administration significantly reduced plasma taurine-coupled BAs, including tauro-α-muricholic acid (T-α-MCA) and T-β-MCA levels, to activate FXR in the liver, which enhanced SHP expression and further inhibited the activity of SREBP-1c and its downstream genes associated with lipid synthesis. On the other hand, blueberry extract reduced the abundance of *Desulfovibrio* and increased colonic Mucin 2 and ZO-1 levels, thereby attenuating gut permeability. In conclusion, blueberry extract may ameliorate MAFLD by modulating the gut microbiota and bile acid pool.

Eugenia jambolana Lam., commonly known as jamun, is an edible berry that has been shown to have antioxidant, anti-inflammatory and hepatoprotective activities (Chhikara et al., 2018). Xu et al. (Xu et al., 2019) found that jamun fruit extract not only ameliorated HFD diet-induced obesity, hepatic steatosis and insulin resistance but also regulated the gut microbiota and SCFA metabolism. Jamun fruit extract supplementation increased the relative abundance of *Bacteroides*, *Prevotella* and *Alloprevotella*, not only promoting the content of total SCFAs and acetic, propionic and butyric acids but also mitigating the imbalance in the major components of SCFAs, especially the propionic/n-butyric acid ratio. In addition, jamun fruit extract also reduced the level of *Clostridium XlVb*, which has been reported to be associated with cognitive impairment in patients with Parkinson's disease (Qian et al., 2018). In conclusion, jamun fruit extract may ameliorate MAFLD by modulating the gut microbiota and SCFA production.

Myrciaria dubia (Kunth) McVaugh, commonly known as camu camu, is an Amazonian fruit rich in vitamin C and polyphenols and is considered a "superfruit" with various properties including antioxidant, antihyperglycemic and anti-obesity properties (García-Chacón et al., 2023). A recent study found that camu camu extract was able to significantly increase the number of

Akkermansia muciniphila, *Bifidobacterium* and *Barnesiella* and decrease the abundance of *Lactobacillus*. Meanwhile, mice receiving camu camu extract feces showed the same trend, suggesting that *Akkermansia muciniphila* and *Lactobacillus* may be important drivers of increased energy expenditure and decreased weight gain. In addition, camu camu extract not only increased the levels of chenodeoxycholic acid, deoxycholic acid and ursodeoxycholic acid to activate TGR5, which enhances the response of brown adipocytes to thyroid hormones and activates nonshivering thermogenesis in this tissue, but also reduced IL-1β, IL-6 and LPS levels to prevent gut inflammation (Anhê et al., 2018). In conclusion, camu camu extract has the potential to prevent and treat MAFLD.

Psidium guajava L., also known as guava, is an edible fruit that is widely used in folk medicine as an adjunct in the treatment of diabetes (Jiao et al., 2017). It was found that polysaccharides extracted from guava have hypolipidemic effects, and the mechanism of action is highly correlated with its role in regulating the gut microbiota and metabolites (Li Y. et al., 2022). Supplementation with guava polysaccharides increased the relative abundance of *Enterorhabdus* and *Clostridium XlVa*, thereby contributing to the restoration of total SCFA levels and increased levels of acetic acid, propionic acid and butyric acid. The increase in SCFA levels was able to activate hepatic AMPKα and inhibit the expression of peroxisome proliferator-activated receptor γ, thereby suppressing HFD-induced hepatic steatosis and insulin resistance. In addition, guava polysaccharides reduced the relative abundance of *Mucispirillum* to alleviate TNF-α release and NF-κB signaling activation. In conclusion, guava polysaccharides are potential prebiotics with beneficial effects on obesity and MAFLD.

Morinda citrifolia L. (noni) is an evergreen tree or shrub of the Rubiaceae family, found primarily in tropical and subtropical regions. In recent years, it has been found that noni fruit polysaccharide not only has physiological activities, such as antitumor, antioxidant and anti-inflammatory activities, but also can play an antilipidemic role by regulating the gut microbiota (Yang X. et al., 2020; Mo et al., 2023). On the one hand, noni fruit polysaccharide increased the relative abundance of *Eubacterium coprostanoligenes*, *Lactobacillus*, *Ruminococcaceae UCG 014*, *Parasutterella*, and *Ruminococcus 1* and decreased the relative abundance of *Prevotella 9*, *Collinsella*, and *Bacteroides*. The altered composition of the gut microbiota increased SCFA production and maintained colonic barrier permeability, thereby reducing serum LPS levels and hepatic lipid accumulation and ultimately ameliorating HFD-induced hepatic oxidative stress and inflammation (Yang X. et al., 2020). On the other hand, noni fruit polysaccharide also increased fecal excretion of fecal-coupled BAs, especially T-α-MCA and T-β-MCA, by modulating the structure of the gut microbiota and activating hepatic and colonic FXR receptors, thus increasing lipid oxidation and energy digestion (Mo et al., 2023). In summary, noni fruit polysaccharide modulates the gut microbiota and its metabolites, which may be its mechanism of action in alleviating MAFLD.

Polygala japonica Houtt. is a commonly used herbal medicine that has been shown to possess anti-obesity (Jee et al., 2021) and anti-inflammatory properties (Wang et al., 2007). In addition, Liao et al. (Liao et al., 2023) found that *Polygala japonica Houtt.* may alleviate NASH by regulating gut microbiota composition and amino acid metabolism. On the one hand, *Polygala japonica Houtt.* administration decreased the relative abundance of *Faecalibaculum* and *Lachnospiraceae NK4A136 group* and increased the relative abundance of *Dubosiella*, *Turicibacter* and *Akkermansia*, with the increase in *Akkermansia* modulating the decrease in fecal L-tryptophan, thereby ameliorating the disorders of tryptophan metabolism. In contrast, *Polygala japonica Houtt.* significantly reduced histamine levels as well as fecal and hepatic glutamate accumulation in NASH mice, suggesting that *Polygala japonica Houtt.* not only reduces inflammatory responses by modulating histamine levels but also ameliorates glutamate metabolism disorders. In conclusion, *Polygala japonica Houtt.* treatment significantly altered the composition of the gut microbiota as well as histidine and tryptophan metabolism, which may play a key role in the treatment of NASH.

Quzhou Fructus Aurantii is the dried unripe fruit of *Citrus aurantium* L that is used as a folk medicine and food for treating liver disease; it has hepatoprotective (Lan et al., 2023) and anti-inflammatory effects (Li L. et al., 2020). Recently, Bai et al. (Bai et al., 2019) found that the mechanism of action of Quzhou Fructus Aurantii extract in the treatment of liver disease may be related to its regulation of the gut microbiota. Quzhou Fructus Aurantii extract administration increased the relative abundance of *Akkermansia* and *Alistipes*, which may contribute to the reduction in gut permeability and LPS leakage. On the one hand, Quzhou Fructus Aurantii extract decreased LPS levels and effectively inhibited the TLR4/NF-κB signaling pathway and subsequent proinflammatory cytokine expression. On the other hand, it restored the expression of the gut tight junction proteins Claudin3 and Occludin and improved gut permeability. Furthermore, Quzhou Fructus Aurantii extract reduced the relative abundance of *Dubosiella*, *Faecalibaculum* and *Lactobacillus*. Thus, Quzhou Fructus Aurantii extract ameliorated MAFLD and gut inflammation, at least in part, by altering the gut microbiota.

3.3 TCM prescriptions treat MAFLD by regulating the gut microbiota

TCMs, which mainly originated in China, is used to prevent, diagnose and treat diseases or regulate human body functions under the guidance of TCM theories and has been widely used to treat liver diseases, including MAFLD. The treatment of MAFLD by TCM is based on liver protection via multiple mechanisms of action, such as antioxidative stress effects, regulation of lipid metabolism, anti-inflammation effects, anti-fibrosis effects, and regulation of the gut microbiota. Among them, TCM prescriptions have multicomponent and multitarget pharmacological effects, which are compatible with the complex pathogenesis of MAFLD and can alleviate the progression of MAFLD. In recent years, as the gut microbiota has gradually become a new target for the treatment of MAFLD, whether TCM prescriptions exert their therapeutic effects through the gut

microbiota has also been gradually investigated. Among them, 12 TCM prescriptions, such as the Salvia-Nelumbinis naturalis formula and Qiang-Gan formula, have been shown to regulate gut microbiota metabolites to improve MAFLD, as shown in Table 2.

The Salvia-Nelumbinis naturalis formula, initially called a lipid-lowering granule, is an herbal compound designed based on TCM theories and has been used clinically for the treatment of MAFLD and alleviation of liver steatosis with significant beneficial effects and few side effects. The alleviation of MAFLD by Salvia-Nelumbinis naturalis may be achieved by regulation of the gut microbiota and BAs (Li C. et al., 2021; Cao et al., 2022). Salvia-Nelumbinis naturalis supplementation restored the relative abundances of the beneficial bacteria *Lactobacillus* and *Alloprevotella*, increased (3α,5β,12α)-3,12-dihydroxy-24-norcholan-23-oic acid levels, and activated the FXR signaling pathway, which helped counteract LPS-induced impairment of the gut epithelial barrier and ameliorated metabolic disorders and liver disease (Li C. et al., 2021). In addition, Salvia-Nelumbinis naturalis counteracts the progression of NASH by increasing the proportion of secondary BAs and activating the endogenous vitamin D receptor (Cao et al., 2022).

The Qianggan formula is a traditional formula used in China for the treatment of liver diseases. Studies have shown that Qianggan formula treatment reverses gut microbiota disturbance and elevates the relative abundances of *Bacteroides* and *Clostridium*, which are involved in the BSH uncoupling process and therefore lead to an increase in secondary bile acid lithocholic acid (LCA) levels. In addition, the expression of TGR5 was increased in the livers of QGE-treated mice with NASH, which could be attributed to the increased LCA activation by TGR5, which ameliorated metabolic disorders and blocked nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3 (NLRP3) inflammasome-dependent inflammation in mice (Li Q. et al., 2020). Thus, the therapeutic effect of the Qianggan formula for MAFLD may be related to the interaction between the gut microbiota and BA metabolism.

Zuogui-Jiangtang-Qinggan-Fang, a new herbal formulation, has been clinically used to treat T2DM and fatty liver disease. Zuogui-Jiangtang-Qinggan-Fang reportedly elevated the relative abundances of Muribaculaceae and Lactobacillaceae and reduced the relative abundances of Lachnospiraceae and Desulfovibrionaceae (Zou et al., 2023). Furthermore, Zuogui-Jiangtang-Qinggan-Fang promoted the growth of the SCFA-producing bacterium *Akkermansia*, which repaired the gut epithelial barrier and alleviated insulin resistance by increasing the concentration of SCFAs (Zou et al., 2022). Thus, Zuogui-Jiangtang-Qinggan-Fang treatment may prevent and alleviate MAFLD by regulating the gut microbiota composition.

Yinchen Linggui Zhugan decoction is a classic combination of two well-known herbal prescriptions, namely, Linggui Zhugan and Yinchenhao decoctions, and has been used to treat hepatobiliary diseases and metabolic syndrome (Jiang et al., 2022a). Yinchen Linggui Zhugan decoction can enrich the SCFA-producing bacteria Christensenellaceae, Muribaculateae and Prevotellaceae to increase the gut SCFA content, especially Christensenellaceae, which is closely correlated with acetic acid, butyric acid and total SCFAs. The increase in butyric acid not only provides energy to gut epithelial cells but also activates the silent information regulator 1/Nrf2 signaling pathway and increases the expression of downstream antioxidant factors, which in turn ameliorates oxidative stress (Jiang et al., 2022b). These results suggest that Yinchen Linggui Zhugan decoction can alleviate MAFLD by modulating the gut microbiota and butyric acid levels.

Zhishi Daozhi decoction, a water decoction of herbs prescribed for use in Zhishi Daozhi pills, has been shown to have hepatoprotective and lipid-lowering effects. The effect of the Zhishi Daozhi decoction in the treatment of MAFLD may be achieved by modulating the gut microbiota (Bi et al., 2022). Zhishi Daozhi decoction may restore gut microbiota imbalance by promoting the growth of *Faecalibacterium* and *Bacteroidetes* and inhibiting the growth of *Brautia* and *Colidex*. The reconstituted gut microbiota also increases the amount of SCFAs in the gut, which not only reduces energy and fat deposition in the body but also provides energy to gut epithelial cells and ensures adequate expression of gut transepithelial resistance and tight junction proteins, thus improving the gut barrier and preventing gut inflammation.

Shenling Baizhu powder is a classic herb with a history of clinical use for thousands of years. Shenling Baizhu powder may alleviate MAFLD by regulating the gut microbiota. PSP not only increased the abundances of *Bifidobacterium* and *Anaerostipes* but also promoted the growth of SCFA-producing bacteria, accelerating the production of SCFAs that maintain normal gut permeability and protect the gut mucosa (Zhang et al., 2018). On the other hand, Shenling Baizhu powder can reduce LPS levels and inhibit TLR4 expression to suppress NLRP3 inflammatory vesicle activation and interleukin-1β (IL-1β) release (Pan et al., 2021). In addition, Shenling Baizhu powder can increase lipocalin levels in the liver and serum and inhibit SREBP-1c expression, thereby regulating systemic lipid metabolism and reducing hepatic lipid accumulation (Tang et al., 2020).

Hongqijiangzhi Fang is a spleen-strengthening herbal formula. Studies have shown that Hongqijiangzhi Fang can alleviate lipid metabolism disorders and reduce liver fat deposition, suggesting that it has a therapeutic effect on MAFLD. Liang et al. (Liang et al., 2018) reported that Hongqijiangzhi Fang may enhance gut barrier integrity by reducing the relative abundances of Enterobacteriaceae and *F. rapa*, thereby reducing LPS levels and suppressing gut inflammation. In addition, Hongqijiangzhi Fang may inhibit hepatic steatosis by reducing Enterobacteriaceae translocation and inhibiting NLRP3 inflammasome activation.

Qushi Huayu decoction is derived from Yinchenhao decoction, a classic prescription dating back to the Han Dynasty documented in the book Treatise on Cold Damage Disorders, which is one of the popular formulas for the treatment of MAFLD in China. In recent years, it has been shown that Qushi Huayu decoction can elevate the relative abundances of SCFA-producing bacteria *Butyricimonas* and *Eubacterium* to increase the SCFA content, which can provide energy to local gut epithelial cells and help maintain the gut epithelial barrier (Leng et al., 2020). Feng et al. (Feng et al., 2017) also reported that Qushi Huayu decoction treatment suppressed the

TABLE 2 Mechanism of action of TCM prescriptions in the treatment of MAFLD by modulating the gut microbiota and its metabolites.

Name	Composition of TCM	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
Salvia-Nelumbinis naturalis Formula	*Salvia miltiorrhiza* Bge., *Gynostemma pentaphyllum* (Thunb.) Makino, Polygonum cuspidatum Sieb. et Zucc., *Artemisia capillaris* Thunb., *Nelumbo nucifera* Gaertn	Male C57BL/6 mice	MCD diet supplemented with Salvia-Nelumbinis naturalis Formula extract, 750 mg/kg for 4 weeks	*Lactobacillus*, *Alloprevotella* ↑	BA metabolism; VDR expression; Gut FXR-FGF15 signaling pathway; TLR4/NF-κB signaling pathway; SIRT1/AMPK signaling pathway	(Li C. et al., 2021; Cao et al., 2022)
Qiang-Gan Formula	*A. capillaris*, *Isatis tinctoria* L., *Angelica sinensis* (Oliv.) Diels., *Paeonia lactiflora* Pall., *S. miltiorrhiza*, *Curcuma wenyujin* Y.H. Chen et C. Ling., *Astragalus membranaceus* (Fisch.) Bge., *Codonopsis pilosula* (Franch.) Nannf., *Alisma orientale* (Sam.) Juz., *Polygonatum kingianum* Coll.et Hemsl., *Rehmannia glutinosa* (Gaertn.) DC., *Dioscorea oppositifolia* L., *Crataegus pinnatifida* Bunge., *Medicated Leaven Massa Medicata Fermentata, Gentiana macrophylla Pall.*, *Glycyrrhiza uralensis* Fisch.	Male C57BL/6 mice	MCD diet supplemented with Qiang-Gan Formula, 400 mg/kg for 4 weeks	*Bacteroides*, *Clostridium* ↑	BA metabolism; BSH activity; TGR5 expression; TGR5/NLRP3 inflammatory pathway	(Li Q. et al., 2020)
Zuogui-Jiangtang-Qinggan-Fang	*Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey., *A. membranaceus*, *D. oppositifolia*, *Cormus officinalis* Sieb.et Zucc., *Coptis chinensis* Franch., *S. miltiorrhiza*, *A. capillaris*, *P. cuspidatum*, *C. wenyujin*, *Citrus reticulata* Blanco	Wild-type FVB inbred mice	HFD with Zuogui-Jiangtang-Qinggan-Fang, 7.5 or 15 g/kg for 8 weeks, and ZGJTQG was administered from the ninth week	Muribaculaceae, Lactobacillaceae, *Akkermansia* ↑ Lachnospiraceae, Desulfovibrionaceae ↓	SCFA production; Gut barrier; Insulin resistance	(Zou et al., 2023; Zou et al., 2022)
Yinchen Linggui Zhugan Decoction	*A. capillaris*, *Gardenia jasminoides* Ellis, *Rheum palmatum* L., *Poria cocos* (Schw.) Wolf, *Cinnamomum cassia* (L.) C. Presl., *Atractylodes macrocephala* Koidz., *G. uralensis*	Male Sprague–Dawley rats	HFD with Yinchen Linggui Zhugan Decoction, 19.2 g/kg/day for 4 consecutive weeks, and YLZD was administered from the eleventh week	SCFA-producing bacteria Christensenellaceae, Muribaculateae, Prevotellaceae ↑	SCFA production; Oxidative stress; SIRT1/Nrf2 signaling pathway	(Jiang et al., 2022b)
Zhishi Daozhi Decoction	*Citrus aurantium* L., *R. palmatum* L., *C. chinensis*, *Scutellaria baicalensis* Georgi, *Massa Medicata Fermentata*, *A. macrocephala*, *P. cocos*, *A. orientale*	Male C57BL/6 mice	HFD with Zhishi Daozhi Decoction, 14.5 g/kg/d for 4 consecutive weeks, and Zhishi Daozhi Decoction was administered from the eleventh week	*Faecalibacterium*, *Bacteroidetes* ↑ *Brautia*, *Colidextribacter* ↓	SCFA production; Gut barrier; Gut inflammation	(Bi et al., 2022)

(Continued)

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TABLE 2 Continued

Name	Composition of TCM	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
Shenling Baizhu powder	*Panax ginseng* C.A.Mey., *P. cocos*, *A. macrocephala*, *D. oppositifolia*, *Dolichos lablab* L., *N. nucifera*, *G. uralensis*, *Coix lacryma-jobi* L.var.*ma-yuen* (Roman.) Stapf, *Platycodon grandiflorum*(Jacq.)A.DC., *Amomum villosum* Lour.	Male Sprague–Dawley rats	Fed a HFD and intragastrically administered 30 g/kg/day Shenling Baizhu powder for 16 weeks	SCFA-producing bacteria; *Bifidobacterium*, *Anaerostipes* ↑	LPS production; LPS/TLR4/NLRP3 inflammatory signaling pathway; Gut permeability and gut mucosa; Lipid metabolism	(Zhang et al., 2018; Pan et al., 2021; Tang et al., 2020)
Hongqijiangzhi Fang	*A. membranaceus*, *Semen oryzae cum monasco*, *A. capillaris*, *Lycium barbarum* L., *Curcuma Longa* L., *N. nucifera*, *Magnolia officinalis* Rehd.et Wils	Specific pathogen-free male Sprague–Dawley rats	HFD with Hongqijiangzhi Fang, 19.05 g/kg/day for 16 weeks	Enterobacteriaceae *F. rappini* ↓	LPS production; NLRP3 inflammatory pathway; Gut inflammation	(Liang et al., 2018)
Qushi Huayu Decoction	*A. capillaris*, *G. jasminoides*, *Fallopia japonica*, *C. longa*, *Hypericum japonicum* Thunb. ex Murray	Male C57BL/6 mice	HFD with Qushi Huayu Decoction, 9.3 g/kg/d for 4 consecutive weeks, and Qushi Huayu Decoction was administered from the thirteenth week	SCFA-producing bacteria *Butyricimonas*, *Eubacterium*, *Collinsella*; *Escherichia*/*Shigella* ↑	Gut epithelial barrier; LPS production; Gut inflammation	(Leng et al., 2020; Feng et al., 2017)
Huazhi-Rougan Formula	*A. capillaris*, *Cassia obtusifolia* L., *R. palmatum*, *A. orientale*, *Polyporus umbellatus* (Pers.) Fries., *C. pinnatifida*, *Atractylodes lancea* (Thunb.) DC., *A. macrocephala*, *C. reticulata*, *Trichosanthes kirilowii* Maxim., *Ligustrum lucidum* Ait., *Eclipta prostrate* L., *L. barbarum*, *Cirsium setosum* (Willd.) Besser, *Bupleurum chinense* DC., *G. uralensis*	Male C57BL/6J mice	MCD with Huazhi-Rougan Formula, 3 or 6 g/kg/d for 4 weeks	Lactobacillaceae, Bifidobacteriaceae, Clostruduaceae ↑	Gut barrier; BA metabolism; BA-transporting portal transport molecules (MRP2/3, OSTα/β)	(Li C. et al., 2022)
Er-Chen Decoction	*Pinellia ernate* (Thunb.) Breit., *C. aurantium*, *Smilax glabra Roxb.*, *G. uralensis*	Specific pathogen-free-grade male Sprague–Dawley rats	HFD with Er-Chen Decoction, 4.5 or 9 g/kg/d for 12 weeks	*Lactobacillus*, *Dubosiella*, *Akkermansia*, *Intestinimonas* ↑ *Alloprevotella*, *C.saccharimonas* ↓	Vitamin B6 metabolism; Taurine and hypotaurine metabolism, cysteine and methionine metabolism;LPS translocation; Oxidative stress	(Liu H. et al., 2021; Miao et al., 2022)

(Continued)

10.3389/fcimb.2024.1323261

TABLE 2 Continued

Name	Composition of TCM	Experimental subject	Experimental design	Modulation target (gut microbiota structure)	Modulation target	Ref.
Sheng-Jiang Powder	*Bombyx batryticatus*, *Cicadae periostracum*, *C. Longa*, *R. palmatum*	Male C57BL/6 mice	HFD was fed for 12 weeks, and Sheng-Jiang Powder was administered from the seventh week for 6 weeks	SCFA-producing bacteria Erysipelotrichaceae *Roseburia* ↑ *Desulfovibrio* ↓	SCFA production; LPS production; Akt/mTOR/S6 signaling pathway; Gut inflammation	(Li J. et al., 2020)
Sanwei Ganjiang Powder	*Zingiber officinale* Rosc., *Amomum kravanh* Pierre ex Gagnep., *Myristica fragrans* Houtt.	Specific pathogen-free inbred BALB/c mice (half male and half female)	Subcutaneous injection of 40% CCl_4 oil solution (5 mL/kg was first injected, followed by 3 mL/kg) twice a week for 8 weeks, and Sanwei Ganjiang Powder, 0.165 or 0.66 g/kg was given from the third week	Promoted beneficial bacteria and inhibited harmful bacteria, reduced the Firmicutes/Bacteroidetes ratio	BA reabsorption and excretion; Gut barrier; Gut inflammation	(Li N. et al., 2019)

MAFLD, Metabolic-associated fatty liver disease; TCM, traditional Chinese medicine; HFD, high-fat diet; MCD, methionine/choline-deficient diet; BA, bile acid; SCFA, short-chain fatty acid; LPS, lipopolysaccharide; BSH, bile salt hydrolase; FXR, farnesoid X receptor; TLR4, Toll-like receptor-4; TGR5, Takeda G-protein-coupled receptor 5; MRP2/3, multidrug resistance-associated protein 2/3; OSTα/β, organic solute transporter alpha/beta; NF-κB, nuclear factor kappa-B; Nrf2, nuclear factor erythroid 2-related factor 2; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3; SIRT1, silent information regulator 1; AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; FGF15, recombinant fibroblast growth factor 15; Akt, serine/threonine kinase Akt; mTOR, mammalian target of rapamycin.
"↑" indicates an increase in the relative abundance of bacteria; "↓" indicates a decrease in the relative abundance of bacteria.

10.3389/fcimb.2024.1323261

HFD-induced increase in *Escherichia/Shigella* and elevated the relative abundance of the SCFA-producing bacteria *Collinsella*. In addition, Qushi Huayu decoction was able to reduce the serum LPS concentration, which may be associated with an improvement in gut inflammation. Therefore, Qushi Huayu decoction may alleviate MAFLD by repairing the gut barrier and modulating the gut microbiota composition.

The Huazhi-Rougan formula contains flavonoids, alkaloids and lactones as the main components and has been widely used to treat MAFLD and its complications. Studies have shown that Huazhi-Rougan formula treatment counteracts MCD-induced hepatic steatosis and inflammation by regulating gut ecological dysregulation and promoting fecal BA excretion (Li C. et al., 2022). The Huazhi-Rougan formula decreased the enrichment of HFD-associated bacteria and elevated the enrichment of Lactobacillaceae, Bifidobacteriaceae, and Clostridiaceae, which are involved in the uncoupling, oxidation and isomerization of BAs and contribute to the formation of secondary BAs. In addition, the Huazhi-Rougan formula alleviated gut barrier damage and alleviated MAFLD by inhibiting the expression of the intestinal BA transporter and the BA-transporting portal transport molecules multidrug resistance-associated protein 2/3 and organic solute transporter alpha/beta, promoting fecal BA excretion and reducing secondary BA accumulation in the gut.

Er-Chen decoction, a common TCM formula originating from the Taiping Huimin Formula Bureau, is a basic herbal formula for treating dry dampness and phlegm and has been used to treat various phlegm-dampness diseases, such as MAFLD. Many studies have investigated the mechanism by which Er-Chen decoction improves lipid metabolism disorders, and Liu et al. (Liu H. et al., 2021) reported that Er-Chen decoction could reduce LPS translocation and reduce liver inflammation by reversing HFD-induced gut barrier dysfunction through regulation of the gut microbiota. Miao et al. (Miao et al., 2022) showed that Er-Chen decoction could alleviate MAFLD by inhibiting oxidative stress, reducing inflammatory responses, ameliorating gut microbiota dysbiosis, and increasing pyridoxal (vitamin B6) levels. The increase in pyridoxal concentrations not only inhibited lipid synthesis but also reduced oxidative and inflammatory stress. Thus, the effect of Er-Chen decoction on MAFLD may be achieved through the gut-liver axis pathway involving the gut microbiota.

Sheng-Jiang powder, derived from the "wan bing hui chun" compiled by Ting-Xian Gong of the Ming Dynasty, is a typical herbal formula for restoring the "ascending and descending abnormalities" of the spleen qi and has been used to treat MAFLD. Sheng-Jiang powder supplementation increased the relative abundances of the SCFA-producing bacteria Erysipelotrichaceae and *Roseburia* and decreased the relative abundance of the harmful bacteria *Desulfovibrio* (Li J. et al., 2020). These findings suggest that Sheng-Jiang powder may alleviate gut leakage by increasing SCFA levels to ameliorate gut barrier damage and may also ameliorate gut inflammation by inhibiting LPS production or entry into the gut. Thus, the protective effect of Sheng-Jiang powder against HFD-induced MAFLD may be partially attributed to its regulation of the gut microbiota.

Sanwei Ganjiang powder, also known as Jia Ga Song Tang, not only has antipyretic and antidiarrheal effects and promotes gas circulation but also has anti-inflammatory effects, protects the gastrointestinal mucosa, and enhances antioxidant and hepatoprotective pharmacological effects. Li et al. (Li N. et al., 2019) reported that Sanwei Ganjiang powder could alleviate liver injury by regulating the gut microbiota and restoring BA homeostasis. Sanwei Ganjiang powder may regulate the Firmicutes/Bacteroidetes ratio, promote beneficial bacteria and inhibit harmful bacteria. Its modifying effects on the gut microbiota lead to upregulation of the expression of specific BA metabolic enzymes, as well as reabsorption and efflux transporters, thus promoting the detoxification, reabsorption and excretion of TBAs and reversing disrupted BA homeostasis. In addition, Sanwei Ganjiang powder increased the expression of gut tight junction proteins such as ZO-1 and Occludin and repaired the gut barrier to inhibit LPS-induced gut inflammation. Therefore, treatment with Sanwei Ganjiang powder may be a potential strategy for treating MAFLD by maintaining the gut-liver axis.

4 Perspective

MAFLD has become the most common chronic liver disease in the world, and its incidence is increasing annually (Lin et al., 2021). However, due to the complexity of the pathogenesis of MAFLD, pathogenic processes such as insulin resistance, lipotoxicity, oxidative stress, altered immune or cytokine or mitochondrial function, and apoptosis, are collectively involved in the development and progression of MAFLD. At the same time, the pathogenetic process of MAFLD is often accompanied by a variety of complications; therefore, drug therapy needs to simultaneously account for the prevention and treatment of hepatic steatosis, inflammation, hepatocellular injury and fibrosis, as well as the treatment of complications, which is very difficult. Second, NASH requires a liver biopsy to determine if the patient has NASH and what stage of liver fibrosis the patient is currently in. However, liver biopsies are invasive and not easily accepted by patients. In clinical practice, it is not very feasible to repeat liver biopsies to assess whether liver injury and liver fibrosis have improved. While it is feasible that current research on NASH drug therapy focuses on improvements in MAFLD activity scores, the scores are subjective. Therefore, there is an urgent need to develop and validate a set of noninvasive methods for assessing NASH fibrosis and documenting its progression or reversal to help enhance accelerated new drug development for MAFLD and NASH. For these and other reasons, there are currently no FDA-approved therapeutic drugs. Therefore, it is essential to find safe and effective anti-MAFLD drugs. In recent years, the relationship between the gut microbiota and metabolic diseases has been widely recognized (Boulangé et al., 2016). An imbalance in the gut microbiome exacerbates disease progression, and targeted modulation of the gut microbiome has emerged as a viable strategy for the prevention and treatment of MAFLD. Plant natural products involve multiple pathways, multiple targets and few side effects. Plant natural products exert their effects in the prevention and treatment of MAFLD not only by regulating

lipogenic pathways, downregulating inflammatory pathways, improving insulin sensitivity, and ameliorating oxidative stress but also by regulating the gut microbiota and its metabolites, for example, via the gut-liver axis (Wang L. et al., 2023).

Modern pharmacokinetic studies have shown that most plant natural products are difficult to absorb or have poor absorption rates. Berberine, for example, is less than 1% orally available, but it has been shown to have therapeutic activity against a variety of diseases. This may be because berberine can be converted to dihydroberberine by enzymes produced by the gut microbiota, which increases its absorption and subsequently utilization. In addition, some "zero absorption" natural ingredients whose main function may be to provide nutrients for the gut microbiota, improve the activity of the gut microbiota and exert their effects through the release of active molecules by the gut microbiota. The contribution and function of the gut microbiota through biotransformation of the components of plant natural products or through enhancement of the activity of the gut microbiota cannot be ignored. Therefore, targeting the gut microbiota for the treatment of MAFLD may be more conducive to ensuring the efficacy of plant natural products in controlling the development of this disease.

Recent research on the relationships among the gut microbiota, gut permeability and metabolic disorders has provided new perspectives on the treatment of MAFLD. In the clinical application of plant natural products for MAFLD treatment, many patients are treated based on the principle of "strengthening the spleen" from the perspective of "spleen deficiency", which is often accompanied by the improvement of gut symptoms such as diarrhea and defecation. Numerous studies have shown that plant natural products can support the growth of beneficial bacteria in the gut, inhibit the growth of harmful bacteria, maintain the balance of beneficial and harmful bacteria, and improve gut permeability. The study of "gut-liver" axis interactions provides a reference and method for the treatment of MAFLD by plant natural products and the gut microbiota, which allows exploration of the pharmacological mechanism of plant natural products in the treatment of MAFLD from the perspective of gut microenvironment and gut barrier regulation.

Although the gut microbiome is a hot topic and a new entry point for studying the mechanism of action of plant natural products, the mechanism cannot be simply attributed to alterations in the gut microbiome. Alterations in the gut microbiota may be one result of plant natural product interventions in the organism, but not all the pathways involved in these effects are known, and additional experimental evidence needs to be obtained. Based on the above, in addition to using 16S ribosomal DNA identification to determine the changes in the gut microbiota, we need to further explore the role of the gut microbiota in the efficacy of plant natural products through FMT, testing with germ-free mice, antibiotic intervention and other methods.

In addition, the changes in the gut microbiota are unstable. The gut microbiota is easily affected by the external environment. Although the self-repair ability of the gut microbiota can restore its balance after short-term disturbance, long-term disturbance often causes gut microbiota disorders; that is, differences in experimental environments and conditions may lead to changes in the gut microbiota. Therefore, it is particularly important and more convincing to identify stable and changing bacterial species, such as pathogenic bacteria or beneficial bacteria, through the instability of the gut microbiota. Crossomics data need to be integrated and analyzed with data from additional high-throughput omics technologies, such as metagenomics, metabolomics, and bioinformatics. From the perspective of the composition and function of the gut microbiota and the interaction between the gut microbiota and host metabolism, key strains and their related metabolites will need to be explored in depth.

Numerous plant natural compounds, such as berberine, resveratrol, curcumin, and puerarin, have been found to alleviate MAFLD. However, TCM prescriptions often achieve better therapeutic effects and have the characteristics and advantages associated with the holistic use of Chinese medicine in the treatment of complex diseases. TCM prescriptions are safe and effective modern Chinese medicines with a clear mechanism of action formed on the basis of TCM enhanced by modernization and pharmacological research and represent one of the future development directions of Chinese medicine. However, there are some limitations in the current development of plant natural products for the treatment of MAFLD. One of the limitations is that the mechanisms of action of plant natural products are complex, and it is difficult to clarify the specific mechanisms of action. Second, the limitation of plant natural products emphasizes the influence of the place of origin on their quality and efficacy. Authentic herbs not only have unique environmental conditions in terms of their origin but also are subjected to specific natural factors and artificial management during their growth process, which makes them of superior quality. In conclusion, the interaction between plant natural products and the gut microbiota provides a safe, effective, and feasible therapeutic approach and modality for treating MAFLD.

5 Conclusion

This narrative review systematically summarizes the effects of plant natural products on the prevention of MAFLD by regulating the gut microbiota and its metabolite-related pathways, which provides feasible ideas for further exploring safer and more effective plant natural products as drugs for the prevention and treatment of MAFLD. Moreover, on the basis of this review, it is necessary to strengthen the study of the interactions among plant natural products, the gut microbiota and the human body. In the future, more attention should be given to the role of the gut microbiota in the pharmacodynamic effect of Chinese medicine and the contribution of the effects of the gut microbiota on host metabolism to the development of disease or pharmacodynamic effects.

Author contributions

TC: Writing – original draft, Writing – review & editing. XS: Writing – original draft, Writing – review & editing. XX: Software, Visualization, Writing – review & editing. LD: Software, Visualization, Writing – review & editing. SL: Software, Visualization, Writing – review & editing. MX: Software, Visualization, Writing – review & editing. YH: Software, Visualization, Writing – review & editing. LZ: Investigation, Resources, Writing – review & editing. TL: Investigation, Resources, Writing – review & editing. XW: Investigation, Resources, Writing – review & editing. YF: Investigation, Resources, Writing – review & editing. ZX: Conceptualization, Writing – review & editing. CW: Conceptualization, Writing – review & editing. MW: Conceptualization, Writing – review & editing. JL: Conceptualization, Writing – review & editing. YZ: Project administration, Supervision, Writing – review & editing. WS: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. LL: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

MAFLD	metabolic-associated fatty liver disease
NAFLD	nonalcoholic fatty liver disease
T2DM	type 2 diabetes mellitus
TCM	traditional Chinese medicine
NAFL	simple fatty liver
NASH	steatohepatitis
HCC	hepatocellular carcinoma
FDA	Food and Drug Administration
BA	bile acid
LPS	lipopolysaccharide
SCFA	short-chain fatty acid
TMAO	trimethylamine-N-oxide
FXR	farnesoid X receptor
VDR	vitamin D receptor
PXR	pregnane X receptor
TGR5	Takeda G protein-coupled receptor 5
BSH	bile salt hydrolase
TBA	total bile acid
SHP	small heterodimer partner
SREBP-1c	sterol regulatory element-binding protein 1c
PPAR-α	peroxisome proliferator-activated receptor alpha
CYP7A1	cytochrome P450 7A1
AgRP	Agouti-related protein
NPY	neuropeptide Y
GPR41/ GPR43	mammalian G protein-coupled receptors 41/43
GPR109A	G protein-coupled receptor 109 A
AMPK	adenosine 5'-monophosphate (AMP)-activated protein kinase
TLR4	Toll-like receptor 4
TMA	trimethylamine
VD	vitamin D
NF-κB	nuclear factor kappa-B
HFD	high-fat diet
TNF-α	tumor necrosis factor alpha
Nrf2	nuclear factor erythroid 2-related factor 2
LXR-α	liver X receptor alpha
ZO-1	zonula occludens protein 1
CYP27A1	cytochrome P450 27A1
BSEP	bile salt export pump
NTCP	Na^+-taurocholate cotransporting polypeptide
FGF15	recombinant fibroblast growth factor 15
T-β-MCA	Tauro-β-muricholic acid
T-α-MCA	Tauro-α-muricholic acid
MCD	methionine–choline-deficient
FMT	fecal microbiota transplantation
TCDCA	taurochenodeoxycholic acid
TUDCA	tauroursodeoxycholic acid
LCA	lithocholic acid
NLRP3	nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3
IL-1β	interleukin-1β

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Research progress on the microbiota in bladder cancer tumors

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The microbiota, also referred to as the microbial community, is a crucial component of the human microenvironment. It is located predominantly in various organs, including the intestines, skin, oral cavity, respiratory tract, and reproductive tract. The microbiota maintains a symbiotic relationship with the human body, influencing physiological and pathological functions to a significant degree. There is increasing evidence linking the microbial flora to human cancers. In contrast to the traditional belief that the urethra and urine of normal individuals are sterile, recent advancements in high-throughput sequencing technology and bacterial cultivation methods have led to the discovery of specific microbial communities in the urethras of healthy individuals. Given the prevalence of bladder cancer (BCa) as a common malignancy of the urinary system, researchers have shifted their focus to exploring the connection between disease development and the unique microbial community within tumors. This shift has led to a deeper investigation into the role of microbiota in the onset, progression, metastasis, prognosis, and potential for early detection of BCa. This article reviews the existing research on the microbiota within BCa tumors and summarizes the findings regarding the roles of different microbes in various aspects of this disease.

KEYWORDS

bladder cancer, microbiota, review, prognosis, mechanism

1 Introduction

Bladder cancer (BCa) ranks among the top ten most common urinary tract malignancies and is the second-most prevalent malignancy of the urinary system. It accounts for 4.6% of all new cancer diagnoses annually, with approximately 400,000 new cases and 160,000 deaths worldwide each year, and has a five-year mortality rate of 30% (Richters et al., 2020). BCa can be divided into muscle-infiltrating bladder cancer (MIBC) and nonmuscle-infiltrating bladder cancer (NMIBC) based on its invasion into the muscular layer of the bladder wall (Lindskrog et al., 2021). Approximately 70% of BCa

cases are NMIBC, while the remaining 30% are MIBC, which has a greater potential for invasion and metastasis (Grayson, 2017). The majority of NMIBC patients undergo transurethral resection of the bladder tumor (TURBT), yet the recurrence rates range between 40% and 80%. Additionally, 25% of NMIBC patients progress to MIBC or distant tumor metastases. MIBC, characterized by few early symptoms, rapid progression, and poor prognosis, remains a significant clinical challenge (Antoni et al., 2017).

The incidence of BCa correlates with age, peaking in individuals aged 75-84 years, who represent 30% of new cases annually (Richters et al., 2020). The disease is 3.7 times more common in men than in women (Sung et al., 2021; Yacouba et al., 2022), a disparity attributed to greater exposure to smoking and chemical carcinogens in men, as well as hormonal differences between the sexes (Madeb and Messing, 2004; Yacouba et al., 2022). Despite an increase in smoking among women, the incidence of BCa in this demographic population remains comparatively low, suggesting additional contributing factors beyond established risk factors (Marcon et al., 2018; Yacouba et al., 2022).

Genetic mutations and alterations in specific pathways have been implicated in BCa. Tumor suppressor genes such as TP53, RB1, and PTEN are frequently mutated in carcinoma *in situ* (CIS) (Castillo-Martin et al., 2010). Oncogenes such as FGFR3, PIK3CA, and RAS, which promote tumor cell development, are characteristic of NMIBC (Castillo-Martin et al., 2010; Knowles and Hurst, 2015). Diet, particularly meat consumption, has been reviewed for its role in bladder carcinogenesis due to the formation of carcinogenic chemicals during meat cooking and processing (Aveta et al., 2022).

Contrary to previous beliefs that healthy bladders are sterile (Han et al., 2014), advancements in urine collection techniques have revealed a distinct bladder microbiota in healthy individuals (Wolfe et al., 2012; Horwitz et al., 2015; Whiteside et al., 2015a; Thomas-White et al., 2016; Thomas-White et al., 2018). Early research by Hicks RM et al. linked bacteria to schistosomiasis-induced BCa through N-nitrosamine formation (Mansour et al., 2020; Chen et al., 2022). BučevićPopović et al. demonstrated microbial infiltration in 20% of malignant tumor tissues, suggesting bacterial involvement in BCa (Bu˘cevi´c Popovi´c et al., 2018). With improvements in detection methods, researchers are increasingly identifying microorganisms in BCa tissues, and exploring their sequencing, functions, and mechanisms. An increasing number of studies indicate that the intratumoral microbiota plays a crucial role in the onset and progression of BCa (Figure 1). This article concentrates on various microbial phyla and reviews the diverse roles played by the intratumoral microbiota in BCa.

2 Materials and methods

A comprehensive literature search was performed using PubMed, Embase and the China National Knowledge

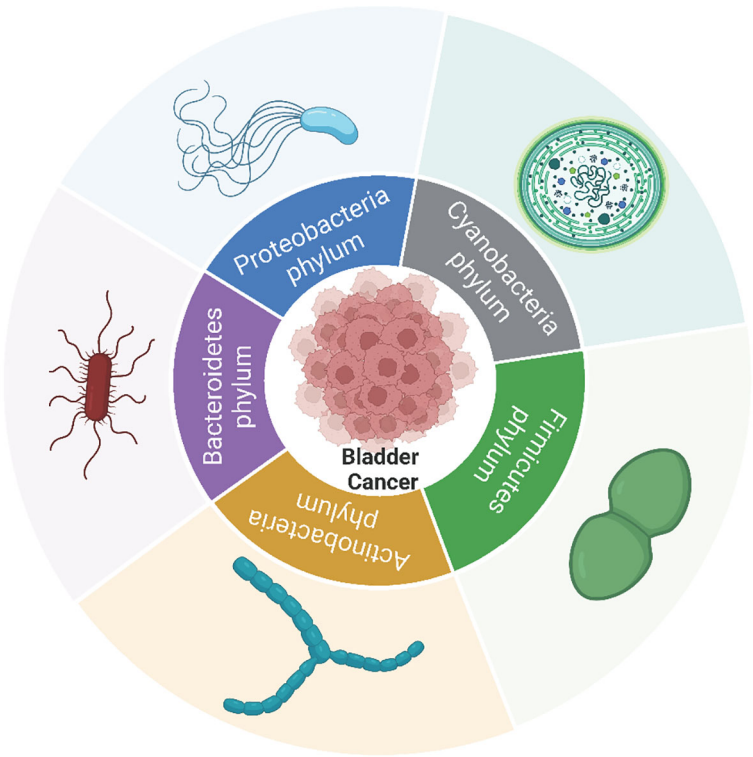

FIGURE 1
The microbial phyla linked to the onset, progression, metastasis and prognosis of bladder cancer within bladder cancer tumors. Among these, the Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria phyla exhibit the highest abundance in bladder cancer as well as adjacent or normal tissues in the majority of studies.

Infrastructure (CNKI) to October 2023. The search terms included 'bladder cancer tissue', 'microbiota' and 'microbiome' (the 'microbiota' is defined as the totality of microorganisms living in a specific environment, and the term 'microbiome' refers to the totality of microbial genes in a specific biological niche. These terms are often used interchangeably in the literature (Berg et al., 2020). The following criteria were used to include studies in this review: (1) included BCa and control groups (case-control studies), (2) provided information on the presence or abundance of microbial taxa, and (3) provided information on the promotion or inhibition of microorganisms in the BCa and/or control groups. A total of 10 relevant articles were screened, including 8 clinical trial papers and 2 bioinformatics analyses (Table 1).

3 Phylum Proteobacteria

Using data from the TCGA database, Rebecca M. Rodriguez et al. analyzed microbial diversity and species differences in 56 paired tumor and adjacent normal tissue samples from 28 patients. They discovered that Proteobacteria was the most prevalent phylum, comprising 93% of the total reads, with *Stenotrophomonas maltophilia* being the most common species (accounting for 61% of the total reads) (Rodriguez et al., 2020). Wei Li conducted genomic sequencing of bacterial species in cancer and adjacent tissues from 18 BCa patients without urinary tract infections and with negative urine cultures. This study corroborated the existence of microbial flora in bladder tissue, revealing that the most abundant bacteria in both cancer and adjacent bladder tissues belonged to the phylum Proteobacteria (Li, 2021). Jian-Xuan Sun's team employed 2bRAD-M microbiome sequencing technology to analyze tumor tissue samples from 22 BCa patients, focusing on the differences in the microbial community between NMIBC and MIBC. Echoing the findings of previous studies, they noted similar microbial compositions in both tumor types, with *Ralstonia_sp000620465* being the predominant species (Sun et al., 2023).

Proteobacteria are currently the largest phylum within the domain Bacteria. A common trait of Proteobacteria is the gram-negative staining and, thus, the presence of lipopolysaccharide in the outer membrane. Many common human pathogens are found in the Proteobacteria phylum, such as *Brucella* genera, *Rickettsia* genera, *Neisseria*, *Escherichia*, *Shigella*, *Salmonella* and *Helicobacter* genera (Human Microbiome Project Consortium, 2012). Proteobacteria, which are typically gut commensals with pathogenic potential, have been studied extensively (Everard et al., 2011; Geurts et al., 2011; Zhang et al., 2012). Recent research highlighted significant variations in the abundance of Proteobacteria in mucosa-associated microbiomes of ileal and rectal biopsies (but not in stool samples) between Crohn's disease patients and control subjects (Gevers et al., 2014). Similar shifts in gut proteobacterial communities have been observed in patients with colitis-associated colorectal cancer (Bonnet et al., 2014). These findings collectively suggest that an imbalance in Proteobacteria could play a crucial role in bladder carcinogenesis. The increase in the abundance of Proteobacteria might serve as a potential diagnostic indicator for dysbiosis, potentially increasing the risk of BCa.

3.1 *Cupriavidus* in BCa

Fei Liu et al. examined tissue samples of cancerous bladder mucosa from patients diagnosed with BCa, consisting of 22 carcinoma tissues and 12 adjacent normal tissues. This study confirmed the occurrence of bladder microbiota dysbiosis in BCa patients. The researchers observed that Proteobacteria was the predominant phylum in both cancerous and noncancerous tissues, with a significantly greater abundance in cancerous tissues. In cancerous tissues, the presence of *Cupriavidus*, *Acinetobacter*, and *Escherichia-Shigella* increases markedly (Wang et al., 2007). Previous research has indicated that certain trivalent pesticide-related chemicals can induce protein carbonylation and oxidative DNA damage in human urothelial cells, potentially leading to BCa (Liu et al., 2019). Interestingly, the enrichment of harmful chemical products, which are subject to metabolic processes, might correlate with the significantly elevated abundance of the genus *Cupriavidus*. This genus was the most abundant bacteria found in both cancerous and noncancerous bladder tissues, despite notable differences in their levels. *Cupriavidus*, known from prior studies as an organophosphorus pesticide-degrading microorganism, is thought to break down harmful substances absorbed by the body, subsequently excreting them into the bladder through specific enzymes (Wang et al., 2007).

3.2 Biofilms in BCa development

Naomi Nadler et al. collected tissue samples from ten patients undergoing TURBT and analyzed them for bacterial aggregates using Fluorescence *in situ* Hybridization (FISH). Dense biofilms were identified in the urothelial cancer tissue of two samples, and spherical bacteria were confirmed in one sample. Notably, both patients had negative preoperative urine cultures. These findings suggest a potential link between biofilm formation and BCa (Nadler et al., 2021).

Bacterial aggregates, commonly referred to as biofilms, often attach to the apical epithelial cells of the bladder and are known as umbrella cells. These cells typically have a protective layer of sulfated polysaccharide aminoglycans. Disruption of this layer may lead to pathological bladder changes and chronic inflammation in the genitourinary system (Garrett, 2015). During biofilm formation, bacteria such as *Aeromonas hydrophila* and *Pseudomonas aeruginosa* can augment the production of extracellular proteases, including serine proteases, metalloproteinases, and elastases (Swift et al., 1999). Some pathogens, such as *Streptococcus pyogenes*, may cause invasive diseases by degrading intercellular junctions in conjunction with the host cysteine protease calpain (Sumitomo et al., 2013).

Studies on bacterial biofilms in colorectal cancer have shown that certain bacteria, such as *Escherichia coli*, can drive cancer development. *Escherichia coli* produces the genotoxin colibactin, which causes mutagenic DNA damage through covalent DNA binding to a chemical moiety known to promote tumorigenesis (Garrett, 2015). Further research is required to understand this relationship, particularly whether bacterial dysbiosis and biofilm

TABLE 1 Characteristics of studies included in the systematic review.

Author	Article type	Population	Stage	Method	High-abundance Microorganisms			Cohorts, Diversity, Abundance and Other Findings
Liu, F.; et al. (Liu et al., 2019)	Original research	22 carcinoma tissues samples (male) and 12 adjacent normal tissues samples	NMIBC 5 MIBC 17	16S rRNA	**Phylum** Proteobacteria (54.1%) Firmicutes (23.7%) Bacteroidetes (13.4%) Actinobacteria (4.4%)	**Genera** *Cupriavidus*(16.9%) *Brucellaceae* (6.0%) *Ralstonia* (5.5%) *Lactobacillus* (5.3%)		1. Verified the occurrence of bladder microbiota dysbiosis in bladder cancer. 2. A statistically significant difference in the Shannon diversity index was observed the abundance of microbiota between cancerous and noncancerous tissues (P = .0417), indicating a significantly lower diversity in cancerous tissues compared to noncancerous tissues. 3. *Lactobacillus* and *Prevotella_9* were enriched in noncancerous tissues, while *Acinetobacter* and *Escherichia-Shigella* were significantly increased in cancerous tissues. 4. There were no significant differences in patients at different grades and different biologically relevant subtypes with respect to the α-diversity or β-diversity-associated bladder cancer taxa.
Rodriguez, R.M.; et al. (Rodriguez et al., 2020)	Bioinformatics analysis	Total of 56 paired tumor and adjacent normal samples from 28 cases	/	TCGA cancer database	**Phylum** Proteobacteria	**Species** *Stenotrophomonas maltophilia*		1. No statistically significant differences between paired tumor and adjacent normal samples total number of reads, relative abundance, or positivity ratio. 2. In the fully adjusted model, there were no significant differences in alpha diversity. No statistically significant differences were observed in the analyses of paired tumor and adjacent normal samples when stratifying by sex, race, anatomical site, or tumor stage.
Mansour, B.; et al. (Mansour et al., 2020)	Original research	14 tissue samples collected from ten bladder cancer patients (male 5, female 5)	NMIBC 6 MIBC 4	16S rRNA	**Phylum** Firmicutes(34%) Actinobacteria (23%) Proteobacteria (22%) Bacteroidetes (15%) Cyanobacteria (8%)	**Genera** *Bacteroides* *Akkermansia* *Klebsiella* *Clostridium* *Enterobacter*		1. *Akkermansia*, *Bacteroides*, *Clostridium sensu stricto*, *Enterobacter* and *Klebsiella*, referred to as "five suspect genera", were found to be over-represented in tissue samples compared to the urine. 2. The abundance of microbiota showed significant difference only in the median of genus richness and Shannon diversity between female and male tissue samples.
Pederzoli, F.; et al. (Pederzoli et al., 2020)	Original research	Total of 87 paired urine, neoplastic and nonneoplastic tissues samples from 29 cases (male 21, female 8)	/	16S rDNA	**Genera** *Burkholderia*			1. In tissue samples, no differences were detected in α- and β-diversity between males and females. The genus *Burkholderia* was more abundant in the neoplastic versus the non-neoplastic tissue in both sexes.
Nadler, N.; et al. (Nadler et al., 2021)	Original research	Total 10 BCa samples (male 8, female 2)	MIBC	16S rRNA	/			1. In two (1 male, 1 female) out of ten patients, the analysis showed abundant bacterial aggregation on the surface epithelium, and one sample even indicated what might be considered a submucosal intracellular bacterial community of coccoid bacteria. 2. Interestingly, urothelial cancer tissue from the two patients that exhibited bacterial aggregation did not have a history of positive urine cultures.

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10.3389/fcimb.2024.1374944

TABLE 1 Continued

Author	Article type	Population	Stage	Method	High-abundance Microorganisms			Cohorts, Diversity, Abundance and Other Findings
Li, W. (Li, 2021)	Original research	Total of 36 paired tumor and adjacent normal samples from 18 cases	/	16S rDNA	**Phylum** Proteobacteria Firmicutes Bacteroidetes Tenericutes			1. Bacteria are present in the outer mucosa, mucosal layer, submucosa, and even the smooth muscle layer of both bladder cancer and tissue adjacent to bladder cancer, with the highest distribution observed in the outer mucosa.. 2. Compared with adjacent tissues, bladder cancer tissues exhibit lower species richness and diversity, but these differences are not statistically significant. Bladder cancer tissues and adjacent tissues have a similar bacterial community structure. 3. At the genus level, the genus with statistical differences in the bladder cancer group is *Staphylococcus*.
Parra-Grande, M.; et al. (Parra-Grande et al., 2022)	Original research	Total of 58 corresponding to 26 patients with paired samples and 6 patients with only tumor tissue samples (male 27, female 5)	MIBC	16S rDNA	**Phylum** Firmicutes (41.46%) Bacteroidetes (28.23%) Proteobacteria (22.78%) Actinobacteria (6.06%)	**Genera** *Bacteroides* (16.24%) *Escherichia.Shigella* (6.07%) *Staphylococcus*(5.43%) *Enterococcus*(4.25%)		1. Significant differences were found in microbial richness at the genus level, with a higher richness observed in the non-tumor mucosa compared to the tumor mucosa (26 samples). Actinobacteria were significantly more enriched in the non-tumor mucosa compared to the tumor mucosa, supporting the hypothesis that a higher abundance of Actinomycetes is associated with a lower rate of bladder cancer in women. 2. In the multivariate analysis, significant differences were found in microbial composition according to tumor grade, as low-grade tumors exhibited a microbial profile that was characterized by a higher enrichment for *Enterococcus*. 3. In the multivariate analysis using the PERMANOVA test, no significant differences were observed between the tumor and non-tumor mucosa regarding microbial composition.
Li, W.T.; et al. (Li et al., 2021)	Bioinformatics analysis	Total 405 BCa samples	MIBC	TCGA legacy archive	/			1. A variety of microbes, including *E. coli*, *butyrate-producing bacterium SM4/1*, and a species of *Oscillatoria*, were associated with expression of classical EMT-associated genes, such as E-cadherin, vimentin, SNAI2, SNAI3, and TWIST1. 2. There are significant correlations between microbial abundance and the expression of genes in the ECM, specifically collagens and elastin.
Mansour, B.; et al. (Mansour et al., 2022)	Original research	55 bladder cancer tissue samples and 12 prostatic hyperplasia tissue samples as control	/	16S rRNA	**Phylum** Firmicutes(46%) Proteobacteria (23%) Actinobacteria (13%) Bacteroidetes (11%)			1. Pronounced differences were observed in both alpha and beta microbiome diversity between the tumor (bladder cancer) and non-tumor (prostatic hyperplasia) tissue samples. 2. The microbiome β-diversity of the 32 male and 14 female tumor samples also showed significant differences. 3. The combined increase in urine HBD2 and HBD3 levels reduces the abundance of non-tumor specific genera (*Bacteroides*, *Parabacteroides*, *Faecalibacterium*) and increases the abundance of more common in-tumor tissue genera (*Staphylococcus*, *Corynebacterium*).

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TABLE 1 Continued

Author	Article type	Population	Stage	Method	High-abundance Microorganisms			Cohorts, Diversity, Abundance and Other Findings
					MIBC			
					Phylum Proteobacteria (68.45%) Firmicutes (14.76%) Actinobacteriota (12.98%)	**Genera** *Ralstonia* (56.29%) *Cutibacterium* (9.82%) *Enterococcus* (6.91%) *Sphingomonas* (5.77%) *Metamycoplasma*(4.60%)	**Species** *Ralstonia_mannitolilytica* *Ralstonia_pickettii* *Ralstonia_sp000620465*	
					NMIBC			
					Phylum Proteobacteria (39.09%) Firmicutes (19.17%) Actinobacteriota (14.92%) Firmicutes_A (13.13%) Bacteroidota (11.55%)	**Genera** *Ralstonia* (22.16%) *Cutibacterium* (6.60%) *Bacteroides* (5.51%) *Staphylococcus* (5.27%) *Acinetobacter* (5.07%)	**Species** *Acinetobacter_guillouiae* *Anoxybacillus_A_rupiensis* *Brevibacillus_agri* *Staphylococcus_lugdunensis*	
Sun, J.X.; et al. (Sun et al., 2023)	Original research	Total 22 BCa samples (mainly male)	NMIBC 7 MIBC 15	2bRAD-M				1. The microbial diversity of NMIBC tissues was significantly higher than that in MIBC tissues. The microbial composition of the two tumor tissues was similar, with *Ralstonia_sp000620465* was the most dominant species. 2. Functional annotation analysis showed 3011 different COGs and 344 related signaling pathways between MIBC and NMIBC microbiomes.

formation in the bladder exhibit oncogenic features similar to those in colorectal cancer. Establishing a causal link could make microbiome modulation a significant therapeutic area.

3.3 Microbial associations with the epithelial-mesenchymal transition in BCa development

Wei Tse Li et al. identified a range of microbes, including *E. coli*, the butyrate-producing bacterium SM4/1, and a species of *Oscillatoria*, that were correlated with the expression of classic EMT-associated genes, such as E-cadherin, vimentin, SNAI2, SNAI3, and TWIST1. Notably, the *Escherichia coli str. K-12 substr.* showed the most significant correlations with EMT-related gene expression. They also observed significant correlations between microbial abundance and the expression of extracellular matrix (ECM) genes, particularly those related to collagens and elastin. The presence of the *Escherichia coli O157:H7* strain exhibited a significant correlation with the expression of ECM proteins (Li et al., 2021).

EMT is a key process in several cancers that enhances metastatic potential by increasing cell mobility and decreasing cell–cell adhesion (Franzen et al., 2015; Ogawa et al., 2020). During EMT, epithelial cells transform into mesenchymal cells, enabling detachment from the basement membrane and invasion into adjacent tissues. These mesenchymal cells can migrate to distant sites and revert back to epithelial cells through mesenchymal–epithelial transition, initiating metastasis. In MIBC, the importance of EMT is highlighted by the upregulation of mesenchymal cell markers, such as N-cadherin and P-cadherin, and the downregulation of epithelial cell markers, such as E-cadherin (Yun and Kim, 2013; Franzen et al., 2015). ECM proteins play a vital role in cancer cell invasion and metastasis, with proteins such as collagens, laminins, and fibronectins associated with survival in urothelial bladder cancer (Brunner and Tzankov, 2007). During metastasis and invasion, ECM proteins degrade and integrins rearrange, facilitating EMT (Brunner and Tzankov, 2007; Jiang et al., 2017). Microbes, known for releasing proteases including collagenases, are thought to influence ECM protein turnover, although this has not yet been confirmed in BCa studies (Alfano et al., 2016).

Interactions between tumor cells and the extracellular microenvironment are critical in cancer development and progression. The interplay between ECM components and bacterial products regulates tissue homeostasis, dysregulation of these processes may create protumorigenic niches, potentially contributing to disease relapse. Understanding the relationship between *Escherichia coli* and the extracellular microenvironment offers valuable insights into the pathogenesis and progression of BCa.

3.4 Role of *Burkholderia* in BCa pathobiology

Filippo Pederzoli and his team conducted a study on paired samples from 21 male and 8 female patients, including urine, neoplastic (Npl), and non-neoplastic (non-Npl) tissues. Their findings revealed a greater abundance of the genus *Burkholderia*

in tumor tissues than in non-tumor tissues, regardless of the patient's gender (Pederzoli et al., 2020). In a parallel study, Bassel Mansour's research group corroborated these results. They investigated microbiome differences in urine and tissue samples from BCa patients and noted a greater abundance of *Burkholderia* in cancerous tissues (Mansour et al., 2020). This observation implies a potential role for this genus in the pathobiology of BCa, similar to recent findings in colorectal cancer versus healthy colon mucosa (Alomair et al., 2018). However, the role of this taxon appears to extend beyond merely acting as a trigger for neoplasia. Recent studies have suggested *Burkholderiales* as a potential "anticancer probiotic." For instance, in an animal sarcoma model, it was demonstrated that the efficacy of immunotherapy with CTLA-4 antibody was influenced by the composition of the microbiota, particularly by *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, and *Burkholderiales* (Vétizou et al., 2015). Additionally, the transplantation of these bacteria into antibiotic-conditioned animals showed a protective effect against colitis induced by CTLA-4 blockade.

4 Phylum Cyanobacteria

Cyanobacteria, also known as blue-green algae, are an ancient, highly diverse group of photoautotrophic organisms, that evolve a wide variety of morphologies reaching from unicellular to filamentous organization and thereby represent one of the most diverse prokaryotic phyla. In the past, they were frequently grouped under algae; however, Cyanobacteria exhibited significant distinctions from eukaryotic organisms. Devoid nuclear membranes and organelles and, with their genetic material, DNA that is not organized into chromosomes are characteristic features of bacteria. Consequently, they are now classified within the domain Bacteria (Schirrmeister et al., 2011). Cyanobacteria are considered the inventors of oxygenic photosynthesis, they employ photosynthetic pigments such as carotenoids, phycobilins, and various forms of chlorophyll to capture light energy (Sagan, 1967). Apart from performing oxygenic photosynthesis, many cyanobacteria are able to fix atmospheric nitrogen, adding to their importance in natural ecosystems (Welsh et al., 2008). Cyanobacteria serve as important model organisms with potential biotechnological applications. They are utilized in bioethanol production, edible pigments, human and animal food, nutritional supplements, and raw materials (Tan, 2007; Demay et al., 2019). Cyanobacteria are also known to produce various toxins, termed cyanotoxins, which may pose risks to humans and animals (van Apeldoorn et al., 2007).

4.1 *Oscillatoria* and EMT-associated gene expression

Wei Tse Li et al. discovered a strong negative correlation between the presence of *Oscillatoria*, a Cyanobacteria member, and the expression of EMT-promoting genes (Li et al., 2021). Although Cyanobacteria are less characterized in humans, their presence has been detected in the gut (Almeida et al., 2019). Importantly, *Oscillatoria* species produce butylated hydroxytoluene, a natural antioxidant, which might explain its association with reduced EMT (Babu and Wu, 2008). This connection is significant as oxidative stress and reactive oxygen species are known to regulate ECM proteins and the EMT process (Jiang et al., 2017).

4.2 Cyanobacteria in BCa: microbiota composition and human β-defensins response

Bassel Mansour et al. compared the microbiota composition in cancerous tissues and urine samples from the same patient set. They noted a surprisingly high presence of Cyanobacteria—7% and 8% in urine and tissue samples, respectively—a finding previously undocumented (Mansour et al., 2020).

Mansour, Ádám Monyók, and their team subsequently investigated the tissue microbiome in BCa patients compared to that in benign prostatic hyperplasia patients and healthy volunteers. The authors focused on the mRNA levels of hBDs in tissue and the levels of defensin in urine. *Oxyphotobacteria*, a Cyanobacterium genus linked to tumor formation (Miao et al., 2016), exhibited a significantly greater abundance in the BCa group, especially in patients with low urinary hBD-1 levels (Mansour et al., 2022).

Defensins, notably hBDs, are critical antimicrobial peptides that play a role in tumor cell dissolution, immune cell attraction, and interactions with complement factors (Adyns et al., 2023). Human β-Defensin-1 (hBD-1) can modify HER2 signal transduction and has been shown to suppress BCa growth (Sun et al., 2019). The ability of hBD-1 to attract cells expressing C-C chemokine receptor 6 (CCR6) suggests its potential for recruiting immune cells, such as Th cells, Tregs, imDCs, and neutrophils (Yang et al., 1999; Biragyn et al., 2001; Wu et al., 2003). The dual antitumor and antibacterial activities of hBD-1, along with the influence of bacterial presence on the production of hBD-2 and hBD-3 (Adyns et al., 2023), highlight the significance of understanding the role of hBDs in antitumoral immune responses and their potential impact on immunotherapy effectiveness.

5 Phylum Firmicutes

Generally, the phylum Firmicutes is recognized for comprising low G + C gram-positive microorganisms characterized by rigid or semi-rigid cell walls containing peptidoglycan. The cells of Firmicutes microorganisms typically take the form of rods or spheres and primarily reproduce through binary fission. Some members can form endospores and exhibit motility facilitated by flagella (Seong et al., 2018). Firmicutes are abundant in soil and aquatic environments and play a crucial role in the decomposition and recycling of organic matter (Baik et al., 2008). Nevertheless, certain genera within this phylum constitute normal flora in the mammalian intestine or act as pathogens for humans, animals, and plants (Lee et al., 2009; Nguyen and Götz, 2016). Additionally, some members of the Firmicutes phylum hold industrial significance for their role in the production of antibiotics, enzymes, and dairy products (Kwak et al., [[NoYear]]; Liu et al., 2012).

5.1 Lactic acid bacteria (LAB) in BCa

LAB, a group of gram-positive bacteria within the phylum Firmicutes, are primarily categorized into the genera *Lactobacillus*, *Streptococcus* and *Lactococcus* (Sharma et al., 2020).

Fei Liu's research indicated a notable difference in the abundance of *Lactobacillus* between cancerous and noncancerous tissues, with a greater prevalence in noncancerous tissues (Wang et al., 2007). *Lactobacillus*, a well-studied probiotic, is known for its health-promoting mechanisms, such as colonizing resistance, acid production, and pathogen exclusion. It has also been linked to cancer, as cell-free supernatants from *Lactobacillus* can reduce the invasion ability of metastatic tumor cells *in vitro* (Siddiqui et al., 2012; Pearce et al., 2014). The predominant strain *Lactobacillus* in women's bladders and its potential protective role against urothelial bladder cancer have not been determined, although there is evidence suggesting its role in reducing chronic inflammation and enhancing immune responses (Kato et al., 1984; Kato et al., 1988; Takagi et al., 2001; Matsumoto et al., 2009; Shida and Nomoto, 2013).

5.2 *Staphylococcus* and its relationship with BCa

Wei Li's study sequenced and analyzed bacterial genomes from cancer and adjacent tissues of 18 BCa patients with no urinary tract infection and negative urine culture. The research has revealed the presence of bacteria in various bladder layers, predominantly in the outer layer of the mucosa. Linear discriminant analysis Effect Size (LEfSe) revealed significant differences in microbial communities, highlighting *Staphylococcus* at the genus level as significantly more abundant in BCa tissues. This finding aligns with multiple cases of *Staphylococcus* in the urine cultures of BCa patients (Li, 2021). Similarly, Bassel Mansour's team noted a greater abundance of *Staphylococcus* in tumor tissues, correlating with elevated levels of hBD-2 and hBD-3 in urine (Mansour et al., 2022). The inducible production of antibacterial HBD2 and HBD3 is affected by bacteria. Elevated levels of HBD2 were shown to cause treatment failure in anticancer immunotherapy (Adyns et al., 2023). *Staphylococcus*, particularly saprophytic *Staphylococcus*, is commonly associated with urinary tract infections. Its role in chronic infections and potential link to the development of BCa warrant further investigation. The impact of saprophytic *Staphylococcus* on bladder epithelial and cancer cells could be a valuable direction for future research.

6 Phylum Actinobacteria

In terms of the number and variety of identified species, the phylum Actinobacteria represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria (Stackebrandt et al., 1997). Actinobacteria display a wide variety of morphologies, ranging from coccoid or rod-coccoid to fragmenting hyphal forms or permanent and highly differentiated branched mycelia. They also exhibit diverse physiological and metabolic properties, including the production of extracellular enzymes and the formation of a wide variety of secondary metabolites. Many of these secondary metabolites are potent antibiotics (Ventura et al., 2007).

6.1 Actinobacteria: diverse roles in BCa dynamics

Since 1976, BCG (*Mycobacterium bovis*, Bacillus Calmette–Guérin) has been used as one of the most successful antitumor immunotherapies, particularly for the treatment of BCa (Morales et al., 2017). This specific strain from the Actinobacteria phylum and *Mycobacterium* genus not only activates the immune system against BCa but also directly induces tumor cell apoptosis (Yu et al., 2015), necrosis (See et al., 2009), or oxidative stress (Shah et al., 2014). The lower incidence of BCa in women might be partially explained by the significantly greater presence of Actinomycetes, including the *Mycobacterium* genus, in the female urinary microbiome (Lewis et al., 2013).

Mónica Parra-Grande et al. observed a significantly greater abundance of Actinobacteria in nonneoplastic bladder mucosa than in tumor tissues ($P = 0.014$). Although not statistically significant at the genus level, *Propionibacterium* was more abundant in the non-tumor mucosa ($P = 0.08$) (Parra-Grande et al., 2022). *Propionibacterium freudenreichii*, notable for its probiotic potential and commercial relevance, has been suggested to be a protective agent against colorectal cancer. Research by Casanova et al. indicated that this bacterium could be useful as a probiotic for early-stage colorectal cancer prevention (Casanova et al., 2018). This finding supports the hypothesis that a microbiota rich in Actinomycetes might be linked to the lower incidence of BCa in women (Raoult, 2017), suggesting that a preventive effect similar to that of the BCG vaccine (composed of Actinomycetes), known for its protective role in BCa treatment and relapse prevention (Whiteside et al., 2015b).

Conversely, the research team led by Bassel Mansour and Ádám Monyók reported a greater abundance of another Actinobacteria phylum member, the *Corynebacterium* genus, in tumor specimens than in non-tumor specimens. This increased presence correlated with elevated levels of hBD-2 and hBD-3 in the urine (Mansour et al., 2022), suggesting a possible role of *Corynebacterium* in BCa pathogenesis.

6.2 Health-promoting bacterium: *Bifidobacterium* in BCa

The *Bifidobacterium* genus is a strictly anaerobic, gram-positive, pleomorphic rod-shaped bacterium that belongs to the Actinobacteria phylum. Owing to their morphological and physiological similarities with *Lactobacillus*, they were historically classified as members of the *Lactobacillus* genus for a significant portion of the 20th century. Only recently have they been

acknowledged as a distinct genus separate from *Lactobacillus* (Turroni et al., 2011). Many bifidobacteria are used as active ingredients in a variety of so-called functional foods due to their perceived health-promoting or probiotic properties, such as protection against pathogens mediated through the process of competitive exclusion, bile salt hydrolase activity, immune modulation, and the ability to adhere to mucus or the intestinal epithelium (Liévin et al., 2000; Ouwehand et al., 2002; Stanton et al., 2005).

Mónica Parra-Grande et al. reported an increased abundance of *Bifidobacterium* in NMIBC tissues (Whiteside et al., 2015b). *Bifidobacterium* plays a crucial role in microbial homeostasis and anti-inflammatory responses in the mucosa (Di Giacinto et al., 2005; Turroni et al., 2011), and can inhibit IL-6 secretion (Trikha et al., 2003), indicating its possible influence on tumorigenesis. Vyara Matson's study revealed a link between enriched *Bifidobacterium* and improved immunotherapy response in melanoma patients (Morales et al., 2017). Parra-Grande's findings suggest that a lower abundance of *Bifidobacterium* in MIBC than in NMIBC could indicate bladder mucosal damage and a protective factor against BCa (Parra-Grande et al., 2022).

7 Phylum Bacteroidetes

The phylum Bacteroidetes consists of more than 7000 different gram-negative bacteria, primarily falling within the genera *Bacteroides*, *Prevotella*, *Parabacteroides*, and *Porphyromonas* (Wexler, 2007). Bacteroidetes, particularly species within the *Bacteroides* and *Prevotella* genera, are major degraders of complex carbohydrates, and possess a variety of polysaccharide and glycoside hydrolases capable of breaking down polysaccharides. They play a crucial role in the breakdown of dietary fiber and starch, release energy, and may act as a primary source of propionate (Tuson et al., 2018). Bacteroidetes are actively involved in immunomodulation, with certain members contributing to the suppression of inflammatory activities, while others may promote inflammation, and some are recognized as opportunistic pathogens. Furthermore, the phylum Bacteroidetes plays a role in the regulation of metabolic syndrome and the gut-brain-axis, with intriguing therapeutic implications for mood impairment and neurologic disorders (Gibiino et al., 2018).

7.1 Implications of *Prevotella_9* for cancer research

In their study, Fei Liu et al. reported that *Prevotella_9* was more prevalent in non-cancerous tissues (Wang et al., 2007). *Prevotella*, an integral part of the gut microbiome, plays a crucial role in the digestion of fiber and complex carbohydrates. Its abundance is closely linked to dietary habits, particularly when it is high in fiber. Members of the *Prevotella* genus have been implicated in various cancers, including colorectal, oral, and stomach cancers (Feng et al., 2023). For instance, an increase in *Prevotella* has been noted in the gut microbiome of colorectal cancer patients (Bonnet et al., 2014). Some *Prevotella* species may contribute to cancer development by affecting the tumor microenvironment, for example, through modulating local immune responses or producing metabolic byproducts. There is also evidence suggesting that *Prevotella* may impact the host immune system, potentially playing a role in tumor immune evasion (Chen et al., 2022). While the general research on *Prevotella* highlights its potential significance in cancer development, additional studies are needed to elucidate the specific role and mechanisms of *Prevotella_9* in cancer. The greater abundance of *Prevotella_9* in non-cancerous tissues, as found by Fei Liu, might indicate distinct characteristics and roles of this subtype within the *Prevotella* genus.

8 Others

8.1 Diversity and discrepancies in BCa microbiota studies

Several studies included in this summary have conducted comparative analyses of BCa tissues, adjacent normal tissues, or non-tumor tissue samples. Bassel Mansour's team highlighted significant differences in microbial composition between tumor and non-tumor tissue samples (Mansour et al., 2022). Fei Liu's team found that, compared with noncancerous tissues, cancerous tissues had lower species richness and diversity, with notable differences in beta-diversity (Wang et al., 2007). Similarly, Mónica Parra-Grande's team observed greater microbial diversity in non-tumor bladder mucosa than in tumor tissues, aligning with global indicators of microbiome diversity and richness (Parra-Grande et al., 2022). However, Rebecca M. Rodriguez's team reported no significant statistical differences in total reads, relative abundance, or positivity ratio between paired tumor and adjacent normal samples (Rodriguez et al., 2020), a finding echoed by Wei Li et al (Li, 2021).

Regarding microbiota variations in BCa tissues of different stages and grades, Mónica Parra-Grande's team noted significant differences in microbial composition among different tumor grades (Parra-Grande et al., 2022). Jian-Xuan Sun's team reported similar microbial compositions in MIBC and NMIBC tumor tissues, but with notably greater microbial diversity in NMIBC tissues (Sun et al., 2023). These findings suggest a potential link between the microbiome and tumor biology. Conversely, Bassel Mansour et al. observed no differences in beta-diversity across different tumor grades and stages or in relation to diabetes and hypertension (Mansour et al., 2022). Similarly, Rebecca M. Rodriguez et al. reported no significant differences when paired tumor and normal tissues were stratified according to sex, race, anatomical site, or tumor stage (Rodriguez et al., 2020).

In the context of sex differences, Bassel Mansour et al.'s studies indicated significant diversity variations between male and female tissue samples (Mansour et al., 2020; Mansour et al., 2022), whereas Filippo Pederzoli et al.'s research showed no differences in alpha- and beta-diversity based on sex (Pederzoli et al., 2020). The study of the BCa tumor microbiota is a burgeoning field, with discrepancies

among studies highlighting the need for larger sample sizes and further research.

9 Discussion

By reviewing the current research on the intratumoral microbiota of BCa, we identified potential mechanisms through which microorganisms can impact the onset, progression, and prognosis of this disease. Possible mechanisms that may promote Bca include: *Cupriavidus*, which may lead to the enrichment of metabolizable harmful chemical products within the bladder, inducing protein carbonylation and oxidative DNA damage in human urothelial cells. Biofilms may augment the production of extracellular proteases, degrade intercellular junctions in conjunction and cause mutagenic DNA damage. *Escherichia coli* may exhibit a significant correlation with the expression of EMT-related genes and ECM proteins. *Burkholderi* may play a role contrary to other studies where it acts as an "anticancer probiotic" affecting the immune therapeutic effects of CTLA-4 antibodies. *Oxyphotobacteria* may influence the levels of hBD-1 in urine, affecting its anticancer effect on the HER2 signaling pathway and its antimicrobial effect on recruiting immune cells. *Corynebacterium* and *Staphylococcus* may be associated with the elevated levels of hBD-2 in urine, leading to failure of anticancer immunotherapy. Besides, *Staphylococcus* may play a potential role in chronic urinary tract infections, increasing the risk of bladder cancer.

Moreover, there are potential anticancer mechanisms, for example, *Oscillatoria* may negatively regulate the EMT and ECM processes by producing a natural antioxidant. *Lactobacillus* may reduce chronic inflammation, enhance immune responses and reduce the invasion ability of metastatic tumor cells. *Bifdobacterium* may play a role in microbial homeostasis, anti-inflammatory responses of the mucosa, and may inhibit IL-6 secretion. *Propionibacterium* may exert a protective role in the treatment and prevention of bladder cancer recurrence, similar to the effects of the BCG vaccine. *Prevotella_9* may play a role contrary to what is observed in other cancers, where it influences the host immune system and participates in immune escape in tumor immunity (Figure 2).

Simultaneously, we have contemplated several questions: How do microorganisms enter BCa tumors and in what form? What role does the intratumoral microbiota play in antitumor immunity? Does it have a positive, negative, or surveillance effect, and to what extent is it an instigator, collaborator, or passive observer? Is the intratumoral microbiota detected in BCa a result of purposeful selection by the human body? Does the intratumoral microbiota influence the neural innervation of BCa tumors? What is the extent of the association between the intratumoral microbiota and the recurrence and prognosis of BCa? What kinds of interactions occur among different intratumoral microbiota groups within the tumor? These unresolved questions may lead to new research ideas and directions in the future.

Furthermore, we have identified several limitations that impede research progress. First, ethical considerations prevent the use of healthy samples as controls, thereby hindering comprehensive comparisons. Second, many studies often feature small sample sizes, and lack strong representativeness of the broader population. Third, ensuring complete sample contamination-free processes, such as collection, transportation, storage, and detection, poses a significant challenge, as contamination can potentially impact experimental results. Fourth, different surgical procedures may introduce intergroup differences. Fifth, the small volume of tumor tissue, low bacterial biomass within the tumor, and uncultivable characteristics of some microbial species increase the difficulty of detection. Moreover, tumor samples exhibit a very high host-to-bacterial DNA ratio, potentially causing bias in amplicon-based sequencing results. Additionally, the intrinsic limitations of amplicon-based microbiome methods and incomplete reference

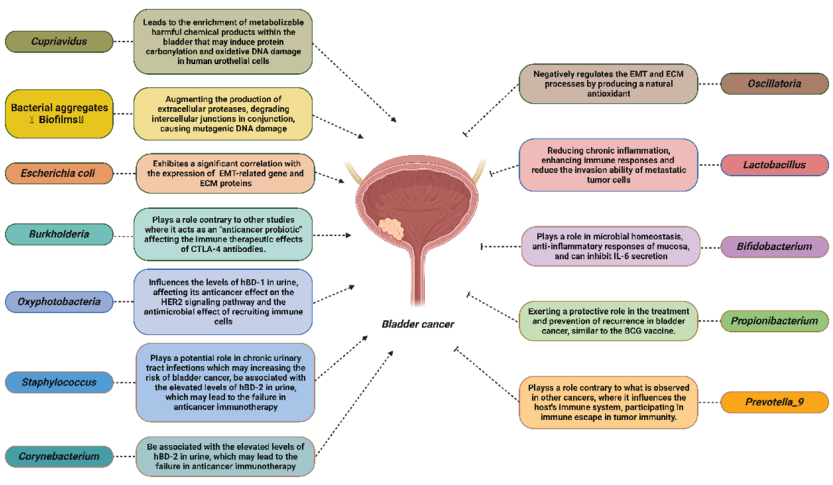

FIGURE 2
Potential mechanisms of different intratumoral microbiota in the onset, progression, metastasis, prognosis and other aspects of bladder cancer.

databases decrease the extrapolation reliability of these methods. Patient-specific factors such as age, race, sex, breastfeeding status, diet, socioeconomic status, epidemiological factors, genetics, exposure to environmental carcinogens, or disease-specific factors such as pathological TNM stage and focality remain uncontrollable variables. Finally, potential bias in samples introduced by previous BCa diagnostic procedures (e.g., cystoscopy) or prior antibiotic therapies (>4 weeks) cannot be excluded, although the impact of these procedures on the bladder and urinary microbiome is currently unclear.

There are still significant gaps in clinical and laboratory research on the intratumoral microbiota of BCa, and there are several contradictory conclusions among different studies. Addressing these challenges and undertaking innovative or in-depth explorations in this field remains a long journey for researchers. In the future, delving into the molecular mechanisms of the microbiota's role in BCa tumors, identifying new treatment targets and biomarkers, and exploring how to manipulate the BCa intratumoral bacterial community for cancer patient treatment are crucial. This finding suggests that the biological contribution of the microbiota to BCa is likely to occupy an increasingly prominent position in future BCa research.

10 Conclusions

In conclusion, this article reviews the existing research on the microbiota within BCa tumors, summarizes the findings regarding the roles of different microbes in various aspects of this disease and reflects on the current challenges and future research directions in this field. It is hoped that this study will provide effective assistance for a better understanding of BCa and offer some ideas for further innovative development of the field of intratumoral microbiota in BCa.

Author contributions

KL: Data curation, Formal analysis, Software, Writing – original draft. JC: Methodology, Software, Writing – original draft. JW: Investigation, Methodology, Writing – original draft. JM: Project administration, Resources, Writing – original draft. SL: Supervision, Validation, Writing – original draft. YC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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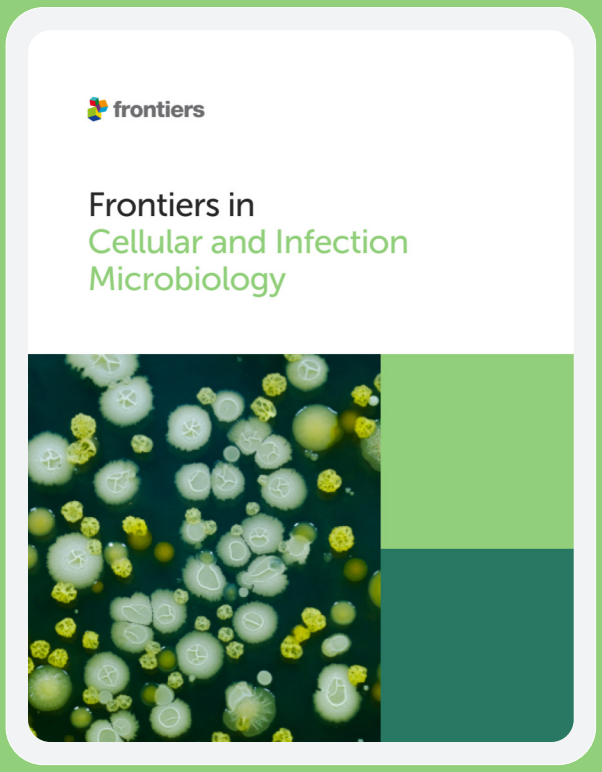

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