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Reviews in the impact of gut microbiota in health and disease

Edited by
Junling Shi, Karolina Skonieczna-Żydecka and Muhammad Shahid Riaz Rajoka

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Reviews in the impact of gut microbiota in health and disease

Topic editors

Junling Shi — Northwestern Polytechnical University, China

Karolina Skonieczna-Żydecka — Pomeranian Medical University, Poland

Muhammad Shahid Riaz Rajoka — Tohoku University, Japan

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Yongqun Oliver He,
University of Michigan, United States

*CORRESPONDENCE
Junling Shi
✉ sjlshi2004@nwpu.edu.cn

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Editorial: Reviews in the impact of gut microbiota in health and disease

Junling Shi*

School of Life Sciences, Northwestern Polytechnical University, Xi'an, China

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Editorial on the Research Topic
Reviews in the impact of gut microbiota in health and disease

This topic consists of 15 articles, authored by scholars from 17 different countries, such as China, Canada, Italy, Tunisia, France, the United States, Romania, Spain, Turkey, Pakistan, Croatia, Slovenia, Poland, Brazil, South Korea, Bydgoszcz, and South Africa.

Up to now, the articles have been downloaded 5,241 times and viewed over 23,000 times. The most frequently viewed article is "Human gut microbiota in health and disease: Unveiling the relationship," which has received 5,423 views.

The published reviews predominantly focus on two aspects: the pathogenic effects of gut microbiota on various diseases, and the application of gut microbiota in therapy. Eleven articles reviewed the correlation between different diseases, including infectious diseases, cerebrovascular diseases, diabetes, Parkinson's disease, and rosacea. These reviews emphasized the pathogenic effects of specific bacteria, such as Helicobacter pylori and Streptococcus. Additionally, an emerging neurometabolic facet of the gut microbiome, known as neuromicrobiology, was highlighted. The methodologies utilized in these reviews included manual summary and analysis, as well as data analysis and visualization using software such as Microsoft Excel 2020, VOSviewer, CiteSpace 5.8.R3, and Co-Occurrence 9.94. Metabolomic analysis was recommended as a powerful tool for exploring the mechanisms underlying the functions of prebiotics and probiotics in the gut health of poultry.

The correlation between gut microbiota and infectious diseases can be explained through the perspective of the human immunological response. The reviews made by Maciel-Fiuza et al. and Afzaal et al., summarized the association between the gut microbial community and the development and progression of various infectious and inflammatory diseases. They also discussed the mechanisms by which disease development is correlated with gut microbiota, specifically focusing on the human immunological response.

Xu et al. summarized the role of the gut microbiome and its metabolites in cerebrovascular diseases. They identified specific gut microbiota and downstream-related metabolites that not only participate in various physiological processes of the human body but also directly or indirectly affect the occurrence and development of cerebrovascular diseases through systemic inflammatory immune response. They further discussed the molecular mechanisms through which gut microbial metabolites regulate the expression of specific interleukins in inflammatory immune responses.

The link between gut microbiota and diabetes has been extensively researched and confirmed globally. Zhang et al., through bibliometrics and visualized studies on publications from 2001 to 2021, found that the understanding of the physiology and pathology of diabetes has been deepened through the lens of gut microbiota.

Papić et al. reviewed the accumulating evidence supporting the identification of microbiota as a potential factor in the earliest, prodromal phases of Parkinson's disease. However, they noted that the link between gut microbiota and neurodegeneration is complex and dependent on various factors. Further research is needed to focus on the metabolic function of gut microbiota in relation to not only motor but also non-motor symptoms of this disease.

Cai et al. reviewed the relationship between gut microbiota and male reproduction. They highlighted how gut microbiota supports male reproduction through nutrition, immunity, and signaling by producing key molecules. They also discussed how gut microbiota helps maintain the integrity of the testes and regulates testicular immunity to protect the spermatogenic environment.

Zhu et al. emphasized the important role of both skin microbiota and intestinal microbiome in rosacea. They indicated *Demodex folliculorum*, *Staphylococcus epidermidis*, *Bacillus oleronius*, *Cutibacterium acnes*, and *Helicobacter pylori* had been identified as pathogens associated with the development of rosacea. Antibiotics and probiotics are commonly used in clinical treatment, and the mechanisms of these treatments were also introduced.

Neuromicrobiology, an emerging aspect of the gut microbiome, highlights the production of neuroactive metabolites by the gut microbiota, particularly neurotransmitters and their precursors. These metabolites stimulate the local nervous system, including the enteric and vagus nerves, which in turn influence brain function and cognition. Miri et al. discussed microbiome-targeted interventions as promising adjunctive treatments using pre-, pro-, post-, and synbiotics. They reviewed the major classes of microbial neuroactive metabolites and emphasized their effects on the microbiome, gut environment, and brain. The authors also discussed the biosynthesis, absorption, and transport of gut microbiota-derived neuroactive metabolites to the brain, as well as their implications in mental disorders.

In addition to the correlation between gut microbiome and diseases, specific pathogens can also play a significant role in the occurrence and development of diseases. *Helicobacter pylori*, as a widely recognized pathogen, has been associated with various gastric diseases, including gastric ulcers, chronic progressive gastritis, and gastric cancer. He et al. elucidated the potential pathogenic role of *H. pylori* in COVID-19, atherosclerosis, hyperemesis gravidarum, and other extragastric diseases. The possible pathogenic mechanisms may involve chronic systemic inflammation and molecular mimicry. Zi et al. summarized the relationship between *Streptococcus* and gastric cancer, as well as the possible carcinogenic mechanisms of *Streptococcus*.

Furthermore, gut microbiota has been explored for its potential therapeutic applications. Four articles discussed the application of gut microbiota in therapy. Fecal microbiota transplantation (FMT) emerged as the most widely used method. The production of butyrate by gut bacteria and the role of *Akkermansia muciniphila* as therapeutic agents were extensively studied. Additionally, "athletic microbiome" is emerging as potential application in therapy.

Wang et al. conducted a bibliometric and visualization study on global research trends and hotspots regarding fecal microbiota transplantation. They identified a total of 57 hotspots related to FMT. Singh et al. suggested that butyrate producers have potential as microbial therapeutics. They explained that these producers generate butyrate from carbohydrates through the butyryl-CoA: acetate CoA-transferase pathway and butyrate kinase terminal enzymes, as well as from amino acids via glutamate and lysine pathways. Butyrate acts as an energy source for colonocytes and maintains an anaerobic environment in the gut. It also helps maintain gut barrier integrity, limit pro-inflammatory cytokines, and inhibit oncogenic pathways. Additionally, colonic butyrate producers shape the gut microbial community by secreting various antimicrobial substances and maintain gut homeostasis by releasing anti-inflammatory molecules.

Akkermansia muciniphila is considered a promising "next-generation beneficial microbe." Li et al. conducted a comprehensive review on this bacterium, which has been extensively studied worldwide since 2004. Clinical uses of *A. muciniphila* have increased over time, and research has been deepened and developed to a more precise level. Oxidative stress has been a prominent focus in related studies.

The concept of the "athletic microbiome" has recently emerged to highlight the potential role of microbiomics in swimmers. As reviewed by Puce et al., training volume/intensity can influence the athlete's microbiome, particularly the non-core or peripheral microbiome, in terms of its architecture, composition, richness, and diversity. Power-/sprint- and endurance-oriented activities, acute and chronic exercise, and anaerobic/aerobic energy systems have differential impacts on the athlete's microbiome. Exploiting microbiomics may have clinical implications, such as assessing the effects of exposure to swimming pools and developing potential pharmacological strategies to address skin infections and inflammation, including acne.

In conclusion, the published articles provided recently reported results on the correlation between gut microbiome and different diseases, as well as the mechanisms and potential application in therapy. The articles may provide useful information for further studies.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of interest

The author declares 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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EDITED BY
Muhammad Shahid Riaz Rajoka,
Tohoku University, Japan

REVIEWED BY
Mohsin Khurshid,
Government College University,
Faisalabad, Pakistan
Samad Azari,
Iran University of Medical Sciences,
Iran

*CORRESPONDENCE
Nicola Luigi Bragazzi
bragazzi@yorku.ca

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Swimming and the human microbiome at the intersection of sports, clinical, and environmental sciences: A scoping review of the literature

Luca Puce[1], Jarrad Hampton-Marcell[2,3], Khaled Trabelsi[4,5], Achraf Ammar[6,7,8], Hamdi Chtourou[4,9], Ayoub Boulares[10], Lucio Marinelli[1,11], Laura Mori[1,11], Filippo Cotellessa[1,11], Antonio Currà[12], Carlo Trompetto[1,11] and Nicola Luigi Bragazzi[13]*

[1]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, Italy, [2]Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, United States, [3]Biosciences Division, Argonne National Laboratory, Lemont, IL, United States, [4]Institut Supérieur du Sport et de l'Éducation Physique de Sfax, Université de Sfax, Sfax, Tunisia, [5]Research Laboratory: Education, Motricité, Sport et Santé, EM2S, Sfax University, Sfax, Tunisia, [6]Department of Training and Movement Science, Institute of Sport Science, Johannes Gutenberg-University Mainz, Mainz, Germany, [7]Institute of Sport Science, Otto-von-Guericke University Magdeburg, Magdeburg, Germany, [8]Interdisciplinary Laboratory in Neurosciences, Physiology and Psychology: Physical Activity, Health and Learning (LINP2), Université Paris Lumières, Paris Nanterre University, Nanterre, France, [9]Activité Physique, Sport et Santé, UR18JS01, Observatoire National du Sport, Tunis, Tunisia, [10]Higher Institute of Sports and Physical Education of Ksar-Said, University of Manouba, Tunis, Tunisia, [11]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ospedale Policlinico San Martino, Genoa, Italy, [12]Department of Medical-Surgical Sciences and Biotechnologies, A. Fiorini Hospital, Sapienza University of Rome, Latina, Italy, [13]Laboratory for Industrial and Applied Mathematics, Department of Mathematics and Statistics, York University, Toronto, ON, Canada

The human microbiota is comprised of more than 10–100 trillion microbial *taxa* and symbiotic cells. Two major human sites that are host to microbial communities are the gut and the skin. Physical exercise has favorable effects on the structure of human microbiota and metabolite production in sedentary subjects. Recently, the concept of "athletic microbiome" has been introduced. To the best of our knowledge, there exists no review specifically addressing the potential role of microbiomics for swimmers, since each sports discipline requires a specific set of techniques, training protocols, and interactions with the athletic infrastructure/facility. Therefore, to fill in this gap, the present scoping review was undertaken. Four studies were included, three focusing on the gut microbiome, and one addressing the skin microbiome. It was found that several exercise-related variables, such as training volume/intensity, impact the athlete's microbiome, and specifically the non-core/peripheral microbiome, in terms of its architecture/composition, richness, and diversity. Swimming-related power-/sprint- and endurance-oriented activities, acute bouts and chronic exercise, anaerobic/aerobic energy systems have a differential impact on the athlete's microbiome.

Therefore, their microbiome can be utilized for different purposes, including talent identification, monitoring the effects of training methodologies, and devising *ad hoc* conditioning protocols, including dietary supplementation. Microbiomics can be exploited also for clinical purposes, assessing the effects of exposure to swimming pools and developing potential pharmacological strategies to counteract the insurgence of skin infections/inflammation, including acne. In conclusion, microbiomics appears to be a promising tool, even though current research is still limited, warranting, as such, further studies.

KEYWORDS

microbiome, swimming, sports microbiomics, clinical microbiomics, scoping review

Introduction

The human microbiota is comprised of more than 10–100 trillion microbial (bacterial, and non-bacterial, such as archaeal, viral, fungal, eukaryal, and parasitical) *taxa* and symbiotic cells (Ursell et al., 2012), the majority of which reside in the gut (Thursby and Juge, 2017). The human microbiome, a term coined by Dr. Joshua Lederberg in 2001, is the comprehensive catalog of genes harbored by these microbial communities (Lederberg and McCray, 2001; Liu, 2016): more than three million genes constitute the intestinal microbiome. Reflecting the mixture of microbes and the diversity of the microbial ecosystem, this consists of several components or compartments (Matijašic et al., 2020): namely, the bacteriome (Donaldson et al., 2016), the archaeome (Borrel et al., 2020), the virome (Liang and Bushman, 2021), the mycobiome (Chin et al., 2020), the eukaryome (Hamad et al., 2016), and the parasitome (Marzano et al., 2017).

Two major human sites that are host to microbial communities are the gut and the skin (De Pessemier et al., 2021). Both microbiomes are extremely heterogeneous, dynamic, and plastic, consisting of a highly diverse population of microbes that can have both beneficial and detrimental impacts on human health (Ogunrinola et al., 2020). In particular, the gut microbiome is composed of more than 1,200 species of bacteria (Jandhyala et al., 2015), including *Bacteroides*, *Actinomycetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*. It plays different immunometabolic functions, ranging from nutrient absorption (in particular, micro-nutrient uptake), and processing to vitamin synthesis, energy harvest, and metabolic homeostasis (including promoting and favoring insulin sensitivity), and fine-tuning/modulation of the immune system and of the inflammatory response at the host level, protecting especially newborns from respiratory and intestinal infections and pathogen invasion (Belkaid and Hand, 2014). It can also provide the individual with sources of energy, by fermenting and processing short-chain fatty acids (SCFAs), like butyrate, acetate, and propionate (den Besten et al., 2013; Portincasa et al., 2022).

The skin microbiome is complex, dynamic, and heterogeneous as well (Stacy and Belkaid, 2019). Skin represents the body's first line of defense against invading microorganisms. The skin microbiome has been shown to provide immunity against exogenous bacterial colonization (Byrd et al., 2018). Some environmental (terrestrial, marine, and freshwater) exposures, including, for instance, recreational water exposures, may alter the skin microbiome and potentially induce skin infections (Nielsen and Jiang, 2019; Patra et al., 2020).

Physical exercise has favorable effects on the structure of gut microbiota and metabolite production in sedentary subjects (Cella et al., 2021; Clauss et al., 2021). The body of currently available evidence is mostly from animal studies: microbial community architecture has been found to exert beneficial effects in terms of microbial composition, structure, richness, and diversity, favoring and promoting the establishment of commensal bacteria, and an anti-inflammatory *milieu* and counteracting/mitigating against pro-inflammatory effects, and optimizing performance-related outcomes. Moreover, it can interact with diet and other lifestyles to further enhance performance (Donati Zeppa et al., 2019; Cella et al., 2021). Of note, alterations in the microbiome can also be a consequence of sports and physical activity (such as swimming) (Barton et al., 2018; Mohr et al., 2020).

Recently, the concept of "athletic microbiome" (Barton et al., 2018; Mohr et al., 2020) has been introduced. Whereas some reviews have synthesized current state-of-art concerning endurance exercise (Mach and Fuster-Botella, 2017) and competitive sports (Wegierska et al., 2022), to the best of our knowledge, there exists no review specifically addressing the potential role of microbiomics for swimmers, since each sports discipline requires a specific set of techniques, training protocols, and interactions with the athletic infrastructure/facility (in this case, the swimming pool)

(Xu et al., 2022). Research has shown that swimming can exert a plethora of regulatory effects on the microbiome, in terms of immunometabolic and neuroimmunological ones, as demonstrated by a number of animal studies (Huang et al., 2019; Xie et al., 2022). However, little is known about the impact of training protocols on the microbiome among swimmers and whether adjustments in an athletic program impact overall changes in the gut microbiome in swimmers, with a particular focus on high-level/elite athletes. Also, there is a lack of prospective, longitudinal studies on the temporal changes and trends at the microbiome level. Therefore, to fill in this gap of knowledge, the present scoping review was undertaken.

Materials and methods

We devised the present review as scoping review, in that the research question was broad and intersectional, across several disciplines (sports sciences, microbiology, biotechnology, and molecular biology). A scoping review is an innovative technique to rapidly synthesize and map the literature on a designated topic in terms of major concepts, sources, and types of evidence (Arksey and O'Malley, 2005; Khalil and Tricco, 2022; Pollock et al., 2022). Several methodologies and guidelines exist: in particular, we leveraged Arksey and O'Malley's six-stage approach (Arksey and O'Malley, 2005). Firstly, we identified the research question and we built and developed our multidisciplinary team. We used the "population/participants-concepts-context" (PCC) mnemonic. "Population/participants" were athletes of any competitive level, national or international, short- or long-distance, and the main concept was about the potential applications of microbiomics within this specific sports discipline. The "context" was worldwide (our search was not confined to a particular territory/geographic location). Based on a preliminary literature search, an *a priori* protocol was devised. MEDLINE, a major scholarly, electronic biomedical database, was accessed *via* PubMed, a freely available interface. No time or language restrictions were applied. The search string consisted of two major components: microbiome and swimmers, with synonyms/variants properly linked by using Boolean operators [(microbiome OR microbiota OR "bacterial community" OR "bacterial communities" OR "bacterial flora") AND (swimming OR swimmer)]. "Medical subject headings" (MeSH) terms and wild-card (truncated words) options were used. Extensive cross-referencing was carried out. Further, specific target journals were hand-searched. Moreover, also gray literature was consulted, by mining Google Scholar. Then, studies were selected for inclusion based on pre-specified inclusion and exclusion criteria, which were formulated based both on the PCC mnemonic and the "population/participants-intervention-comparator/comparison-outcome-study design" (PICOS) components. Studies were included if focusing on a population of swimmers (P), of any competitive level, subjected to a particular training protocol (I). Studies were deemed eligible if comparing swimmers against the general population. Other comparisons of interest included gender- and age-specific comparisons or related to a particular swimming style (C). Outcomes of interest were the quantification of the changes in the microbiome, in terms of architecture/composition, richness, or diversity (O) (see **Tables 1, 2**). Any study design was eligible for inclusion: retrospective, prospective, quantitative, observational, interventional, randomized, or non-randomized (S). Included studies were synthesized in a narrative fashion. Major topics/themes were identified by means of thematic analysis and overviewed qualitatively. Furthermore, we followed the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) extension for scoping reviews (PRISMA-Scr) (Tricco et al., 2018). Finally, a formal quality appraisal was not conducted given that is not a mandatory component of scoping reviews.

Results

The initial search yielded 195 items. One hundred eighty-six studies were discarded after reading the title and/or the abstract, as they were irrelevant to the topic under study. Nine studies were screened in full text. Five studies were excluded with reason, since they did not meet our PICOS criteria (the population consisted of non-athletes). Finally, four studies were included in the present scoping review. Three of them focused on the gut microbiome, and one addressed the skin microbiome.

TABLE 1 Microbiome-related terms/expressions.

Microbiome-related term/expression	Explanation
Richness	The total number of microbial species in a given microbiome
Diversity	The amount of individual microbes from each species present in a given microbiome
Alpha diversity	A measure of microbiome diversity related to a single sample (within-sample diversity)
Beta diversity	A measure of similarity/dissimilarity of different communities/populations (between-sample diversity)

TABLE 2 Search strategy adopted in the present scoping review.

Search strategy component	Related item(s)
Searched databases	PubMed/MEDLINE, Google scholar
Search string used	(microbiome OR microbiota OR "bacterial community" OR "bacterial communities" OR "bacterial flora") AND (swimming OR swimmer)
Inclusion criteria	PCC
	Population/participants: athletes of any competitive level, national or international, short- or long-distance
	Concept: the potential applications of microbiomics within the sports discipline of swimming
	Context: worldwide
	PICOS
	Population/participants: swimmers, of any competitive level
	Intervention: any training protocol
	Comparator/comparison: swimmers against the general population; gender- and age-specific comparisons or comparison related to a particular swimming style
	Outcome(s): quantification of the changes in the microbiome, in terms of architecture/composition, richness, or diversity
	Study design: any study design (retrospective, prospective, quantitative, observational, interventional, randomized, or non-randomized)
Time restriction	None applied
Language filter	None applied

Sports microbiomics in swimmers: Effects of training and probiotic consumption

Bielik et al. (2022) sampled from a longitudinal prospective study and recruited 17 and 7 young competitive male and female swimmers, respectively, aged 16–25 years. The authors assessed the impact of a 7-week, high-intensity training (HIT) program with or without probiotic (*Bryndza* sheep-cheese) consumption (30 g, 3–4 times *per* week) on swimming performance-related outcomes during the Slovak Swimming National Championship over a long course (being the pool 50 m in length). The probiotic contains 3 microbial families, 24 *genera*, and 44 species. Total DNA was extracted from stool samples and amplified utilizing primers that specifically target the V1-V3 regions of 16SrDNA. 300 bp pair-end reads were obtained, collected, and processed. The HIT program was comprised of swimming lengths of 12.5, 25, 50, and 100 m, carried out at an intensity of > 90% of maximum speed. The authors were able to find a HIT-induced increase in alpha diversity [in terms of operational taxonomic units (OTUs), Shannon index, but not Simpson index], independently of probiotic consumption. In particular, in the HIT group, among the most represented *phyla*, *Firmicutes* decreased from 80.2 to 76.3%, whereas *Bacteroidota* and *Actinobacteriota* increased from 17.7 to 21.6% and from 0.99 to 1%. In the HIT + probiotic consumption (HITB), *Firmicutes* and *Actinobacteriota* decreased from 82.3 to 77.7% and from 2.1 to 1.1%, respectively, whilst *Bacteroidota* increased from 14.1 to 19.9%. The *phyla Proteobacteria*, *Verrucomicrobiota*, *Cyanobacteria*, *Desulfobacterota*, *Fusobacteriota*, *Fibrobacterota*, *Patescibacteria*, and *Campylobacterota* were detected with an abundance lower than 1% in the HIT group. Similarly, in the HITB group, these *phyla* (with the exception of *Fusobacteriota*, and *Fibrobacterota*) could be reported. In terms of families, the *Lachnospiraceae* family was abundant both in the HIT and HITB groups. It was found to increase in the former group (from 41.5 to 43.5%) and to decrease in the latter (from 47.6 to 45.4%). Other abundant families in both groups were *Ruminococcaceae*, *Bacteroidaceae*, *Prevotellaceae*, and *Oscillospiraceae*. Furthermore, in terms of *genera*, *Faecalibacterium*, *Blautia*, *Bacteroides*, *Roseburia*, *Subdoligranulum*, *Ruminococcus*, *Prevotella_9*, *Agathobacter*, *Coprococcus*, and the *Ruminococcus torques* group could be identified in both groups. In terms of statistical significance, *Bacteroidiota* increased in both groups (p = 0.005 in HIT, p = 0.0260 in HITB). Concerning lactic acid bacteria, the order *Lactobacillales* (p = 0.015) and the family *Streptococcaceae* (p = 0.019) were significantly different pre vs. post in the HITB group. *Lactococcus* spp. was found to be increased in both groups (p = 0.046 in HIT, p = 0.008 in HITB), with a higher effect size in the probiotic consumers (12.8-fold vs. 5-fold change). The increase in HIT was reflected in the increase in anaerobic metabolism (namely, increased concentrations of pyruvate, and lactate, and decreased levels of acetate, and butyrate) as well as in the increase of bacterial species producing SCFA metabolites, such as *Butyricimonas* (p = 0.028) and *Alistipes* (p = 0.010). The latter increased also in the HITB group, but only in a borderline fashion (p = 0.060). Finally, by means of a machine-learning approach (random forest), the authors were able to build a set of parameters (acetate, pyruvate, *Butyricimonas*, butyrate, *Bacteroidetes*, *Alistipes*, and α-diversity measured by means of the Shannon index; pyruvate, lactate, acetate, α-diversity/Shannon index, and butyrate) able to differentiate pre- vs. post-intervention in HIT and HITB, respectively, with Area under the Curve (AUC) values of 0.78 and 0.99.

Sports microbiomics in swimmers: Effects of detraining

Hampton-Marcell et al. (2020) recruited a sample of 13 (8 women and 5 men) collegiate swimmers aged 18–24 years from a Division 1 university. Microbial community small-subunit (SSU) rRNA genes were amplified using barcoded

PCR primers targeting the V4 region and barcoded SSU rRNA amplicons were, subsequently, cleaned and processed. 150 nt sequences were obtained from the pooled DNA, and 79 samples were collected, totaling 395,000 16S rRNA sequences and 7,684 OTUs. The most abundant bacterial *phyla* were *Bacteroidetes* (46.5%) and *Firmicutes* (46.6%) *phyla*, with an average ratio of *Firmicutes*: *Bacteroidetes* of 2:1 at the peak of the training program. The most represented families were *Bacteroidaceae* (39.5%), *Lachnospiraceae* (16.6%), and *Ruminococcaceae* (14.0%) over the entire study period. *Porphyromonas* (9.2%), *Sutterella* (7.9%), and unclassified *genera* within the families *Lachnospiraceae* and *Ruminococcaceae* (5.8%) were identified as the commonest *taxa*. Whilst no differences in terms of body composition and anthropometric measurements (fat mass, fat-free mass, or weight) could be computed, in terms of Bray-Curtis dissimilarity between study training phases, microbial community diversity and structure were impacted by changes in training volume and shifted 43% on average. Along with changes in beta diversity, alpha diversity changed too, positively correlating with yardage *per* week, decreasing and paralleling decreases in training volume, as quantitatively assessed utilizing both the Shannon index and community evenness (the inverse Simpson index). This ratio gradually decreased to 1:1, with the decreases in training. Detraining was reflected in reduced energy harvesting and expenditure/consumption by *Firmicutes*-derived microbes. A "core" component of the microbiome could be identified, with 82% of the OTUs being consistent over time and the different study phases, and being similar among the swimmers. Finally, two major families (*Lachnospiraceae* and *Ruminococcaceae*), and two major *genera* (*Coprococcus* and *Faecalibacterium*) robustly correlated with short-term changes in training volume.

Genetic and allelic regulation and sports microbiomics in swimmers: Correlations with performance outcomes

The GALANTL6 gene, at 4q34.1, consists of 21 exons and is expressed mainly in adult testis, brain, spinal cord, and cerebellum, as well as at the level of the skeletal muscle tissue. It encodes the enzyme polypeptide N-acetylgalactosaminyltransferase like type 6, which plays a key role in the metabolic homeostasis (specifically of lactate) and regulation of the gut microbiota *via* O-glycosylation and degradation of glycans. In particular, the gene can modulate the cycle (synthesis and resynthesis) and properties (anti-inflammatory effects) of the microbial species processing and producing SCFAs. Li et al. (2015) and Zmijewski et al. (2021) assessed the hypothesis that the T allele of the GALNTL6 rs558129 single-nucleotide polymorphism (SNP) could positively impact anaerobic metabolism and athletic performance in a sample of 147 Polish short-distance and 49 long-distance swimmers, taking part into national or international competitions. These elite athletes (aged 20.31 ± 2.67 years) were matched with 379 sedentary students, acting as controls (aged 22.6 ± 2.8 years). The study cohort was genotyped using the real-time polymerase chain reaction (real-time PCR). The SNP was in Hardy-Weinberg *equilibrium* in controls and in the entire study population. When compared to their CC homozygote counterparts, carriers of the T allele (CT + TT) displayed a 1.56 times higher likelihood of being short-distance swimmers. There was an overrepresentation of the T allele among swimmers, even though this correlation did not achieve statistical significance in long-distance swimmers. Finally, no significant effect of sex and gender could be computed.

Clinical microbiomics in swimmers

Cutibacterium acnes (*C. acnes*, formerly known as *Propionibacterium acnes*) is an opportunistic pathogen that plays a major role in the etiopathogenesis of acne. Swimmers should be protected against this dermatological disease, in that they regularly have immersion in antimicrobial chlorine, even though some reports have shown that chlorine in the pools can affect the swimmer's microbiome and metabolome (van Veldhoven et al., 2018; Morss-Walton et al., 2022). However, it is a commonly reported clinical observation that adolescent swimmers may suffer from acne and even develop standard therapies-resistant acne. Besides some potential mechanisms (such as skin dryness, and impaired barrier function) that can be hypothesized, another driver of the so-called "swimmer's acne" could be the presence of microorganisms, such as the family *Pseudomonadaceae* (a family of gram-negative *bacteria*, including *Pseudomonas aeruginosa*), associated with recreational water, hot tubs, and swimming facilities. Morss-Walton et al. (2022) investigated the microbial dynamics of *C. acnes* and *Pseudomonadaceae* pre- vs. post-swimming in a sample of 16 swimmers (8 girls and 8 boys, 75% whites), belonging to a local competitive swimming club, seven of which suffering from acne. Coproporphyrin III (CPIII), the main porphyrin produced by *C. acnes*, was measured by means of fluorescence photography to quantify the absolute abundance of the pathogen on the face of each participant. The technique of 16S rRNA gene sequencing using primers targeting the V4 region was exploited to characterize the skin microbiome, after the collection of skin swabs. CPIII fluorescence levels were found to be reduced after 1 h of swimming (p-value < 0.001), whereas the relative abundances

of *C. acnes* and of *Pseudomonadaceae* were stable (slightly increasing from 15.0 to 19.0%) and increased (p = 0.027, from 0.4 to 1.7%), respectively. The relative abundances of *Gemellales*, *Lactobacillales*, *Pasteurellales*, *Pasteurellaceae*, *Streptococcus*, and *Lautropia* significantly decreased. Of note, after swimming, alpha diversity of the skin microbiome decreased in terms of the Shannon index, the Chao1 index, and observed OTUs (p-value $<$ 0.001 for all three metrics). On the contrary, beta diversity (in terms of the OTU Bray-Curtis distance) increased after swimming. In conclusion, the authors found that decolonization and colonization of *C. acnes* and *Pseudomonadaceae* may result in skin dysbiosis and acne.

Discussion and conclusion

Microbiomics represents an emerging field (Neu et al., 2021), with increasing applications in the sports arena. Microbial metrics can well characterize an athlete's energy utilization, even when changes in physical activity levels and adjustments of training protocols do not reflect in biochemical (such as total cholesterol, insulin, or glucose) (Bielik et al., 2022), body composition and anthropometric (like fat mass, fat-free mass, or weight), or fitness measures (Hampton-Marcell et al., 2020). The human microbiome is an excellent predictor of changes in host phenotype and, more generally speaking, in phenome (Ursell et al., 2012; Neu et al., 2021), explaining up to 20% of host adaptation and related cellular/molecular phenomena, whilst the genome can explain up to less than 2% of host-related modifications.

Comprehensive sophisticated approaches, including high-throughput quantitative polymerase chain reaction (qPCR)/real-time PCR, amplicon and shotgun genomic DNA sequencing, as well as 16S rRNA gene sequencing, can be exploited to thoroughly characterize the human microbiome in athletes (Han et al., 2020).

Whereas 70–80% of the microbiome (defined as the "core microbiome") remains stable over time, the so-called non-core or peripheral microbiome is susceptible to environmental/external *stimuli* and exposures. A "core microbiome" can be defined as "any set of microbial *taxa*, or the genomic and functional attributes associated with those *taxa*, that are characteristic of a host or environment of interest" (Neu et al., 2021).

Several exercise-related variables, such as training volume/intensity, impact the athlete's microbiome, and specifically the non-core/peripheral microbiome, in terms of its architecture, composition, richness, and diversity. Swimming-related power-/sprint- and endurance-oriented activities, acute bouts, and chronic exercise, anaerobic and aerobic energy systems have a differential impact on the athlete's microbiome, specifically in the swimmers (Li et al., 2015; Hampton-Marcell et al., 2020; Zmijewski et al., 2021; Bielik et al., 2022). Therefore, their microbiome can be utilized for different purposes, including talent identification, monitoring the effects of training methodologies, and devising *ad hoc* conditioning protocols, including the administration of supplements and probiotics.

Moreover, given the marked inter-individual variability in microbial changes and shifts, microbiomics could be a valuable tool to monitor athletes' response to exercise and diet, personalizing training protocol as well as sports nutrition to enhance performance-related outcomes (Hughes, 2020; Sorrenti et al., 2020; Hughes and Holscher, 2021). Microbiomics can be exploited also for clinical purposes, assessing the effects of exposure to water facilities (swimming pools) and developing potential pharmacological strategies to counteract the insurgence of skin infections and inflammation, including acne.

In conclusion, microbiomics appears to be a promising tool to investigate the impact of training, detraining, dietary intake and supplements/probiotics use among swimmers, as well as clinical effects of interactions with swimming facilities, even though current research is still limited, warranting, as such, further studies.

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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EDITED BY
Muhammad Shahid Riaz Rajoka,
Tohoku University, Japan

REVIEWED BY
A. K. M. Humayun Kober,
Chittagong Veterinary and Animal Sciences University, Bangladesh
Ahmad Ud Din,
Sichuan University, China

*CORRESPONDENCE
Ping Li
lp25920150@126.com
Changjiang Hu
hcj888@tmmu.edu.cn

†These authors have contributed equally to this work

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Helicobacter pylori and unignorable extragastric diseases: Mechanism and implications

Junjian He[1†], Yunyi Liu[1†], Qin Ouyang[2], Rongxing Li[3], Jie Li[1], Weiyan Chen[1], Weichao Hu[1], Lijiao He[1], Qiyu Bao[1], Ping Li[4*] and Changjiang Hu[1*]

[1]Department of Gastroenterology, Xinqiao Hospital, Army Medical University, Chongqing, China, [2]Department of Medicinal Chemistry, College of Pharmacy, Army Medical University, Chongqing, China, [3]Department of Foreign Languages, Army Medical University, Chongqing, China, [4]Institute of Cardiovascular Diseases, Xinqiao Hospital, Army Medical University, Chongqing, China

Considered as the most popular pathogen worldwide, *Helicobacter pylori* is intensively associated with diverse gastric diseases, including gastric ulcers, chronic progressive gastritis, and gastric cancer. Aside from its pathogenic effect on gastric diseases, growing evidences reveal that *H. pylori* may be related to numerous extragastric diseases. In this article, we reviewed recent studies and systematically elucidated that *H. pylori* may interfere with many biological processes outside the stomach and influence the occurrence of various extragastric diseases. Many epidemiological studies have indicated that *H. pylori* plays a pathogenic role in COVID-19, atherosclerosis, hyperemesis gravidarum and several other extragastric diseases, while the effect of *H. pylori* is currently under investigation in gastroesophageal reflux disease, asthma, and inflammatory bowel disease. Moreover, we also summarized the possible pathogenic mechanisms of *H. pylori* that may be related to chronic systemic inflammation and molecular mimicker. Taken together, this review provides a new perspective on the role of *H. pylori* in extragastric diseases and explores the possible mechanisms, which may help guide clinical treatment.

KEYWORDS

***Helicobacter pylori*, extragastric diseases, pathological mechanism, systemic inflammation, molecular mimicry**

Introduction

Helicobacter pylori is recognized as the most popular human pathogen, which infects nearly half of the population worldwide (approximately 4.4 billion people) (Hooi et al., 2017). Exposure to *H. pylori* may bring about lifelong chronic progressive gastritis, and 1–10% of infected individuals will have clinical complications, including gastric intestinal metaplasia, peptic ulcer disease, atrophy of gastric mucosa, gastric cancer (GC), and mucosa-associated lymphoid tissue (MALT) lymphoma (Yamaoka, 2018). The World Health Organization (WHO) has categorized *H. pylori* as one of the Class 1 carcinogens (No authors listed, 1994; Plummer et al., 2015). Previous studies have mostly focused on the role of *H. pylori* in inflammation and tumor development of the stomach. Several clinical trials have proven that the eradication of *H. pylori* reduces the incidence of GC (Lee et al., 2016) and atrophic gastritis (Choi et al., 2018). Management of epithelial precancerous conditions and lesions in the stomach (MAPS II) guideline in 2019 (Pimentel-Nunes et al., 2019) recommended prevention aims for *H. pylori* due to its role in gastric carcinogenesis, precancerous and early cancer lesions. Almost all previous clinical studies on *H. pylori* have suggested *H. pylori* eradication for patients suffering from gastric and intestinal metaplasia or chronic atrophic gastritis.

However, growing evidences reveal that *H. pylori* infection may be related to numerous extragastric diseases of various systems throughout the human body in addition to the pathogenetic effects on gastric diseases. For example, *H. pylori* has been described to be related to some blood system diseases. A separate meta-analysis of 15 observational studies proved that iron deficiency anemia (IDA) was more common among *H. pylori*-positive individuals than *H. pylori*-negative controls (OR = 2.2; 95% CI = 1.5–3.2) (Qu et al., 2010). *H. pylori* infection was also found to be more prevalent in adolescents suffering from IDA (Xia et al., 2012). In the reproductive system, a more significant incidence of *H. pylori* in pregnant women suffering from hyperemesis gravidarum was observed in a meta-analysis (Li et al., 2015). Some endocrine and metabolic diseases are also closely related to *H. pylori*. As shown in a meta-analysis, *H. pylori*-positive subjects with type 1 diabetes had a higher level of glycosylated hemoglobin than uninfected patients (Dai et al., 2015). Apart from the diseases mentioned above, *H. pylori* infection may also cause disorders in many other human systems (Razuka-Ebela et al., 2018). Moreover, studies on pathogenic mechanisms have shown that *H. pylori* can stimulate macrophages, T cells, B cells and other inflammatory cells to accelerate chronic systemic inflammation, interfere with normal physiological processes and ultimately becomes a crucial risk factor for atherosclerosis, insulin resistance, etc. (Franceschi et al., 2014). Similar antigens between *H. pylori* and human tissues may also lead to vitamin B deficiency, pernicious anemia and atherosclerosis (Chmiela and Gonciarz, 2017). The latest American College of Gastroenterology (ACG) Clinical Guideline in 2017 proposed associations between numerous extragastric disorders and *H. pylori* infection, aiming at raising the concern amid clinical workers to attach great importance on *H. pylori* and confronting these diseases in clinical practice (Chey et al., 2017).

In this article, we aim to elucidate the correlation of *H. pylori* and many extragastric diseases, which is necessary to refine the understanding of the pathogenic processes of *H. pylori* and help improve clinical prognosis and guide management. We reviewed latest studies and found that *H. pylori* may be associated with several extragastric diseases of various systems throughout the human body. In addition, we also explored the promising pathogenic mechanisms of *H. pylori* infection. Ultimately, we sought to improve and refine clinical guidelines and benefit patients suffering from the mentioned extragastric diseases and *H. pylori* infection.

Respiratory disease

The relation of *H. pylori* infection with asthma has attracted extensive attention. For example, Zuo et al. (2021) found that *H. pylori* had a protective effect on allergic asthma by regulating Thl7/Tregs and the Th1/Th2 balance, reducing HSP70 and DCs, stimulating TLRs, and inhibiting gastroesophageal reflux. There are three well-known hypotheses related to the pathogenesis, including the gut-lung axis theory, the "disappearing microbiota" hypothesis and the hygiene hypothesis, all of them supporting the protective effect of *H. pylori*. In addition, therapeutic products made by *H. pylori* (such as *H. pylori* extract) have also been utilized to treat and prevent asthma. Perinatal *H. pylori* exposure reduced inflammation of the allergic airway in the offspring as well, providing a promising target for interventional therapy of asthma (Zuo et al., 2021). *H. pylori* can modulate anti-Th2 inflammation activity through neutrophil-activating protein (NAP) and contribute to allergic asthma, and purifying rNAP before sensitization can significantly reduce the accumulation of eosinophils in the lung tissue of asthmatic mice. It is worth noting that *H. pylori* treatment decreases the levels of IL-4, IL-13, and serological IgE, and increases the levels of IL-10 and IFN-γ (Zhou et al., 2017). This study suggests that eradication of *H. pylori* may have a preventive effect on the suppression of allergic asthma. However, it was not supported that *H. pylori* or its specific antigens provided protective antigens that reduced the occurrence of allergic asthma in a meta-analysis (Miftahussurur et al., 2017). Similarly, another cohort study published in 2017 showed that *H. pylori* was significantly associated with a 1.38-fold increased risk of asthma in adults. In addition, the risk of asthma in adults with *H. pylori* infection was still 1.85 times higher than that in *H. pylori* uninfected people (Wang et al., 2017). Thus, the protective effect of *H. pylori* on allergic asthma is controversial.

Helicobacter pylori may also promote the progression and evolution of chronic obstructive pulmonary disease (COPD). *H. pylori*-positive subjects showed a lower FEV1 (L) at baseline than *H. pylori*-negative patients, although no significant discrepancy in the decline rate between the two groups (p-value = 0.35) was shown (Sze et al., 2015). Socioeconomic status (SES) is a prognostic indicator for COPD. Interestingly, this study also found that years of education (on behalf of SES during childhood) were intensively associated with *H. pylori* status and might have effects on adult height. However, no significant difference was found in *H. pylori* seropositivity between individuals with GOLD 1 (global initiative for chronic obstructive lung disease) and GOLD 2 severity (Sze et al., 2015). A cohort study involving 3,619 subjects showed that neither *H. pylori* infection nor eradication treatment was related to COPD progression or lung dysfunction on a general population health screen. In summary, *H. pylori* may not be an intensively aggravated factor in lung function or COPD (Lee et al., 2020).

It is worth noting that *H. pylori* infection may also be associated with COVID-19. A large number of emerging results show that people infected with *H. pylori* may be more vulnerable to severe form of COVID-19 (Balamtekin et al., 2021). Besides, the inflammatory activation caused by *H. pylori* infection may enhance the respiratory inflammatory response of COVID-19, recruit inflammatory cells and promote sustained production of TNF-α, IL-8, and IL-1β, as well as endothelial dysfunction markers such as V-CAM and ICAM, leading to subsequent virus-mediated acute lung injury. *H. pylori* may also aggravate acute respiratory distress syndrome (ARDS), which is a serious complication threatening numerous COVID-19 patients (Gonzalez et al., 2022). However, there was no significant difference in loss of smell, dyspnea, fever, and dry cough between COVID-19 patients with or without *H. pylori* infection. At present, there is no evidence showing that *H. pylori* infection significantly increases the risk of chronic pulmonary fibrosis and COPD among patients with COVID-19 (Balamtekin et al., 2021). The possible reason may be that *H. pylori* infection only affects the acute progression of COVID-19, but not the chronic course.

Studies have found that *H. pylori* pathogen-derived proteins (such as VacA) are found in lung biopsy specimens and bronchoalveolar lavage fluid of lung cancer. These proteins can aggravate the progress of airway diseases, promote the *H. pylori* infection inflammatory status (anti-*H. pylori* IgG and IgM) and recruit B cells, and finally accelerate the occurrence of lung cancer. Besides, eradication of *H. pylori* was significantly correlated with the decrease of lung cancer marker CEA. This explained that *H. pylori* may be of benefit for the treatment of lung cancer (Xu et al., 2018a). Of concern, there is a currently ongoing clinical trial investigating the association between *H. pylori* strain specific blood biomarkers and lung cancer risk (PLCO2019-1026), which may help understand of *H. pylori* infection and lung cancer risk, identify markers for lung cancer risk, and provide new information for a feasible cancer prevention strategy.

Although recent studies suggested an association between *H. pylori* infection and respiratory diseases, further studies are necessary to confirm a causal relationship. Moreover, the roles of other risk factors, such as air pollution or smoking habits, as well as the latent molecular mechanisms should also be considered (GonzAlez et al., 2018; **Supplementary Table S1**).

Heart and circulatory disease

The association of *H. pylori* infection with coronary artery disease has also been investigated. One study showed that *H. pylori* infection significantly reduced endothelium-dependent flow-mediated vasodilation in a young group and strongly repressed acetylcholine-induced endothelium-dependent aortic relaxation without altering nitroglycerin-induced endothelium-dependent vascular relaxation in mice. In addition, *H. pylori* eradication in both human subjects and mice obviously improved endothelium-dependent vasodilation (Xia et al., 2020). Infection with serum CagA+ *H. pylori* can induce cardiovascular disease and coronary heart disease (Sharma and Aggarwal, 2015). Mechanisms by which CagA+ *H. pylori* causes atherosclerosis include increasing the production of COX-1/2 from the vascular endothelium, thereby stimulating the synthesis of thromboxane A2 (TXA2) and prostaglandin to induce platelet aggregation. In addition, *H. pylori* releases many cytokines, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), IL-1 and free radicals, causing atherosclerosis and oxidative stress. Furthermore, an aberrant immune reaction is considered to play a role in atherosclerotic plaque rupture and destabilization by the cross-reactivity between antibodies and CagA vascular wall antigens (de Boer et al., 2000; Byrne et al., 2003; Guo et al., 2007; Feletou et al., 2011). Therefore, as *H. pylori* infection can lead to endothelial dysfunction, dyslipidemia and hyperhomocysteinemia, *H. pylori* eradication therapy is recommended as a possible secondary cardiovascular prevention strategy (Zuin et al., 2016).

Myocardial infarction (MI) is the most dire and serious outcome for patients with CAD due to its fatal influence on survival quality. A meta-analysis including more than 20,000 subjects and 26 studies found that *H. pylori* infection is a risk factor for MI, even among young participants (Liu et al., 2015).

A cohort study that included 12,836 participants showed that *H. pylori* may also significantly increase the risk of carotid atherosclerosis in Chinese men under 50 years old (Zhang et al., 2019). Another study indicated that non-alcoholic fatty liver disease (NAFLD) caused by infection with *H. pylori* increases the formation of carotid artery plaques (Yu L. Y. et al., 2019).

After adjusting for potential cofactors, a trial that included 5,168 study participants revealed an association between high blood pressure and *H. pylori*. In this study, *H. pylori* was

related to an increased risk of hypertension (95% CI = 1.04–1.46; OR = 1.23). Compared with individuals without *H. pylori* infection, infected subjects showed a 0.735 mmHg increase in diastolic blood pressure (95% CI = 0.101–1.369) and a 0.723 mmHg increase in mean arterial pressure (95% CI = 0.034–1.413) (Wan et al., 2018; **Supplementary Table S1**).

Digestive disease

Eosinophilic esophagitis (EoE) is a kind of disease mediated by the immune response. A meta-analysis by Doulberis et al. (2020a) revealed that *H. pylori* infection is one of the protective factors against EoE. However, in 2018, a prospective case–control study conducted in 23 centers reported that *H. pylori* was not negatively associated with EoE, neither in adults nor in children (Molina-Infante et al., 2018). Thus, the effect of *H. pylori* infection on EoE still needs further study.

In developing countries, esophageal squamous cell carcinoma is a prevalent esophageal disorder. Currently, there is no definite evidence showing that *H. pylori* infection contributes to the incidence of esophageal squamous cell carcinoma. A meta-analysis of 35 studies with 345,886 participants indicated that there was no crucial association between esophageal squamous cell carcinoma and *H. pylori* infection (Gao et al., 2019). However, a study that included 95 esophageal squamous cell carcinoma patients showed a statistically significant negative association between esophageal squamous cell carcinoma and *H. pylori* infection via testing gastric biopsy materials from the patients (Poyrazoglu et al., 2017).

Some studies have proposed a different relationship between *H. pylori* and gastroesophageal reflux disease (GERD). An analysis of GERD patients found a higher prevalence of *H. pylori* infection among patients with peptic ulcers (Jie et al., 2019). In contrast, a prospective clinical study of 124 patients with GERD, revealed that *H. pylori* infection reduced esophageal acid exposure, enhanced lower esophageal sphincter pressure, and improved esophageal peristalsis. Thus, *H. pylori* may be protective factors for GERD (Liu et al., 2018). However, interestingly, *H. pylori* eradication did not increase the incidence of GERD. In summary, more studies are needed to determine this pathogenesis.

Several clinical trials have found a relationship between hepatocellular carcinoma (HCC) and *H. pylori*, which was detected in liver samples from individuals with HCC, but this presence cannot support a definite causal relationship (Okushin et al., 2018).

Cholelithiasis and chronic cholecystitis are quite prevalent worldwide. A meta-analysis found that the chronic cholecystitis/cholelithiasis group was more prevalent in *H. pylori* infected gallbladder than the control group in 17 studies (Wang et al., 2021).

The supposed role of *H. pylori* infection in gallstones and gallbladder polyps is still debated. A retrospective study showed that *H. pylori* infection was related to gallstones and gallbladder polyps in a Chinese population (Xu et al., 2018b), whereas this relation was not supported in another case–control matched study of a Chinese population (Zhang et al., 2020). Thus, the role of *H. pylori* in cholecystic polyps and gallstones requires further research.

Non-alcoholic fatty liver disease is a kind of liver injury that is induced by metabolic stress. A meta-analysis of 21 studies indicated that *H. pylori* infection was one of the factors contributing to NAFLD progression in the Asian population (Liu et al., 2019), but *H. pylori* infection was not an independent risk factor for NAFLD revealed by a cross-sectional study in China (Fan et al., 2018). One hypothesis is that *H. pylori* infection may cause chronic low-level systemic inflammation, which increases the concentration of inflammatory cytokines, such as IL-6 and TNF-α, stimulating IKK/NF-κB signaling and leading to insulin resistance. *H. pylori* infection may also restrain leptin release from white adipose tissue, which in turn leads to liver stearoyl-CoA desaturase, thereby stimulating fat and VLDL-C deposition in liver tissue. Another hypothesis is that *H. pylori* infection may cause dysbiosis of gastrointestinal flora, increase serum lipopolysaccharide, accelerate the systemic inflammatory response and increase the expression of IL-6, TNF-α, and C-reactive protein, which results in reduced lipoprotein activity followed by dyslipidemia (Cheng et al., 2017). Notably, an ongoing clinical study may contribute to reveal the risk of NAFLD due to *H. pylori* infection by investigating the genome-wide association of *H. pylori* infection (PLCO-989).

Helicobacter pylori infection might play a protective role in inflammatory bowel disease (IBD) reported by a meta-analysis (Imawana et al., 2020). Besides, another meta-analysis of clinical studies including 1,748 individuals, also indicated an association between CagA seropositivity and lower odds of IBD (Tepler et al., 2019; **Supplementary Table S2**).

Viral hepatis has also been found to be related to *H. pylori* infection. Esmat et al. (2012) found the existence of CagA gene of *H. pylori* in liver samples of patients with hepatitis C virus (HCV)-related chronic hepatitis. A multivariate analysis further indicated that positive anti-*H. pylori* antibody was independently and significantly related to cirrhosis in individuals with HCV-related chronic hepatitis (Queiroz et al., 2006). Moreover, clinical reports also suggested an association between *H. pylori* and HBV-related liver diseases. A meta-analysis of a Chinese population demonstrated that the infection rate of *H. pylori* in patients with HBV-related liver diseases had a positive relation with the increase of disease severity. In addition, the rate of *H. pylori* positivity in chronic HBV patients was 2.44-fold higher than that in healthy controls (Wang et al., 2016). Therefore, the prevalence of *H. pylori* may promote the progression of HBV-related liver diseases. However, the

relationship between *H. pylori* infection and HAV is usually overestimated by confounding factors such as socio-economic status and age, and eliminating interference of these factors would reduce this correlation (BinSaeed, 2010).

It has been found that *H. pylori* can interact with the gut microbiome and affect extragastric diseases progression. Heimesaat et al. (2014) found that with long-term *H. pylori* infection, gut microbiome showed a lower level of *Lactobacillus* spp. and a significant higher loads of *E. coli*, *Bacteroides/Prevotella* spp., and *Enterococcus* spp. than *H. pylori*-negative subjects. In addition, *H. pylori* permits more microorganisms to pass through the gastric acid barrier and colonize the distal gut, increasing gut microbiota diversity through hypergastrinemia and hypochlorhydria (Lopetuso et al., 2014). Subsequently, low level of beneficial gut bacteria (such as *Lactobacillus* spp.) may lead to the proliferation of some harmful bacteria and damage gut barrier function. This also causes the immune imbalance and mediates several chronic inflammatory diseases mentioned above (Sanders et al., 2019). Furthermore, *H. pylori* infection-related gut microbiome alternation may decrease insulin sensitivity and lead to diabetes, and may also lead to abnormal lipid metabolism, increasing the risk of NAFLD (He et al., 2016). Recovery of gut microbiome balance was observed after *H. pylori* eradication (Chen et al., 2021). Taken together, *H. pylori* can induce the gut microbiome alternation and lead to the progression of several extragastric diseases (**Figure 1**).

Blood system disease

It is found that *H. pylori* infection is closely related to MALT lymphoma. *H. pylori*-induced T cells can promote macrophages to secrete APRIL, which is an important cytokine that promotes the progression of MALT lymphoma (Planelles et al., 2008; Zhang et al., 2015). *H. pylori* may also directly drive CagA protein into B cells, leading to increased Bcl-2 expression, activating extracellular signal-regulated kinase and inhibiting apoptosis, which finally promote MALT lymphoma progression (Kuo et al., 2014). Besides, CagA+ *H. pylori*-infected

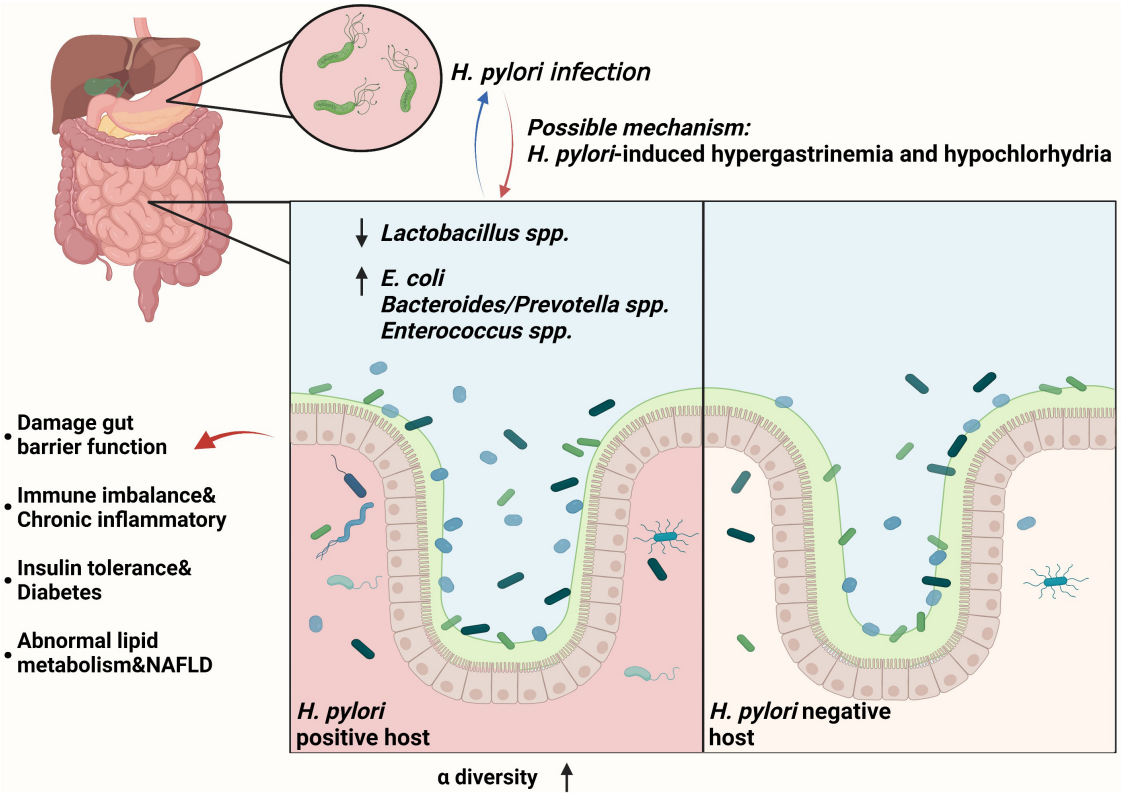

FIGURE 1
Helicobacter pylori infection can interact with the gut microbiome and affect extragastric diseases progression. With chronic *H. pylori* infection, gut microbiome showed a lower level of *Lactobacillus* spp. and a significant higher loads of *E. coli, Bacteroides/Prevotella* spp., and *Enterococcus* spp., increasing α-diversity of gut microbiota (Heimesaat et al., 2014). For the mechanism, gut microbiota changes may be triggered by *H. pylori*-induced gastric immune pathogenesis, including hypergastrinemia and hypochlorhydria (Lopetuso et al., 2014). Subsequently, low level of beneficial gut bacteria (such as *Lactobacillus* spp.) may lead to the proliferation of some harmful bacteria and damage gut barrier function (Sanders et al., 2019). This also leads to the immune imbalance and chronic inflammatory, insulin tolerance and diabetes, and abnormal lipid metabolism and NAFLD (He et al., 2016).

MALT lymphoma patients significantly delayed the progression of MALT lymphoma after *H. pylori* eradication treatment (Kuo et al., 2014). Infected by *H. pylori*, normal B cells are driven into malignant clones by three kinds of chromosomal translocations, t (14;18) (q32; q21), t (1;14) (p22; q32), and t (11;18) (q21; q21), activating NF-κB signaling and regulating apoptosis, inflammation, and immunity (Bertoni and Zucca, 2006; Ruskone-Fourmestraux et al., 2011; Bautista-Quach et al., 2012; Zullo et al., 2014). Among them, t (Dai et al., 2015; Miftahussurur et al., 2017) (q21; q21) may be conducive to the occurrence of MALT lymphoma (Streubel et al., 2006).

Many studies have proven that *H. pylori* infection leads to IDA. The Maastricht III European guidelines for people with unknown sarcopenic anemia recommend an *H. pylori* infection test and germ eradicate therapy (Malfertheiner et al., 2007). Flores et al. (2017) found that CagA protein is significant in alteration of iron metabolism in gastric adenocarcinoma cells of *H. pylori*-infected humans, and this is mediated by transferrin endocytosis and increasing iron uptake.

It has been reported that the lack of vitamin B12 absorption contributes to pernicious anemia and *H. pylori* also plays a role in this process. *H. pylori* infection changes intragastric pH, leading to vitamin B12 malabsorption (Cohen et al., 2000). In addition, *H. pylori* may also evoke an antigen similar to antibodies against the H+K+-adenosine triphosphate protein to inhibit vitamin B12 absorption (Claeys et al., 1998). Besides, an ongoing clinical study may help reveal the risk of vitamin B12 deficiency due to *H. pylori* infection by investigating the genome-wide association of *H. pylori* infection (PLCO-989).

The role of *H. pylori* in Idiopathic or Immune Thrombocytopenic Purpura (ITP) has also been investigated. A meta-analysis of six studies involving 241 patients proved that *H. pylori* eradication is an effective treatment for ITP patients (Kim B. J. et al., 2018). Lei et al. (2021) reported that *H. pylori* can promote platelet destruction in mice, and the mechanisms may be related to activating NF-κB/IL-17 signaling.

Antiphospholipid syndrome is characterized by both venous and arterial thrombosis, and often leads to abortions, premature birth, and preeclampsia. Cicconi et al. (2001) reported that after the eradication of *H. pylori*, the antiphospholipid syndrome of a case disappeared (**Supplementary Table S3**).

Endocrine and metabolic disease

Diabetes is the most prevalent metabolic disorder worldwide, killing approximately four million people each year. A meta-analysis of 9,559 individuals found that the effects of *H. pylori* on type 1 and 2 diabetes and diabetes mellitus (both types) were 1.19 (95% CI = 0.98–1.45), 1.43 (95% CI = 1.11–1.85) and 1.17 (95% CI = 0.94–1.45), respectively, indicating that *H. pylori*-infected individuals would have a higher risk of diabetes. According to an analysis of geographical subpopulation regions, the infection risk of *H. pylori* in the Asian population was slightly higher than that in other populations (Mansori et al., 2020). In contrast, a cross-sectional study showed that there was no significant correlation between *H. pylori* and diabetes, though it has been estimated that *H. pylori* may be associated with an increased risk of diabetes in Chinese females (Man et al., 2020). Moreover, an ongoing clinical study may help reveal the association between diabetes and *H. pylori* infection by investigating the genome-wide association of *H. pylori* infection (PLCO-989).

Obesity has become a crucial public health problem. The impact of *H. pylori* on obesity or overweight is still unclear. A meta-analysis including 22 articles and 178,033 samples showed that obesity was associated with *H. pylori*, which may increase the risk of obesity (OR = 1.2) (Xu et al., 2019). However, from a retrospective study of 3,039 subjects, *H. pylori* was not related to obesity or overweight observed in a Chinese population (P = 0.321) (Xu et al., 2017). More investigation of the relationship between *H. pylori* infection and obesity are still needed.

The relation of *H. pylori* with autoimmune thyroid diseases (AITDs) also needs more research to clarify. A meta-analysis of 15 articles that included 3,046 cases showed that *H. pylori* was positively correlated with HT and GD (HT: 95% CI: 1.44–3.23, OR = 2.16; GD: 95% CI: 1.68–4.61, OR = 2.78), and CagA+ *H. pylori* was positively related to AITD (95% CI: 1.07–3.70, OR = 1.99) (Hou et al., 2017). Nevertheless, another study proposed that this pathogenesis might be caused by molecular mimics and an increased inflammatory state (Figura et al., 2019; **Supplementary Table S3**).

Nerve disease

Alzheimer's disease (AD), as a kind of nerve disease characterized by neurodegeneration, has also been studied for a possible association with *H. pylori* infection. Beydoun et al. (2018) found a direct relationship between AD mortality and *H. pylori* seropositivity in their retrospective cohort study that included 16,970 participants. In addition, a systematic study also revealed that AD may be associated with gastrointestinal microbiota dominated by *H. pylori* (Katsinelos et al., 2019).

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world. Although the pathogenesis of PD remains unclear, *H. pylori* eradication was found to intensively improve the clinical symptoms of PD in a prospective cohort study. *H. pylori* eradication not only increased the normal motor function time (also known as 'on' time) of the day, but also improved gastrointestinal symptoms and reduced fatigue symptoms (Lolekha et al., 2021). Another case–control study found that the positive serum of *H. pylori* was related to the adverse reaction and higher dosage of levodopa, and *H. pylori* eradication improved the prognosis

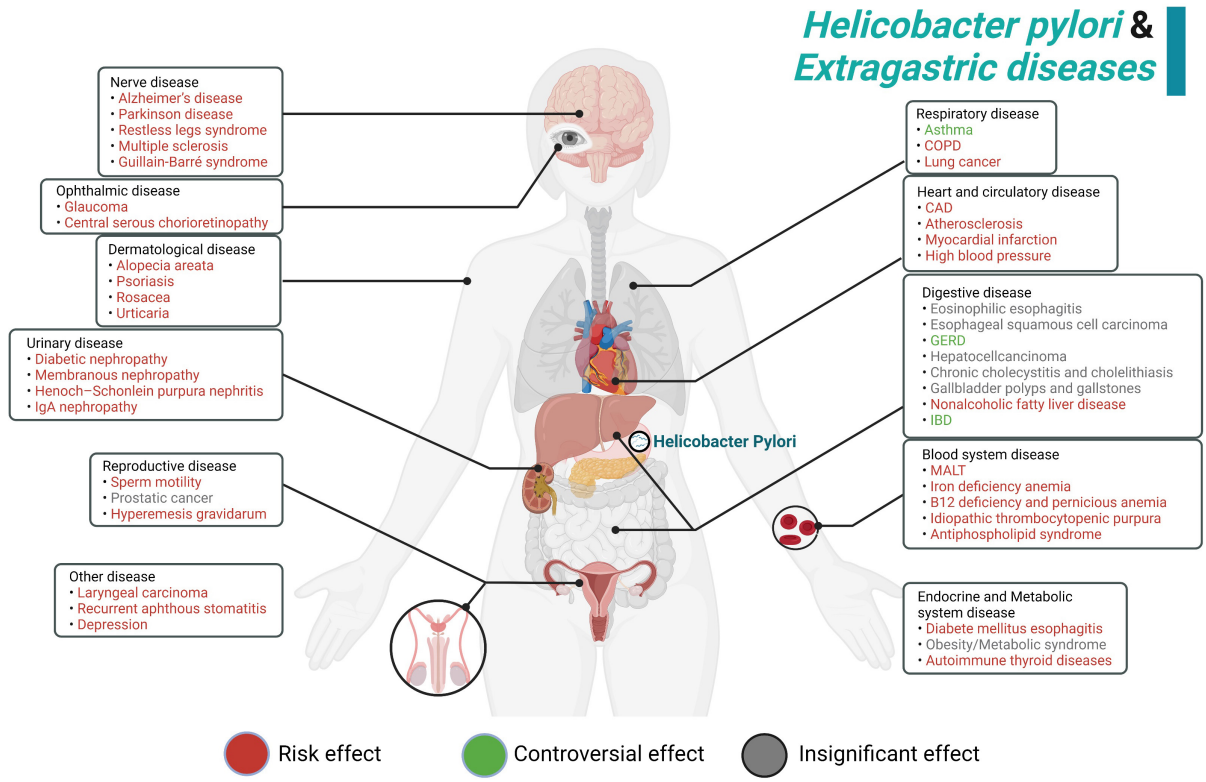

FIGURE 2
Summary schematic of the systematic effect of *H. pylori* infection. In red, the manifestations for which *H. pylori* infection represents a risk effect. In green, although some studies revealed that *H. pylori* showed a positive effect on these diseases, there are still many controversial aspects and further research is needed. In gray, we show the manifestations for which *H. pylori* infection shows an insignificant effect.

of patients (Mridula et al., 2017). A meta-analysis of 13 studies also found that *H. pylori* infection was significantly associated with adverse drug response, higher levodopa equivalent daily dose (LEDD) and severer motor symptoms in PD patients (Zhong et al., 2022).

A descriptive analytical cross-sectional study in Iran showed that *H. pylori* was related to the etiology of restless legs syndrome (RLS). Proinflammatory cytokines released by *H. pylori* infection, such as IL-6, have been shown to increase production of hepcidin, which affects iron transport in healthy human, resulting in an iron deficiency in the CNS and causing RLS (Rezvani et al., 2018).

The etiology of multiple sclerosis (MS) is the complex interaction of environmental and genetic factors. Bacterial exposure has been identified as one of the many pathogenic factors of MS (Cossu et al., 2018). As shown in a meta-analysis conducted in Western countries, the presence of bacteria was negatively correlated with MS (Jaruvongvanich et al., 2016). In Asian countries, *H. pylori* antigen antibodies were more common in patients with aquaporin 4 antibody-positive neuromyelitis optica spectrum disorders (NMOSDs) but negative in patients with MS (Yoshimura et al., 2013). The above results suggested that *H. pylori* may be a protective factor by manipulating pattern-recognition receptors (PRRs) (Efthymiou et al., 2017) and inhibiting Th1/Th17-cell responses (Salama et al., 2013). A recent seroprevalence study showed that antibodies against VacA were frequently detected in patients with secondary progressive MS (Efthymiou et al., 2017). Aside from the local role of *H. pylori*, the direct regulation was observed in the brain-intestinal axis (Kountouras et al., 2015).

Guillain–Barré syndrome (GBS) is a serious peripheral nerve autoimmune demyelinating disease that often occurs after bacterial infection. A meta-analysis revealed that there was an intensive relationship between GBS and *H. pylori* antibodies, especially in cerebrospinal fluid, suggesting that *H. pylori* is significant in GBS pathophysiology (Dardiotis et al., 2020; **Supplementary Table S4**).

Ophthalmic disease

Glaucoma is a leading cause of blindness worldwide. A meta-analysis that included 15 studies and 2,664 participants found that *H. pylori* infection was associated with non-heterogeneous glaucoma (Doulberis et al., 2020b). Following *H. pylori* eradication therapy, a significant ($p = 0.005$) reduction

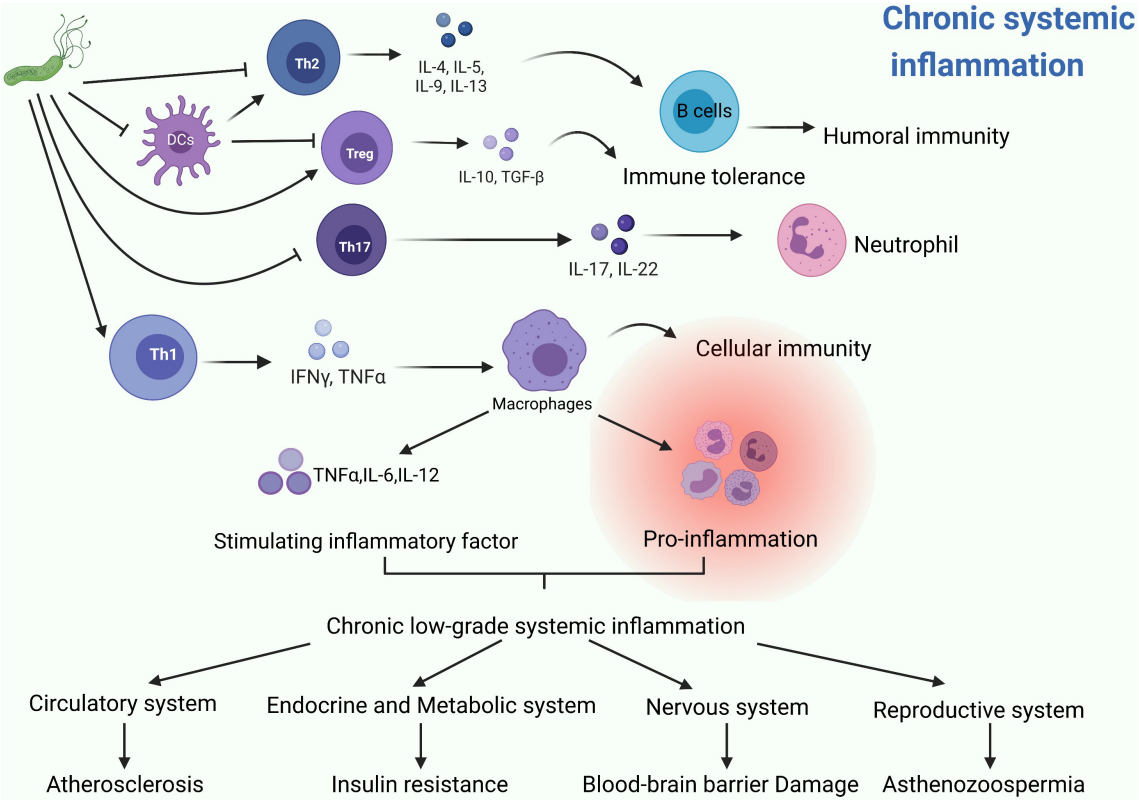

FIGURE 3
The possible common mechanisms by which *H. pylori* induces these systemic diseases can be summarized into two promising hypotheses: Hypotheses 1. Since *Helicobacter pylori* can induce several inflammatory factors, such as IL-1/2/6/8/10, TNF-α and IFN-γ, these factors may lead to chronic low-level systemic inflammation in the human body and ultimately represent diseases (Franceschi et al., 2014). Typical disorders due to *H. pylori*-induced inflammatory factors turbulence include: atherosclerosis (de Boer et al., 2000), insulin resistance (Cheng et al., 2017), blood-brain barrier damage, brain neurodegenerative disease (Efthymiou et al., 2017), and decreased sperm motility (Figura et al., 2002).

in intraocular pressure (IOP) was found after 2 months of follow-up, showing that *H. pylori* eradication may be positive in glaucoma therapy (Ala et al., 2020).

A meta-analysis found a higher *H. pylori* prevalence among central serous chorioretinopathy (CSR) patients (Bagheri et al., 2017). In addition, some studies have indicated that CagA antigen antibodies might cross-react with vascular endothelial antigens to promote the occurrence of vascular wall injury and atherosclerosis (Franceschi et al., 2002). As atherosclerosis is one of the most significant risk factors for CSR, *H. pylori* may play a pathogenic role in CSR and injure the vascular endothelium through similar antigens and cross-reactivity (**Supplementary Table S5**).

Dermatological disease

Alopecia areata is an inflammatory alopecia mediated by immunity that appears in all age and ethnic groups. The results of a case–control study including 162 examples showed that *H. pylori* infection may have a pathogenic effect on alopecia areata (Behrangi et al., 2017). *H. pylori* can promote chronic immune responses and local inflammatory, leading to sustained release of inflammatory mediators including PAF, LTC4, IFN-γ, TNF-α, and IL-1. These mediators may contribute to the occurrence of alopecia areata.

Besides, a meta-analysis of 11 studies and 1,741 examples revealed that *H. pylori* was also associated with psoriasis and that *H. pylori*+ individuals had a higher score on the Psoriasis Area and Severity Index (PASI) (Yu M. et al., 2019). However, a population-based longitudinal cohort study found no correlation between *H. pylori* and psoriasis (Wu et al., 2020). Thus, more studies are necessary to determine the relationship between psoriasis and *H. pylori*.

Similarly, a meta-analysis of 27 studies confirmed that *H. pylori* was related to the rosacea process (Yang, 2018), and *H. pylori*-infected individuals had a higher risk of suffering from rosacea.

Urticaria, a prevalent dermatological disease has also been reported to have a relation with *H. pylori*. Some studies found that the level of *H. pylori* antigens in individuals with chronic urticaria was significantly higher than that in

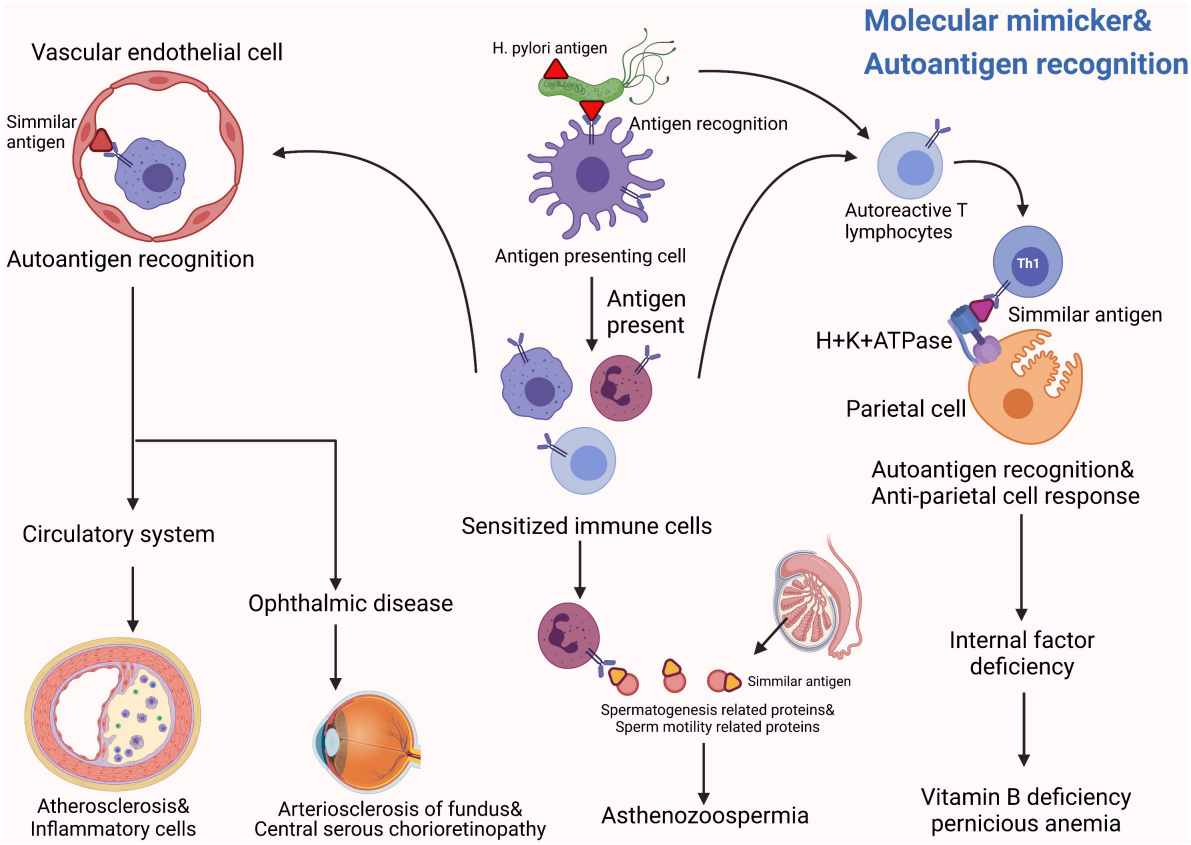

FIGURE 4
The possible common mechanisms by which *H. pylori* induces these systemic diseases can be summarized into two promising hypotheses: Hypotheses 2. *H. pylori* antigen, like the antigen components of host leads to molecular mimicker and cross-antigen reactions, which cause autoimmune attacks and relevant diseases (Chmiela and Gonciarz, 2017). Typical diseases that chiefly rely on this mechanism include: a cross reaction between the CagA antibody and the vascular wall induces atherosclerosis (Guo et al., 2007); *H. pylori* and gastric H+K+ATPase cross antigen contributes to vitamin B12 deficiency (Claeys et al., 1998); arteriosclerosis of fundus for autoimmune reaction induces central serous choroidal retinopathy (CSR) (Franceschi et al., 2002); cross-antigen reactivity between spermatogenesis-related proteins, sperm motility related proteins and *H. pylori* contributes to hypomotility of sperm (Figura et al., 2002).

controls. The eradication of *H. pylori* alleviated the symptoms of these patients, which supported an impact of *H. pylori* on pathogenesis (Erdem et al., 2020; **Supplementary Table S5**).

Urinary disease

Helicobacter pylori is significantly related to immunoglobulin A (IgA) nephropathy, membranous nephropathy, Henoch–Schonlein purpura nephritis, diabetic nephropathy and other urinary diseases (Moriyama et al., 2007). *H. pylori* antigens were found in pathological tissues of these diseases (Li et al., 2013). A study indicated that *H. pylori* was probably a risk factor for kidney damage in patients with *H. pylori*+ peptic ulcers, and eradication of *H. pylori* may alleviate kidney damage and prevent chronic processes (Pan et al., 2019). Another study revealed that *H. pylori* infection may lead to a strong mucosal immune response and play a pathogenic role in IgA nephropathy based on renal tubular injury (Zhu et al., 2016; **Supplementary Table S5**).

Reproductive disease

Previous studies have revealed that in men with fertility problems, the prevalence of *H. pylori* was much higher. Some immunocytochemical studies emphasized that serum samples from infected men (as well as anti-*H. pylori* hyperimmune serum) reacted with the equatorial segment and the flagella (especially abundant in tubulin) of sperm (Figura et al., 2002). However, in 2020, a cross-sectional study found that there was no difference in anti-Müllerian hormone (AMH) levels and sperm parameters in Chinese patients based on *H. pylori* infection history (Feng et al., 2020).

A cohort study showed that there was no significant relationship between subsequent prostate cancer risk and

H. pylori-infected peptic ulcers (Fang et al., 2020). To date, the relationship of prostate cancer (PCa), benign prostatic hyperplasia (BPH), and *H. pylori* needs to be further studied.

Hyperemesis gravidarum (HG) is characterized by excessive vomiting and severe nausea that begins before the end of 22 weeks of pregnancy (World Health Organization, 2016). A study showed that in the stomach of women with HG, *H. pylori* was more prevalent, and there was a significant positive correlation between *H. pylori* serum levels and HG symptoms (Bustos et al., 2017; **Supplementary Table S5**).

Other diseases

Laryngeal cancer is a serious disease threatening human health. A prospective controlled study found that in cases of *H. pylori* ureA gene-positive laryngeal cancer, 46.7–49.3% of 75 were also CagA positive. The CagA gene in laryngeal cancer greatly reduced the survival rate and increased the possibility of recurrence (Burduk, 2013).

Some studies revealed an association between oral diseases and *H. pylori* infection. Okuda et al. (2000) found the expression of *H. pylori* in the dental plaques in 12 of 54 *H. pylori* infected subjects. Moreover, a study reported that some oral samples expressed the *H. pylori* ureA gene, and the primary host of oral infection was identified as dental pulp (Iwai et al., 2019). The presence of *H. pylori* may be harmful to the oral environment. Recurrent aphthous stomatitis (RAS) is regarded as a recurrent painful ulcerative disease that regularly impacts mucosa in the oral cavity. Gao et al. (2021) reported a RAS case with a history of 24 years that was cured after treatment for *H. pylori*, indicating that eradication of *H. pylori* might relieve RAS symptoms and is a promising RAS therapy.

In addition to the standard drug regimen, the clinical practice of appending antidepressants to the treatment of *H. pylori* eradication is not quite explicit. A meta-analysis that included three RCTs, two review articles, one cohort study, four prospective studies, and eight cross-sectional studies found that individuals with functional dyspepsia who did not improve after *H. pylori* eradication (Al Quraan et al., 2019). Another study found that stress/anxiety/depression (SAD) and *H. pylori* infection were significantly prevalent in patients with functional dysplasia (FD) (Kabeer et al., 2017). A cohort study showed that in the general Chinese adult population, *H. pylori* infection was related to depressive symptoms in women but not men (Gu et al., 2019; **Supplementary Table S5**).

Discussion

Previously, *H. pylori* infection was mostly considered as a risk factor for gastric disorders. However, growing evidences show that *H. pylori* infection presents more complexity and tends to be associated with almost every system in the human body. From our perspective, *H. pylori* can produce many kinds of bacterial toxins and induce numerous extragastric diseases in the human body, such as asthma, COPD, ITP and psoriasis. We summarized these diseases in terms of the human system, listed them methodically in this article and showed a schematic diagram (**Figure 2**). Interestingly, recent studies mentioned in this review partially elucidate the potential pathogenesis of these extragastric diseases caused by *H. pylori* infection. We synthesized the results of these studies and proposed two promising hypotheses. (i) Since *H. pylori* can induce several inflammatory factors, such as IL-1/2/6/8/10, TNF-α and IFN-γ, these factors may lead to chronic low-level systemic inflammation in the human body and ultimately represent diseases. Typical disorders due to *H. pylori*-induced inflammatory factor turbulence include atherosclerosis, insulin resistance, blood-brain barrier damage, brain neurodegenerative disease and decreased sperm motility (**Figure 3**). (ii) *H. pylori* antigen, like the antigen components of host leads to molecular mimicker and cross-antigen reactions, which cause autoimmune attacks and relevant diseases. Typical diseases chiefly rely on this mechanism, including cross reaction between CagA antibody and vascular wall inducing atherosclerosis; *H. pylori* and gastric H+K+ATPase cross antigen contributes to vitamin B12 deficiency; arteriosclerosis of fundus for autoimmune reaction induces central serous choroidal retinopathy (CSR); and cross-antigen reactivity between spermatogenesis related proteins, sperm motility related proteins and *H. pylori* contributes to hypomotility of sperm (**Figure 4**). In a word, it is believed that the two hypotheses contribute to deciphering the reasons why *H. pylori* is associated with disorders in many systems of the human body (Franceschi et al., 2014; Chmiela and Gonciarz, 2017).

There are still some limitations of current studies that need to be improved. First, at present, the sample size of *H. pylori*-related extragastric diseases in most studies is generally insufficient. Larger sample sizes and broader clinical trials are beneficial to decipher the correlation between various clinical diseases and *H. pylori*, and the control of confounding factors is necessary. In addition, the pathogenic effect of *H. pylori* in some extragastric diseases, such as gastroesophageal reflux disease, asthma, and IBD, are still controversial (**Figure 2**). Some studies even proposed that *H. pylori* may have a certain protective effect on some diseases (such as GERD) (Scida et al., 2018). And most studies are only correlation studies without explanation of causality. The proof of Evidence-based medicine is not strong enough. It also needs to clarify causality with the help of animal model research of disease and in-depth molecular mechanism research. What's more, the hypothesis (i) about systemic inflammation is limited for the heterogeneity of participants and the control of confounding

factors is often incomplete (Kim T. J. et al., 2018). Therefore, the establishment of *H. pylori* infection model based on specific inflammatory markers (such as CRP and PLR) and the study of inflammatory-activated pathways are of great significance to reveal the systemic effects of *H. pylori*. Furthermore, there is high heterogeneity in the research on the relationship between *H. pylori* and the development of autoimmune diseases, and the differences of their distribution patterns make the research results controversial. At present, it is found that *H. pylori* infection may increase susceptibility to autoimmune diseases by stimulating cell damage, chronic inflammatory, and polyclonal lymphocyte activation (Youssefi et al., 2021). Aside from that, several intervention variables, including antibiotic treatment, microbiota, and host genome polymorphism may also be involved in the self-recognition of anti-*H. pylori* antibodies. The pathogenesis of gastric immunity induced by *H. pylori*, including hypergastrinemia and hypochlorhydria, may lead to changes in gastrointestinal microbiome. Nevertheless, the exact potential mechanism needs to be further clarified to confirm the systematic effects of *H. pylori* infection. In addition, in current clinical practice, the first-line treatment for most *H. pylori*-related extragastric diseases remains *H. pylori* eradication. However, *H. pylori* treatment to prevent allergic asthma and coronary artery disease has showed promising clinical outcomes (Zuin et al., 2016; Zhou et al., 2017). Thus, it is worth exploring that *H. pylori* preventive control strategies may be valuable for the contribution of other extragastric diseases. In general, most of previous articles on the extragastric diseases caused by *H. pylori* infection have limitations on the finite sample size, unclear pathogenic mechanism, and the limitation of *H. pylori* detection means (**Supplementary Table S6**).

Helicobacter pylori infection can induce several extragastric diseases through many pathways, and different types of *H. pylori* may contribute to different kinds of diseases because of their specific bacterial toxins and pathogenies. It is generally accepted that the systemic effects of *H. pylori* infection should not be neglected. Although *H. pylori* has been discovered over more than 100 years ago, many aspects of *H. pylori* still need further studies. For clinical practitioners, the impact of *H. pylori* infection on extragastric diseases should be taken into more consideration.

Author contributions

PL and CH contributed to the conception of the study. JH and YL were responsible for searching the literature, creating graphical illustrations, and writing the manuscript. QO, WC, WH, and LH contributed to manuscript review and read the submitted version. All authors contributed to the review 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.972777/full#supplementary-material

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EDITED BY
Karolina Skonieczna-Żydecka,
Pomeranian Medical University, Poland

REVIEWED BY
Agata Mulak,
Wrocław Medical University, Poland
Yong Zhao,
Chinese Academy of Agricultural Sciences (CAAS), China

*CORRESPONDENCE
Sha Peng
pengshacxh@nwafu.edu.cn

†These authors have contributed equally to this work

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Gut microbiota supports male reproduction *via* nutrition, immunity, and signaling

Hui Cai[1†], Xuanhong Cao[1†], Dezhe Qin[2], Yundie Liu[1], Yang Liu[1], Jinlian Hua[1] and Sha Peng[1*]

[1]Shaanxi Centre of Stem Cells Engineering and Technology, College of Veterinary Medicine, Northwest A&F University, Shaanxi, China, [2]State Key Laboratory for Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Gut microbiota (GM) is a major component of the gastrointestinal tract. Growing evidence suggests that it has various effects on many distal organs including the male reproductive system in mammals. GM and testis form the gut-testis axis involving the production of key molecules through microbial metabolism or *de novo* synthesis. These molecules have nutrition, immunity, and hormone-related functions and promote the male reproductive system *via* the circulatory system. GM helps maintain the integral structure of testes and regulates testicular immunity to protect the spermatogenic environment. Factors damaging GM negatively impact male reproductive function, however, the related mechanism is unknown. Also, the correlation between GM and testis remains to be yet investigated. This review discusses the complex influence of GM on the male reproductive system highlighting the impact on male fertility.

KEYWORDS

gut microbiota, testis, male fertility, gut-testis axis, probiotics

Introduction

Gut microbiota (GM) is an indispensable regulator of host metabolism, immunity, and endocrine functions. Its composition, abundance, metabolites, and signaling pathways significantly impact organ development starting from the local intestine to distal organs. The metabolic outcomes of GM determine key processes like lipid and bile metabolism, vitamin and short-chain fatty acids production, pathogens resistance, DNA expression and detoxification (Walter and Ley, 2011). The genetic and chemical diversity of GM is far greater than that of the host genome as GM includes trillions of symbiotic bacteria, virus, and fungi in the intestine (Lam et al., 2022; Schupack et al., 2022). As for bacteria, intestinal microenvironment is mainly conducive to the growth of six major bacterial phyla, including *Firmicutes*, Bacteroides, Proteobacteria, Actinomycetes, Verrucomicrobia, and Fusobacterium (Eckburg et al., 2005). Among them, *Bacteroides*

and *Firmicutes* account for >90% (Qin et al., 2010), and their proportions change dynamically during different stages of animal life. Although a large part of GM remains conserved, evidence suggests that the microbial abundance of GM changes dynamically at the species level depending on the host's age or health conditions (Yatsunenko et al., 2012). These features allow GM to work much better in different phases/health conditions. Although GM is dynamic, it has some basic functions regulating immunity, metabolism, and nervous system impacting the general physical and mental health of the host (Adak and Khan, 2019).

In GM mediated digestion of nutrients, the main end product of carbohydrates is short-chain fatty acids (SCFAs), which play a role in the metabolism and circulation of glucose and lipid. Three kinds of SCFAs, propionate, acetate, and butyrate, have roles in maintaining intestinal integrity and relieving inflammation (Morrison and Preston, 2016). Amino acids and short peptides produced in the digestive tract after proteolysis are used by GM to synthesize other kinds of proteins. Moreover, GM can enzymatically decompose protein products to generate energy or produce signaling molecules to regulate the physiological state of the host (**Figure 1**; Nicholson et al., 2012). The GM balance is very critical to the homeostasis of the host's immune system. Beneficial strains strengthen the tight junctions of the intestine. In case of disturbed GM, immune responses are generated in the local intestine, which can gradually advance to inflammatory bowel disease (IBD). The integrity of the intestine is impaired by inflammation induced by bacteria-produced lipopolysaccharides (LPS) and inflammatory cytokines, which can circulate and spread to other organs (Ulluwishewa et al., 2011).

The health status or balance of the GM also affects the development and health of the male reproductive system of in mammals (Martinot et al., 2021). This effect could be positive or negative in nature (Guo et al., 2020; Liu et al., 2022). Certainly, the reproductive ability of male animals directly determines the survival and reproduction of organisms, which can become vulnerable at the time of imbalanced GM. Also, the testicles, wrapped in the scrotum outside the body cavity, are easily vulnerable to changes in the internal and external environment. Heat and cold stress, hormone levels and endocrine disruptors, dietary structure, exercise, growth and development, and congenital factors all have effects on the function of testis (Belloc et al., 2014; Tian et al., 2019; Qin et al., 2021). According to the World Health Organization (WHO), 15% of couples worldwide struggle with infertility, of which, 50% of cases of infertility are due to men having troubles such as varicoceles and azoospermia (Jensen et al., 2017; Wu et al., 2021).

The growth of the testis relies on germ and somatic cells. A mature testis produces sperm. Spermatozoa accumulate in the epididymis and are then discharged out of the penis through the deferens to complete the jaculation process.

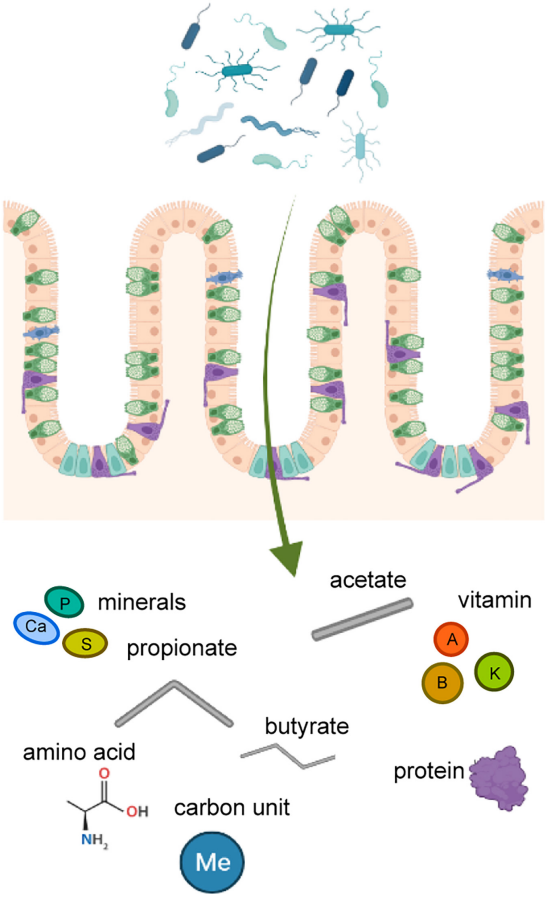

FIGURE 1
The nutrient substance produced by gut microbiota. Products secreted by gut microbiota in the intestine affect the distal parts of body through the circulatory system. These substances include amino acids, proteins, vitamins, minerals, fatty acids, and so on.

This process requires nutrients such as water, amino acids, lipids, carbohydrates, vitamins, and minerals. Germ cells achieve differentiation and maturation through the process of exchanging nutrients and metabolic wastes with Sertoli cells (Dance et al., 2015). In addition, the erection of the penis requires stimuli from various gas signaling molecules, which are majorly produced by cyclic metabolism in GM (Zmora et al., 2019). Recent studies found a small number of bacteria in testicles are similar to gut bacteria and semen can regulate certain male reproductive diseases (Altmae et al., 2019; Godia et al., 2020). From the perspective of the male reproductive system, the testes and penis protect the germ cells, while GM absorbs and metabolizes nutrients to ensure the functioning of male reproductive organs. This review aims to expound on the regulation of GM that in multiple ways promotes the growth and development of the male reproductive system.

Gut microbiota supports testis by metabolizing nutrients

Testes cannot *de novo* synthesize nutrients. The blood vessels in the testis transport nutrients, including those synthesized or metabolized by GM, from the digestive system to the testicular interstitium *via* the convoluted seminiferous tubules through Sertoli cells and their intercellular connections. Nutrients such as vitamins and minerals synthesized or metabolized by GM are essential for testes (**Table 1**). The changed microbiota may disturb the original nutritional structure and function of the testis.

Vitamin A

Vitamin A is an indispensable nutrient for the reproductive system and embryonic development. One of the metabolic forms of vitamin A is retinoic acid (RA), which prompts the stagnant spermatogonial stem cells in the G0/G1 stage in the embryo to initiate meiosis for differentiation into mature sperms. Vitamin A deficiency leads to the failure of type A spermatogonial stem cells differentiation into type A1; the stratified squamous keratinizing epithelium replaces epithelia of the prostate, epididymis, and seminal vesicle, slowing sperm production (Clagett-Dame and Knutson, 2011). In humans and mice, the expression of two genes related to spermatogenesis (*Stra8* and *Rec8*) is promoted by RA. Without the expression of *Stra8*, undifferentiated spermatogonia are difficult to accumulate and differentiate, which causes the failure of meiosis. In Sertoli cells, RA binds to retinoic acid receptor (RAR) recruiting retinoid X receptors (RXRs), which promotes the transcription process. Interference of RAR or RXRs in Sertoli cells blocking the RA-RAR/RXR signaling causes the failure of the blood-testis barrier (BTB), which forbids sperms to mature and release from Sertoli cells (Schleif et al., 2022). GM plays an important role in regulating the intestinal absorption and metabolism of vitamin A. Proteins produced by *Escherichia coli* like RXRs and farnesoid X receptors have been linked to the transport of vitamin A to intestinal cells, where retinal dehydrogenase (RALDH) converts retinal into RA. Also, *Clostridia* directly modulates the RA concentration. Moreover, GM inhibits the activity of the cytochrome P450 (CYP) family of protein, which can degrade vitamin A. In addition, intestinal microbial enzymes promote the production of retinoic acid from b-carotene (Stacchiotti et al., 2021). In sheep, diet-induced metabolic disorders lead to the imbalance of GM reducing the production of bile acids and the absorption of vitamin A, a kind of fat-soluble vitamin. Consequently, it significantly increases the ratio of undifferentiated spermatogonia in the testis but decreases the number of mature sperms (Zhang et al., 2022).

TABLE 1 The summary of gut microbiota produced nutrients affecting the male reproductive system.

Nutrients	Function in the male reproductive system	Main bacteria producer
Vitamin A	Promotes spermatogonial stem cells differentiation into sperms	*Escherichia coil*, *Clostridia* (Stacchiotti et al., 2021)
Folic acid	Promoting germ cell differentiation, resistance of oxidative stress and inflammation; prevention of hyperhomocysteinemia.	*Lactobacillus*, *Bifidobacterium*, *Acidobacillus* (Kadry and Megeed, 2018; Wu et al., 2022)
Calcium	Improves sperm motility and sperm capacitation; activates acrosome reaction, and signal transduction in germ cells	*Bifidobacteria*, *Lactobacillus* (D'Amelio and Sassi, 2018)
Vitamin K	Resistance against inflammatory response; promotes serum testosterone and the blood-testis barrier	*Bacteroides fragilis* (Stacchiotti et al., 2021)

Folic acid

Folic acid (vitamin B9) is necessary for DNA and RNA synthesis and methylation. It can affect chromatin structure by affecting histone methylation which is necessary for cell division. Folic acid in the diet improves semen quality and testicular tissue structure, especially if the animal is exposed to reproductive toxic substances. Folic acid helps germ cells to resist oxidative stress and inflammation to prevent DNA damage and apoptosis. Also, it protects the proliferation and differentiation of germ cells from the accumulation of oxidative substances (Rad et al., 2021). Methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes in folic acid metabolism, which participates in the biosynthesis of tetrahydro folic acid (THFA) and vitamin B12 and can re-methylate homocysteine to methionine, an essential amino acid. The low levels of these two vitamins lead to hyperhomocysteinemia, a disease related to the failure of *in vitro* fertilization (IVF) and decreased sperm density, vitality, and DNA integrity. The circulating homocysteine and degree of oxidative stress are positively correlated (Fowler, 2005). Human *MTHFR* gene polymorphisms 677CT and 1298AC can cause a 70% reduction in folate metabolism and hyperhomocysteinemia. *MTHFR* 677T allele is an important factor for male infertility in Asia. Folic acid treatment for 3 months can significantly alleviate the semen oxidative stress due to *MTHFR* 677TT gene carriers, and decrease malondialdehyde and sperm DNA breakage index, improving the natural pregnancy rate and live birth rate (Huang et al., 2020). Folic acid is obtained mainly from dietary supplements and bacterial synthesis. THFA is synthesized by intestinal bacteria from GTP, erythrose 4-phosphate, and phosphoenolpyruvate, which is directly absorbed through

the proton-coupled folate transporter of colon cells and distributed through the circulatory system. Metagenomic analysis showed that *Bacteroides fragilis* and *Prevotella copri* of Bacteroidetes, *Clostridium difficile*, *Lactobacillus plantarum*, *L. reuteri*, *L. delbrueckii* ssp., *bulgaricus* and *Streptococcus thermophilus* of Firmicutes, part of *Bifidobacterium* spp. of Actinobacteria, *Fusobacterium varium* of Fusobacteria, and *Salmonella enterica* of Proteobacteria genera play a role in THFA synthesis (Yoshii et al., 2019). In GM, *Lactobacillus* and *Bifidobacterium* of GM are the main folic acid-producing and metabolizing bacteria (Wu et al., 2022). The production of folic acid can be detected in the culture system of human fecal microbiota *in vitro*. A study showed that oral *Lactobacillus* or *Acidobacillus* in cadmium-poisoned mice reduced testicular cadmium poisoning and promoted germ cell formation, which is a similar effect to folic acid supplementation (Kadry and Megeed, 2018).

Calcium

Calcium plays a decisive role in the fertilization process. It regulates sperm motility in mammals, which directly determines the occurrence of sperm-egg fusion. The process of sperm capacitation is dependent on the activation of the calcium ion channels on the sperm flagellum for sperm motility into the female reproductive tract (Vyklicka and Lishko, 2020). This chemotactic behavior determines acrosome reaction, including hyper activated motility (HAM) like progressive motility and flagellar asymmetric motility. For the acrosome reaction, the sperm needs a sustained increase in intracellular Ca^{2+} levels until F-actin is released from the plasma membrane. Ca^{2+} induces HAM by regulating F-actin, and its influx is mainly controlled by CatSper, which is a sperm-specific Ca^{2+} channel. Ca^{2+} influx can also produce cAMP through a cascade signal reaction promoting active protein kinase A (PKA) causing protein tyrosine phosphorylation. Ultimately, signal transduction in sperm is promoted. Calcium ions accumulate in the epididymis and prostate fluid against the concentration gradient, which is 2–3 times higher than that of the circulatory blood levels (Finkelstein et al., 2020). Blood calcium concentration is sustained by the dissolution of calcium salts *via* osteoclasts in bones. GM is the main regulator of mammalian bone mass, which regulates Ca^{2+} levels in the reproductive system by regulating the conversion between blood and bone calcium. In GM, *Bifidobacteria* and *Lactobacillus* affect the absorption of food calcium. The short-chain fatty acids (SCFAs) in the colon are the regulator of bone cell metabolism. GM produces SCFAs by decomposing dietary fiber. SCFAs reduce the formation of calcium phosphate and promote calcium absorption by lowering the intestine Ph (D'Amelio and Sassi, 2018). A study showed that the levels of IL-6, RANKL, and TNF-α in bone tissues decreased in germ-free mice lowering the number of osteoclasts than SPF mice. SCFAs increase calcium transport through regulations of signaling pathways. Additionally, SCFAs promote the synthesis of serotonin (5-HT), which interacts with bone cells *via* the activation of 5-HT1B receptors on pre-osteogenic cells to inhibit the proliferation of osteoblasts and reduce the formation of bone calcium. This ensures the blood calcium content (Sjogren et al., 2012). A study in GF (germ-free) mice showed an increase in bone mass, while the number of osteoclasts on the surface of bone decreased lowering the concentration of free Ca^{2+}. Re-colonization of the GM in GF mice could normalize the bone mass (Ding K. et al., 2020). This modulating effect of GM on the calcium salt status either promotes or inhibits the survival and motility status of sperm.

Vitamin K

There are two sources of natural vitamin K, plant-derived phylloquinone (vitamin K1), and menaquinone (vitamin K2 or MK-n) produced by microorganisms. In mammals, GM synthesizes menaquinone and transports it through the circulatory system. Vitamin K1 must be converted into vitamin K2 to play important physiological functions such as blood coagulation, fibrinolysis, and bone homeostasis. A vitamin K-rich diet can improve the inflammatory resistance ability of the testes. It can also upregulate the cholesterol and steroid hormone synthase genes, such as *Cyp11a*, thereby increasing the concentration of serum testosterone. In the testicular inflammation rat model induced by LPS, inflammatory mediators such as Nuclear Factor kappa B (NF-κB) and pro-inflammatory factors reduced the transcriptional activity of steroidogenic factor 1 and cyclic AMP response element-binding protein that regulate *Cyp11a*. Consequently, the reduced expression of *Cyp11a* decreased inhibited the synthesis of testosterone in the testis. In all, vitamin K inhibited the activation of NF-κB, increased the expression of *Cyp11a* after LPS treatment, and reduced the inhibitory effect of inflammatory stimulation on testosterone synthesis (Takumi et al., 2011). Vitamin K, as a cofactor, helps γ-glutamyl carboxylase (GGCX) to carboxylate glutamic acid residues into γ-carboxyglutamic acid residues, which then activates vitamin K-dependent proteins. GGCX in testis may promote vitamin K-dependent γ-carboxylation of androgen receptor in Sertoli cell, which helps maintain the BTB structure, and facilitates the development of germ cells and sperm release (Shiba et al., 2021). In idiopathic non-obstructive azoospermia (iNOA) patients, vitamin K epoxide reductase complex subunit 1 (VKORC1), the substrate of vitamin K cycle metabolism, was found abnormally deleted in Leydig cells and extracellular matrix (Alfano et al., 2019). In addition, the relative proportion of vitamin K and D also significantly

impact calcium metabolism affecting the development and motility of sperm (Khalil et al., 2021). Human or mice GM can add or reduce the side chain of dietary supplement vitamin K precursor to remodel to menaquinone 4, 10, 11, and 12 for further utilization (Ellis et al., 2021). GM like *Bacteroides fragilis* can produce vitamin K, mainly the menaquinones. MK4 promotes the genes related to testosterone synthesis. Also, MK7 works with Vitamin D to regulate the level of calcium (Stacchiotti et al., 2021).

Gut microbiota regulates the immune microenvironment of testis

Testes are immune privilege organs. Notably, the male haploid germ cells are not produced until the time of puberty, a long time after birth, which makes these new cells prone to the self-immune system (Qu et al., 2020). Therefore, these germ cells, which are self-antigens, are isolated from the environment to prevent attacks from the immune system. The seminiferous tubules are surrounded by a basement membrane, which is composed of supporting cells and intercellular connections in the blood-testis barrier, specialization of basal exoplasm, and muscle-like tubule cells. The seminiferous tubules create independent cavities, which block the attack from the immune system. Sertoli cells also phagocytose and digest apoptotic germ cells and their remnants to prevent autoimmunity. Androgens synthesized by interstitial cells, corticosterone secreted by testicular macrophages, and prostaglandins, activin, and 25-hydroxycholesterol present in the interstitium inhibit the function of macrophages in the testis. The secretion of corticosterone induces the differentiation of macrophages into immunosuppressive M2 type, promotes the secretion of anti-inflammatory cytokine IL-10, inhibits the expression of TNFα, IL-6, and other pro-inflammatory factors, and reduces the level of the immune response (Wang et al., 2017). The regulatory T lymphocytes (Treg cells) present in the testis upregulate the anti-inflammatory factors IL-10, IL-35, and TGF-β, creating an immunosuppressive microenvironment. A higher number of effector T cells over Treg cells in the testis disturbs the immune-suppressed environment and the autoimmune response is activated (Jacobo, 2018). Although GM promotes maintenance of the immune privileged microenvironment of testis in multiple ways, it can also break it in adverse situation (**Figure 2**).

Gut microbiota and peripheral immunity

The crosstalk between GM and the peripheral immune system influences the balance of pro- and anti-inflammatory cells and maintains the tolerance of the immune exemption department in testis. *Bacteroides Fragilis* produces Polysaccharide A, activates TLR2 signal to induce the production of Foxp3 + Treg cells, promotes the secretion of anti-inflammatory factor IL-10, and inhibits the effect of pro-inflammatory Th17 cells. All this enhances the organ resistance against inflammation (Round and Mazmanian, 2010). SCFAs, an important product of gut microbes, especially butyrate, can facilitate peripheral naive CD4 + T cells of extrathymus to differentiate into Foxp3 + Treg cells (Arpaia et al., 2013). Also, butyric acid promotes the differentiation of M2 macrophages (Ji et al., 2016). SCFAs inhibit the NF-κB pathway by inhibiting lipopolysaccharide-induced macrophages to produce nitric oxide and pro-inflammatory cytokines TNFα, IL-1β, and IL-6 while promoting the secretion of IL-10 (Liu et al., 2012). Finally, the produced immune cells reach the testis *via* mesenteric the lymph system, hepatic portal vein and testicular artery, and affect the immune microenvironment of the testis. In the case of disturbed GM, the secretion of pro-inflammatory factors increases activating macrophages and dendritic cells in the testis. When these innate immune cells enter the epididymis, sperms are recognized as non-self substances attacked, affecting their survival and function (Zheng et al., 2021).

Gut microbiota and androgen

GM regulates the development of gonads through the gut-brain axis, promotes androgen synthesis, and protects the testicular immune tolerance. Androgens ensure the level of Treg cells, inhibit the proliferation of NK cells, and also protect the structure of BTB to prevent pathogenic substances (Kabbesh et al., 2021). A study showed that gut microbes have a strong ability to promote testosterone levels. In adult mice, the level of dihydrotestosterone (DHT) in feces is >20 times higher than that in serum (Collden et al., 2019). Furthermore, compared with sterile mice, the normal concentration of free DHT in the intestine of normal mice was higher. Also, the levels of testosterone, serum gonadotropins luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were higher in the testes of normal mice or mice colonized with probiotics than those in sterile mice. In addition, genes controlling the testosterone production in GF mice such as *Hsd3b1*, *Hsd17b11*, *Cyp11a1*, and *INSL3* were down regulated (Al-Asmakh et al., 2014). GM-produced LPS and pro-inflammatory factors degrade testicular IkB and promote the expression of upstream kinase IKK, which promotes nuclear translocation of NF-κB and inhibits transcription. Phosphorylated NF-κB inhibits the transcription of SF-1 and CREB in testis decreasing the expression of steroid producing gene Cyp11a and testosterone levels. This process can be reversed by increasing the colonization of GM synthesizing vitamin K (Takumi et al., 2011).

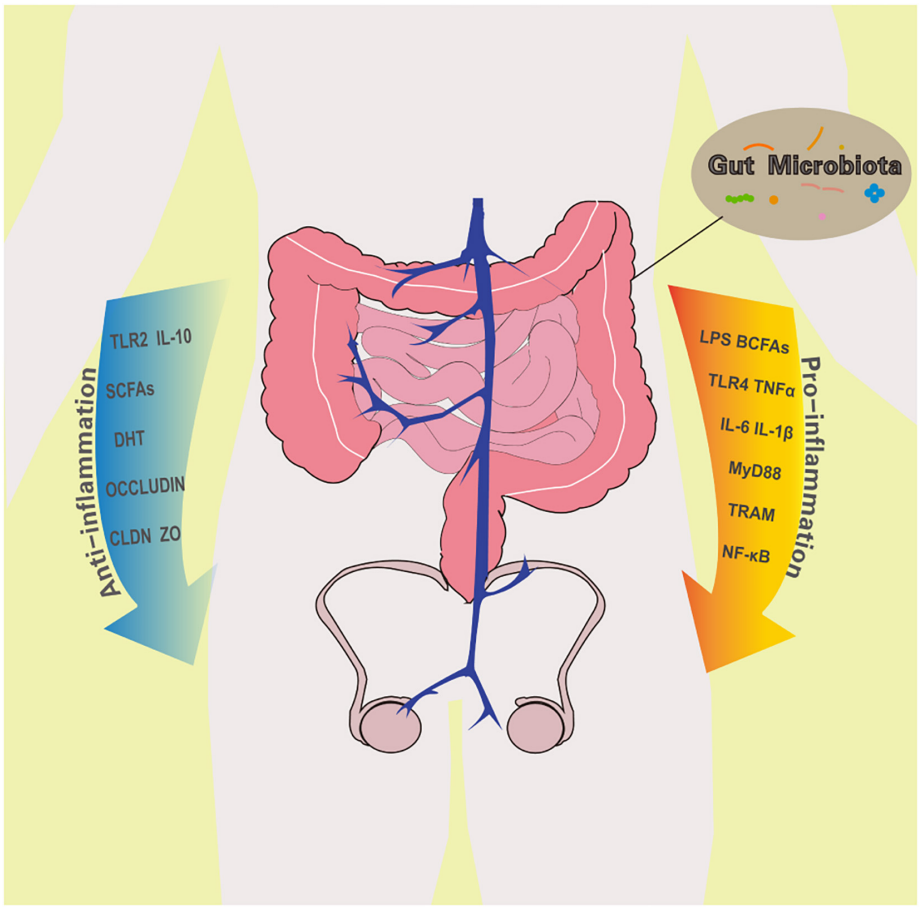

FIGURE 2
The effect of gut microbiota on testes immune privileged microenvironment. A healthy GM promotes anti-inflammatory cells and factors. However, when abnormal bacteria multiply in large numbers, they increase the concentration of pro-inflammatory molecules in the intestine and body fluids. Both positive and negative changes in GM affect the status of the testicular immune microenvironment. TLR2, Toll-like receptor 2; IL-10, Interleukin-10; SCFAs, short chain fatty acids; DHT, dihydrotestosterone; CLDN, claudins; ZO, Zona occludens; LPS, lipopolysaccharide; BCFAs, branched chain fatty acids; TLR4, Toll-like receptor 4; TNFα, tumor necrosis factor α; IL-6, Interleukin-6; IL-1β, Interleukin-1β; MyD88, myeloid differentiation factor 88; TRAM, translocation associated membrane protein; NF-κB: nuclear factor kappa B.

Gut microbiota and blood-testis barrier

A healthy GM improves the integrity of the BTB by upregulating intercellular connections and reducing permeability. The BTB is composed of Sertoli cells and adhesion junction (AJ) and tight junction (TJ) proteins between the cells, such as occludin, claudins (CLDN), JAM, Zona occludens (ZO-1, ZO-2, ZO-3) (Mruk and Cheng, 2015). GM promotes the development of Sertoli cells and their tight junctions, thereby ensuring the formation of seminiferous tubules and the safety of the microenvironment. In 15–16 days old prepubertal mice, SPF mice showed more complete seminiferous tubule development and lumen formation than GF mice. Due to underdeveloped Sertoli cells and low quantity, the lumen of the seminiferous tubules of GF mice was more atresia, showing no attachment of mature luminal co-germ cells. The expression of adhesion links and tight junction proteins, such as occludin, ZO-2, and E-cadherin, was also lower in GF mice. The re-colonization of probiotics in the intestines of GF mice improved the above situation. Due to the loss of Sertoli cells and their intercellular connections, the BTB of GF mice showed higher permeability than SPF mice. The Evans Blue (EB) perfusion test showed higher fluorescence intensity in the seminiferous tubules of GF mice, while after probiotics colonization, the fluorescence only appeared in the interstitium outside the seminiferous tubules (Al-Asmakh et al., 2014). The colonization with normal GM promotes the development of the BTB by improving the secretion of androgen. Testosterone binds to the testosterone receptor on Sertoli cells and promotes the expression of Claudin3 protein in Sertoli cells to increase the tightness of the BTB (Meng et al., 2005). Bacterial translocation induced by abnormal intestinal permeability leads to oxidative stress, activates testicular LPS/TLR4, and transfers NF-κB and

mitogen-activated protein kinase to the nucleus through the MyD88 and TRAM pathways. This activates the innate immunity damaging testicular endothelium and the BTB (Wang and Xie, 2022).

Gut microbiota and testicular immune environment

The altered composition of GM can change gut permeability and immune status through its metabolites, endotoxins, and pro-inflammatory factors, thereby, affecting the immune environment of testis and damaging the reproductive system. A study showed that boars with highly abnormal sperm rates and lower semen utilization contained higher plasma endotoxin and pro-inflammatory factors such as TNF- α and IL-6, and lower anti-inflammatory factor such as IL-10 (Guo et al., 2020). Also, the concentration of fecal branch chain fatty acids (BCFAs), and the markers of proteolysis in the colon were significantly higher than that in boars with high-quality semen. Meanwhile, zonulin and diamineoxidase, which destroy the integrity of the intestine, were also higher in the plasma of boars with low-quality semen. Eventually, the study found that *Sphingobium*, a genus of bacteria that destroys the integrity of the intestine, was enriched in the GM of boars with low-quality semen. The abundance of *Sphingobium* had a strong positive correlation with plasma endotoxin. simultaneously, the abundance of gram-negative *Proteobacteria* in the intestine of boars also increased with low semen utilization. BCFAs, the product of abnormal protein breakdown by *Proteobacteria*, showed higher enrichment in the feces of boars with poor semen quality, which is an indicator of increased intestinal permeability. *Proteobacteria* use amino acids to produce BCFAs while other toxic metabolites are produced in the process. This suggests that higher levels of *Sphingobium* and *Proteobacteria* in the intestine may cause inflammatory responses decreasing semen quality. Increased intestinal permeability promotes LPS leakage into the blood, activates Toll-like receptors, and triggers the immune system to produce IL-6 and TNFα and other pro-inflammatory factors causing immune attacks on the testis (Vaarala et al., 2008; El-Baz et al., 2021). Eventually, the sperm cell membrane in such boars is damaged by lipid peroxidation, the vitality is reduced, and the damage to sperm DNA increases. Also, testosterone synthesis is reduced lowering reproductive ability.

Gut microbiota regulates testis by releasing signaling molecules

The growth, development, and functional regulation of the male reproductive system are also affected by various signaling molecules. For example, 5-hydroxytryptamine (5-HT, serotonin), γ-aminobutyric acid (GABA), and dopamine

TABLE 2 The summary of GM secreted signaling molecules regulating the male reproductive system.

Signaling molecules	Regulation of the male reproductive system	Main bacteria producer
GABA	Promotes sperm capacitation and acrosomal reaction; reduces the excessive activation of sperm; increases libido and sexual behavior	*Bacteroides*, *Parabacter* and *Escherichia coli* (Strandwitz et al., 2019)
5-HT	Balances androgens; reduces the weight and volume of the testis; inhibits ejaculation	*Escherichia coli*, *Streptococcus*, *Enterococcus*, *Bacillus*, Spore-forming microbes, *Clostridium ramosum* and *Corynebacterium* spp. (Yano et al., 2015; Mandic et al., 2019; Liu et al., 2020)
NO	Induces penis erection	*Lactobaillus* spp., *Bifidobacterium* spp., *Staphylococcus aureus*, *Bacillus* spp. (Dai et al., 2015)
H_2S and SO_2	Induce penis erection	*Desulfovibrio*, *Desulfobacter*, *Desulfobulbus* and *Desulfotomaculum* (Gibson et al., 1993; Ran et al., 2019)
LH, FSH and T	Promote testicular cell growth and function; support gonadal development and reproductive function	*Prevotellaceae*, *Cytophagaceae*, *Fibrobacteriaceae*, *Sphingobacteriaceae*, *Idiomarinaceae*, etc. (Markle et al., 2013)

can regulate androgen levels and the process of sperm capacitation. Nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), and sulfur dioxide (SO_2) are important signal molecules synthesized from arginine, glycine, and cysteine, respectively (**Table 2**). These activate guanylate cyclase to produce cGMP, which regulates vascular smooth muscle cell relaxation, hemodynamics, neurotransmission, and cell metabolism through cGMP-dependent protein kinases. H_2S is also an important regulator of nerve function and endothelium-dependent relaxation, regulating membrane KATP channel stimulation and intracellular cAMP signal transmission. In addition, NH_3 is the main product of amino acid catabolism in bacteria and profoundly affects the function of neurons and the vascular system through glutamine-dependent inhibition of NO synthesis (Li et al., 2009).

GABA and 5-HT

GM has been shown to produce various neurotransmitters, such as GABA, 5-HT, dopamine, and norepinephrine by

metabolizing proteins and amino acids or by *de novo* synthesis (Dai et al., 2015). Experiments in mammals show that a large number of neurotransmitters molecules produced by GM play a role in maintaining and changing the physiological functions of animals (Strandwitz, 2018; Huang and Wu, 2021). The presence of 5-HT in the testis balances the production of androgens. In rat interstitial cells, 5-HT binds to 5-HT2 receptors to stimulate the secretion of corticotropin-releasing factor (CRF), which inhibits the synthesis of cAMP and gonadotropin-induced androgen (Tinajero et al., 1993). 5-HT (four times a day, 10 mg/kg) injected into the abdominal cavity of rats reduced the weight and volume of the testis, and lowered the concentration of inhibin and serum testosterone (Hedger et al., 1995). Also, 5-HT inhibits ejaculation and adjusts penile flaccidity and detumescence *via* the control of vascular resistance, blood pressure, hemostasis and platelet function. 5-HT binding to 5-HT2C and 5-HT1B receptors increases ejaculatory latency and delays orgasm, while 5-HT binding to 5-HT1A receptor decreases ejaculatory latency. The testis itself can produce endogenous 5-HT, while the rest is mainly used from the peripheral circulation (Berger et al., 2009). The gut is the main source of 5-HT; >90% of the total 5-HT is gut-derived, which is transported to the whole body through platelets. Enterochromaffin cells (ECs), mucosal mast cells, and myenteric neuron cells mainly synthesize 5-HT in the intestine. Studies have shown that nearly 10% of ECs synthesis peripheral 5-HT rely on GM. The concentration of serum 5-HT in adult GF mice decreased, and correspondingly, the concentration of 5-HT in the colon and feces decreased significantly. Spore-forming microbes (Sp) from the healthy mouse and human microbiota promote local and peripheral 5-HT concentration through its metabolites to promote the expression of tryptophan hydroxylase 1 (*Tph1*), an important gene for 5-HT synthesis in ECs (Yano et al., 2015). Cellular components of *Clostridium ramosum* have also been shown to stimulate host ECs to secrete 5-HT and modify the colonic stem cells to differentiate into lineages that secrete 5-HT (Mandic et al., 2019). Some bacteria in culture, including *Corynebacterium* spp., *Streptococcus* spp. and *Escherichia coli*, were reported to synthesize 5-HT (Yano et al., 2015). Damage GM induces local inflammation, which lowers the number of 5-HT transporters (Stasi et al., 2019). A study showed that male Brandt's voles reared in high density stress environments exhibited a higher abundance of *Streptococcus* and *E. coli* in the intestine, which possibly increases the serum cortisol and 5-HT concentrations. Both of these increased the serum testosterone levels of Brandt's voles *via* the hypothalamic-pituitary-gonadal axis making the animal more aggressive (Liu et al., 2020).

The GM genome metabolism model showed that *Bacteroides*, *Parabacter* and *E. coli* actively express GABA. Also, the isolation and culture of *Lactobacillus* and *Bifidobacterium* in the intestine could produce GABA. The GABA concentration is related to the process of sperm capacitation in the vagina. GABA promotes the tyrosine phosphorylation of sperm protein, which is an indicator of sperm capacitation. GABA also promotes the acrosome reaction, which is inhibited by selective GABA receptor antagonists (Kurata et al., 2019). A study in hamsters showed that GABA reduces the excessive activation of sperm by inhibiting the binding of 5-HT to 5-HT2 receptors, thereby co-regulating sperm activation with 5-HT (Fujinoki and Takei, 2017). GABA can also regulate the sexual behavior of male mammals. Treatment with Moxidectin, an anthelmintic drug, in rats lowered their libido and sexual behavior by reducing GABA secretion, which hindered penile erection (Rodrigues-Alves et al., 2008).

Nitric oxide and hydrogen sulfide

Arginine amino acid has nutritional effects on male reproductive function. Although bacteria in the small intestine can decompose arginine and affect the use of arginine by the reproductive system, some bacteria such as *Lactobaillus* spp., *Bifidobacterium* spp., *Staphylococcus aureus*, *Bacillus* spp. affect the NO production *via* arginine metabolism (Dai et al., 2015). NO synthesizing bacteria Bacillus and Paenibacillus were found in the GM of obese girls, and the NO synthesis was positively correlated with the level of FSH (Li Y. et al., 2021). Physiological levels of NO, a signaling molecule, also play an important role in the male reproductive system. In the brain, NO promotes the release of neurotransmitters to maintain libido and the secretion of luteinizing hormone-releasing hormone (LHRH) and GnRH to increase sex hormone levels. In testis, NO dilates blood vessels, allowing the testes to regulate local temperature. In the reproductive system, NO is released at the nerve endings of the cavernous body to activate guanosine cyclase. Activated guanylate cyclase produces cGMP to relax the vascular smooth muscle congesting the corpus cavernosum which leads to penis erection (Gratzke et al., 2010). H_2S can also act as a physiological vasodilator, which directly affects erectile function. A study showed an increase in penis length after H_2S injection into the penile cavernous body; the efficiency of penis lengthening was similar to the effect of 20 μg prostaglandin E1 (D'Emmanuele di Villa Bianca et al., 2011). Intestinal sulfate-reducing bacteria (SRB) such as *Desulfovibrio* spp., can use H_2, lactic acid, and acetate as electron donors, and sulfate or sulfite as electron acceptors to produce H_2S. There are also some bacteria in large intestines, such as *E. coli*, *Salmonella enterica*, *Clostridium* spp., and *Enterobacter aerogenes*, that can metabolize sulfur-containing amino acids to produce H_2S (Gibson et al., 1993; Ran et al., 2019).

Sex hormone

The type and abundance of gut microbes can affect the level of sex hormones in animals. Bacterial overgrowth in the

small intestine may trigger an increase in intestinal permeability and systemic circulation, and a decrease in serum testosterone, which impairs testicular function (Tremellen and Pearce, 2020). This effect may be achieved by interfering with the steroid cycle metabolism and affecting the hormone-HPG axis. The level of sex steroid hormones is related to the composition and diversity of gut microbes. Individuals with more diverse gut microbes have higher levels of sex steroids (Shin et al., 2019). Estrogens (such as estradiol), progesterone, and their receptor exist in male sexual glands maintain male fertility. In women, estrogen production requires GM-secreted β-glucuronidase to covert conjugated estrogens to deconjugated forms. The increase in the abundance of β-glucuronidase-producing bacteria can promote in the level of circulating estrogen. A study showed that the α diversity of GM negatively correlates with the concentration of estradiol and positively correlates with the proportion of estrogen metabolites in the urine of women, however, the same needs to be verified in men and male animals (Qi et al., 2021). As mentioned earlier, GM can alter the expression of steroid-producing genes HSD3β1, Cyp11a, etc., which changes the levels of sex hormones (Takumi et al., 2011; Ding N. et al., 2020). Compared with SPF male mice, GF male mice had lower serum levels of testosterone (T), LH, and FSH, however, colonizing their intestine with probiotics significantly increased the serum levels of these hormones (Al-Asmakh et al., 2014). A study in the O-PLS mice model showed that testosterone levels were positively associated with *Prevotellaceae*, *Cytophagaceae*, *Fibrobacteriaceae*, *Sphingobacteriaceae*, and *Idiomarinaceae*, and negatively associated with *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Verrucomicrobia* phylum (Markle et al., 2013). In adolescent males, the level of testosterone was found to be associated with *Adlercreutzia*, *Ruminococcus*, *Dorea*, *Clostridium* and *Parabacteroides* genus (Yuan et al., 2020). Besides, a part of GM converts androgen precursors into active androgens (Pernigoni et al., 2021). GM promotes the deglucuronidation of testosterone and DHT, and increases the levels of free testosterone and DHT, which contribute to the development of secondary sexual characteristics in male animals. GC-MS (Gas chromatography-tandem mass spectrometry) analysis revealed that the intestinal levels of free testosterone and DHT were higher in segments with a high microbial density such as the cecum and colon than in a low microbial density segment such as the proximal small intestine. The free DHT level in feces is >70 times higher than in serum. Compared to normal mice, the concentration of free DHT is much lower in the distal intestine of GF mice, which contained a lot of glucuronidated T and DHT (Collden et al., 2019). Transplantation of fecal microbes from high-fat diet mice were into the intestine of normal mice increased the intestinal abundance of *Bacteroidaceae* and *Prevotellaceae* in the transplanted mice decreasing the expression of the Hsd3β1 gene encoding DHT synthase in testis (Ding N. et al., 2020).

Conclusion and perspectives

GM metabolizes nutrients in animals regulating their immune state. GM has great research value for its effect on far distal organs. Experimental and clinical evidence from different species indicate that the main ways through which microbiota affects the development and function of the reproductive system include: providing nutrients like SCFAs, vitamins, and minerals to transform the function and gene expression status of the reproductive system, regulating the testicular immune microenvironment, controlling physiological processes through signal transduction, and affecting hormone levels (Dai et al., 2015; Li et al., 2017; Li X. et al., 2021; Zhang et al., 2022). The metabolic processes of GM provide crucial nutrients such as vitamins and minerals to the reproductive system; and regulate the development and functions of testes to maintain their immune privileged state. GF animals, which had no microbial abundance in the gut, exhibited decreased testosterone levels and abnormal BTB structure than the normal ones (Al-Asmakh et al., 2014). An altered GM negatively affects the function of the testis under on various stresses or the influence of toxic substances (Liu et al., 2022). Instead of providing nutritional molecules and support to the reproductive system, the abnormal microbiota produces pro-inflammatory factors and creates an oxidative environment that disrupts the spermatogenic process in the testis (Tian et al., 2019; Ding N. et al., 2020; Zhao et al., 2020). The effect of GM on distal organs is a fascinating prospect that requires more research. It may also provide a new promising way to regulate reproduction. Improving dietary structure, recolonizing healthy fecal microbes, and supplementing health products like probiotics have been shown to alleviate infertility in men and male animals, which further proves that altering the composition of GM can regulate the physiological functions of the testis, or even reverse the alterations to the aging effect on reproductive system (Poutahidis et al., 2014; Xie et al., 2019). Studies have shown that the decreased number of germ cells and low-quality semen in high-fat diet male animals are largely induced by GM disturbances which cause an accumulation of harmful metabolites such as sphingosine. Remodeling their GM by feeding melatonin or transplanting alginate oligosaccharides-improved fecal microbiota effectively alleviates the above conditions (Hao et al., 2022a; Sun et al., 2022). Zhang C. et al. (2021), Zhang P. et al. (2021), and Hao et al. (2022b) also found that transplantation of fecal microbiota from mice supplemented with alginate oligosaccharide to mice treated with busulfan or streptozotocin (a type 1 diabetes inducer) could rescue germ cell loss and improve semen quality through metabolic pathways. Although GM metabolites have an impact on fertility, basic phenomena yet remain to be defined completely. The physiological changes and specific consequences of this phenomenon are difficult to quantify, track and locate in real-time. For now, it is unknown how

many metabolites from the circulatory system pass the BTB directly affect the male reproductive system. Existing research trends indicate that using multi-omics technology can delineate the interactions between GM and the host organs/tissues (Tilocca et al., 2020). With the establishment of gene expression profiles and metabolomics, researchers can now locate the transverse spatial organization and longitudinal phase states of GM (Tropini et al., 2017; Mars et al., 2020). The intricate networks between GM as well as the breaking and rebuilding of microbial balance are other research challenges. In the following research, scholars need to pay attention to the effect of partial and/or the entire function of the GM on toward the reproductive capacity in males and design a series of microbial complex agents to promote or inhibit fertility without affecting normal health (Alfano et al., 2018). Research targeting the treatment and development of GM will generate more emphasis in the near future to improve the health status of humans and animals.

Author contributions

HC wrote the manuscript. SP investigated and supervised the manuscript. XC and DQ designed the tables and figures and edited the manuscript. JH, YDL, and YL edited the manuscript. All authors read and approved the final version of the 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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EDITED BY
Karolina Skonieczna-Żydecka,
Pomeranian Medical University,
Poland

REVIEWED BY
Igor Łoniewski,
Pomeranian Medical University,
Poland
Jarosław Biliński,
Medical University of Warsaw,
Poland

*CORRESPONDENCE
Youcheng Zhang
zhangychmd@126.com

†These authors have contributed equally to this work and share first authorship

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Global research trends and hotspots of fecal microbiota transplantation: A bibliometric and visualization study

Mancai Wang[1†], Xiaofeng Xie[2†], Songbo Zhao[1], Wei Han[1] and Youcheng Zhang[1*]

[1]Department of General Surgery, Lanzhou University Second Hospital, Lanzhou, China, [2]Medical College, Northwest Minzu University, Lanzhou, China

Introduction: Fecal microbiota transplantation (FMT) has gained considerable attention in a variety of clinical research areas, and an increasing number of articles are being published. It is very critical to reveal the global status, future research trends, and hotspots in the FMT research and application.

Methods: We searched the Web of Science Core Collection up to May 10, 2022, and only articles and review articles about FMT were included finally. CiteSpace 5.8.R3, VOSviewer 1.6.18, Scimago Graphica and Microsoft Office Excel 2019 were used for data analysis and visualization. The results included publication characteristics, Co-authorships analysis, Co-cited analysis, Co-occurrence analysis, and burst analysis.

Results: Eleven thousand nine hundred seventy-two records were used for the analysis and visualization finally, these records were published between 1980 and 2022, and the publication about FMT is increasing year by year. Co-authorship analysis shown that the USA played a key role in this field. After data analysis and visualization, a total of 57 hotspots about FMT were produced. We summarized these hotspots and classified them into 7 grades according to the number of evidence sources. The evidence sources included top 25 of Web of Science categories, top 30 most Co-cited references, top 10 clusters of references, top 25 references with the strongest citation bursts, top 25 keywords with the most occurrence frequency, major 15 clusters of keywords, top 25 keywords with the strongest citation bursts, and top 35 disease keywords.

Conclusion: This bibliometric analysis is expected to provide overall perspective for FMT. FMT has gained increasing attention and interest, there are many hotspots in this field, which may help researchers to explore new directions for future research.

KEYWORDS

fecal microbiota transplantation, research trends, research hotspots, bibliometric analysis, visualization analysis

Introduction

Fecal microbiota transplantation (FMT) is an old and non-conventional therapy comes of age (Kelly, 2013), in which fecal materials from healthy donors are given to patients attempt to cure disease or relieve symptoms (Aroniadis et al., 2019). The concept of FMT is not new in the literature. Some scholars thought that this idea is possibly first proposed in veterinary medicine by the Italian anatomist Fabricius Aquapendente in the 17th century (Borody et al., 2004; Brandt et al., 2012). However, Zhang et al. firmly believes that it is Ge Hong, a well-known traditional Chinese medicine doctor in China, described the use of human fecal suspension by mouth for patients who had food poisoning or severe diarrhea during the Dong-jin dynasty in the 4th century (Zhang et al., 2012). The earliest reports of FMT in the modern literature can be traced back to 1958, in which fecal enema was used as an adjunct in the treatment of pseudomembranous enterocolitis (Eiseman et al., 1958). However, because of the lack of sufficient evidences, FMT has not become a routine therapy in the past few decades (Zhang et al., 2012).

Numerous studies have proved that gut microbiota dysbiosis is closely related to the occurrence and development of various diseases (Aron-Wisnewsky et al., 2021; Chen et al., 2021; Yang et al., 2021). Sufficient evidences shown that FMT is an efficient way of modulating the gut microbiota and introducing a balanced conglomerate of microorganisms (Browne et al., 2021; Du et al., 2021). FMT is already widely practiced as a highly effective treatment for recurrent *Clostridium difficile* infection (CDI; Hui et al., 2019; Hvas et al., 2019; Green et al., 2020; Tixier et al., 2022). A wealth of researches also supported that it may be used to treat other health conditions, including gastrointestinal (Caldeira et al., 2020; Green et al., 2020; Wu et al., 2022), oncological (McQuade et al., 2020; Lythgoe et al., 2022), cardiovascular (Hu et al., 2019; Zhong et al., 2021), autoimmune (Engen et al., 2020; Liang et al., 2021), metabolic (Aron-Wisnewsky et al., 2019; Proença et al., 2020; Hanssen et al., 2021; Manrique et al., 2021), and neuropsychiatric (Evrensel and Ceylan, 2016; Vendrik et al., 2020) diseases, etc. As expected, FMT may herald the puberty of a broad and exciting new branch of human therapeutics (Kelly, 2013).

In recent years, FMT has gained considerable attention in a variety of clinical research areas as described above, and an increasing number of articles are being published. We speculated that there may be many hotspots and focuses in the field of FMT research. However, few attempts have been made to thoroughly assess the scientific output and current status in this topic from a worldwide viewpoint. Therefore, it is very critical to reveal the global status, future research trends, and hotspots in the FMT research and application.

Bibliometric analysis is a statistical method used for the analysis and visualization of key characteristics and research trends in a specific field using online literature databases (Ellegaard and Wallin, 2015; Donthu et al., 2021), it has been widely applied in a variety of fields. Bibliometric analysis is also an effective tool to qualitatively and quantitatively analyze the publications and identify significant research hotspots and trends (Gu et al., 2021a). In this study, we aimed to conduct a comprehensive bibliometric analysis of publications related to FMT, and gain the research hotspots and potential trends, and finally provide useful reference guideline for future researches.

Materials and methods

Data search and selection

We systematically searched the electronic database Web of Science Core Collection (WoSCC) up to May 10, 2022. This search was performed using topic term. Search terms included fecal, faecal, feces, faeces, stool, microbiota, microbiome, microflora, bacteria, transplantation, transplant, transfer, enema, infusion, bacteriotherapy. The full search syntaxes were supplied in Supplementary Table 1. Only articles and review articles were included for the analysis and visualization finally.

Data analysis and visualization

We exported the full records and cited references of records from WoSCC. In this study, CiteSpace 5.8.R3, VOSviewer 1.6.18, Scimago Graphica and Microsoft Office Excel 2019 were used for data analysis and visualization. The flowchart of study identification and data analysis/visualization was shown in Figure 1.

Microsoft Office Excel 2019 and Scimago Graphica were used for the analysis and visualization of publication characteristics, which included total publication, annual publication and trend, document types, and Web of Science categories. VOSviewer 1.6.18 and Scimago Graphica were used for Co-authorships analysis and visualization, which included country/region Co-authorships, institution Co-authorships, and author Co-authorships. CiteSpace 5.8.R3 was used for Co-cited analysis, Co-occurrence analysis, and burst analysis. Burst analysis included burst references and keywords analysis. All data in tables was extracted by the VOSviewer 1.6.18.

Results

Over characteristics of publication

A total of 13,679 publication records met the search criteria primitively, of which 11,972 records were articles and review articles that were used finally for the analysis and visualization (Figure 1). As shown in Figure 2, these records were published between 1980 and 2022, a growing trend in publication was observed, indicating the increasing attention and interest in the FMT field. The annual publications began rapidly growing from 1991, more than 1,000 papers were published annually from 2019. Of these records, articles accounted for around 83% of document type (Figure 2), indicating a larger emphasis on original studies in the field of FMT.

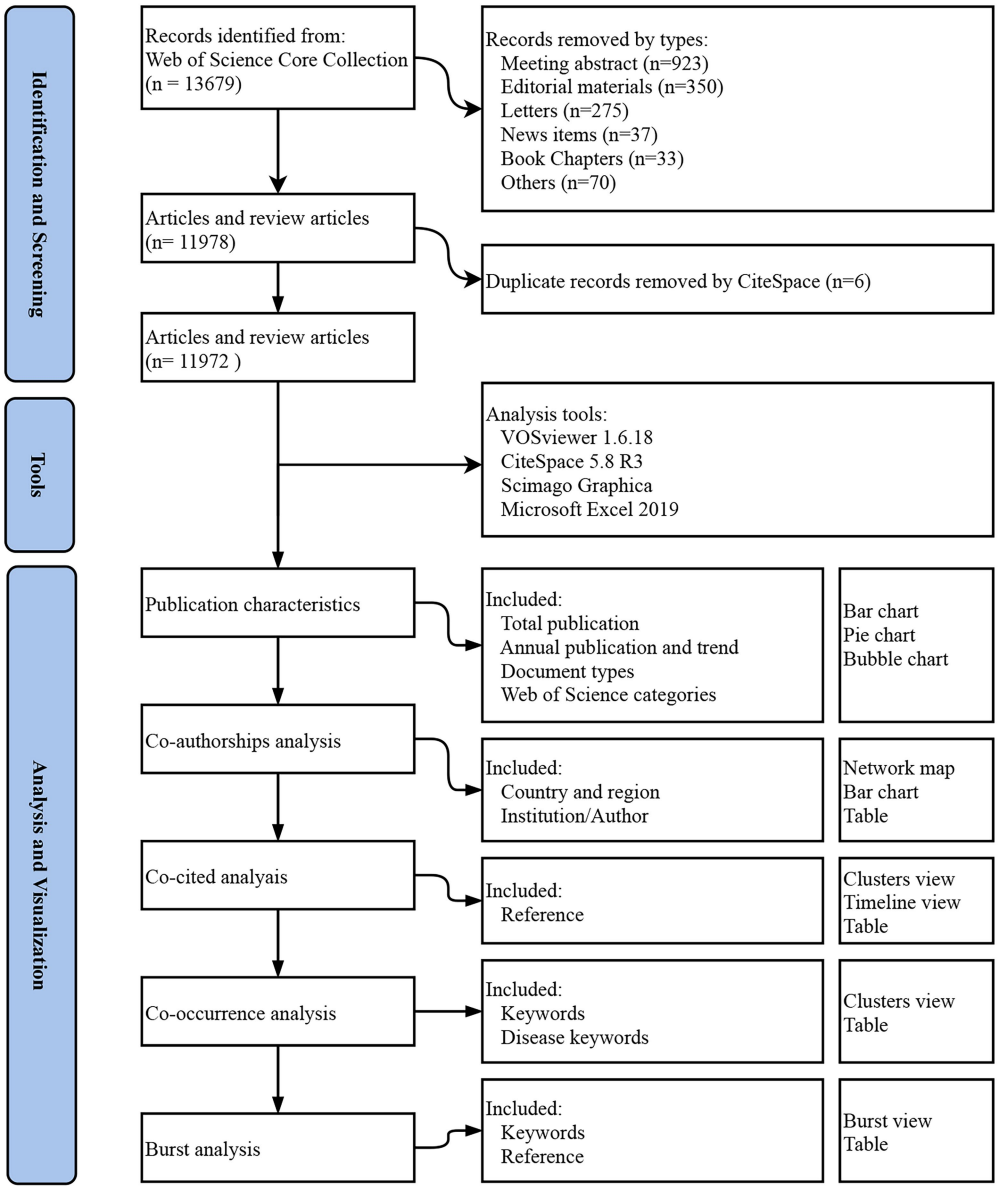


FIGURE 1
The flowchart of study identification and data analysis/visualization.

Web of Science categories

All the analyzed records were divided into 173 entries of the Web of Science categories, among which gastroenterology hepatology was the largest, accounting for 15.5% of the records, followed by microbiology, surgery, pharmacology pharmacy, and immunology, etc. Top 25 categories were shown in Figure 3A, and the trend of their annual publications was shown in Figure 3B. In the remaining 148 entries, 38 were closely related to clinical medicine, and the trend of their annual publications was shown in Figure 3C. From these figures above, we could clearly find that most of the top 25 categories were the most classic and persistent research fields and also the hotspots of FMT research at the present. In addition, the number of publications in neuroscience, clinical neurology, psychiatry had increased significantly in the past three years, which may has become new research hotspots in the fields of FMT.

Distribution and Co-authorship analysis of countries/regions

All publications in the field of FMT were distributed among 147 countries/regions, the global distribution and cooperation of these major countries were shown, respectively, in Figures 4A,B. The production of the USA ranked the first with 3,880 documents by far, followed by the China, United Kingdom,

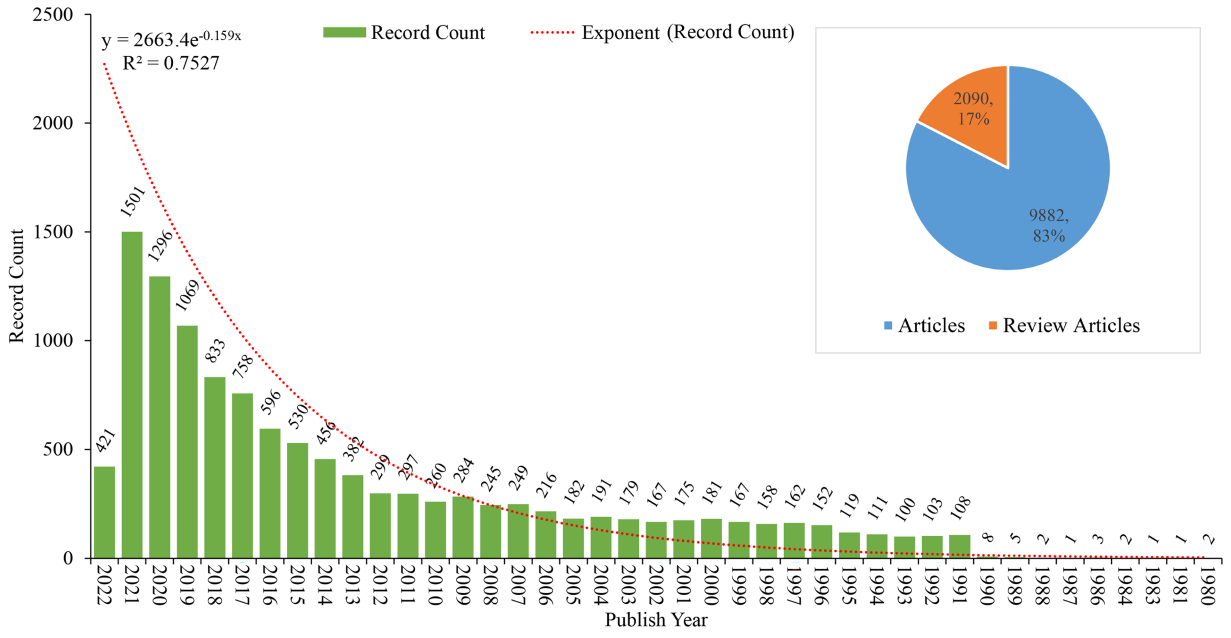


FIGURE 2
The publication characteristics of FMT.

and Germany. The top 20 countries with the most publications and their total link strength were shown in Table 1, the USA was also one of the most cooperative countries in the FMT research, and it cooperated closely with China and other countries.

The trends of the annual publication of the top 10 countries were shown in Figure 4C. The USA was one of the earliest countries to study FMT, and its publications increased significantly since 1991, which make it the country with the most annual publications between 1991 and 2020. As a rising star, China's research boom on FMT mainly started after 2014, and its annual publications surpassed that of the USA in 2021. The trends of the annual publication relation to medicine of the top 10 countries were shown in Supplementary Figure 1, it was similar compared with Figure 4C.

The total citations of the USA were extremely outstanding, followed by the United Kingdom, France, and China, etc. (Figure 4D; Table 1). As shown in Figure 4D, the United States was the only country marked with purple circles and had strongest betweenness centrality (0.39), which means it played a key role in the field of FMT. Europe was not only one of the regions with the largest number of countries conducting FMT research (Figure 4A), but also had highest average citations in many countries, such as the Finland (Chen et al., 2019), Sweden (El-Salhy et al., 2020), Netherlands (Ianiro et al., 2018a), and United Kingdom (Wilson et al., 2019), etc. (Table 1). Although the number of publications in China had increased rapidly in recent years, the total citations, especially the average citations, were relatively low, and its betweenness centrality is 0. These results indicated that the quality of China research needs to be improved further.

Distribution and Co-authorship analysis of institutions

A total of 10,019 institutions contributed to the research on FMT. The characteristics of the top 20 institutions with most publications was shown in Table 2, and ten of them located in the United States, 3 in China, 2 in Canada, and others located in the Denmark, Finland, Netherlands, France, and Brazil. The institution with the most publications (128) was the Univ Minnesota, and the institution with the highest average citations (115) was the Harvard Univ, both of which are located in the USA. The Co-authorship network of major institutions (1%) was shown in Supplementary Figure 2. The institutions marked with purple circles, including the Harvard Med Sch (0.18) and Univ Helsinki (0.1) had strongest betweenness centrality, which means they played key roles in the field of FMT.

Distribution and Co-authorship analysis of authors

A total of 58,460 authors contributed to the research on FMT. The characteristics of the top 20 authors with most publications was shown in Table 3, eight of them in the USA, 5 in China, 3 in Italy, 2 in Netherlands, and others in United Kingdom and Canada. Among them, the author with highest average citation was De Vos WM (159), who worked in the Wageningen Univ of Netherlands, followed by Nieuwdorp M (116) and Sadowsky MJ (116), they worked, respectively, in the Univ Amsterdam of Netherlands and Univ Minnesota of the USA.

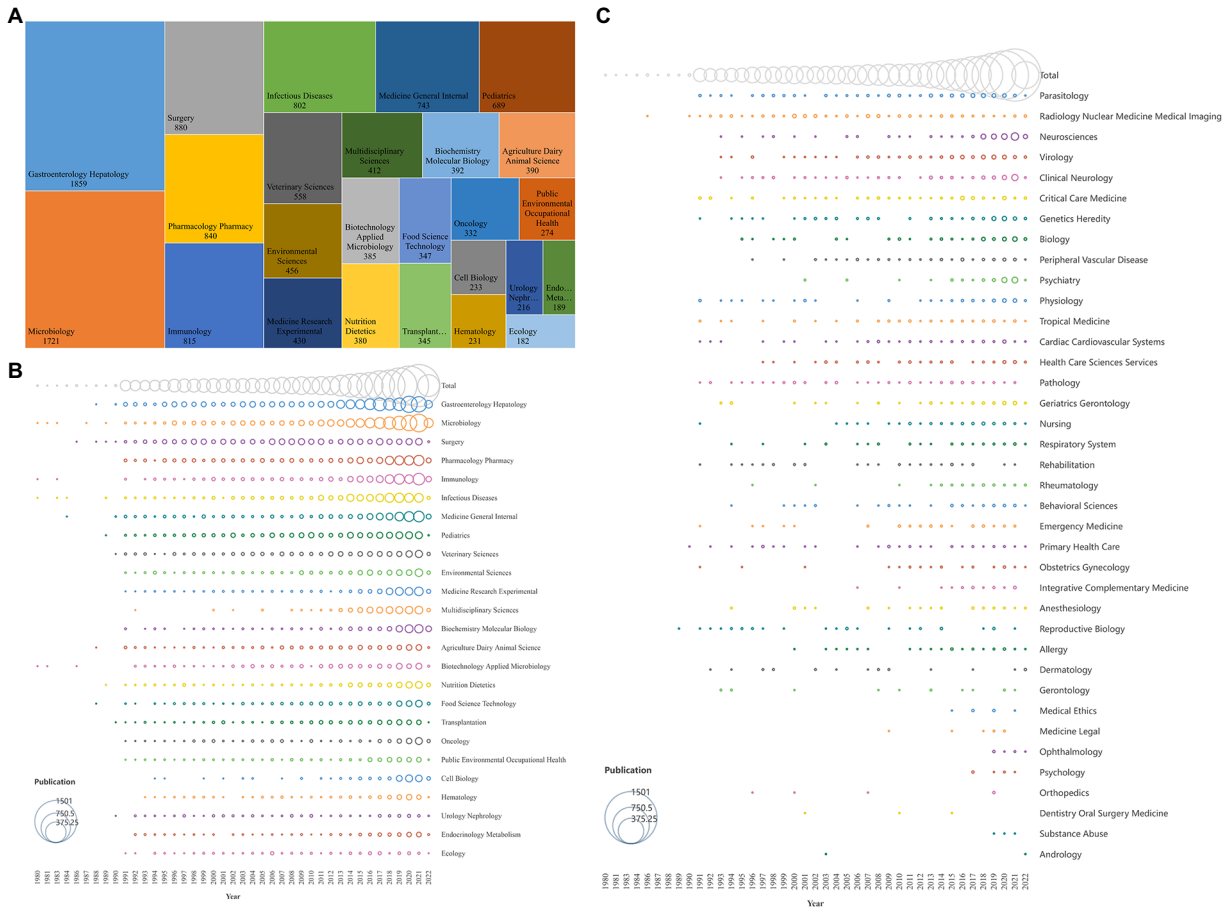


FIGURE 3
The Web of Science categories and the trend of their annual publications. **(A)** Top 25 categories of publication about FMT. **(B)** Annual publications and trend of the top 25 categories. **(C)** Annual publications and trend of other categories related to clinical medicine. The size of the circle represents the number of annual publications in each category.

The collaborations among the lead authors (1%) and their teams on FMT were shown in the Figure 5A. We found that most of the top 20 authors had cooperative relationships with each other (Figure 5B). The main cooperative networks of the top 20 authors with other researchers were shown, respectively, in Supplementary Figure 3.

Active journals analysis

A total of 2,790 journals have published documents on the subject of FMT. The characteristic of the top 20 journals with most publications was shown in Supplementary Table 2. Journal with the most publications was the Plos One (158), followed by the Frontiers in Microbiology (148), Journal of Pediatric Surgery (143), and Scientific Reports (118). Of the top 20, journal with the highest average citations was the Gastroenterology (185), followed by the American Journal of Gastroenterology (124), and Gut (104). In recent years, the following journals have begun to focus on the FMT research, including the Frontiers in Immunology, Frontiers in Microbiology, Gut Microbes, and Microbiome, etc. (Figure 6).

Co-cited references analysis

A total of 327,028 references cited by 11,972 publications were identified by the software of VOSviewer. The top 10 most-cited references (Caporaso et al., 2010; Bakken et al., 2011; Gough et al., 2011; Vrieze et al., 2012; Kassam et al., 2013; Surawicz et al., 2013; van Nood et al., 2013; Moayyedi et al., 2015; Rossen et al., 2015; Paramsothy et al., 2017) were shown in Table 4, they were published between 2011 and 2017, and four of them were reviews. Five (Bakken et al., 2011; Gough et al., 2011; Kassam et al., 2013; Surawicz et al., 2013; van Nood et al., 2013) of the top 10 references were on the topic of FMT for the treatment of *Clostridium difficile* infection (CDI), and they were all published before 2013. Three (Moayyedi et al., 2015; Rossen et al., 2015; Paramsothy et al., 2017) of them was for ulcerative colitis (UC), one (Vrieze et al., 2012) for metabolic syndrome, and one

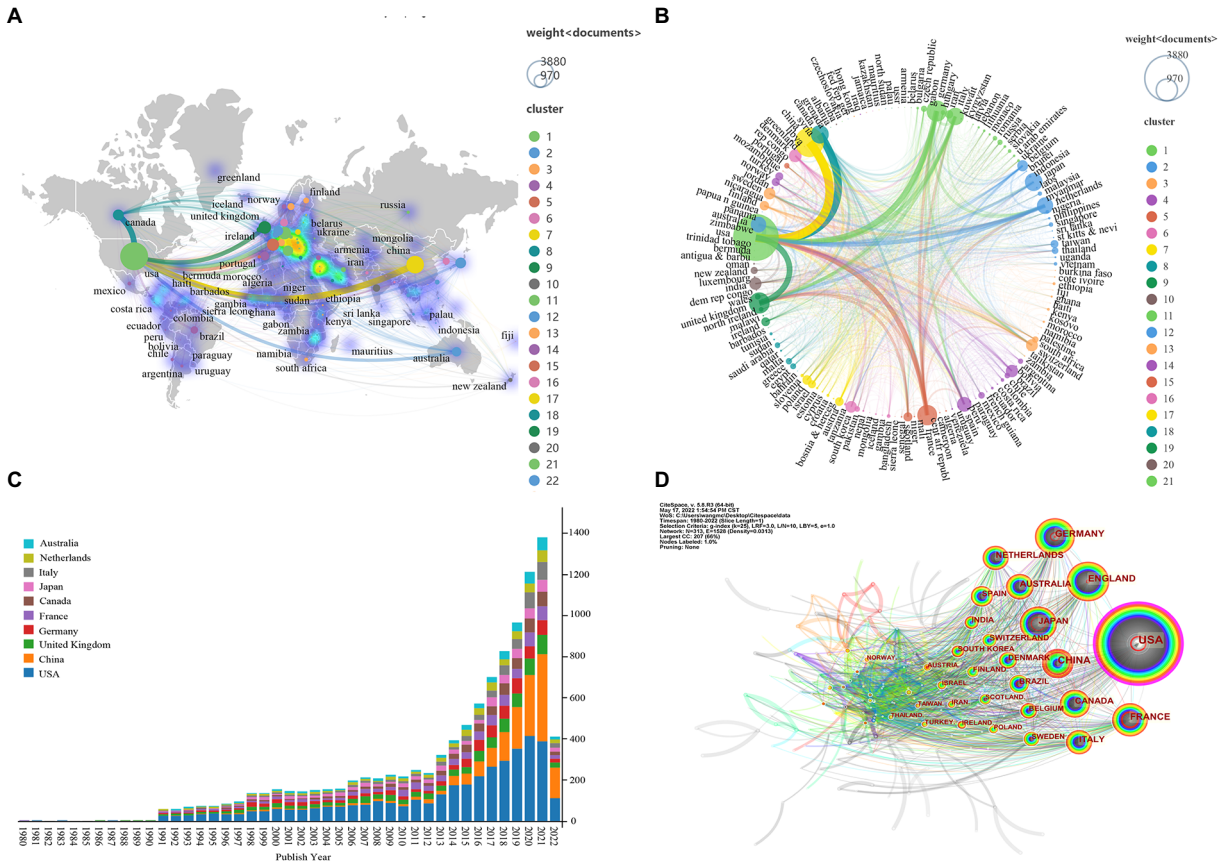


FIGURE 4
The global distribution and cooperation network of FMT research. **(A)** The global distribution of FMT research. The size of the circle represents the number of total publications in different countries, the width of the lines between different countries represents the strength of their cooperation. **(B)** The cooperation network of FMT research in different countries. The size of the circle represents the number of total publications in different countries, the width of the lines between different countries represents the strength of their cooperation. **(C)** The annual publications and trends of the top 10 countries. **(D)** The total citations of publications in different countries. The overall size of the circle represents the number of publications in different countries. Each colored circle (tree ring history) represents the number of publications published by that country in a single time slice. The width of the lines between different countries represents the strength of their cooperation; The outermost purple circle represents the country has a significant role in the FMT field.

(Caporaso et al., 2010) for QIIME, which was an analysis tool for high-throughput community sequencing data.

Considering that the top 10 most-cited references were published in an older time, we analyzed and summarized the top 20 most-cited references (DeFilipp et al., 2018, 2019; Gopalakrishnan et al., 2018; Halkjær et al., 2018; Ianiro et al., 2018a,b; Routy et al., 2018; Smillie et al., 2018; Suez et al., 2018; Taur et al., 2018; Wang et al., 2018; Zhang et al., 2018; Zuo et al., 2018; Allegretti et al., 2019; Bolyen et al., 2019; Costello et al., 2019; Kang et al., 2019; Paramsothy et al., 2019; Wilson et al., 2019; El-Salhy et al., 2020) published in the last 5 years, which were shown in Table 5. Most of them were clinical trial and were published between 2018 and 2020. It is remarkable that their topics were completely different from those above (Table 4). Some new topics about FMT may have become hotspots and potential trends in recent years, which included drug-resistant bacteremia (safety of FMT; DeFilipp et al., 2019), tumors (Gopalakrishnan et al., 2018; Routy et al., 2018), irritable bowel syndrome (Halkjær et al., 2018; El-Salhy et al., 2020), antibiotics-associated dysbiosis (Suez et al., 2018; Taur et al., 2018), autism (Kang et al., 2019), allogeneic hematopoietic cell transplantation (DeFilipp et al., 2018), super-donor (Wilson et al., 2019), bacterial engraftment (Smillie et al., 2018), and bacteriophage transfer (Zuo et al., 2018), etc. However, the topics that have not changed included *Clostridium difficile* infection (Ianiro et al., 2018a,b), ulcerative colitis (Costello et al., 2019; Paramsothy et al., 2019), and QIIME (Bolyen et al., 2019).

Total 10 major clusters ($Q = 0.82$, $S = 0.90$, $Q/S = 0.89$) were generated from the co-citation networks of references after cluster analysis by the software of CiteSpace (Figure 7A), and the cluster nomenclature may reflect the study hotspots and frontiers in FMT field. The largest cluster (2,695 Nodes, 44%) was #0 *Clostridium difficile* infection, followed by #1 gut microbiota, #2 irritable bowel syndrome, #3 difficile infection, #4 inflammatory bowel disease, #6 versus-host disease, #10 colorectal cancer, #12 liver diseases, #16 fecal microbiota transplantation, and #20 cardiovascular disease.

TABLE 1 Characteristics of the top 20 countries with the most publications.

Num	Country	Publications	Citations	Average citations	Total link strength	Betweenness centrality
1	United States	3,880	176,555	46	1834	0.39
2	China	1,539	28,653	19	552	0.00
3	United Kingdom	794	37,777	48	969	0.07
4	Germany	770	28,102	36	843	0.04
5	France	737	34,413	47	740	0.07
6	Canada	592	26,256	44	650	0.02
7	Japan	576	13,241	23	245	0.02
8	Italy	537	18,377	34	606	0.02
9	Netherlands	492	27,099	55	647	0.03
10	Australia	445	17,727	40	438	0.02
11	Spain	391	13,181	34	450	0.02
12	Denmark	295	14,000	47	365	0.03
13	Brazil	278	5,734	21	130	0.01
14	India	272	4,713	17	178	0.01
15	Switzerland	260	10,783	41	395	0.05
16	Sweden	245	14,843	61	390	0.04
17	South Korea	240	4,819	20	136	0.01
18	Belgium	218	9,391	43	315	0.02
19	Finland	162	12,255	76	256	0.02
20	Poland	159	2,577	16	151	0.00

Total link strength, generated by VOSviewer 1.6.18 software, it indicates the strength or closeness of the country's cooperation with other countries in the field of FMT; Betweenness centrality, generated by CiteSpace 5.8 software, it represents the influence or contribution of the country in the FMT field, and greater than 0.1 means that the country has an important contribution or a great influence.

TABLE 2 The characteristics of the top 20 institutions based on publications.

No.	Institutions	Country	Publications	Citations	Average citations	Total link strength	Betweenness centrality
1	Univ Minnesota	United States	128	9,750	76	229	0.04
2	Harvard Med Sch	United States	120	7,423	62	398	0.18
3	Univ Copenhagen	Denmark	119	7,674	64	243	0.04
4	Univ Helsinki	Finland	111	6,853	62	194	0.10
5	Mayo Clin	United States	111	8,203	74	225	0.05
6	Zhejiang Univ	China	100	2,245	22	109	0.05
7	Univ Amsterdam	Netherlands	99	7,921	80	204	0.01
8	Univ Washington	United States	97	6,767	70	211	0.01
9	Harvard Univ	United States	95	10,920	115	200	0.05
10	Univ Michigan	United States	94	5,938	63	166	0.06
11	Univ Alberta	Canada	92	4,562	50	276	0.06
12	Univ Toronto	Canada	92	3,370	37	238	0.05
13	Univ Calif Davis	United States	83	2,432	29	115	0.02
14	Baylor Coll Med	United States	82	5,627	69	171	0.00
15	Inra	France	80	6,105	76	115	0.00
16	Chinese Acad Sci	China	79	2010	25	160	0.00
17	Nanjing Med Univ	China	79	1,631	21	115	0.03
18	Massachusetts Gen Hosp	United States	76	5,188	68	212	0.01
19	Univ Calif San Francisco	United States	76	4,924	65	186	0.03
20	Univ São Paulo	Brazil	72	941	13	54	0.00

Total link strength, it indicates the strength or closeness of the institution's cooperation with other institutions in the field of FMT; Betweenness centrality, it represents the influence or contribution of the institution in the FMT field, and greater than 0.1 means that the institution has an important contribution or a great influence.

TABLE 3 The characteristics of the top 20 authors based on publications.

No.	Author	Country	Institutions	Publications	Citations	Average citations	Total link strength
1	Khoruts, Alexander	United States	Univ Minnesota	51	4,994	98	161
2	Gasbarrini, Antonio	Italy	Univ Cattolica Sacro Cuore	41	1,389	34	148
3	Khanna, Sahil	United States	Mayo Clin	40	925	23	72
4	Kassam, Zain	United States	MIT	39	2,668	68	150
5	Zhang, Faming	China	Nanjing Med Univ	39	1,062	27	224
6	Allegretti, Jessica R.	United States	Harvard Med Sch	37	1,171	32	157
7	Ianiro, Gianluca	Italy	Univ Cattolica Sacro Cuore	36	1,298	36	149
8	Nieuwdorp, Max	Netherlands	Univ Amsterdam	35	4,064	116	76
9	Cammarota, Giovanni	Italy	Univ Cattolica Sacro Cuore	34	1,114	33	145
10	Cui, Bota	China	Nanjing Med Univ	31	826	27	186
11	Fischer, Monika	United States	Indiana Univ	31	812	26	123
12	Sadowsky, Michael J.	United States	Univ Minnesota	31	3,605	116	107
13	Kelly, Colleen R.	United States	Brown Univ	30	1967	66	97
14	De Vos, Willem M.	Netherlands	Wageningen Univ	29	4,625	159	68
15	Li, Ning	China	Nanjing Univ	28	727	26	99
16	Wei, Hong	China	Third Mil Med Univ	28	999	36	70
17	Levitt, Marc A.	United States	Cincinnati Childrens Hosp Med Ctr	27	835	31	49
18	Mullish, Benjamin H.	United Kingdom	Imperial Coll London	26	819	32	126
19	Zhang, Ting	China	Nanjing Med Univ	26	595	23	136
20	Kao, Dina	Canada	Univ Alberta	25	1,160	46	113

Total link strength, it indicates the strength or closeness of the author's cooperation with other authors in the field of FMT.

Timeline view of the 10 major clusters was shown in Figure 7B, which presented the cluster topics at different intervals over time. We found that most of the references in the largest cluster #0 *Clostridium difficile* infection were cited before 2016, but the references of another similar cluster #3 difficile infection were widely cited in recent years. In addition, references in these clusters, such as #2 irritable bowel syndrome, #3 difficile infection, #4 inflammatory bowel disease, #6 versus-host disease, #10 colorectal cancer, and #12 liver diseases, have also been widely cited in recent years.

The top 25 references with the strongest citation bursts were also identified *via* bursts analysis with the CiteSpace (Figure 7C), which was another method for determining research hotspots. The details of these 25 references were listed in Supplementary Table 3. Among them, 15 references were for the topics of *Clostridium difficile* infection, 2 for ulcerative colitis, 1 for metabolic syndrome, 1 for drug-resistant bacteremia, 2 for the practice guideline of fecal microbiota transplantation, and 4 for others.

Keyword Co-occurrence analysis

There were 326 keywords with occurrence frequency greater than 5, which were extracted from the author keywords by using CiteSpace. After combining the synonyms and analogous keywords, fecal microbiota transplantation was the keyword with the most occurrence frequency. Besides, the other top 25 keywords were gut microbiota, clostridium difficile, inflammatory bowel disease, ulcerative colitis, antibiotic resistance, fecal incontinence, clostridium difficile infection, colorectal cancer, crohns disease, escherichia coli, short-chain fatty acid, irritable bowel syndrome, gut-brain axis, hepatitis virus, bile acid, stem cell transplantation, biliary atresia, graft versus host disease, liver transplantation, anorectal malformation, metabolic syndrome, quality of life, risk factor, and antegrade continence enema.

Total 43 clusters ($Q = 0.81$, $S = 0.95$, $Q/S = 0.88$) were generated after cluster analysis, the major 15 clusters were shown in Figure 8A. The largest cluster was #0 fecal microbiota transplantation (4,388 Nodes, 61%), followed by #1 inflammatory bowel disease, #2 fecal incontinence, # 3 escherichia coli, #4 colorectal cancer, #5 amino acids, #6 primary production, #7 hepatitis e virus, #8 gastrointestinal tract, # 9 reverse cholesterol transport, #10 short bowel syndrome, and #11 risk factors, etc.

The top 25 keywords with the strongest citation bursts were shown in Figure 8B. Keyword fecal microbiota transplantation had the strongest burst strength (strength = 310.96), which begun from 2017 up to now. Followed by gut microbiota (strength = 223.88,

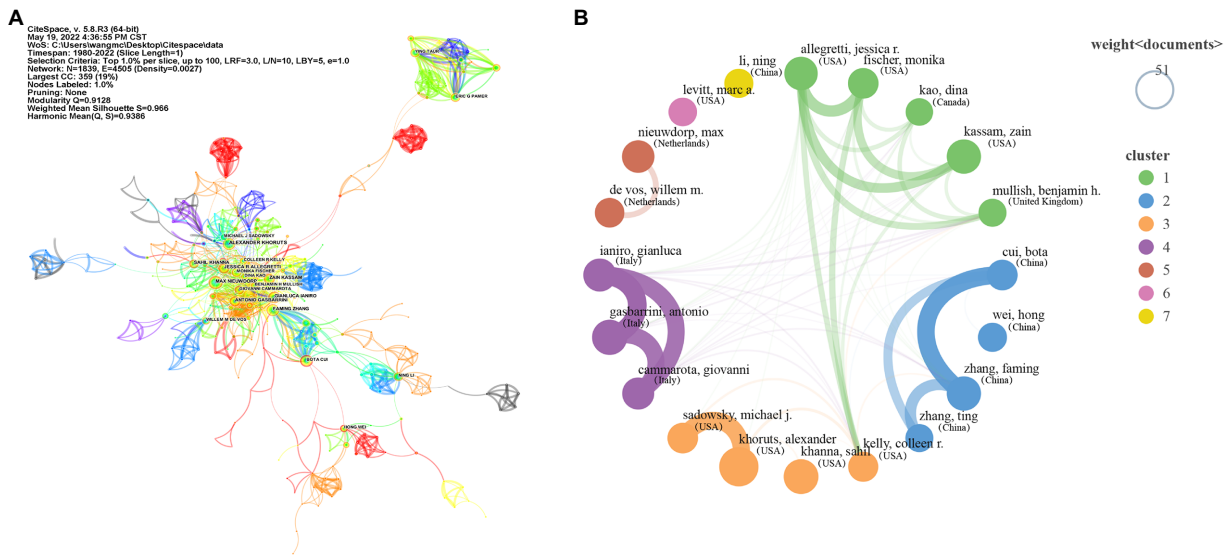


FIGURE 5
Distribution and Co-authorship analysis of authors. **(A)** The collaborations among the main authors and their teams on FMT. Each dot or circle represents an author, authors with the same color may be from the same research team; the line between them represents a collaborative relationship, and the width of the lines represents the strength of their cooperation. **(B)** The cooperative relationships of the top 20 authors with each other. The size of the circle represents the number of publications of different authors; the width of the lines between different authors represents the strength of their cooperation; authors with the same color may be from the same research team.

2018–2022), inflammatory bowel disease (strength = 57.37, 2017–2022), and clostridium difficile (strength = 52.23, 2015–2019), etc. In addition, up to 2022, keywords with strongest citation bursts included ulcerative colitis (strength = 39.30, 2017–2022), clostridium difficile infection (strength = 34.49, 2017–2022), antibiotic resistance (strength = 25.57, 2017–2022), short-chain fatty acid (strength =18.72, 2019–2022), gut-brain axis (strength = 18.68, 2018–2022), and others.

Disease keywords analysis

Keywords were extracted from all keywords by using VOSviewer software. In order to further understand the application status of FMT in different diseases, we combined keywords related to disease names and their synonyms, and then sorted them according to frequency of occurrence. Supplementary Table 4 shown the top 35 diseases for which FMT was most frequently applied. Among them, *Clostridium difficile* infection was the most common disease, followed by inflammatory bowel disease, organ transplantation, and diarrhea, ulcerative colitis, gastritis and enteritis, infectious disease, Crohn's disease, cell transplantation, and hepatitis, etc.

Summary of hotspots evidences

We summarized the hotspots above and classified them into different grades according to the number of evidence sources. The evidence sources included top 25 of Web of Science categories, top 30 most Co-cited references, top 10 clusters of references, top 25 references with the strongest citation bursts, top 25 keywords with the most occurrence frequency, major 15 clusters of keywords, top 25 keywords with the strongest citation bursts, and top 35 disease keywords. The summary of hotspots evidences was shown in Figure 9, a total of 57 hotspots on FMT research were divided into 7 grades. Hotspots in grade 1 included fecal microbiota transplantation, *Clostridium difficile* infection, and colorectal cancer/other cancer. Grade 2 included irritable bowel syndrome, ulcerative colitis, metabolic syndrome, and inflammatory bowel disease. Grade 3 included gut microbiota, graft versus host disease, and hepatitis virus. Other hotspots and their grades were shown in Figure 9.

Discussion

FMT, as a non-conventional therapy with great potential, is being applied in many clinical fields. In this study, we conducted a comprehensive bibliometric analysis of publications related to FMT, and finally gained the research hotspots and potential trends. The bibliometric analysis was performed based on publication characteristics analysis, Co-authorships analysis, Co-cited analysis, Co-occurrence analysis, and burst analysis.

After the publication characteristics analysis, we found that the researches on FMT was still in the ascendant, the number of publications was increasing year by year, and more than 1,000 papers were published annually from 2019. In this part,

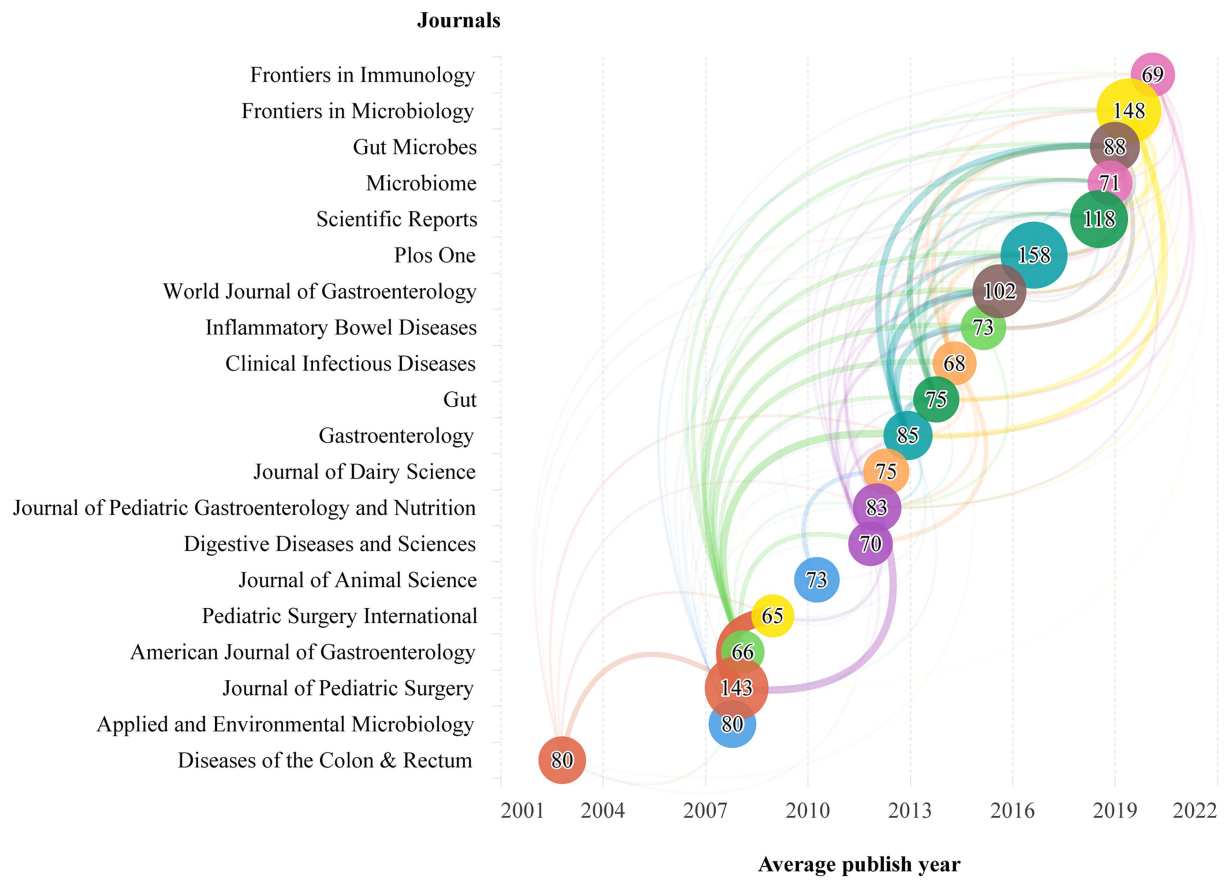


FIGURE 6
The average publish year of the top 20 journals with most publications. The size of the circle represents the total number of publications about FMT in different journals; the width of the lines between different journals represents the strength of cited each other.

we analyzed the categories of all publications in the Web of Science, and regarded the top 25 categories as one of the evidence sources of hotspots on FMT (Figure 3). All publications were divided into 173 categories in the Web of Science, and most of them (51%) were in gastroenterology hepatology, microbiology, surgery, pharmacology pharmacy, and immunology.

Co-authorships analysis shown that the United States was the center of FMT research, it played a key role in the field, it was also one of the most cooperative countries with others. Although publications in China had increased rapidly in recent years, and the annual publications surpassed that of the USA in 2021, the total citations and average citations were relatively low, the quality of research needs to be improved further. Europe was another center for FMT research, with the highest average citations in many countries, such as the Finland, Sweden, Netherlands, and United Kingdom, etc. (Table 1). In addition, Frontiers in Immunology, Frontiers in Microbiology, Gut Microbes, and Microbiome were among the journals that have published many papers on the subject of FMT in recent years.

After Co-cited analysis, Co-occurrence analysis, and burst analysis, we produced another seven evidence sources and total 57 hotspots on FMT research, and these evidence sources included the top 30 most Co-cited references, top 10 clusters of references, top 25 references with the strongest citation bursts, top 25 keywords with the most occurrence frequency, major 15 clusters of keywords, top 25 keywords with the strongest citation bursts, and top 35 disease keywords. All 57 hotspots were finally divided into 7 grades according to the number of evidence sources (Figure 9).

Hotspots in grade 1 included fecal microbiota transplantation, *Clostridium difficile* infection, and colorectal cancer/other cancer, which were all given seven different evidence sources (Figure 9). Fecal microbiota transplantation (FMT) itself was still one of the hotspots, mainly due to the following reasons: (Kelly, 2013) FMT have been successfully used in a limited number of diseases, such as *Clostridium difficile* infection, and it is being eagerly attempted for the diagnosis and treatment of other diseases (Allegretti et al., 2019; Aroniadis et al., 2019; Aron-Wisnewsky et al., 2019; Kang et al., 2019; Green et al., 2020; Proença et al., 2020; Wu et al., 2022). Many factors such as characteristics of donors, types of stool material, administration routes, stool dose and frequency may affect the effectiveness and safety of FMT, but the sufficient evidences are still on the way (Borody et al., 2004; Halkjær et al., 2018; Ramai et al., 2021). The concept, methodology and strategy

TABLE 4 The top 10 most-cited references.

No.	Authors	Year, journal, title	Citations	Topics	Types
1	Van Nood E	2013, N Engl J Med, Duodenal infusion of donor feces for recurrent *Clostridium difficile*	1,065	Recurrent *Clostridium difficile*	Clinical trial
2	Moayyedi P	2015, Gastroenterology, Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial	542	Ulcerative colitis	Clinical trial
3	Kassam Z	2013, Am J Gastroenterol, Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis	404	*Clostridium difficile* infection	Meta analysis (Review)
4	Vrieze A	2013, Gastroenterology, Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome	404	Metabolic syndrome	Clinical trial
5	Gough E	2011, Clin Infect Dis, Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection	400	Recurrent *Clostridium difficile*	Review
6	Surawicz CM	2013, Am J Gastroenterol, Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections	396	*Clostridium difficile* infection	Review
7	Rossen NG	2015, Gastroenterology, Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis	395	Ulcerative colitis	Clinical trial
8	Caporaso JG	2010, Nat Methods, QIIME allows analysis of high-throughput community sequencing data	385	QIIME	Analysis method
9	Paramsothy S	2017, Lancet, Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomized placebo-controlled trial	391	Ulcerative colitis	Clinical trial
10	Bakken JS	2011, Clin Gastroenterol Hepatol, Treating *Clostridium difficile* infection with fecal microbiota transplantation	373	*Clostridium difficile* infection	Review

for its modernization are being updated and standardized (Table 5; Supplementary Table 3; Zhang et al., 2018; Cammarota et al., 2019).

Clostridium difficile infection is the second hotspot in grade 1, it is the most common disease for FMT applying (Supplementary Table 4). Sufficient evidences shown that FMT is highly efficacious for recurrent *Clostridium difficile* infection with response rates of around 90% (Rokkas et al., 2019; Tixier et al., 2022). In recent years, the researches of FMT on *Clostridium difficile* infection mainly focused on the following aspects: (1) Efficacy of different FMT protocols for *Clostridium difficile* infection (Table 5; Supplementary Table 3; Youngster et al., 2014; Ianiro et al., 2018a). (2) Comparison of FMT with other treatments, such as fixed bacterial mixture (Cold et al., 2022), vancomycin (Table 5; Cammarota et al., 2015; Ianiro et al., 2018b). (3) For special populations with *Clostridium difficile* infection, such as pediatric patients (Bernard et al., 2021), immunocompromised patients (Supplementary Table 3; Kelly et al., 2014), and severe or fulminant *Clostridium difficile* infection (Tixier et al., 2022). (4) The mechanisms and pharmacology of FMT for *Clostridium difficile* infection (Mullish et al., 2019; Jan et al., 2021; Khoruts et al., 2021).

The third hotspot in grade 1 was colorectal cancer/other cancer. Gut microbiota may have a close relationship with the development of colorectal cancer (Wieczorska et al., 2020), and targeted treatment of the gut microbiota could be a promising

TABLE 5 The top 20 most-cited references published in the last 5 years.

No.	Authors	Year, journal, title	Citations	Topics	Types
1	Defilipp Z	2019, N Engl J Med, Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant	226	Drug-Resistant bacteremia	Case report
2	Costello SP	2019, JAMA, Effect of fecal microbiota transplantation on 8-Week remission in patients with ulcerative colitis: a randomized clinical trial	214	Ulcerative colitis	Clinical trial
3	Routy B	2018, Science, Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors	203	Tumor	Clinical trial
4	Gopalakrishnan V	2018, Science, Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients	171	Tumor	Clinical trial
5	Halkjaer SI	2018, Gut, Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomized, double-blind placebo-controlled study	98	Irritable bowel syndrome	Clinical trial
6	Wilson BC	2019, Front Cell Infect Microbiol, The super-donor phenomenon in fecal microbiota transplantation	94	Super-donor	Review
7	Suez J	2018, Cell, Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT	87	Antibiotics-associated dysbiosis	Clinical trial
8	Wang YH	2019, Nat Med, Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis	87	Inhibitor-associated colitis	Case report
9	Bolyen E	2019, Nat Biotechnol, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2	85	QIIME	Analysis method
10	allegretti jr	2019, Lancet, The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications	77	Faecal microbiota transplantation	Review
11	Paramsothy S	2019, Gastroenterology, Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis	76	Ulcerative colitis	Clinical trial
12	Ianiro G	2018b, Aliment Pharmacol Ther, Randomized clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory *Clostridium difficile* infection-single versus multiple infusions	73	*Clostridium difficile* infection	Clinical trial
13	Ianiro G	2018a, United European Gastroenterol J, Efficacy of different faecal microbiota transplantation protocols for *Clostridium difficile* infection: a systematic review and meta-analysis	73	*Clostridium difficile* infection	Meta analysis (Review)
14	Smillie CS	2018, Cell Host Microbe, Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation	73	Bacterial Engraftment and efficacy	Clinical trial

(*Continued*)

TABLE 5 Continued

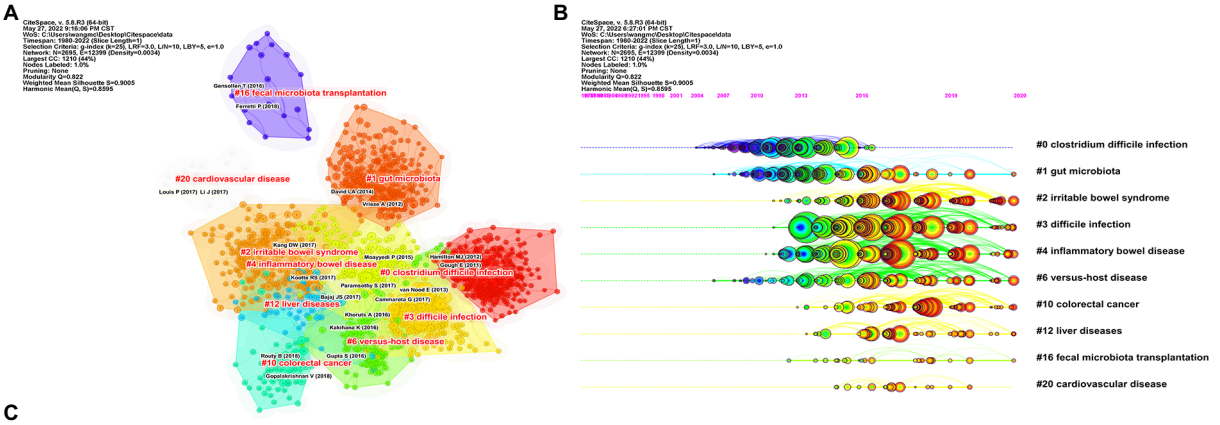
No.	Authors	Year, journal, title	Citations	Topics	Types
15	Zuo T	2018,Gut, Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome	73	Bacteriophage transfer and efficacy	Clinical trial
16	Defilipp Z	2018, Blood Adv, Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity	70	Allogeneic hematopoietic cell transplantation	Clinical trial
17	Kang DW	2019, Sci Rep, Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota	70	Autism	Clinical trial
18	Zhang Fm	2018, Protein Cell, Microbiota transplantation: concept, methodology and strategy for its modernization	70	Faecal microbiota transplantation	Review
19	El-salhy M	2020, Gut, Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomized, double-blind, placebo-controlled study	69	Irritable bowel syndrome	Clinical trial
20	Taur Y	2018, Sci Transl Med, Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant	69	Antibiotics-associated dysbiosis	Clinical trial

strategy for patients with colorectal cancer (Ma and Chen, 2019). In addition, mounting evidences have demonstrated that gut microbiota plays a critical role in cancer patients' therapeutic responses to chemotherapy, radiotherapy, especially immunotherapy, including clinical efficacy and sensitivity to toxicity, and FMT is being used to modulate gut microbiota in cancer patients (Supplementary Table 3; Gopalakrishnan et al., 2018; Chen et al., 2019; Ma and Chen, 2019; Wu et al., 2019; McQuade et al., 2020).

Hotspots in grade 2 were all given six different evidence sources (Figure 9), which included inflammatory bowel disease, ulcerative colitis, irritable bowel syndrome, and metabolic syndrome. Inflammatory bowel diseases were second only to *Clostridium difficile* infection for FMT applying (Supplementary Table 4), it included ulcerative colitis and Crohn's disease, and the Crohn's disease was also a hotspot in the grade 4 (Figure 9). FMT is being explored as a therapeutic option for the patients with inflammatory bowel diseases and irritable bowel syndrome. The current studies mainly focus on the follow two aspects. First, many randomized controlled trials (RCTs) are being conducted in recent years, positive effects in various degrees were obtained in some RCTs, while there was no effect in the others, so the results from these RCTs are inconsistent (Zhao et al., 2020; El-Salhy et al., 2021). At the same time, almost all RCTs are small sample size studies (Aroniadis et al., 2019; Costello et al., 2019; El-Salhy et al., 2020; Zhao et al., 2020). Therefore, carrying out RCTs with large samples will be one of the research trends and hotspots in the future. Secondly, the changes of gut microbiota after FMT and the determination of disease-specific microbiota or biomarkers are of great significance for the treatment of these diseases. However, there is no consistent conclusion at present, so these will still be the hotspots and trends of future researches.

FMT has emerged as a new promising therapeutic approach in metabolic diseases, included metabolic syndrome (Vrieze et al., 2012; grade 2), obesity (Aron-Wisnewsky et al., 2019; grade 6), diabetes (Aron-Wisnewsky et al., 2019; grade 6), and cardiovascular diseases (Mehmood et al., 2021; grade 6), etc. (Supplementary Table 4). Researches of FMT in these diseases are still in the early stages, and the efficacy and mechanisms of FMT are still controversial (Aron-Wisnewsky et al., 2019). Vrieze et al. (2012) found that transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals with metabolic syndrome. Ng et al. (2022) proved that repeated FMTs enhanced the level and duration of microbiota engraftment in obese patients with T2DM, and combining lifestyle intervention with FMT led to more favorable changes in recipients' microbiota and improvement in lipid profile and liver stiffness.

Hotspots in grade 3 were all given five different evidence sources (Figure 9), which included gut microbiota, graft versus host disease, and hepatitis virus. It is generally accepted that many diseases are characterized by gut microbiome dysbiosis (Chen et al., 2021; Yang et al., 2021), but it is difficult to identify the specific microbial patterns that could characterize different diseases. The relationship between the gut microbiome and the etiology of diseases still remains unsolved (Duvallet et al., 2017). It is also accepted that FMT could alter gut microbiota in patients with different diseases and introduce a balanced conglomerate of



C

References	Year	Strength	Begin	End	1980 - 2022
van Nood E, 2013, NEW ENGL J MED, V368, P407, DOI 10.1056/NEJMoa1205037, DOI	2013	174.14	2013	2018	
Hamilton MJ, 2012, AM J GASTROENTEROL, V107, P761, DOI 10.1038/ajg.2011.482, DOI	2012	65.17	2012	2017	
Brandt LJ, 2012, AM J GASTROENTEROL, V107, P1079, DOI 10.1038/ajg.2012.60, DOI	2012	63.68	2012	2017	
Louie TJ, 2011, NEW ENGL J MED, V364, P422, DOI 10.1056/NEJMoa0910812, DOI	2011	59	2011	2016	
Borody TJ, 2012, NAT REV GASTRO HEPAT, V9, P88, DOI 10.1038/nrgastro.2011.244, DOI	2012	42.87	2012	2017	
Petrof EO, 2013, MICROBIOME, V1, P0, DOI 10.1186/2049-2618-1-3, DOI	2013	42.02	2013	2018	
Gough E, 2011, CLIN INFECT DIS, V53, P994, DOI 10.1093/cid/cir632, DOI	2011	99.69	2012	2016	
Bakken JS, 2011, CLIN GASTROENTEROL H, V9, P1044, DOI 10.1016/j.cgh.2011.08.014, DOI	2011	77.32	2012	2016	
Kassam Z, 2013, AM J GASTROENTEROL, V108, P500, DOI 10.1038/ajg.2013.59, DOI	2013	70.33	2014	2018	
Cohen SH, 2010, INFECT CONT HOSP EP, V31, P431, DOI 10.1086/651706, DOI	2010	70.04	2011	2015	
Surawicz CM, 2013, AM J GASTROENTEROL, V108, P478, DOI 10.1038/ajg.2013.4, DOI	2013	69.7	2014	2018	
Vrieze A, 2012, GASTROENTEROLOGY, V143, P913, DOI 10.1053/j.gastro.2012.06.031, DOI	2012	62.67	2013	2017	
Khoruts A, 2010, J CLIN GASTROENTEROL, V44, P354, DOI 10.1097/MCG.0b013e3181c87e02, DOI	2010	57.06	2011	2015	
Moayyedi P, 2015, GASTROENTEROLOGY, V149, P102, DOI 10.1053/j.gastro.2015.04.001, DOI	2015	52.11	2016	2020	
Kelly CR, 2014, AM J GASTROENTEROL, V109, P1065, DOI 10.1038/ajg.2014.133, DOI	2014	50.35	2015	2019	
Youngster I, 2014, JAMA-J AM MED ASSOC, V312, P1772, DOI 10.1001/jama.2014.13875, DOI	2014	48.29	2015	2019	
Qin JJ, 2010, NATURE, V464, P59, DOI 10.1038/nature08821, DOI	2010	41.95	2011	2015	
Huttenhower C, 2012, NATURE, V486, P207, DOI 10.1038/nature11234, DOI	2012	39.28	2013	2017	
Debast SB, 2014, CLIN MICROBIOL INFEC, V20, P1, DOI 10.1111/1469-0691.12418, DOI	2014	39.26	2015	2019	
Ridaura VK, 2013, SCIENCE, V341, P1079, DOI 10.1126/science.1241214, DOI	2013	38.99	2014	2018	
McDonald LC, 2018, CLIN INFECT DIS, V66, P0	2018	56.82	2019	2022	
Lessa FC, 2015, NEW ENGL J MED, V372, P2369	2015	38.32	2016	2019	
DeFilipp Z, 2019, NEW ENGL J MED, V381, P2043, DOI 10.1056/NEJMoa1910437, DOI	2019	78.74	2020	2022	
Costello SP, 2019, JAMA-J AM MED ASSOC, V321, P156, DOI 10.1001/jama.2018.20046, DOI	2019	50.32	2020	2022	
Cammarota G, 2019, GUT, V68, P2111, DOI 10.1136/gutjnl-2019-319548, DOI	2019	38.15	2020	2022	

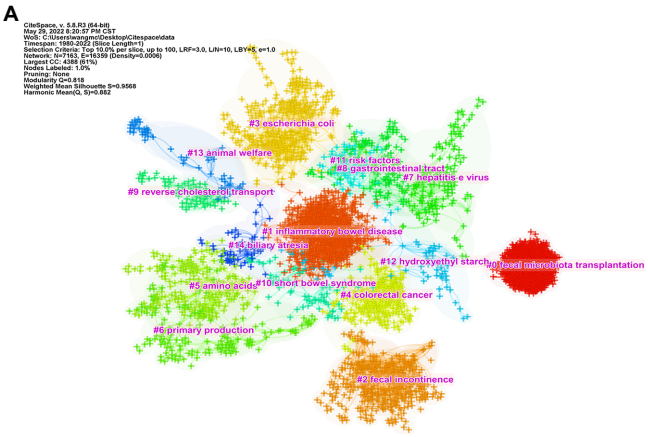
FIGURE 7
Co-cited references analysis. **(A)** The 10 major clusters of references. Each circle represents a reference, and circles with the same color represent a cluster with the same topic. **(B)** Timeline view of the 10 major clusters. Each circle represents a reference, and the circle on the same line represents a cluster with the same topic; The position of each circle represents the time when it was first cited, and the size of the circle represents the total number of it was cited. Each colored circle (tree ring history) represents the citations in a single time slice. **(C)** The top 25 references with the strongest citation bursts. The "Strength" represents the strength of citation bursts, the strength value is proportional to the bursts.

microorganisms. However, the relationship and the mechanisms between the gut microbiome and the effect of FMT are still unclear. Research shown that microbiota-derived metabolites, such as bile acids (grade 6), short-chain fatty acids (grade 6), and amino acids (grade 7), are proposed as possible etiological factors of some diseases, and they may provide some new avenues for the diagnosis and treatment.

Graft-versus-host disease (GvHD) is one of the life-threatening complications after allogenic hematopoietic stem cell transplant (allo-HSCT; grade 4), it is associated with up to 25% mortality (Zhang et al., 2021). Biliński et al. (2022) review shows that in the published studies to date, the overall response rate of FMT in the treatment of gastrointestinal acute GvHD could reach even 74%, with complete response accounting for 50%. At present, the clinical studies of FMT for GvHD are mainly small sample studies, the total number of patients is less than 200 (Biliński et al., 2022), and larger clinical studies are required to confirm the safety and efficacy of FMT for GvHD (Zhang et al., 2021).

FMT has therapeutic effects on various liver diseases (Gu et al., 2021b), such as viral hepatitis (grade 3), liver cirrhosis (grade 6), and other liver diseases (grade 5). In addition, there is an altered microbial composition in liver transplantation patients (grade 4) and a distinct signature of microbiota associated with the perioperative period (Lai et al., 2022), so FMT may be an intervention strategy to improve transplant outcomes.

Except for these hotspots above, others included biliary atresia, autism, psychosis, autoimmune disease, antibiotics-associated dysbiosis, gut-brain axis, drug-resistant bacteremia, HIV, Covid-19, risk factor, super-donor, and stool banking, etc. They were located in grade 5, grade 6 and grade 7 based on the



B

Keywords	Year	Strength	Begin	End	1980 - 2022
fecal incontinence	1980	28.95	1996	2010	
imperforate anus	1980	7.91	1996	2010	
colorectal cancer	1980	18.7	1997	2010	
ct colonography	1980	8.85	2006	2013	
virtual colonoscopy	1980	7.41	2006	2011	
reverse cholesterol transport	1980	7.47	2008	2013	
hepatitis virus	1980	7.43	2009	2013	
clostridium difficile	1980	52.23	2015	2019	
crohns disease	1980	23	2016	2022	
fecal microbiota transplantation	1980	310.96	2017	2022	
inflammatory bowel disease	1980	57.37	2017	2022	
ulcerative coliti	1980	39.3	2017	2022	
clostridium difficile infection	1980	34.49	2017	2022	
antibiotic resistance	1980	25.74	2017	2022	
irritable bowel syndrome	1980	11.24	2017	2022	
gastrointestinal microbiome	1980	10.32	2017	2020	
metabolic syndrome	1980	9.01	2017	2022	
parkinsons disease	1980	8.27	2017	2022	
gut microbiota	1980	223.88	2018	2022	
gut-brain axi	1980	18.68	2018	2022	
gut dysbiosis	1980	7.72	2018	2022	
short-chain fatty acid	1980	18.72	2019	2022	
intestinal flora	1980	8.22	2019	2022	
immune checkpoint inhibitor	1980	7.16	2019	2022	
autism spectrum disorder	1980	10.66	2020	2022	

FIGURE 8
Keyword co-occurrence analysis. **(A)** The top 15 clusters of keywords. Each cross represents a keyword, and crosses with the same colors represent a cluster with the same topic. **(B)** The top 25 keywords with the strongest citation bursts. The "Strength" represents the strength of citation bursts, the strength value is proportional to the bursts. It also represents the important value of the keyword.

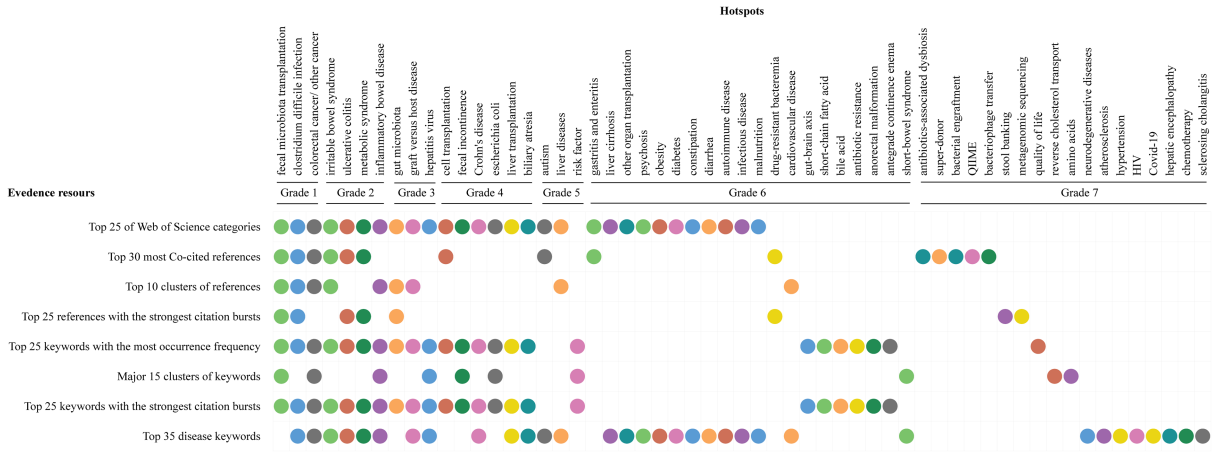


FIGURE 9
The summary of hotspots evidences.

number of evidence sources, but most of them have been or will become the research hotspots in the field of FMT.

This study has some limitations that need to be considered. First, the data used in this study was obtained only from the WoSCC database due to its reliability of the publications and citations. However, compared with other databases, such as PubMed and Embase, the WoSCC has fewer literatures and journals, which may increase the risk of literature selection bias. Second, the generation of hotspots is based on all types of studies. However, different types of studies do have different impacts on the field, such as RCTs, guidelines and recommendations, and the conclusions of these types of studies may be more important. Therefore, data analysis and visualization for different types of studies may be more convincing in future research. Third, research on the mechanisms of FMT is a key topic in this field, and among the 57 hotspots finally obtained, 4 are about mechanism research, which included bile acids, short-chain fatty acids, amino acids, and gut-brain axis, but we are acutely aware that these may be far from comprehensive.

In conclusion, this bibliometric analysis is expected to provide overall perspective for FMT. Based on this study, research on FMT has gained increasing attention and interest since 1991, especially in recent years. There are many hotspots about FMT, and some of them may represent the research trends in the field of FMT. These hotspots can be divided into four categories, one of which is the clinical application of FMT in various diseases. The clinical applications of FMT are comprehensive and multifaceted. Currently, *Clostridium difficile* infection is the only disease for which FMT has a clear therapeutic effect. However, there is still a lack of high-quality evidence on the efficacy and safety of FMT in

other diseases, which will become a hotspot and trend of future research. The second category can be summarized as the mechanism research of FMT. Studies on the mechanism have focused on the role of gut microbiota, microbiota-derived metabolites, gut-brain axis and others, but there are no consistent conclusions at present. This will become the second hotspot and trend in future. The third category can be summarized as the standardization of FMT process, such as selection of stool donor, stool material styles, routes of FMT administration, and stool banking establishment, etc. The last category may include the pharmacology of FMT, FMT product manufacturing, etc., although they are not among the hotspots summarized in this study.

Author contributions

MW and YZ designed the study. MW, XX, and YZ independently assessed studies for possible inclusion and collected the data. XX and SZ analyzed the data. MW and WH drafted the manuscript. YZ proofread the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MW received research grants from the National Natural Science Foundation of China, Gansu Province Youth Science and Technology Fund program, Health Industry Science and Technology plan of Gansu Province, and Cuiying Scientific and Technological Innovation Program of Lanzhou University Second Hospital. XX received research grants from the Fundamental Research Funds for the Central Universities. WH received research grants from Gansu Province Youth Science and Technology Fund program.

The remaining 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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.990800/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The trends of the annual publication relation to medicine of the top 10 countries. The search time is up to July 19, 2022, the number of publication relation to medicine is 9570.

SUPPLEMENTARY FIGURE 2
The Co-authorship network of major institutions. The overall size of the circle represents the number of publications in different institutions. Each colored circle (tree ring history) represents the number of publications published by that institution in a single time slice. The width of the lines between different institutions represents the strength of their cooperation; The outermost purple circle represents the institution has a significant role in the FMT field.

SUPPLEMENTARY FIGURE 3
The main cooperative networks of the top 20 authors with other researchers. The size of the circle represents the number of publications that the author has published, the line between them represents a collaborative relationship. The author's ranking is consistent with that in Table 3.

SUPPLEMENTARY TABLE 3
The top 25 references with the strongest citation bursts.

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EDITED BY
Junling Shi,
Northwestern Polytechnical University, China

REVIEWED BY
Kaiser Mahmood,
Universiti Sains Malaysia (USM), Malaysia
Muawuz Ijaz,
University of Veterinary and Animal Sciences, Pakistan

*CORRESPONDENCE
Muhammad Afzaal
muhammadafzaal@gcuf.edu.pk
Claudia Terezia Socol
clausocol@yahoo.com
Rana Muhammad Aadil
muhammad.aadil@uaf.edu.pk

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Human gut microbiota in health and disease: Unveiling the relationship

Muhammad Afzaal[1]*, Farhan Saeed[1], Yasir Abbas Shah[1], Muzzamal Hussain[1], Roshina Rabail[2], Claudia Terezia Socol[3]*, Abdo Hassoun[4,5], Mirian Pateiro[6], José M. Lorenzo[6,7], Alexandru Vasile Rusu[8,9] and Rana Muhammad Aadil[2]*

[1]Department of Food Science, Government College University Faisalabad, Faisalabad, Pakistan, [2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan, [3]Department of Genetics, University of Oradea, Oradea, Romania, [4]Sustainable AgriFoodtech Innovation & Research (SAFIR), Arras, France, [5]Syrian Academic Expertise (SAE), Gaziantep, Turkey, [6]Centro Tecnológico de la Carne de Galicia, Ourense, Spain, [7]Área de Tecnoloxía dos Alimentos, Faculdade de Ciências de Ourense, Universidade de Vigo, Ourense, Spain, [8]Life Science Institute, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania, [9]Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

The human gut possesses millions of microbes that define a complex microbial community. The gut microbiota has been characterized as a vital organ forming its multidirectional connecting axis with other organs. This gut microbiota axis is responsible for host-microbe interactions and works by communicating with the neural, endocrinal, humoral, immunological, and metabolic pathways. The human gut microorganisms (mostly non-pathogenic) have symbiotic host relationships and are usually associated with the host's immunity to defend against pathogenic invasion. The dysbiosis of the gut microbiota is therefore linked to various human diseases, such as anxiety, depression, hypertension, cardiovascular diseases, obesity, diabetes, inflammatory bowel disease, and cancer. The mechanism leading to the disease development has a crucial correlation with gut microbiota, metabolic products, and host immune response in humans. The understanding of mechanisms over gut microbiota exerts its positive or harmful impacts remains largely undefined. However, many recent clinical studies conducted worldwide are demonstrating the relation of specific microbial species and eubiosis in health and disease. A comprehensive understanding of gut microbiota interactions, its role in health and disease, and recent updates on the subject are the striking topics of the current review. We have also addressed the daunting challenges that must be brought under control to maintain health and treat diseases.

KEYWORDS

human gut microbiota, health, disease, eubiosis, dysbiosis, pathogenic

Introduction

The association of human health with the intestine has been long acknowledged as Hippocrates said, "Death sits in the bowls" in 400 B.C. Many studies worldwide have focused on the significant impact of intestinal microbiota on human health and disease (AboNahas et al., 2022). The human body is colonized by a diversity of bacteria, viruses, archaea, and unicellular eukaryotes. Microbes inhabit all human body surfaces, but a significant number of microbes live in the gastrointestinal tract/gut. The human gut possesses approximately more than one thousand microbial species that form a complex ecological community called gut microbiota (Lagier et al., 2016). The human gut microbiota is carrying about 150 times more genes compared to the entire human genome. It is widely accepted that approximately a hundred trillion microbes live on and inside the human body having a key role in various biological processes including health and disease (Wang et al., 2017). They are the primary mediators of body homeostasis, impacting various physiological activities, such as metabolism, barrier homeostasis, inflammation, and hematopoiesis through both intestinal and extra-intestinal actions. The gut microbiota has recently been classified as a "vital organ" because of its multidirectional and communicational connection or axis with other organs through neural, endocrine, humoral, immunological, and metabolic pathways. Any change in the microbial community not only causes gut-related issues but also influences other organs related diseases, though the actual interaction mechanism between the gut and the organs has yet to be fully understood (Ahlawat and Sharma, 2021).

The interaction between host and microbes plays a pivotal role in both health and disease. Gut microbiota diversity is greatly dependent on various host factors including diet, human lifestyle, age, and environmental factors. However, diet is currently considered one of the major factors (modifiers) in modulating the gut microbiota (Simões et al., 2022). Human microbiota has promising potential in altering appetite, increasing nutrient harvest, and exerting energy from various food components. Microbes have also a fundamental role in xenobiotic metabolism. In xenobiotic metabolism, various gut microbes alter the chemical structures of various diet components, drugs, pollutants, and many pesticides (Nakov and Velikova, 2020).

Many research studies have supported the concept that gut microbiota plays a key role in modulating immunity, weight gain or loss, energy homeostasis, and obesity-related disorders (Piccioni et al., 2022). Likewise, gut microbiota and their metabolites are associated with various non-alcoholic fatty liver diseases (NAFLDs), inflammatory bowels diseases (IBDs), hepatocellular carcinoma, cardiovascular diseases (CVDs), alcoholic liver disease (ALD), chronic kidney diseases (CKDs), and cirrhosis (Hsu et al., 2020; Jansen et al., 2021; Ryma et al., 2021; Wang et al., 2021; Zhou et al., 2021; Philips et al., 2022).

Figure 1 depicts several symbiotic gut microbial strains and the possible negative health consequences of dysbiosis on the gut-organ axis. Hence, the comprehensive understanding of recent gut microbiota interactions, their eubiotic role in health and disease, and other recent updates on this subject are compiled in this review, with a major focus on controlling the challenges to maintain health and treat various diseases.

Significance of human gut microbiota eubiosis

Comprehensive clinical studies are available on microbiota and involvement in their balance, i.e., eubiosis and related pathophysiological aspects. The compositional difference in gut microbiota has been observed in health and disease conditions. Eubiosis conditions are effective in controlling various diseases caused by microbes. Proper intake of a healthy diet and the development of eubiosis acts in favor of human health. The high intake of antibiotics causes an imbalance in the gut microbiota and favors systemic diseases (Santacroce et al., 2021).

Several population-based studies have revealed the highly beneficial role of human gut microbiota in healthy people, as well as the importance of well-understanding its structure and the factors that influence its composition, such as food, age, geography, systemic disorders, and drugs (Wang et al., 2017; Rowland et al., 2018). Phyla Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, and Verrucomicrobia contribute to the significant resident bacterial populations in the gut microbiome (Fava et al., 2019). The first step in identifying the symbiotic interactions between intestinal microbes and their hosts is to describe the balanced composition of gut microbiota and disease-related variations. The microbes reside in a mutual association with the host in a healthy state, affecting the host's health by controlling nutrient metabolism, defending against pathogens, and delivering signals to immune cells to promote host physiology and immunity (Ribaldone et al., 2022). An initial underestimation of the total number of microbial species in the intestine has been described through several *vivo* and *ex vivo* studies due to complications in culturing certain microorganisms (Lagier et al., 2015).

Bacteria and proteobacteria contribute to carbohydrate digestion, gut microbiota, regulation of the immune system, and defense against pathogen colonization (Rosser and Mauri, 2016; Fan and Pedersen, 2021). For survival, microbes in the intestine tract mainly depend on dietary substrates undigested in the upper digestive tract. Saccharolytic bacterial fermentation typically creates advantageous metabolites, while bacteria switch to an alternative energy source if there are insufficient carbohydrates, leading to the development of other metabolites that could be more disadvantageous to human health (Rowland et al., 2018). *Methanobrevibacter smithii* is the human-associated Archaea that plays a vital function in the synthesis of methane

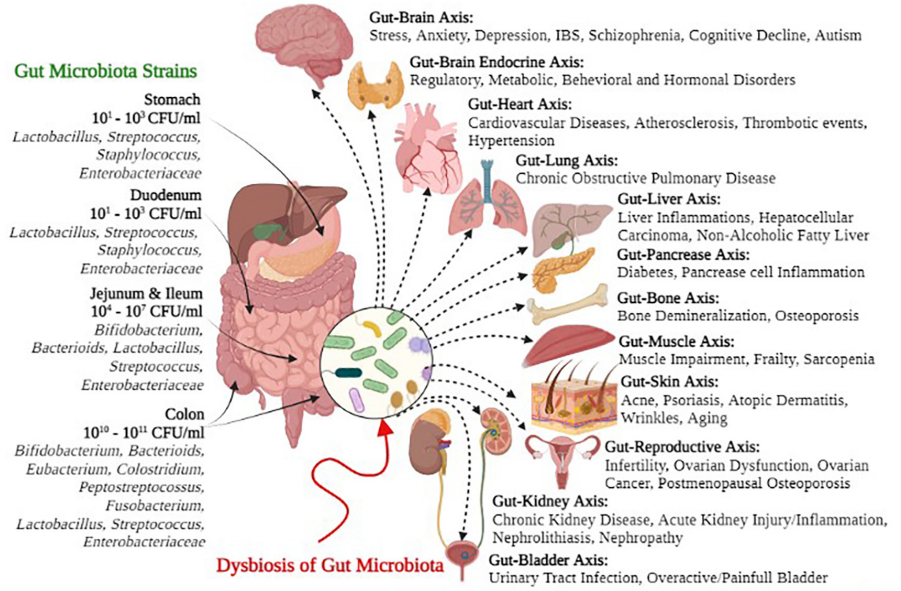

FIGURE 1
Gut microbial strains and negative health outcomes of gut microbial dysbiosis.

from H2 processed by bacterial metabolism. It is a prominent and essential Archean in the gut microbiota (Hoffmann et al., 2013; Berry, 2016). Some of the beneficial functions of gut microbiota for human health are shown in **Figure 2**.

It is considered that diet is a significant factor associated with health and disease control, but some recent studies concluded that diet is pivotal for shaping the gut microbial structure and influencing the metabolism of the host. The gut environment, sequentially, can help reproduce, grow, and survive the microbial community (Browne et al., 2016). Carbohydrates are an essential and significant energy source; also, intestinal microbiota has provided a fermentation stage to deliver vital biomolecules to the host (Conlon and Bird, 2015).

A normal balance between the host and gut flora is essential for human health, while disruption is linked with various human diseases, like hypertension, obesity, cardiovascular disorders, diabetes, and IBD (Von Martels et al., 2017; Kho and Lal, 2018; Szablewski, 2018). However, the human microbiome analysis is still at its initial phase in filling the knowledge gap in the microbiome-host relationship and its role in disease pathogenesis and therapeutical importance. Therefore, further in-depth research is needed to unravel this fascinating yet enigmatic area of study.

Gut microbiota and human metabolism

The diverse human microbiome has substantial metabolic activities essential for the functioning of mammalian enzymes in the gut mucosa and liver and the host metabolism. Gut microbiota influence host health by shaping the biochemical profile of the diet. The significant role of gut microbiota in human immunity has promoted research to investigate the contributions of particular microbes in metabolic pathways, especially in dietary components' metabolism (Cardona and Roman, 2022). Recent studies have found that gut microbiota can metabolize phytochemicals, especially polyphenols, by well-defined paths (Rowland et al., 2018). The human gut microbiota reacts efficiently to major dietary changes. The presence of these fast, diet-induced patterns is confirmed by evidence from individuals switching between plant and meat-based diets, adding to their diet more than 30 g of specific dietary fibers a day or adapting either a high-fiber-low fat diet or a low-fiber-high-fat diet for ten days; in all cases, the structure and composition of microbiome changed over 1–2 days (Wu et al., 2011; David et al., 2014). This flexibility may be an advantageous feature of enlisting microbes as part of the digestive structure, particularly when considering the potential day-to-day variability in food available to foragers. It may also be an inescapable consequence of dealing with such a microbial community that is diverse and competitive and undergoes rapid turnover. Human gut microbiota is associated with the degradation of dietary fibers, proteins, and peptides by fermentation and anaerobic degradation (Yadav et al., 2018).

Carbohydrates and simple sugars are the main components of food metabolized by gut microbiota. Bacterial species, especially the phyla Bacteroidetes and Firmicutes, can ferment fibers (the indigestible carbohydrates) to produce branched-chain and short-chain fatty acids (SCFAs), lactate, ethanol,

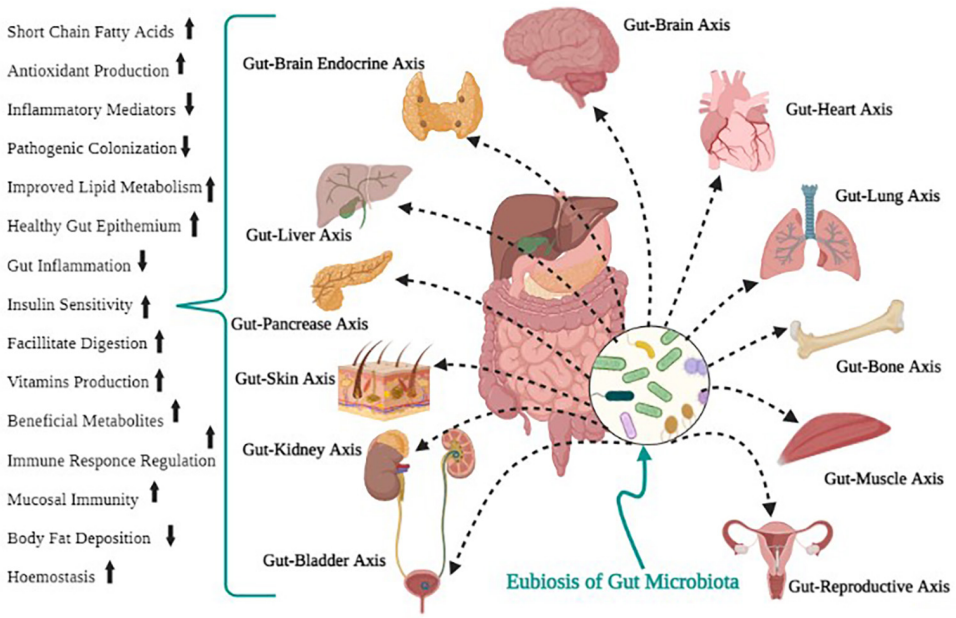

FIGURE 2
Positive health outcomes of gut microbial eubiosis.

hydrogen, and carbon dioxide; these products are further used by the host or excreted (Patrascu et al., 2017). Acetate, propionate, and butyrate are the main short-chain fatty acids (SCFAs) distinguished in human feces, usually found in 3:1:1 to 10:2:1 molar ratio; this ratio is consistent with the values reported in the intestine in early sudden deaths (Rowland et al., 2018). These are the main SCFAs that perform several essential functions in the human body (Rauf et al., 2022). Butyrate is perhaps the essential SCFA for human health, as it is the primary source of energy for human colonocytes (Wang et al., 2019). Butyrate has the potential to act as an anti-carcinogen as it persuades apoptosis of colon cancer cells and regulates gene expression by inhibiting histone deacetylase (Havenaar, 2011; Steliou et al., 2012). Propionate is also an essential energy source for the epithelial cells in the liver; it plays a vital role in gluconeogenesis (Cani, 2018). Acetate helps in the growth of other bacteria as an essential co-factor; for example, *Faecalibacterium prausnitzii* will not grow in pure culture in the absence of acetate (Rowland et al., 2018).

Human gut microbiota can also synthesize essential vitamins, including biotin, folate, and vitamin K, and neutralize carcinogenic compounds, such as pyro lysates (Selber-Hnatiw et al., 2017). Various indications specify that the host metabolism is mainly affected by multiple microbial metabolites that bind to specific host membranes or nuclear receptors (Bhutia et al., 2017). Some of the most important metabolites produced by gut microbiota are described in **Table 1**. The majority of essential functions for host physiology and maintenance are associated with gut microbiota, e.g., the nervous system's development, intestinal development, appetite regulation, etc.

Gut microbiota in immune homeostasis

The contribution of the human gut microbiota to various aspects of human health, especially the immune system, is crucial for providing the host with several essential benefits. Recent studies have found that early development of the gut microbiota is crucial in preventing autoimmune disorders and proper immune functioning (Lazar et al., 2018; Spencer et al., 2019; Elmassry et al., 2020; Schluter et al., 2020). The intestinal microbiome is essential for the maturation of the immune system, which includes adaptive and innate immune responses. Innate immunity deals with the physical barrier of the epithelia, specialized cells, and circulating chemicals to immediately identify a wide assortment of foreign antigens and eradicate them (Thaiss et al., 2016). The mucosal immune system, in particular, mechanisms are primarily independent of the systemic immune system, and after bacterial colonization of the intestinal tract, it undergoes significant changes. For the immune system's growth and development, commensal microorganisms are necessary to distinguish between commensal and pathogenic bacteria. Recent studies have demonstrated that gastrointestinal tract microbiota modulates the movement and role of neutrophils and influences the division of populations of T cells into

TABLE 1 Metabolites produced by gut microbiota and their functions.

Metabolites	Functions	References
Bile acid metabolites; including deoxycholic acid (DCA) and lithocholic acid (LCA)	Regulate bile acid, cholesterol, lipid, glucose, and energy metabolism, show antimicrobial effects, and activate host nuclear receptors and cell signaling pathways.	Ramírez-Macías et al., 2022
Short-chain fatty acids (SCFAs) metabolites such as propionate and butyrate	Regulate food intake and insulin secretion, also aid in maintaining body weight.	Psichas et al., 2015; Larraufie et al., 2018
Branched-chain fatty acids (BCFA) including isobutyrate, isovalerate	Histone deacetylase (HDAC) inhibition, increased histone acetylation.	Mischke and Plösch, 2016
Indole derivatives including indoxyl sulfate and indole-3-propionic acid (IPA)	IPA exhibits neuroprotective effects, acts as a powerful antioxidant, and regulates intestinal barrier function. Indoxyl sulfate is a uremic toxin that accumulates in the blood of individuals with impaired excretion systems.	Hendrikx and Schnabl, 2019
Lipopolysaccharide (LPS), peptidoglycan (PGN), lipoteichoic acid (LTA)	Epigenetic regulation of genes in colorectal cancer, modulation of chromatin structure and transcriptional activity.	Lightfoot et al., 2013; Mischke and Plösch, 2016
Phenolic derivatives include 4-OH phenylacetic acid, urolithins, enterodiol, and 9-prenylnaringenin	Exhibit antimicrobial effects, maintain intestinal health, and protect against oxidative stress.	Larrosa et al., 2010
Choline metabolites include choline, trimethylamine N-oxide (TMAO), and betaine	Regulating lipid metabolism, and glucose synthesis contribute to the development of cardiovascular disease.	Smallwood et al., 2016
Polyamines include putrescine, spermidine, and spermine	Sustaining the high proliferation rate of intestinal epithelial cells enhances intestinal barrier integrity and enhances the systematic adaptive immune system.	Rooks and Garrett, 2016; Tofalo et al., 2019
Vitamins including thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic acid (B5), biotin (B7), folate (B11-B9), cobalamin (B12), and menaquinone (K2)	Help in red blood cell formation, DNA replication, and repair, work as an enzymatic co-factor, and enhance immune functioning.	Nicholson et al., 2012; Forster et al., 2017
Ethanol	Protein fermentation metabolites may be involved in NAFLD progression.	Yao et al., 2016; Wu et al., 2021
Hydrogen sulfide (H2s)	Reduction/neutralization of reactive oxygen species.	Afanas'ev, 2014; Mischke and Plösch, 2016

various forms of T helper cells (Th), respectively: Th1, Th2, and Th17 or into regulatory T cells (Francino, 2014; Owaga et al., 2015; Tomkovich and Jobin, 2016). Th17 cells are a subset of TCD4+ cells that secrete several cytokines, affecting immune homeostasis and inflammation (Rossi and Bot, 2013). Gut microbiota contributes to the stimulation and maturation of the immune system in response to pathogens, and it induces and sustains tolerance (Pickard et al., 2017).

Development of the immune system begins at birth, with the introduction of the microbiota, and can only become fully mature in the presence of commensal microflora. Proper immune system maturation is needed to prevent aberrant immune responses, which can cause chronic inflammation and illness (Tibbs et al., 2019). Various strategies, including the germ-free (GF) model, have been taken to demonstrate the importance of gut flora for forming both innate and adaptive immune systems (Uzbay, 2019). In comparison, gut microbiota modulation with antibiotic treatment also demonstrated its importance for immune homeostasis (Hill et al., 2010; Ubeda and Pamer, 2012). Antigen-presenting cells (APCs), having co-evolved with gut microbiota, a key advantage of intestinal APCs is their potential to defend the body from infection while retaining the immune tolerance to the normal gut microbiota (Wu and Wu, 2012). Gut microbiota plays a significant role in controlling the production of APCs. Gut microbiota is also involved in various intestinal and extraintestinal autoimmune diseases, as demonstrated by multiple studies (Andréasson et al., 2016; Rinninella et al., 2019).

Gut microbiota in malnutrition and fasting

Diets and food supplements have a significant influence on the gut's microbial composition and its variability over time. A high-fat diet is a risk factor for diseases like obesity, metabolic syndrome, and diabetes, all of which are linked to significant gut microbiota composition changes. Disruption of the circadian physiological rhythm increases the probability of intestinal dysbiosis, potentially leading to the pathogenesis of a variety of metabolic and inflammatory disorders, like diabetes, intestinal inflammatory diseases, and even cancer (Reynolds et al., 2017). Studies have also found that gut microbiota responds to malnutrition and fasting (Flint et al., 2015). The impacts of malnourishment on the gut microbiota were only studied under controlled conditions in lab animals due to ethical reasons. In a study, several weeks of nutrient deficiency showed increased microbiome diversity in fish, mice, and toads; geckos showed

a decrease while no change was detected in quails (Kohl et al., 2014). Due to these variations, it is challenging to investigate the influence of human nutrient deficiency, which can only be experienced in particular undernourished people. One of the leading causes of child mortality is malnutrition; nutrient-rich therapeutic foods are used to treat severe malnutrition. Also, children cannot completely recover from body mass improvements, probably due to their immature microbiomes. In children, the early development of the intestinal microbiome is particularly significant because microbiome composition keeps changing as they grow and continue changing their diet (Derrien et al., 2019).

Weight loss is promoted by intermittent fasting (IF) regimens, which contribute to enhanced metabolic health. Through metabolic activities, IF participates in the modulation of the gut flora, allowing ongoing interaction with nutrients to be digested and shaping intestinal immune responses during the development of coronary heart disease, blood pressure, and diabetes mellitus (Matías-Pérez et al., 2022). Microbiota reshaping by antibiotic therapy has extended the survival of children with acute malnutrition; even so, severe malnutrition reappeared when the microbiome remained immature, implying that microbiota maturity would anticipate the long-term therapeutic efficacy of the food (Subramanian et al., 2014). Furthermore, a study found that gut microbiota contributes a beneficial impact to the start of severe malnutrition, which can be regenerated by microbiota transplantation into gnotobiotic mice (Smith et al., 2013). Dietary and lifestyle activity such as fasting, and time-restricted eating influences the makeup of the intestinal microbiota. Various microbial products such as SCFAs, trimethylamine N-oxide, tryptophan, and tyrosine derivatives can significantly change with significant microbiota composition changes. However, there are several promising observational studies on human malnutrition, holding out the hope that therapeutic renovation of the gut microbiota will support eradicating mortality linked to malnutrition.

Gut microbiota in major human diseases

From the findings of recent epidemiological, physiological and omics-based studies, supported by cellular and animal experiments, it is demonstrated that intestinal microbiota plays a significant role in both health and disease (Ding et al., 2019). Although this research area is still at a very initial stage, with less understanding of the functional characteristics of the complex gut microbiota, some promising studies have been reported and indicated an enormous potential for revolutionizing the pathogenesis of diseases and therapeutic approaches (Ding et al., 2019; Yin et al., 2019; Rajoka et al., 2020; Bangar et al., 2022). Several major human diseases are associated with an altered gastrointestinal microbiota, for example, obesity, diabetes, cardiovascular disorders, cancer, hypertension, and IBDs (Ding et al., 2019; Nie et al., 2019; Xu et al., 2019) have been discussed individually later in this review. A state called "dysbiosis" is the variation in gut microbiota composition, which is described in many diseases, as shown in **Table 2**. It is a common problem in the current era because of bacterial infections, diet shifts, and antibiotics (Lindell et al., 2022). It has been challenging to define an appropriate healthy microbiome composition because of inter-individual variation (Lloyd-Price et al., 2016). A well-balanced gut microbial community is essential for the host and the microbiome to co-exist in a mutually beneficial relationship.

Obesity

The global prevalence of obesity has exceeded nearly 650 million people in the last four decades, a total that is six times more than what was reported in the 1990s (Sørensen et al., 2022). That can only be justified by increasing caloric intake and decreasing physical activity (Pascale et al., 2019). Several other diseases, such as diabetes mellitus, coronary heart disease, and cancers, are linked to obesity (Amin et al., 2019; Sun et al., 2019). Thus, weight management and reduction have gained more interest and attention from researchers. The involvement of gut microbiota in obesity is becoming a broad research topic and potentially useful for obesity treatment. Remarkably, the effect of diet on intestinal microbiota composition has become a specific subject of research. In this regard, recent evidence from various studies of humans and mice has demonstrated that changes in gut microbiota composition may play a vital role in the development of obesity (Davis, 2016; Bouter et al., 2017; Stephens et al., 2018; Socol et al., 2022). Several gut microbiota species, called the obesogenic gut microbiota, can significantly contribute to obesity, such as *Firmicutes, Bacteroidetes*, *Rhizobium, Lactococcus*, and *Clostridium* (Cao et al., 2019). In particular, obesogenic gut microbiota could facilitate obesity by producing SCFAs such as butyrate, providing the host with extra energy, and inducing low-grade inflammation caused by intestinal microbiota metabolites (Cao et al., 2019). Genetic aspects and epigenetic variations also play a significant role in the correlation between the composition of the gut microbiota and its contribution to obesity and the production of metabolites.

Some mechanisms have been proposed to define the role of gut microbiota in the development of obesity. Gut microbiota can reduce fatty acid oxidation by suppressing adenosine monophosphate kinase (AMPk) (López, 2017). This enzyme is present in muscle fibers and the liver and serves as a cellular energy indicator. AMPk suppression leads to reduced oxidation of fatty acids and, as a result, increased fat accumulation. By inducing systematic inflammation, intestinal microbiota can also lead to metabolic disturbance observed in obesity

TABLE 2 Diseases associated with gut microbiota abnormalities.

Disease	Features	References
Irritable bowel syndrome	An abundance of Firmicutes and a decrease in Bacteroidetes.	Kennedy et al., 2014
Type 1 diabetes	In genetically predisposed individuals, autoimmune against pancreatic b-cells. Deficient development or alteration of the microbiota may contribute to dysfunctional immunity with the devastation of autoimmune b-cells and increased leakiness of the intestinal epithelial barrier. Variability of microbiomes reduced.	Dunne et al., 2014
Asthma	Outbreaks of *Chlamydophila pneumonia* during bronchitis and pneumonia development affect the airway microbiome. Gut microbiota is influenced by the introduction of microbiota to the environment, particularly in early life, which helps immune function growth and the development of defending against allergic sensitization.	Huang and Boushey, 2015
Food-borne pathogens and food poisoning	Opportunistic pathogens (*Campylobacter*, *Salmonella*, *Escherichia coli*, *Shigella*, etc.) disturb the microbiome's balance leading to dysbiosis.	Josephs-Spaulding et al., 2016
Malnutrition	Decrease or missing species that either process food categories efficiently or produce vitamins may reduce the absorption of nutrients. An overabundance of *Enterobacteriaceae* can lead to epithelial damage, diarrhea, and limited absorption of nutrients.	Kane et al., 2015
Depression	In physiological systems, *Bifidobacterium infantis*, generally found in infants' gastrointestinal tract and administered probiotic drugs, can have antidepressant effects.	Evrensel and Ceylan, 2015
Anxiety	Oral administration of *Campylobacter jejuni* subclinical doses in murine models induced anxiety-like behavior without stimulating immunity. In a marine model, the *Lactobacillus* and *Bifidobacterium* may act as an anxiolytic influencer.	Schnorr and Bachner, 2016

(Pindjakova et al., 2017). Another proposed mechanism is the energy regulation and microbes' potential to ferment dietary polysaccharides that are not digested by humans (Khan et al., 2016). The fermentation of dietary fiber produces SCFAs. SCFA can stimulate lipogenesis after being absorbed and boost triglyceride storage *via* molecular pathways. Also, SCFA has the potential to suppress the fasting-induced adipocyte factor (FIAF), which inhibits lipoprotein lipase (LPL), causing the accumulation of triglycerides in the host adipocytes (Khan et al., 2016). To acknowledge, how intestinal microbiota promotes the development of obesity, more prospective and interventional studies are needed.

Hypertension

Hypertension is becoming a significant threat to public health and an important risk factor for cardiac, stroke, and kidney diseases (Shah et al., 2019). By 2025, it is estimated that the total number of patients with hypertension will rise to 1.56 billion worldwide (Xu et al., 2020). Studies have shown that various genetic and environmental factors, including dietary salt intake, lack of exercise, and alcohol consumption, also contribute to hypertension progression (Booth et al., 2012; Rust and Ekmekcioglu, 2016). Previous research on animal models and human subjects has shown that hypertension progression is also linked to gut microbiota dysbiosis (Jose and Raj, 2015; Miremadi et al., 2016). Moreover, alterations in the composition of the intestinal microbiota can result in the development of novel antihypertensive therapies. The various mechanisms underlying the relation between gut microbiota and hypertension have been proposed, although there is no definite understanding. The ratio of *Bacteroidetes* and *Firmicutes* within intestinal microbiota has been significantly associated with hypertension (Yang et al., 2015). Hypertensive animals and seven hypertensive patients reported an abundance of *Bacteroidetes* and *Firmicutes* in their gut microbiota as sequenced by 16S ribosomal RNA (Moghadamrad et al., 2015). Studies using angiotensin II-infused GF mice have shown that gut microbiota is involved in vascular dysfunction and hypertension induced by angiotensin II (Karbach et al., 2016).

Short-chain fatty acids play a crucial role in maintaining gut microbiome homeostasis and host immunity. Recent studies have found that SCFAs produced by gut microbiota is involved in modulating blood pressure (Kang and Cai, 2018). SCFAs have the potential to stimulate host G-protein-coupled receptor (GPR) pathways that affect the secretion of renin and blood pressure (Pluznick et al., 2013). In another study to investigate the correlation between serum metabolites and hypertension, it was found that lyxose levels (a by-product of intestinal microbial fermentation) were higher in patients with newly diagnosed hypertension compared to healthy controls (Hao et al., 2016). However, these findings are preliminary; it is essential to validate other environmental factors like the diet that might affect the gut microbiota.

Furthermore, a beneficial role of *Lactobacillus* in the regulation of blood pressure has been reported (Gómez-Guzmán et al., 2015). Recent studies and clinical trials

demonstrate a close but complex inter-relationship between gut microbiota and hypertension. However, more studies involving human participants are needed to elaborate on the critical role of gut microbiota in hypertension and to demonstrate promising therapeutical approaches.

Cardiovascular diseases

Even with the existing approaches in atherothrombosis prevention and treatment, heart disease is still a significant cause of death globally. It will constantly rise due to increased incidence in low and middle-income countries (Odutayo et al., 2016). In the pathophysiology and progression of CVDs, the intestine has also been involved, primarily due to decreased perfusion of the intestines leading to intestinal barrier dysfunction. The intestinal endothelial barrier is regulated by many mechanisms of a well well-balanced intestinal microbiota (Sabatino et al., 2015). Recently, due to accumulating evidence, intestinal microbiota has been studied as a contributing factor to heart disease and stroke (Tang et al., 2017; Leustean et al., 2018; Jayachandran et al., 2020). Emerging evidence has shown that gut dysbiosis was correlated with the production of many metabolites from intestinal microbiota and also fostered disruption of the function of the gut endothelial barrier.

Furthermore, an essential correlation between the amount of fecal gut microbiota and the intensity of intestinal permeability was identified in patients with CVDs (Pasini et al., 2016). In contrast, patients who had bacterial DNA in the peripheral blood had considerably high plasma levels of inflammatory markers, particularly highly sensitive C-reactive protein and interleukin-6 levels, compared to those who did not have bacterial DNA in their peripheral blood (Wang et al., 2012). Moreover, an increased abundance of *Streptococcus* and *Enterobacteriaceae* is linked with coronary artery disease (Jie et al., 2017). Patients with coronary artery disease have altered populations of the most prevalent bacterial species that make up the gut microbiota, with a decrease in Bacteroidetes and an increase in Firmicutes. Trimethylamine-N-oxide is a metabolite that plays an important role in atherosclerosis and can help predict cardiovascular risk (Ramírez-Macías et al., 2022).

Various mechanisms have been proposed to understand the crucial role of gut microbiota in the development and prevention of CVDs. Copies of bacterial genes coding for trimethylamine (TMA) lyase and atherosclerotic CVDs have also been found to be associated (Barrington and Lusis, 2017). TMA lyase contributes to the generation of trimethylamine-N-oxide (TMAO), a metabolite derived from the gut microbiota (Witkowski et al., 2022). TMAO has been shown to contribute to the development of cardiovascular atherosclerotic disease in animal studies and seems to be significantly linked in human studies, identifying the primary function that TMAO may perform in developing atherosclerotic CVD (Tang and Hazen, 2014; Jonsson and Bäckhed, 2017). Thus, a rapid increase in cardiovascular and metabolic disorders has concentrated on gut microbiota regulation as an effective treatment option.

Diabetes mellitus

Diabetes mellitus causes a significant adverse effect on the health condition of human populations worldwide. Diabetes-related risk factors include aspects like a family history of diabetes, poor eating habits, and being overweight. Regarding the continuous rise of urbanization, shifts in diet, and the emergence of more unhealthy lifestyles, the growing incidence of diabetes is a global crisis. According to a report, about 463 million people globally reported diabetes in 2019, and future estimates predict that by 2045, the number of diabetic patients will exceed 700 million (Saeedi et al., 2019). Recent studies have demonstrated that the progression of diabetes is closely correlated to the alterations in the composition of intestinal microbiota (Sender et al., 2016; Gurung et al., 2020). Diet is among the key determinants of the composition of the intestinal microbiota and a significant causal factor in the development of diabetes (Meijnikman et al., 2018).

Given that the development and formation of the gut microbiota depend on the availability of nutrients, it is vitally important to demonstrate that metabolite production depends on food consumption. It has been found that, in response to a shift from a low-fat, plant polysaccharide-rich diet to a high-fat, high-sugar diet, the microbiome composition changed rapidly (Turnbaugh et al., 2009). Human eating patterns have evolved over the past few decades, with fats preferred over fibers; in response to recent eating habits, intestinal microbiota has also changed. Therefore, it was suggested that diabetes could be linked to the intestinal microbiota's systematic alterations (Sircana et al., 2018).

It was observed in the diabetes prevention and prediction (DIPP) study that new-onset type-1 diabetes subjects had a distinct composition of gut microbiota compared to controls (Brown et al., 2011). It was found that mucin formation was caused by lactate and butyrate-producing bacteria in the control group to sustain gut integrity. In contrast, mucin synthesis was inhibited by non-butyrate producing lactate-utilizing bacteria contributing to autoimmunity of β-cells and type 1 diabetes (Brown et al., 2011). Also, an increase in the occurrence of *Akkermansia muciniphila* has been observed to be inversely related to the probability of developing type 1 diabetes (Hansen et al., 2012; Navab-Moghadam et al., 2017). *A. muciniphila* may is a potential probiotic in the treatment of type 1 diabetes. Many other studies have reported the variations in the composition of gut microbiota between type 1 diabetes and their matched health controls, illustrating the need for a

better understanding of the function that these bacteria can play in the development of diabetes (Murri et al., 2013; Gülden et al., 2015).

It has been indicated that the influence of microbiota on type 2 diabetes can be mediated through mechanisms involving changes in the butyrate and incretins secretions (Nøhr et al., 2013; Baothman et al., 2016). In patients with type 2 diabetes, a study showed a moderate degree of intestinal microbial dysbiosis, a decrease in bacteria-producing universal butyrate, and an increase in opportunistic pathogens (Baothman et al., 2016). Other studies have also shown the significant influence of gut microbiota on type 2 diabetes pathways, including insulin signaling, inflammation, and glucose homeostasis (Baothman et al., 2016; Cani, 2018). However, more studies are needed to deeply understand the mechanisms and influential role of gut microbiota in the development of diabetes.

Cancer

Cancer is the second most common cause of death globally (Fitzmaurice et al., 2017). Many factors significantly influence cancer risks, such as exposure to pathogens, UV radiation and toxic substances, diet, and lifestyle. However, the risk mainly depends on the dosage, the period, and the combination of such factors, along with the genetic background of the patient (Vivarelli et al., 2019). There is a growing interest in the characterization and functionality of intestinal microbiota due to its complicated relationship with the host (Tao et al., 2020). Different studies have indicated that abrogation or alteration of gut microbiota significantly contributes to developing colorectal carcinoma in genetic and carcinogenic tumorigenesis models (Arthur et al., 2012; Vivarelli et al., 2019). Metabolomics and metagenomics studies have demonstrated the dual role of gut microbiota in cancer risk reduction and tumor growth, and anti-cancer therapies (Bultman, 2014).

A greater abundance of *Bacteroides massiliensis* was found in patients with prostate cancer, while *Eubacterium rectale* and *F. prausnitzii* have been identified in comparatively less abundance, indicating the potential contribution of these specific microorganisms in the pathogenesis of prostate cancer (Chung et al., 2018). It has also been found that the gut microbiota is linked with the development of colorectal cancer, with *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Peptostreptococcus anaerobic* being identified in its development as important players (Hsieh et al., 2018). Gut bacteria, especially *F. nucleatum* and *Clostridium colicanis*, were proposed as indicative markers in gastric cancer's carcinogenesis (Mehta et al., 2017). Recent studies have indicated that *F. nucleatum* can suppress the host's immune response and upgrade cellular proliferation. In contrast, a diet rich in whole grains and dietary fiber have a lower risk of *F. nucleatum* positive cancer, indicating that the gut microbiome may be a significant mediator between dietary and colorectal cancer interactions (Hall et al., 2017). Various preclinical studies using GF mice have proposed the mechanism and considerable impact of gut microbiota on genesis and cancer progression (Arthur et al., 2012). A deeper understanding of the influential role of gut microbiota in the development of cancer has increased the interest in research for microbiome-based therapeutics in cancer treatment. However, more studies involving human participants are required to deeply understand the mechanism of gut microbiota in the development of cancer and its anti-carcinogenic characteristics.

Inflammatory bowel diseases

Inflammatory bowel disease is a significant disease with the highest prevalence in western countries; its incidence has risen rapidly in newly industrialized countries in Asia, the Middle East, Africa, and South America (Kaplan and Ng, 2017). It is also imperative to examine the exact etiology and pathogenesis of IBD. Notable advancements have been achieved in identifying the development of IBD in the last few years. The most significant and clinically beneficial aspect of this advancement was the identification of gut microbiota as a crucial multifunctional inflammatory factor. Recently, the role of intestinal microbiota in the pathogenesis of IBD has been emphasized. Several lines of evidence indicate the essential part of the gut microbiota in intestinal inflammation. Most studies have demonstrated decreased intestinal microbiota diversity in patients with IBD (Willing et al., 2010; Matsuoka and Kanai, 2015). Significant decreases in *Firmicutes* and proteobacteria are the most important observations of altered composition of gut microbiota in patients with IBD. The decreased diversity of intestinal microbiota found in patients with IBD was primarily due to the reduction of *Firmicutes*. A decline in the *Clostridium leptum* groups, particularly *F. prausnitzii*, has been observed among Firmicutes (Wang et al., 2014). In biologically susceptible hosts, alterations of the gut microbiota have been associated with aberrant mucosal immune responses that result in a variety of intestinal and extraintestinal disorders, including IBD. As a result, restoring immunological homeostasis by modifying the gut microbiota is currently considered to be a potential therapeutic strategy to treat IBD patients (Facciotti, 2022).

The majority of discovered human pathogenic bacteria belong to the phylum Proteobacteria, which play an increasingly important role in IBD (Mukhopadhya et al., 2012). Analysis of microbial diversity shows a rise in the number of bacterial species belonging to this phylum, implying an active role in initiating chronic inflammation in patients with IBD (Hold et al., 2014). The abundance of *Ruminococcus gnavus* is also

found to be higher in IBD (Örtqvist et al., 2019). Although more clinical studies are required to examine and deeply understand the mechanism through which gut microbiota contribute to IBD progression.

Eubiosis and food

Dietary effects and influences on our gut microbiome are not new subjects of research. Food causes transient changes in the gut microbiota composition, which are primarily due to fish, meat, and fiber, which have long-term effects (Bajinka et al., 2020). More than two macronutrients can be found in one diet, which alters the gut microbiota while also altering metabolic output (Qiu et al., 2020). The positive benefits of dietary fiber on human metabolism have been explored and found to be significant. Dietary fiber has been shown to alter the microbiota and produce beneficial metabolites like butyrate (Silva et al., 2020). While a balanced nutritional diet is important for overall health, a diet high in fiber is particularly essential to maintain the diversity of the intestinal microbiota (Zhang et al., 2013).

Microbiota ferment complex undigested carbohydrates, also known as microbiota-accessible carbohydrates (MAC), leads to an increase in SCFA levels and, as a result, a positive health effect (Seo et al., 2020). These complex carbohydrates, which include resistant starch, oligosaccharides, and dietary fiber, can positively modulate a variety of gut microbes that are beneficial to health (Yang et al., 2020). Unsaturated plant-based fats in the diet reduce detrimental bacteria while increasing the abundance of *Bifidobacterium* and butyrate-producing bacteria (*Roseburia* and *Faecalibacterium*), all of which have been associated with positive health effects (Muralidharan et al., 2019). Micronutrients, in addition to macronutrients, may play a key role in gut reshaping, according to various studies (Ramos and Martín, 2021). All of these findings point to the importance of dietary factors as modulators of the microbial community, which can therefore have an impact on human physiology and disease processes.

Conclusion

The crucial role of probiotics in health, disease, and nutrition has increased their scientific and marketing significance across the globe. The attention has been shifted from prospective studies to clinical trials to have a better understanding of how microbiota can interplay in human health and disease. Eubiosis is important in exerting the health endorsing benefits of probiotics. An unhealthy diet intake, such low intakes of fruits and vegetables intakes and overuse of antibiotics can result in dysbiosis. In nutshell, probiotics aid in the treatment of various infectious diseases, dysfunctions of the GI tract, and inflammatory disorders as well as in controlling obesity and diabetes. The advances in gut microbiota modeling and analysis will enhance our knowledge of how they influence health and disease, allowing us to adapt current and forthcoming therapeutic and preventive strategies. Understanding the specific roles played by the gut microbiome in our growth and development, as well as how it functions in health and disease, holds the potential to improve many parts of our daily lives, from improving the formula for infants to offering new approaches in fighting obesity and cancer, among others. As gut microbiota is a complex topic, future research should focus on multidisciplinary approaches, taking into consideration recent innovations in various scientific fields.

Author contributions

MA and RA: conceptualization and writing—original draft preparation. MA, FS, YS, MH, RR, AH, AR, MP, JL, and CS: writing—review and editing. RA: supervision. All authors read and agreed to the final version of the 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.

Publisher's note

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EDITED BY
Junling Shi,
Northwestern Polytechnical University, China

REVIEWED BY
Dejian Yu,
Nanjing Audit University, China
Anand Kumar,
Los Alamos National Laboratory (DOE), United States

*CORRESPONDENCE
Lili Zhang
lilizhang369@163.com
Linhua Zhao
melonzhao@163.com

†These authors have contributed equally to this work

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Global research trends on the links between the gut microbiota and diabetes between 2001 and 2021: A bibliometrics and visualized study

Boxun Zhang[1,2†], Zishan Jin[1,3†], Tiangang Zhai[1,3], Qiyou Ding[1,3], Haoyu Yang[1,3], Jia Wang[4], Lili Zhang[1*] and Linhua Zhao[1*]

[1]Institute of Metabolic Diseases, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]Post-Doctoral Research Center, China Academy of Chinese Medical Sciences, Beijing, China, [3]Graduate College, Beijing University of Chinese Medicine, Beijing, China, [4]General Department, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China

Background: Over the past 20 years, evidence has suggested that gut microbiota plays an important role in metabolic homeostasis. The relationship between gut microbiota and diabetes has become the focus of considerable scientific interest. With the sharp increase in publications in this area, it is imperative to analyze the relevant articles using bibliometrics methods.

Methods: Publications on "the gut microbiota and diabetes" were retrieved and downloaded from the Web of Science Core Collection database. Microsoft Excel 2020, VOSviewer, CiteSpace 5.8.R3 and Co-Occurrence 9.94 software were used for data analysis and visualization. Country/academic institution, journal, author, subject category, keyword and reference were analyzed thoroughly. The cutting-edge directions in this field were also determined by analyzing keywords and key articles.

Results: A total of 2,342 documents were included in the analysis; the number of articles in this field has increased yearly, particularly after 2010. China and the University of Copenhagen are the country and research institution associated with the largest number of publications. *Nutrients* have published 191 articles in this field, ranking first among highly productive journals in the number of publications. The researcher *Cani PD* affiliated with the University of Leuven, Belgium, published the greatest number of articles in this field between 2001 and 2021 and was also ranked as the first co-cited author and the largest contributor of highly cited papers in this field. *Endocrinology & Metabolism* was the most common subject category. Three of the most frequently found keywords, besides terms related to "microbiota" and "diabetes," were "obesity," "probiotics,"

and "inflammation." *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, trimethylamine n-oxide and branched-chain amino acids are intestinal bacteria or metabolites that have attracted more attention in recent years. Natural products represented by Chinese herbal medicine and some protein receptors or signaling pathways such as aryl hydrocarbon receptor, farnesoid X receptor and AMP-activated protein kinase were frontiers in this field.

Conclusion: Over the past two decades, the rapid development of research on the gut microbiota has deepened the understanding of the physiology and pathology of diabetes, providing new insights into different approaches to treatment. In the future, further interdisciplinary innovation, clinical transformation, and application may receive more attention.

KEYWORDS

gut microbiota, diabetes, research trend, bibliometrics, visualization

Introduction

Over the past 20 years, significant progress has been made in intestinal microecology, and the relationship between the gut microbiota and multiple inflammation-related diseases has gradually become a research hotspot (Boulangé et al., 2016). Accumulating evidence suggests that diabetes, primarily type 2 diabetes (T2D), is a chronic systemic inflammatory disease (Hotamisligil, 2006), and compared with normal subjects, the intestinal flora of patients with T2D was characterized by a decrease in butyrate-producing bacteria and an increase in various opportunistic pathogens (Qin et al., 2012). Similarly, intestinal flora disturbance is also found in patients with type 1 diabetes (T1D), mainly characterized by differences in *Bacteroides spp.*, *Streptococcus spp.*, *Clostridium spp.*, and *Bifidobacterium spp.* (Jamshidi et al., 2019), and for infants, the α-diversity of the gut microbiota showed a remarkable downward trend before the diagnosis of T1D (Kostic et al., 2015). In addition, the main metabolites/products of the gut microbiota, such as short-chain fatty acids (SCFAs), lipopolysaccharides (LPS) and bile acids (BAs), also play important roles in regulating the metabolic homeostasis of the host (Dehghan et al., 2014; Zhao et al., 2019). Compared with the healthy group, the concentration of SCFA in the feces and circulation of patients with diabetes decreased, whereas the content of LPS increased significantly; these changes can promote damage to the intestinal barrier and low-grade systemic inflammation, subsequently inducing insulin resistance (IR) (Saad et al., 2016).

Diabetes treatment strategies and methods targeting the gut microbiota have also attracted wide attention. Results from a meta-analysis suggested that supplementation with probiotics, prebiotics, or synbiotics could improve metabolic outcomes in patients with diabetes (Bock et al., 2021). Some polyphenols, polysaccharides, or other active substances extracted from functional foods or herbs can also regulate glucose metabolism by modulating gut microbiota (Lyu et al., 2017). The molecular mechanism of intestinal microecological agents in treating diabetes may involve multiple pathways such as anti-inflammatory, antioxidant, intestinal barrier protection, and intestinal hormone regulation (Kim et al., 2018).

In addition, interdisciplinary integration is a significant feature in this field, which further increases the complexity of the knowledge structure. Although there has been a considerable expansion in articles on "gut microbiota and diabetes," to the best of our knowledge, there is no research analyzing the basic information presented in the publications and exploring the changing trends in research topics. Bibliometrics is a subject that applies mathematical and statistical methods to analyze the knowledge structure and development trends of publications (Pritchard, 1969). Data integration and clustering can identify the salient authors, journals, and academic institutions in this field as soon as possible and accurately screen out the frontier research (Agarwal et al., 2016). In recent years, various new methods have emerged in the field of bibliometrics, providing ideas for the in-depth development of related research, at the same time, a large number of bibliometrics articles are also published in an increasing trend year by year (He et al., 2017; Zyoud et al., 2019; Yu and Pan, 2021). This study intends to apply bibliometrics to analyze the relevant information of the articles on "the gut microbiota and diabetes" published between January 2001 and December 2021 to improve understanding of the research history and status of current knowledge in this field, straighten out the publication trend, and explore the research highlights.

Materials and methods

Sources of data and search strategy

In order to ensure the authority of the original documents, data were retrieved and downloaded from the Web of Science Core Collection (WoSCC) (indexes: Science Citation Index Expanded [SCI-E]). To further examine the latest trends developing in this field, the time limit was from January 2001 to December 2021, which is also a period when major breakthroughs have been made in the research of gut microbiota. In order to facilitate the statistical analysis of literature data, we only included English documents. The scope of the retrieval was limited to Web of Science (WOS) database subject words, and the terms of the search strategy are shown in **Table 1**.

Inclusion and exclusion criteria

Screening the retrieved literature is necessary to ensure the reliability of the data used for analysis. Two investigators (Boxun Zhang and Zishan Jin) independently reviewed the document according to the following criteria, and any differences were resolved through consultation with a third party.

Inclusion criteria: (1) the research topic of the article involves both the gut microbiota and diabetes and its related diseases (pre-diabetes, insulin resistance, diabetes complications); (2) the document type is "article" or "review articles"; (3) the document language is limited to "English"; and (4) the publication time is from 1 January 2001 to 31 December 2021.

Exclusion criteria: (1) the theme of the document is other metabolic diseases (such as obesity, non-alcoholic fatty liver, lipid metabolism disorder); (2) the topic of the study is not the gut microbiota but the urine, saliva or vaginal microbiota; (3) withdrawn or duplicate publications; and (4) documents that cannot provide the basic information required for bibliometric analysis.

TABLE 1 Search strategy of Web of Science database.

Step	Search strategy
#1	TS = (gut OR intestin* OR gastrointestin*) AND TS = (microbio* OR microflora OR flora OR bacteri* OR dysbiosis OR microecology OR 16Sr* OR metagenome)
#2	TS = (prebiotic* OR probiotic* OR synbiotic*)
#3	#1 OR #2
#4	TS = (diabetes OR diabetic* OR IDDM OR NIDDM OR MODY OR T1D OR T1DM OR T2D OR T2DM)
#5	#3 AND #4

TS = Topic.

Data collection and analysis

The basic information in the records, such as article title, author, publication year, abstract, keywords, and citation frequency, were extracted and classified to analyze the data better. For some important articles, we searched the official websites of WOS and Scimago Journal & Country Rank (SJR) for their latest impact factors (IF), 5-year impact factor, quartile of a journal category and Hirsch index (H-index). The impact factor (IF) value was a quantitative index representing the influence of journals, which was determined based on the frequency of citations by other scientific publications (Garfield, 1999). The H-index, proposed by *J. E. Hirsch*, is another international evaluation index that can comprehensively quantify the academic contribution of scientists (Hirsch, 2005) and, at the same time, can be used to evaluate the influence of academic journals (Chen et al., 2020). Next, we used Microsoft Excel 2020 (Redmond, Washington, USA), VOSviewer (Leiden University, Leiden, the Netherlands), CiteSpace V 5.8.R3 (Drexel University, Philadelphia, PA, USA) and Co-Occurrence 9.94 (COOC 9.94) to perform data statistics and visual analysis. Specifically, Microsoft Excel was used for managing, screening and ranking documents; VOSviewer was used to create network visualization maps to analyze the collaborative relationships between countries/regions, institutions, journals, authors and keywords, as well as the co-citation network of journals and authors; CiteSpace was used to capture key information with strong bursts during a specific period, to help us identify and further discuss hot topics; COOC was used for making frequency statistics on countries, keywords, journals, and analyzing the changes of subject categories.

Results

General characteristics of the retrieved documents

According to the search strategy, a total of 6,019 documents were retrieved, but after screening according to the inclusion and exclusion criteria, only 2,342 documents could be used for further analysis (**Figure 1**). Of these, 73.5% were original, and 26.5% were review articles. In the past 21 years, the number of papers in this field has been increasing year by year, particularly after 2010.

Country/region and academic institution distribution

The authors of these articles were from 84 countries or regions. A corresponding author in China published a total of 786 articles, ranking first for the number of articles published.

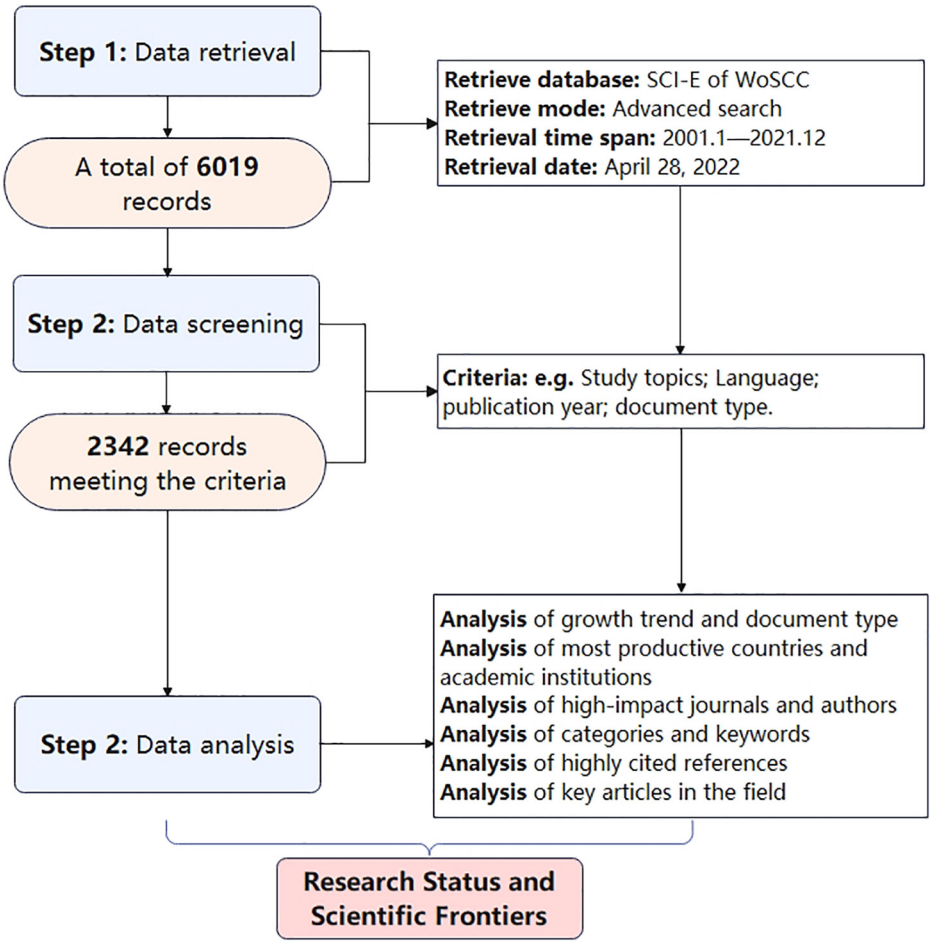

FIGURE 1
Flowchart of document search, screening, and data analysis.

Authors ranking second to fifth were from the US, Iran, Canada, and Japan, respectively. Figure 2A shows the global distribution of research output in this field (based on the nationality of corresponding authors). In addition, we analyzed the change in trends in the annual number of publications from authors based in the countries above (Figure 2C and Table 2). Before 2015, the number of papers published by American scholars ranked first in the world, and over the past 5 years, papers from China increased sharply. However, regarding the citation frequency of each article, China still lags behind most countries (Table 2). In addition, several publications from other countries showed an increasing trend year by year (Figure 2B).

To further explore any cooperation relationship between countries/regions, we used VOSviewer software to perform a co-occurrence clustering analysis. The node's size represented the strength of links to others, the thickness of the line represented the number of cooperation, and the same color meant that these countries or institutions had closer cooperation. As shown in Figure 3A, the USA had the highest total link strength, reflecting the closest level of cooperation with other countries, particularly China and Canada; some European countries/regions, such as England, France, Germany, Belgium, Sweden and Netherlands, jointly formed a blue cluster and also built close cooperation networks; additionally, Italy and Spain led the red clusters (Supplementary Table 1).

These studies included 2,670 academic institutions, with the University of Copenhagen, University of Helsinki, University of Gothenburg, Catholic University of Leuven and Chinese Academy of Sciences (CAS) ranking among the top five institutions for the number of papers published (Supplementary Table 2). Figure 3B shows the leading academic institutions in this field. Co-occurrence cluster analysis was used to explore the cooperative relationship among academic institutions. The results showed that the total link strength of the University of Copenhagen was the highest, indicating that it had the closest level of cooperation with other academic institutions. Furthermore, the University of Helsinki and the University of Gothenburg were key nodes of

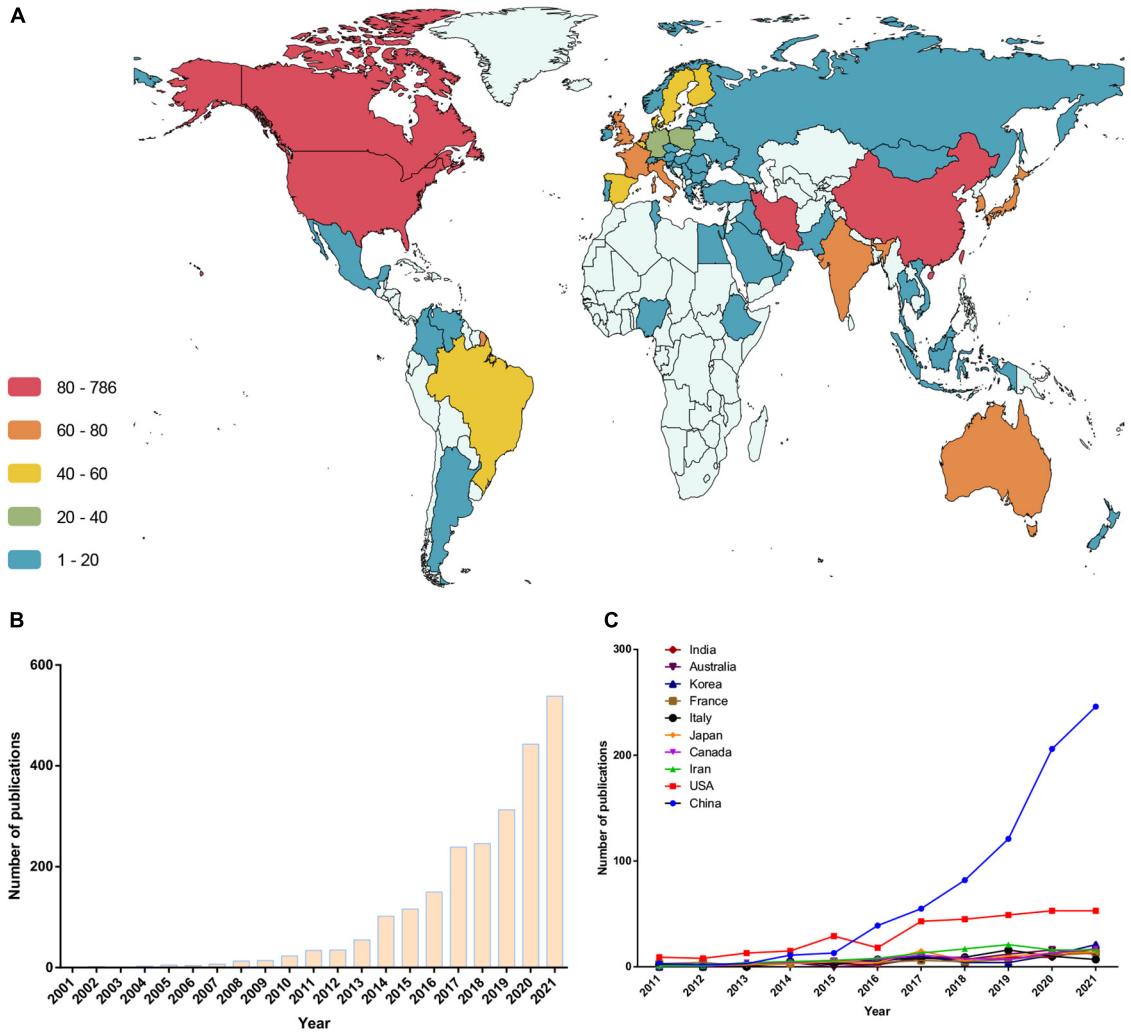

FIGURE 2
Trends in the number of publications and analysis of country/regions in the "gut microbiota and diabetes" area. **(A)** Geographical distribution of publication. **(B)** The trend in the total number of publications in the past 21 years. **(C)** The trend in the number of publications in the top 10 productive countries.

TABLE 2 Top 10 productive countries/regions related to the research field of the gut microbiota and diabetes.

Rank	Countries/ regions	Total number of papers	N/2342	Continents	Total citations	Citations per article
1	China	786	33.6%	Asia	20,076	25.5
2	USA	346	14.8%	North America	19,783	57.2
3	Iran	108	4.6%	Asia	3,639	33.7
4	Canada	81	3.5%	North America	4,187	51.7
5	Japan	74	3.2%	Asia	3,101	41.9
6	Italy	72	3.1%	Europe	3,609	50.1
7	France	67	2.9%	Europe	13,375	199.6
8	Korea	67	2.9%	Asia	2,806	41.9
9	Australia	65	2.8%	Oceania	2,102	32.3
10	India	65	2.8%	Asia	2,046	31.5

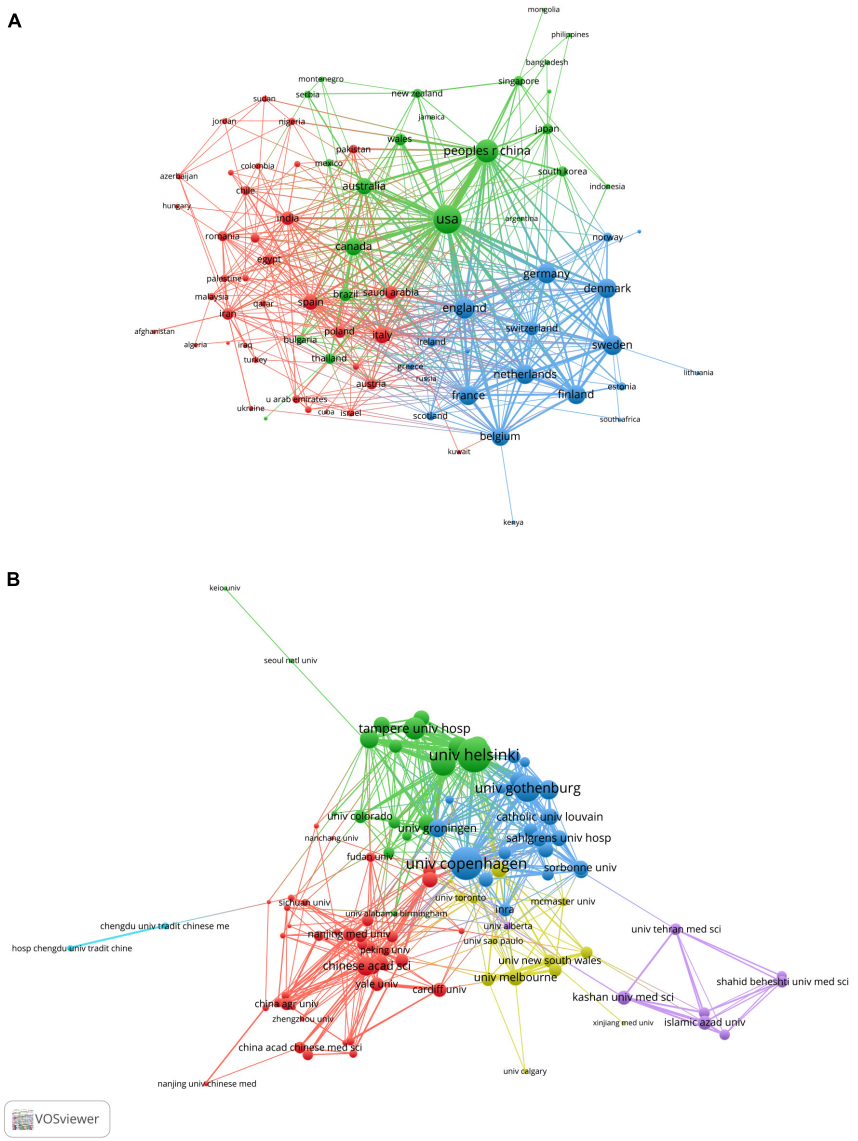

FIGURE 3
VOSviewer network visualization map of countries/regions and academic collaboration in the "gut microbiota and diabetes" area.
(A) Collaboration network map of the most productive countries. **(B)** Collaboration network map of the most productive research institutions.

the collaboration network (Supplementary Table 2). Chinese research institutions represented by CAS, Shanghai Jiao Tong University and Zhejiang University formed the red group, suggesting extensive cooperation.

Journal distribution

The 2,342 papers were published in 1,237 academic journals. Among the ten most productive journals, *PLOS One* was US-based, and the remaining were from Europe (Switzerland, UK, France and Germany). The average journal IF listed in Table 3 was 5.56 (IQR: 4.67–6.38), and their average H-index was 180.5 (IQR: 117.5–241.75). All the journals were classified as Q1 (the top 25% of the IF distribution) or Q2 (between the 50th and 25th percentile).

When two or more journals are cited by one article simultaneously, they form a co-citation relationship. Table 4 lists the top 10 co-citation journals, six from the USA, three from the UK and one from Germany. All journals except the *British Journal of Nutrition* and *PLOS One* were considered as Q1. We used VOSviewer software to perform a co-occurrence clustering analysis. The results showed that these co-citation publications could be divided into three clusters (Figure 4A and Supplementary Table 4): (1) comprehensive scientific journals in the red cluster, such as *Nature*, *PLOS One*, *PANS*, *Science*;

(2) diabetes-related journals in green, such as *Diabetes, Diabetes Care, Diabetologia, Cell Metabolism*; and (3) food and nutrition-related journals in blue, such as *British Journal of Nutrition*, *American Journal of Clinical Nutrition*, *Nutrients*, *Journal of Nutrition*.

Author distribution

A total of 11,414 authors are included in these publications. Table 5 describes the top 10 most productive and co-cited authors' basic information. It is important to note that nine of the most productive and six co-citation authors were from Europe. *Cani PD* of the *University of Leuven in Belgium* published the most publications in this field between 2001 and 2021, followed by *Nieuwdorp M*, *Delzenne NM*, *Bäckhed F*, and *Burcelin R*. If two or more authors appeared in the references of an article at the same time, they were considered as co-citation authors. These authors often had considerable research achievements and may be regarded as leading figures in the field. Table 4 lists the top 10 co-citation authors, and *Cani PD* and *Bäckhed F* also ranked among the top five in the high co-citation authors list, indicating that they published the greatest number of articles and had extensive international influence. Figure 4B shows the network visualization map of the co-cited authors. The node size represented the number of co-citations, and authors in the same color group were co-cited more frequently.

Category analysis

The subject category represents the main research direction of a study. In general, 2,342 papers involved 88 WOS categories, and the top five subjects were identified as Endocrine & Metabolism, Nutrition & Dietetics, Food science & Technology, Biochemistry & Molecular biology, and Microbiology, accounting for 14.7, 10.9, 7.2, 6.8, and 6.7% of the total, respectively (Supplementary Table 4). Next, we paid more attention to the emerging categories in this field in the past 3 years. The weighted average year of occurrence of a specific category (with a frequency of at least five times) is calculated by using COOC software. The results show some categories not belonging to the biomedical category frequently appeared, such as Chemistry, Agriculture and Polymer Science. Clinical disciplines related to diabetes, such as Geriatrics & Gerontology, Urology & Nephrology, and Integrative & Complementary Medicine, started to increase after 2019 (Supplementary Table 5).

Keywords analysis

The 2,342 articles contained a total of 3,415 different keywords. It is noteworthy that several of the most frequently found keywords, besides terms related to "microbiota" and "diabetes," were "obesity," "probiotics," and "inflammation." To further understand the knowledge structure in this field, we performed co-occurrence analysis using VOSviewer software. As shown in Figure 5A, the size of the circle represented the total link strength, and the thickness of the line represented the number of co-occurrences. Finally, high-frequency keywords were clustered into three clusters. The red cluster showed some T2D-related keywords, such as obesity, inflammation, insulin resistance, and fatty acids. The green cluster mainly included T1D-related keywords, such as children, autoimmunity and nod mice. The red cluster mainly included keywords related to intestinal microecological agents, such as probiotics, prebiotics, and some words about clinical research, such as double-blind. The details are shown in Supplementary Table 6.

To better track research hotspots and frontiers, we applied the "overlay visualization" mode to analyze the keywords again. We classified the frequently occurring keywords according to three categories of the gut microbiota and its metabolites, intervention measures and molecular mechanisms. As Figures 5B–D shows, the abscissa represents the score calculated by the VOSviewer software based on the average year

TABLE 3 Top 10 productive journals in the "gut microbiota and diabetes" area.

Rank	Journal	Country	Count (n)	IF (2021)	5-year IF	H-index	Quartile in category
1	Nutrients	Switzerland	191	6.706	7.185	143	Q1
2	Scientific Reports	UK	130	4.996	5.516	242	Q1
3	PLOS One	USA	110	3.752	4.069	367	Q2
4	Food & Function	UK	91	6.317	6.375	89	Q1/Q2
5	Journal of Functional Foods	UK	75	5.223	5.178	97	Q1/Q2
6	International Journal of Molecular Sciences	Switzerland	68	6.208	6.628	195	Q1/Q2
7	Biomedicine & Pharmacotherapy	France	61	7.419	6.581	109	Q1
8	Frontiers in Microbiology	Switzerland	50	6.064	6.843	166	Q1
9	Diabetes	USA	47	9.337	10.509	345	Q1
10	Diabetologia	Germany	46	10.46	10.617	241	Q1

of publication, and the ordinate represents the weight of the keyword, that is, the frequency of occurrence. In recent years, in addition to some beneficial bacteria, such as "*Lactobacillus,*" "*Bifidobacteria,*" "*Akkermansia muciniphila* (*A. muciniphila*)," and "*Faecalibacterium prausnitzii* (*F. prausnitzii*)," metabolites or derivatives derived from the gut microbiota such as "SCFAs," "LPS," and "BAs" also received more attention. Still, after the year 2019, "trimethylamine n-oxide (TMAO)," "branched-chain amino acids (BCAAs)," "p-cresyl sulfate (PCS)," "indoxyl sulfate (IS)," and "succinate" gradually appeared in more scientific papers. Studies on natural products represented by Chinese herbal medicine (TCM) or their extracts have also appeared in large numbers. The keywords related to the mechanism were immune inflammation, protein receptor, glucose metabolism, and oxidative stress, among others. In the recent 3 years, "aryl hydrocarbon receptor (AhR)," "farnesoid X receptor (FXR)," "AMP-activated protein kinase (AMPK)," "gluconeogenesis," and "Peroxisome proliferator-activated receptor-gamma (PPAR-γ)" were getting more attention.

Reference analysis

When two or more publications were cited by one article simultaneously, they constituted a co-citation relationship. References with high co-citation are considered an important knowledge base in this field. We used VOSviewer software to screen out the ten most cited references and found they were published in *Nature* (four articles), *Diabetes* (two articles), *PANS* (three articles) and *PLOS One* (one article). Four articles focused on the relationship between obesity and gut microbiota. All these achievements were made under the guidance of *Gordon JI* of Washington University in the USA. Three articles (Larsen et al., 2010; Qin et al., 2012; Karlsson et al., 2013) reported the characteristics of intestinal flora in diabetic patients. Two articles (Cani et al., 2007a,b, 2008) discussed diabetes, intestinal flora and metabolic inflammation. One article explored the relationship between *A. muciniphila*, intestinal epithelium and diet-induced obesity. **Table 6** provides further details.

In addition, all co-cited references were divided into four clusters. The red cluster mainly involved studies on the gut microbiota characteristics in the diabetes population and its influencing factors (such as metformin and flora transplantation). The green cluster was the knowledge base on the relationship between the gut microbiota and the host metabolism. Most of the studies in the blue cluster are about T1D and autoimmunity. Finally, the yellow cluster mainly included research papers on the application of intestinal microecological agents (**Figure 6**).

Key articles in this field

We ranked the articles according to the number of citations (as of April 28, 2022). We listed the basic information (author, title, article type, year, country, and times cited) of the top 15 highly cited papers in **Supplementary Table 7**. These articles were published between 2007 and 2013 and consisted of nine animal experiments, four clinical studies, and two reviews. The articles were cited more than 1,000 times, and the highest number of citations was 3,474. In addition, according to the corresponding author's country, 11 studies were conducted by research teams from European countries, the US research team mainly completed two studies, and the remaining two were from China and Canada.

Article citations are greatly affected by publication time. The citation times of articles published in recent 5 years are relatively low, even in important research articles. Therefore we used the "burst terms" function of CiteSpace to analyze the references with a sudden increase in citations in nearly 5 years. We have listed the basic information of the top 15 papers with the highest burst strength in **Supplementary Table 8**.

According to the above 30 landmark studies in this field, we sorted out the main research topics and development trends in the past 21 years. One of the hot research points is

TABLE 4 Top 10 co-cited journals in the "gut microbiota and diabetes" area.

Rank	Journal	Country	Count (n)	IF (2020)	5-year IF	H-index	Quartile in category
1	Nature	UK	49.962	69.504	63.58	1,276	Q1
2	Diabetes	USA	3.24	9.337	10.509	345	Q1
3	PLOS One	USA	9.461	3.752	4.069	367	Q2
4	P. Natl. Acad. Sci. USA	USA	11.205	12.779	13.45	805	Q1
5	Diabetologia	Germany	47.728	10.46	10.617	241	Q1
6	Diabetes Care	USA	23.059	17.152	17.242	380	Q1
7	Gut	UK	19.112	31.793	27.827	311	Q1
8	Science	USA	7.045	63.714	59.924	1,229	Q1
9	British Journal of Nutrition	UK	10.122	4.125	4.862	198	Q3
10	Cell Metabolism	USA	22.682	31.373	35.104	292	Q1

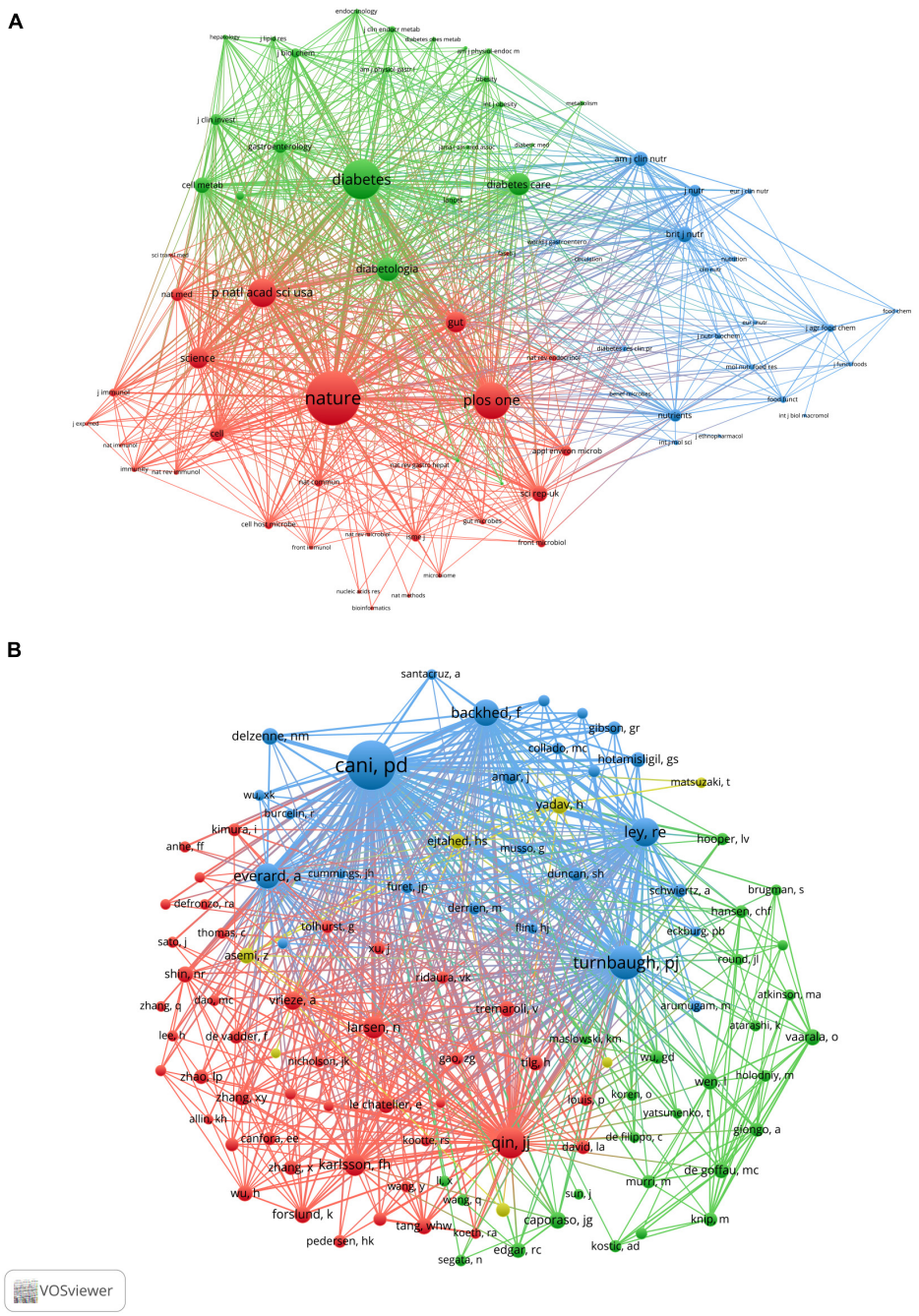

FIGURE 4
VOSviewer network visualization map of co-cited journals and authors of the publications related to the "gut microbiota and diabetes" area. **(A)** Visualization map of co-cited journals. **(B)** Visualization map of co-cited authors.

the gut microbiota characteristics of diabetic patients. Larsen et al. (2010) confirmed differences in the gut microbiota between healthy people and patients with T2D. Subsequently, researchers from China and Europe further identified the gut microbiota characteristics in individuals with T2D using metagenomic sequencing (Qin et al., 2012; Karlsson et al., 2013). A study published in 2015 pointed out that the use of metformin was a confounding factor that could not be ignored when analyzing the specificity of the gut microbiota in diabetic patients (Forslund et al., 2015). Nevertheless, metformin-induced gut microbiota modulation could benefit host metabolism, which has been confirmed in sterile mice (Wu et al., 2017). A study on community-dwelling Colombian adults found that diabetic patients taking metformin had a

TABLE 5 Top 10 productive authors and co-cited authors in the "gut microbiota and diabetes" area.

Rank	Author	Count	Country	Rank	Author	Citations weight	Country
1	Cani PD	44	Belgium	1	Cani PD	2,070	Belgium
2	Nieuwdorp M	34	Netherlands	2	Turnbaugh PJ	1,035	USA
3	Delzenne NM	29	Belgium	3	Qin J	844	China
4	Bäckhed F	29	Sweden	4	Ley RE	734	USA
5	Burcelin R	26	France	5	Bäckhed F	630	Sweden
6	Asemi Z	24	Iran	6	Everard A	580	Belgium
7	Pedersen O	18	Denmark	7	Larsen N	447	Denmark
8	Wong FS	18	UK	8	Karlsson FH	446	Sweden
9	Hansen AK	16	Denmark	9	Vrieze A	307	Netherlands
10	Everard A	16	Belgium	10	Yadav H	274	USA

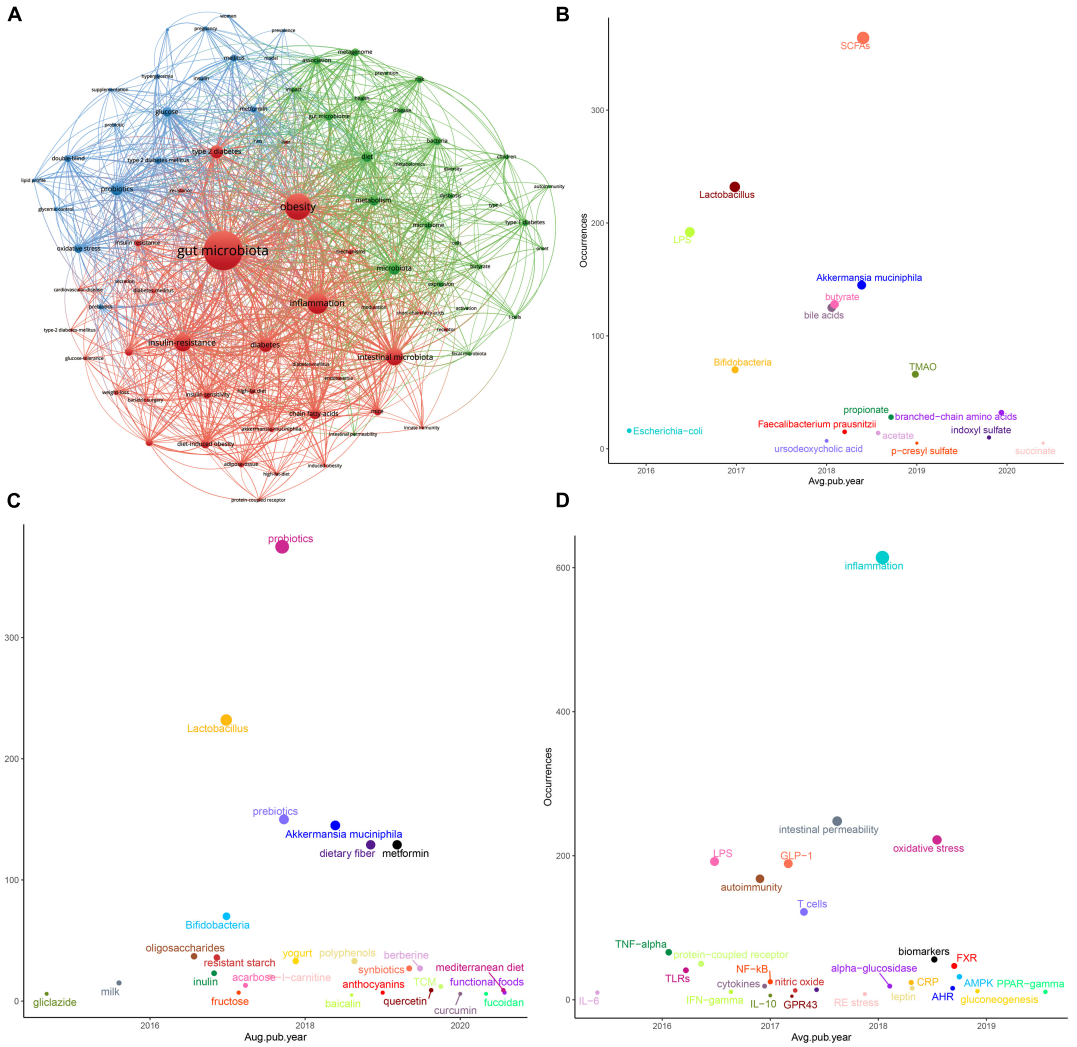

FIGURE 5

Keywords analysis in the "gut microbiota and diabetes" area. **(A)** Clustering analysis of high frequency keywords. **(B)** Coordinate diagram of frequency occurrence of the intestinal bacteria (and their metabolites). **(C)** Coordinate diagram of frequency occurrence of interventions. **(D)** Coordinate diagram of frequency occurrence of molecular mechanisms.

higher abundance of *A. muciniphila* and several gut microbiota known for producing SCFAs (de la Cuesta-Zuluaga et al., 2017). In recent years, research on gut microbiota characteristics has also been extended to people with pre-diabetes and T1D. The understanding of the relationship between the pathogenesis of diabetes and the gut microbiota is deepening (Kostic et al., 2015; Allin et al., 2018; Vatanen et al., 2018).

Functional interactions between the gut microbiota and host metabolism have been the primary research in this field in the past decade. The intestinal mucosal barrier is a research focus area (Tremaroli and Bäckhed, 2012). Cani et al. (2007a, 2008) confirmed that intestinal barrier damage and excessive LPS entering the blood circulation were key links in the onset of diabetes and obesity, and regulating the gut microbiota could reverse the pathological process to a certain extent. Subsequently, the research team of Professor *Cani PD* further confirmed that the mechanism of improving the intestinal barrier by regulating the gut microbiota was related to increasing endogenous GLP-2 production (Cani et al., 2009). In 2018, a research team pointed out hyperglycemia could drive intestinal barrier permeability through GLUT2-dependent transcriptional reprogramming of intestinal epithelial cells and alteration of tight and adherence junction integrity (Thaiss et al., 2018).

Among the gut microbiota metabolites, SCFAs and BAs have attracted more attention. SCFAs are the products of indigestible carbohydrates fermented in the intestine, including acetic acid, propionic acid and butyric acid (Morrison and Preston, 2016). In 2012, *Tolhurst* and his colleagues confirmed that SCFA could trigger the secretion of GLP-1 in intestinal L cells through a G-protein coupled receptor-dependent pathway (Tolhurst et al., 2012). In 2018, a clinical study showed that fiber-rich diets could promote the production of intestinal SCFAs, increase GLP-1 secretion and improve hemoglobin A1c (HbA1c) levels in patients with T2D (Zhao et al., 2018). Another study found acetic acid and butyric acid could also prevent the occurrence of T1D by limiting the frequency of autoimmune T cells (Mariño et al., 2017). BAs are endogenous molecules synthesized by cholesterol in the liver and metabolized by the gut microbiota (Wahlström et al., 2016). Sun et al. confirmed metformin acted in part through a *Bacteroides fragilis* – the bile acid glycoursodeoxycholic acid (GUDCA) – intestinal farnesoid X receptor (FXR) axis to improve metabolic dysfunction, including hyperglycemia (Mariño et al., 2017). In addition, two review articles on intestinal flora metabolites also had a high number of citations, reaching 961 (Morrison and Preston, 2016) and 853 times (Sun et al., 2018), respectively.

The interaction between gut microbiota and the immune system has also aroused the interest of a large number of scholars. Wen et al. (2008) found germ-free MyD88-negative NOD mice would develop robust diabetes, but not the specific pathogen-free mice, which indicated the interaction of the gut microbiota with the innate immune system was a critical epigenetic factor modifying T1D predisposition. Besides, Markle et al. (2013) found that intestinal microflora could also prevent the occurrence of T1D by increasing serum testosterone levels (Vijay-Kumar et al., 2010). Intestinal immunity was also important for the occurrence of T2D. Vijay-Kumar et al. (2010) found that mice genetically deficient in Toll-like receptor 5 (TLR 5) tended to show the characteristics of multiple metabolic disorders, and these metabolic changes were correlated with changes in the composition of the gut microbiota (Markle et al., 2013).

Regulating the gut microbiota to achieve the goal of treating diabetes is also a hot topic in this field. Cani et al. (2007b) and Everard et al. (2013) found that *A. muciniphila* and selective increases of bifidobacteria could repair the damaged intestinal mucosal barrier, inhibiting systemic metabolic inflammation and improving insulin resistance. Subsequently, Plovier et al. (2017) confirmed that *Amuc_1100*, a specific protein isolated from the outer membrane of *A. muciniphila*, could play a therapeutic role in improving the intestinal mucosal barrier by interacting with toll-like receptor 2(TLR 2). Besides, various prebiotics and functional foods were also considered potential intestinal flora regulators (Roberfroid et al., 2010; Anhê et al., 2015), and research has shown blowout growth in the past decade. Fecal microbiota transplantation (FMT) is another feasible strategy. A clinical study published in 2012 found 6 weeks after infusion of microbiota from lean donors, insulin sensitivity of recipients increased along with levels of butyrate-producing intestinal microbiota (Vrieze et al., 2012). After that, another clinical study confirmed the metabolic improvement effects of FMT were closely related to the baseline fecal microbiota composition (Kootte et al., 2017).

Discussion

Research overview

In this study, we conducted a bibliometric analysis of publications with the theme of "gut microbiota and diabetes" between 2001 and 2021. We comprehensively described the distribution of countries/journals/authors/subjects in this field. We summarized the current research hotspots and developing trends by analyzing keywords and highly cited articles. Several published bibliometric articles have recently discussed the relationship between gut microbiota and other diseases such as obesity, irritable bowel syndrome, and brain diseases. This indicates that research progress in the field of gut microbiota is affecting the development of many disciplines (Ejtahed et al., 2019; Zyoud et al., 2019, 2021). In 2017, our team analyzed 100 highly cited articles on "the gut microbiota and diabetes" published between 2007 and 2015 and analyzed the distribution of article types, journals, countries, institutions and authors (Tian et al., 2017). In contrast, the current study included

TABLE 6 Top 10 co-cited references in the "gut microbiota and gut microbiota and diabetes" area.

Authors	Title	Article type	Year of publication	Source	Country of corresponding author
Qin et al	A metagenome-wide association study of gut microbiota in type 2 diabetes	Clinical research	2012	Nature	China
Turnbaugh	An obesity-associated gut microbiome with increased capacity for energy harvest	Animal experiment	2006	Nature	USA
Larsen et al	Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults	Clinical research	2010	PLOS One	Denmark
Cani et al	Metabolic endotoxemia initiates obesity and insulin resistance	Animal experiment	2007	Diabetes	France
Karlsson et al	Gut metagenome in European women with normal, impaired and diabetic glucose control	Clinical research	2013	Nature	Sweden
Cani et al	Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice	Animal experiment	2008	Diabetes	France
Ley et al	Microbial ecology: human gut microbes associated with obesity	Clinical research	2006	Nature	USA
Bäckhed et al	The gut microbiota as an environmental factor that regulates fat storage	Animal experiment	2004	PANS	USA
Ley et al	Obesity alters gut microbial ecology	Animal experiment	2005	PANS	USA
Everard et al	Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity	animal experiment	2013	PANS	Belgium

more important publications, particularly those published in the recent 5 years. Except for some basic bibliometric statistics, this study focused more on the distribution and changing trends in research topics over recent years so that readers can obtain a more comprehensive understanding of this research field.

The following reasons may explain why the gut microbiota has gradually become a hotspot in diabetes research. Firstly, the rise of systemic biology has changed the traditional research model. Compared with studying the function of a single molecule, scientists focus more on the interaction between different elements that affect life activities (Gu et al., 2020). With further research, the gut microbiota, previously regarded as a "forgotten organ," has received renewed attention and is considered a key link in the system network (O'Hara and Shanahan, 2006). The strong demand for medical development has boosted the progress of related technologies. For example, the application of germ-free animals, microbial culture technology, high-throughput sequencing and multi-omics research methods not only enabled researchers to better analyze the composition and structure of the gut microbiota but also promoted the serial research on the functional interaction between the gut microbiota and the host (Allen-Vercoe, 2013; Grover and Kashyap, 2014; Whon et al., 2021). In addition, strong support from the government constitutes a solid foundation for the continuous promotion of the research. For example, the US National Institutes of Health (NIH) invested more than US $1 billion in human microbiome research between 2007 and 2016, including the Human Microbiome Project (HMP) program, with a total investment of US $215 million (NIH Human Microbiome Portfolio Analysis Team, 2019; Proctor, 2019). Similarly, some other countries have also set up research projects in microbiology, such as the Canadian Microbiome Initiative, the Japanese Human Metagenome consortium and the China Microbiome Project. These have extensively promoted the rapid development of microbiology and the cross-integration with other disciplines (NIH Human Microbiome Portfolio Analysis Team, 2019).

Characteristics of publications

Regarding the national distribution of published publications over the past 21 years, the number of corresponding authors from China was the largest, showing a sharp upward trend after 2015. However, at the same time, it should be noted that the citation rate of Chinese papers was relatively low. On the other hand, France had the highest citation frequency among the top ten high-productive countries, which is directly related to the two highly cited papers written by *Burcelin R*. If these two articles are removed, the number of citations per French article decreases to 106.9. From the perspective of the research institutions involved in the article, the University of Copenhagen, the University of Helsinki in

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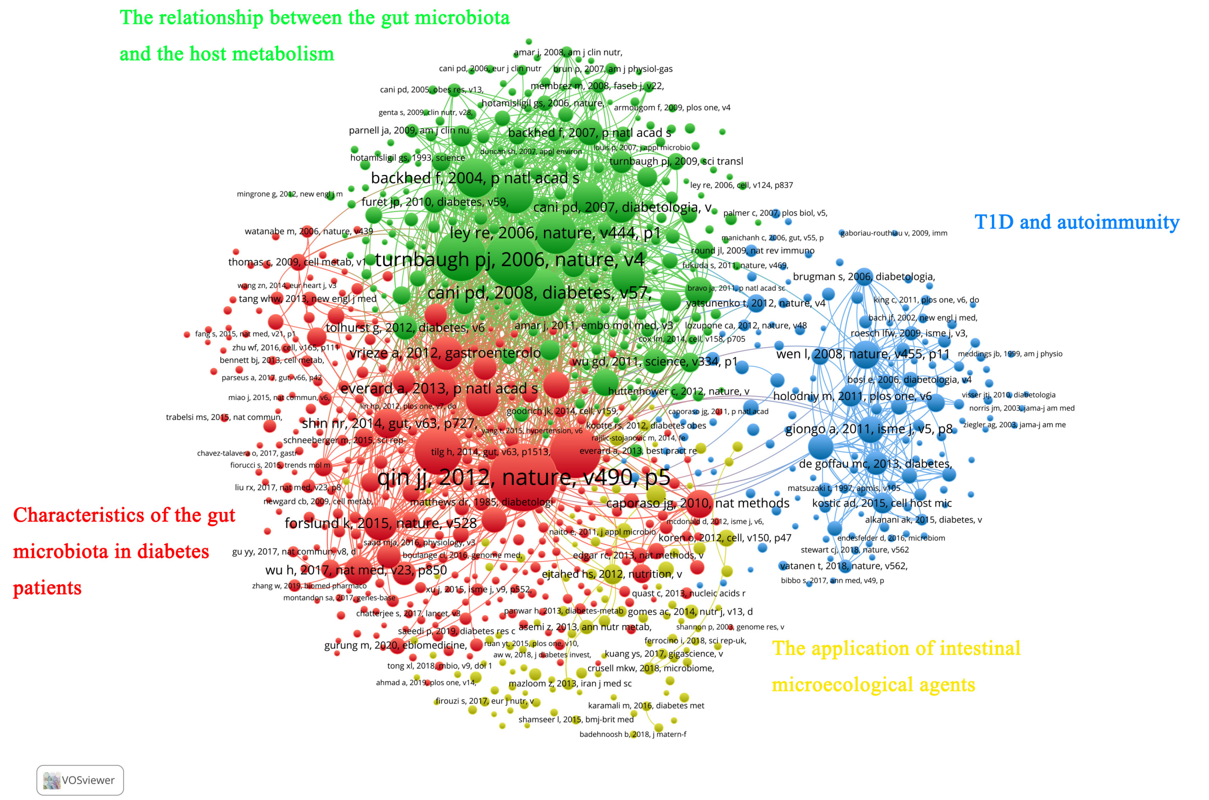

FIGURE 6
VOSviewer network visualization map of co-cited references.

Finland, and the University of Gothenburg in Sweden were among the top five, and have established extensive contact with many international academic institutions, which reflected the fact that Nordic universities had a strong research tradition and global influence in this field. In addition, cluster analysis showed that European countries and European academic institutions have a close cooperation network, which may be related to the promotion of a series of research projects under the EU framework, such as the EU MetaHIT Project (the EU Project on metagenomics of the human intestinal tract) (Proctor, 2019).

Development trend and research hotspots

Probiotics represented by *Lactobacillus*, *Bifidobacteria*, *A. muciniphila* and a variety of prebiotics were the most concerned intestinal flora regulators with the therapeutic effect on diabetes. In addition, the regulative action of some commonly used hypoglycemic drugs, such as metformin and acarbose, has also attracted more attention. But in recent years, more studies have focused on synbiotics, TCM and their extracts. As the new intestinal microecological agent, synbiotics are a mixture of probiotics and prebiotics. With the help of prebiotics, the possibility of probiotics settling in the intestine can be greatly increased, and their survival time can also be significantly prolonged (Markowiak and Śliżewska, 2017). TCM has been used in China for thousands of years. With further research, some extracts from the TCM, such as baicalin, berberine, quercetin, and curcumin, have proved to exert hypoglycemic effects by regulating the gut microbiota.

The results of co-cited references showed that the relationship between obesity and gut microbiota had laid the foundation for diabetes research. Professor *Gordon JI* from the Washington University School of Medicine made outstanding contributions to obesity research. Professor *Gordon JI*'s research team not only found that there excited a close relationship between host metabolic abnormalities and the gut microbiota but also applied some innovative experimental technologies such as germ-free mice to explore the causal link, which had a profound impact on the development of this field in the next decade (Bäckhed et al., 2004; Ley et al., 2005). With the deepening of research, more intestinal bacteria and metabolites closely related to diabetes have been identified. *A. muciniphila* colonization in the intestinal mucosa was a potential probiotic with metabolic regulating effects (Zhang et al., 2019). Its importance to the host included

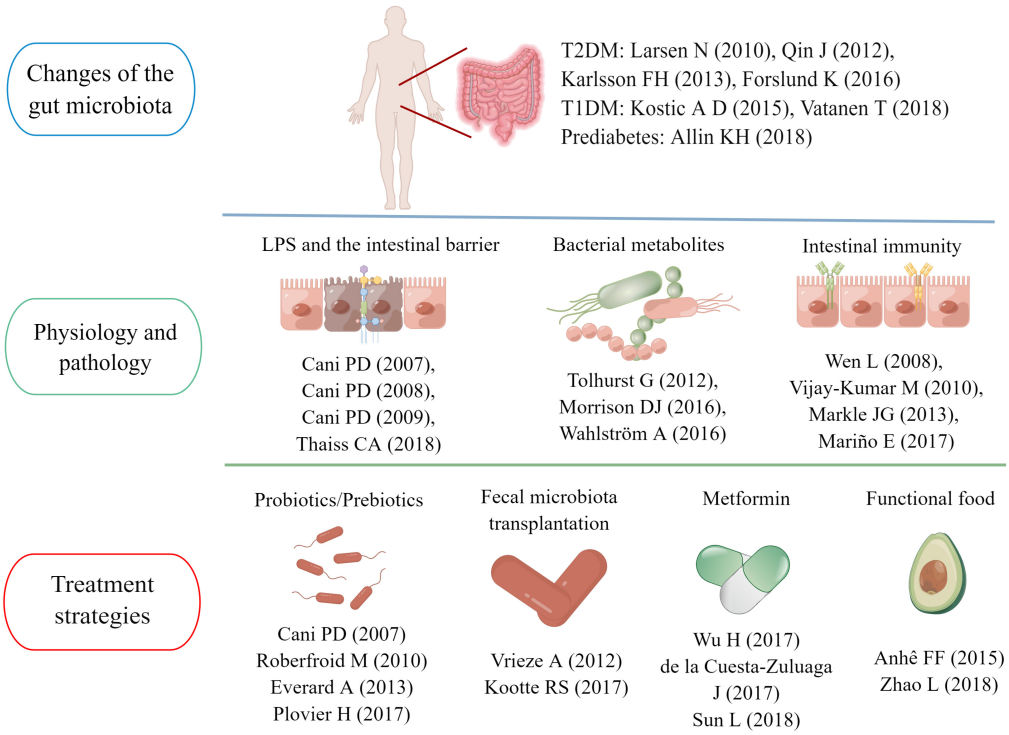

FIGURE 7
Main research topics of representative articles in the "gut microbiota and diabetes" area.

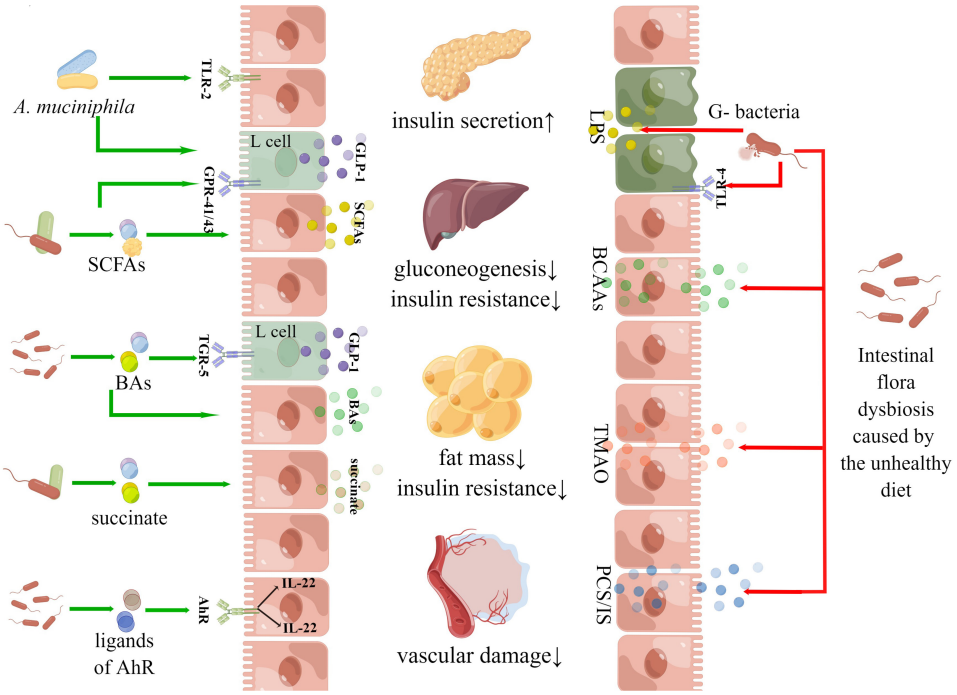

FIGURE 8
Diagram of hotspot mechanisms in the "gut microbiota and diabetes" area.

the uptake and utilization of nutrients, the protection of the intestinal barrier, and the maintenance of intestinal mucosal immune homeostasis (Macchione et al., 2019). Several studies have reported that the decrease in *A. muciniphila* was closely associated with the development of diabetes and obesity (Depommier et al., 2019). Oral administration of *A. muciniphila* could effectively improve glucose and lipid metabolism disorders in patients with metabolic syndrome (Depommier et al., 2019). *F. prausnitzii*, an important butyrate-producing bacteria, could play an anti-inflammatory and intestinal mucosal protection role by regulating intestinal immunity, which was essential to inhibiting the development of chronic systemic inflammation (Xu et al., 2020). TMAO was a metabolite of the gut microbiota closely linked to diabetes and cardiovascular diseases. It could inhibit insulin-related signal pathways, activate inflammatory reactions, promote hyperglycemia and decrease glucose tolerance (Li et al., 2022). Tang et al. (2017) found that the increase of plasma TMAO level in T2DM patients has a good predictive value for adverse cardiovascular and cerebrovascular events and death. BCAAs are a class of essential amino acids which mainly depend on food intake and are also regulated by the gut microbiota. Multiple clinical studies found the level of BCAAs in plasma of patients with diabetes increased significantly (Izundegui and Nayor, 2022), and the accumulation of BCAAs could inhibit the transport and utilization of pyruvic acid and fatty acid, promote glycogen synthesis and eventually lead to hyperglycemia (Cuomo et al., 2022). Succinate is a multifunctional metabolite produced by the interaction between the host and the gut microbiota, which could play a role similar to hormones and signal molecules by binding with the succinate receptor-1 (SUCNR1) (Canfora et al., 2019). De Vadder et al. (2016) found that succinate could activate intestinal gluconeogenesis, reduce the expression of hepatic glucose-6-phosphatase, and maintain blood glucose homeostasis by mediating "gut-liver" crosstalk. PCS and IS were two of the most well-studied uremic retention solutes and were formed from dietary amino acids by colonic bacteria that possess p-cresol- and indole-forming enzymes, respectively (Snelson et al., 2019). A meta-analysis confirmed that elevated levels of PCS and IS were associated with increased mortality in patients with chronic kidney disease. PCS was also related to an increased risk of cardiovascular events (Lin et al., 2015). With the in-depth study of diabetes kidney disease and diabetes-related cardiovascular risk, the attention on these two metabolites has gradually increased.

There were multiple pathways linking the gut microbiota and the host metabolism. In addition to the "intestinal barrier – metabolic inflammation," "SCFA-GPR," "BAs-FXR," and autoimmunity have attracted much attention, AhR, AMPK and PPAR-γwere becoming research hotspots in recent years. The activation of aromatic hydrocarbon receptors could promote the secretion of IL-22 with intestinal mucosal protection and enhance the integrity of the intestinal mucosal barrier (Ning et al., 2019). A study found that mice with intestinal-specific AhR deficiency were more sensitive to DSS-induced intestinal inflammation and epithelial cell apoptosis than the control group, indicating that AhR plays an important role in maintaining intestinal barrier homeostasis (Krishnan et al., 2018). AMPK and PPAR-γboth were key molecular targets of host metabolism. AMPK was called an "energy receptor," and once the ratio of AMP/ATP in cytoplasm increased or other factors activated AMPK, glucose utilization and fatty acid oxidation would be enhanced. Gluconeogenesis and lipid synthesis pathways will be inhibited to maintain the balance of cell energy metabolism (Foretz et al., 2019). PPAR-γplayed a significant role in regulating various biological processes such as lipid metabolism, lipogenesis, cell division and apoptosis. PPAR-γagonist (such as thiazolidinedione) could exert plenty of pharmacological effects contributing to metabolic regulation (Dali-Youcef et al., 2013). **Figure 8** gives these details.

Limitations and future research directions

In this study, we only searched the Web of Science database and only included the English literature, which inevitably caused the omission of the original literature.

In future studies, research on the following issues may become directions for further exploration. First, many studies still need to demonstrate the causal relationship between the intestinal flora and the host phenotype, which also relates to whether the therapeutic methods targeted to the intestinal flora can play ideal therapeutic effects. Second, more attention may shift from intestinal to extraintestinal and bacteria to other microorganisms. A complete set of mature technical methods has been formed in the field of intestinal bacteria, which to a certain extent, has stimulated the curiosity of researchers about the microbiome of other parts of the human body, such as tongue coating, urine, and skin. Besides bacteria, the relationship between viruses, fungi and glucose metabolism is also gradually being explored. Thirdly, disease prediction and individualized precision treatment based on the gut microbiota are the trends for future clinical application. However, there is still a lack of high-quality, evidence-based medical evidence, which needs to be repeatedly verified through large-scale clinical studies. Fourth, the standardized preparation process of intestinal microecological preparations and more convenient and efficient microbiota detection methods are the basis for further development of relevant industries. For clinicians, a standardized guideline on gut microbiota therapies may be the most urgent need.

Conclusion

Research on the association between gut microbiota and diabetes has recently become a hot topic, and the number of articles is increasing yearly. Through bibliometric analysis, we concluded that the countries and institutions with the highest number of publications were China and the University of Copenhagen, respectively. The journal with the most publications is *Nutrients*; Professor *Cani PD* is the most productive scholar and an important contributor to highly cited papers, and *Endocrinology & Metabolism* is the most common subject category. The following keywords represented research frontiers: *A. muciniphila*, *F. prausnitzii* and metabolites of the intestinal flora TMAO and BACCs; natural products represented by the TCM; some metabolite receptors such as AhR, FXR, and signal pathways represented by AMPK and PPAR-γ. In future research, the clinical transformation of theoretical results and interdisciplinary innovation research may receive more attention.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Author contributions

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

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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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Supplementary material

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EDITED BY
Karolina Skonieczna-Żydecka,
Pomeranian Medical University,
Poland

REVIEWED BY
Tomasz M. Karpiński,
Poznan University of Medical Sciences,
Poland
Anastasios Koulaouzidis,
University of Southern Denmark, Denmark

*CORRESPONDENCE
Li Yuan
yuanli2768@zjcc.org.cn
Xiangdong Cheng
chengxd@zjcc.org.cn

†These authors share first authorship

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A literature review on the potential clinical implications of streptococci in gastric cancer

Mengli Zi[1†], Yanqiang Zhang[1,2,3†], Can Hu[1,2,3], Shengjie Zhang[1,2,3], Jinxia Chen[1,2,3], Li Yuan[1,2,3*] and Xiangdong Cheng[1,2,3*]

[1]Department of Gastric surgery, The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Institutes of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, China, [2]Zhejiang Provincial Research Center for Upper Gastrointestinal Tract Cancer, Zhejiang Cancer Hospital, Hangzhou, China, [3]Zhejiang Key Lab of Prevention, Diagnosis and Therapy of Upper Gastrointestinal Cancer, Zhejiang Cancer Hospital, Hangzhou, China

Streptococcus is widely found in nature and the human body, and most species are not pathogenic. In recent years, studies have found that Streptococcus is associated with gastric cancer. Streptococcus was found to be enriched in the oral cavity, stomach and intestine of gastric cancer patients and found to be increased in gastric cancer tissues, suggesting that Streptococcus may be the pathogenic bacteria underlying gastric cancer. This review discusses the discovery of Streptococcus, the relationship between Streptococcus and gastric cancer, and the possible carcinogenic mechanism of Streptococcus and summarizes the progress of the research on the role of Streptococcus in gastric cancer to provide new ideas for the early detection, diagnosis and treatment of gastric cancer.

KEYWORDS

gastric cancer, Streptococcus, Helicobacter pylori, digestive tract, oral

Introduction

Gastric cancer is the fifth most common type of malignancy in the world and the fourth leading cause of death from cancer (Sung et al., 2021). Asia (and mainly China) exhibits the highest number of gastric cancer cases (Torre et al., 2016; Zhao et al., 2017), and the 5-year survival rate of gastric cancer patients is 27.4% in China (Huang et al., 2021), making it one of the major cancers threatening human health. Gastric cancer is a multifactorial and multistep inflammatory disease. It is believed that the development process of gastric cancer is as follows: chronic superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, atypical hyperplasia, gastric adenocarcinoma (Correa, 1992). Studies have found that multiple factors, including host genetic factors (El-Omar et al., 2000; Allison and Ferrero, 2010; Castaño-Rodríguez et al., 2014; Mommersteeg et al., 2018), environmental factors (such as smoking, alcohol consumption, high salt and meat intake, low vegetable/fruit intake) and microbial factors (such as Helicobacter pylori infection and other gastric microorganisms), play an important role in gastric cancer (Correa, 1995; Correa and Houghton, 2007; Moss, 2017). Gastric cancer is mainly treated by surgery but also by chemotherapy, immunization

and targeted drug therapy (Ajani et al., 2022), while anti-Helicobacter pylori infection is currently the only microbial treatment for gastric cancer prevention (Wu et al., 2010).

Some epidemiological studies have shown that gastric microbes are involved in the occurrence of gastric cancer by inducing chronic inflammation or downregulating host immunity (Parsonnet, 1995). For example, Helicobacter pylori (Hp), classified as a class I carcinogen by the World Health Organization, destroys the structure and function of the gastric epithelium by enhancing the inflammatory response (Amieva and Peek, 2016), affects the richness and evenness of other bacterial communities (Bessède and Mégraud, 2022)and plays a key role in the initial steps of gastric cancer. However, studies have found that gastric cancer still occurs after the eradication of H. pylori (Fukase et al., 2008; Ma et al., 2012), less than 3% of people infected with H. pylori develop gastric cancer (Engstrand and Graham, 2020), and the level of H. pylori colonization decreases and its presence eventually disappears with the progression of gastric cancer (El-Omar et al., 1997). At the same time, studies using the INS-GAS mouse model showed that stomach and intestinal microbes could promote the formation of tumor lesions (Bik et al., 2006; Lofgren et al., 2011; Maldonado-Contreras et al., 2011; Lertpiriyapong et al., 2014). These results indicate that bacteria other than H. pylori also play an important role in the occurrence and development of gastric cancer. With the development of molecular biology and metagenomics, scientists have a more comprehensive understanding of gastrointestinal microbes, and it is believed that microbial dysbiosis can promote the occurrence of gastric cancer through different mechanisms, and Streptococcus bacteria are considered to affect the development of cancers in the oral cavity, lungs, colorectum and cervix (Kang et al., 2021; Stasiewicz and Karpiński, 2021; Goto, 2022; Karpiński et al., 2022).

Studies have found that the flora of patients with gastric cancer is in an imbalanced state, and Streptococcus is enriched in gastric cancer tissues (Liu et al., 2019; Shao et al., 2019; Dai et al., 2021), which is significantly different from the flora of healthy people or patients with chronic gastritis (Eun et al., 2014; Coker et al., 2018). Therefore, Streptococcus is considered a potential marker for predicting gastric cancer (Qi et al., 2019). Yu et al. used a random forest model (RF) to produce further evidence of the use of Streptococcus as a marker of gastric cancer (Yu et al., 2021). Both H. pylori and Streptococcus can produce urease, which is the main inducer of the innate immune response and is involved in the occurrence of gastric cancer (Mobley and Hausinger, 1989; MacMicking et al., 1997; Gobert et al., 2002; Suerbaum and Michetti, 2002; Brandi et al., 2006; Osaki et al., 2008); Streptococcus is also involved in the formation of nitroso compounds (NOCs) in the stomach (Ayanaba and Alexander, 1973; Jo et al., 2016; Sohn et al., 2017), and NOCs are associated with an increased risk of gastric cancer (Ayanaba and Alexander, 1973; Mowat et al., 2000; Dicksved et al., 2009; Jo et al., 2016). These results indicate that Streptococcus may affect the occurrence and development of gastric cancer. Moreover, studies on gastric cancer-related microorganisms are not limited to the stomach but have also been conducted on the oral cavity and intestine, and Streptococcus has been found in different studies of the three sites, suggesting the important role of Streptococcus in gastric cancer research.

Streptococcus in gastric microecology

Streptococcus is another common bacterial pyogenic coccus that widely exists in nature. The important Streptococcus encountered in medicine mainly include alpha-hemolytic streptococci, beta-hemolytic streptococci, and non-hemolytic streptococci. Streptococcus belongs to the bacterial domain, Firmicutes phylum, Bacillus class, Lactobacillus order, Streptococcus family, and Streptococcus genus and is further subdivided into different species of Streptococcus. Streptococcus is a microorganism that naturally exists in the human body, especially in the digestive tract. The Streptococcus genus and its different species, such as Streptococcus pneumoniae, Streptococcus pyogenes and Streptococcus agalactiae, have been found in healthy people and patients with gastric cancer (Li et al., 2009; Delgado et al., 2013; Sohn et al., 2017; Coker et al., 2018; Chen et al., 2019).

Due to its highly acidic environment, motility and mucosal mucus layer, the stomach was regarded as a sterile environment until the discovery of Helicobacter pylori (Hp) in 1982, after which Hp was considered the only bacterium that could colonize the stomach. However, in 1981, a few months before the discovery of H. pylori, the Lancet reported that a large number of bacteria, including Streptococcus, Neisseria and Lactobacillus, could be detected in the stomach, and multiple studies have found streptococci in gastric juice. In 1984, Sharma et al. performed bacterial culture using the gastric juice of healthy men and found 9 bacterial genera, including Streptococcus (hemolytic and nonhemolytic) (Sharma et al., 1984); this was the first time Streptococcus was cultured using gastric juice. Sjostedt et al. cultured Streptococcus using the gastric juice of gastric cancer patients in the following year (Sjöstedt et al., 1985). Later, Choi and Hu et al. performed metagenomic analysis of gastric juice and found the presence of Streptococcus, which was significantly increased in gastric cancer patients (Choi et al., 2017; Hu et al., 2018). Multiple studies have found streptococcal overgrowth in gastric juices during proton pump inhibitor (PPI) acid-suppressive therapy (Thorens et al., 1996; Sanduleanu et al., 2001; Rosen et al., 2014; Rosen et al., 2015; Tsuda et al., 2015).

To further confirm the relationship between Streptococcus and gastric cancer, bacterial detection and analysis of gastric mucosa tissues have also been carried out. Sasaki et al. performed Southern blot analysis on surgical specimens of gastric cancers in 1995 and detected DNA fragments of Streptococcus anginosus in 9 (20%) surgical specimens (Sasaki et al., 1995). Three years later, they conducted research in the same way and found the presence of Streptococcus anginosus in the cancerous gastric tissues but not in the adjacent normal tissues (Sasaki et al., 1998). The results of a study by Dicksved et al. showed that the flora observed in gastric cancer mainly comprised different species of Streptococcus,

Lactobacillus, Veillonella and Prevotella (Dicksved et al., 2009). Eun, Jo, and Coker et al. found that the abundance of Streptococcus was significantly increased in gastric cancer patients (Ayanaba and Alexander, 1973; Eun et al., 2014; Coker et al., 2018). The first study of gastric microbiota after subtotal gastrectomy in patients with gastric cancer by Tseng et al. found that Streptococcus remains one of the most abundant bacterial genera (Tseng et al., 2016). The important events in the discovery of Streptococcus in the gastric microecological environment are shown in Figure 1. Streptococcus was found in the gastric juice and gastric mucosa of healthy people and patients with gastric cancer and was enriched in gastric cancer patients, while Streptococcus may be present in the oropharynx and enter the stomach through food swallowing and was found to be a transit bacterium. To further investigate whether Streptococcus colonizes the stomach, in 2009, Li et al. obtained biopsy samples extracted from gastritis patients and healthy controls that were washed in phosphate buffered saline (PBS). After three consecutive washes, more than 90% of bacteria, including Streptococcus, were still attached to the specimens (Li et al., 2009). A high bacterial isolation rate (average 56.5%) observed in a 2014 study suggested that Streptococcus may colonize the stomach, not just pass through it (Khosravi et al., 2014). In 2020, Spiegelhauer et al. used 16S rRNA sequencing for the first time aiming to distinguish between transient and resident bacteria, and the results suggested that Streptococcus may be a resident bacteria (Spiegelhauer et al., 2020). The above studies show that Streptococcus exists in the gastric mucosa and is a persistent bacterium. It is enriched in gastric cancer and may be related to the occurrence and development of gastric cancer, which is worthy of further research.

Relationship between Streptococcus and Helicobacter pylori

The gastric microbiota of patients infected with Helicobacter pylori (Hp) is different from that of noninfected patients, and studies have shown that Hp is the main factor that influences the dysbiosis of the gastric microbiota (Wang L. et al., 2016; Liu et al., 2019; Guo et al., 2020). The relationship between Streptococcus, one of the dominant bacteria in the stomach, and Hp is unclear. Researchers have studied the relationship between intragastric Streptococcus and Hp in nongastric cancer patients in terms of the presence/absence of Hp and bactericidal or acid-suppressive therapy. First, bacteria other than Hp in the gastric mucosa of nongastric cancer patients were analyzed under the premise of the existence of Hp, and it was found that the dominant bacterial species were Neisseria flavescens (13.7%), Streptococcus salivarius (9.5%), Rothia mucilaginosa (8.9%) and Streptococcus pneumonia (6.6%) (Hu et al., 2012). Moreover, the abundances of Streptococcus, Prevotella and Haemophilus in the stomach increased significantly during Hp sterilization treatment or PPI alone, but the bacterial species that were present did not change significantly, and the relative proportion of existing bacteria changed and recovered to the pretreatment level for a period of

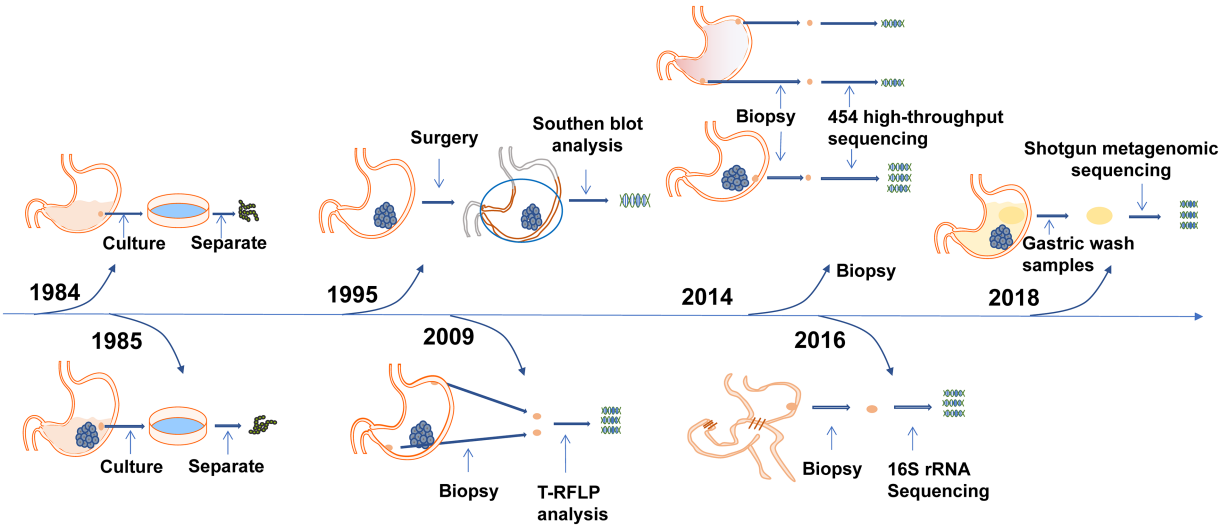

FIGURE 1
Time axis diagram: Events of great significance in the study of Streptococcus and gastric cancer. Pound's team was the first to grow the Streptococcus genus in the stomach juices of healthy men in 1984. Nord's team grew Streptococcus in gastric juice from patients with stomach cancer in 1985. Terraada's team found DNA fragments of Streptococcus anginosus in gastric cancer surgical specimens in 1995. Engstrand's team first used molecular biology techniques to analyze the microbiota of gastric cancer and found that Streptococcus was the dominant bacterium in 2009. Kim's team first found that gastric microbes were different from those of patients with chronic gastritis, and the Streptococcus family was increased in gastric cancer in 2014. Wu's team first studied the gastric microbiota after gastric cancer surgery and found that Streptococcus was still one of the most abundant bacterial genera in 2016. Metagenomic analysis of gastric fluid by Zhang's team found that Streptococcus was the dominant genus and that its abundance was significantly increased in patients with gastric cancer in 2018.

time after treatment (Stark et al., 1996; Thorens et al., 1996; Adamsson et al., 1999; Rosen et al., 2014; Rosen et al., 2015). A study in nongastric cancer patients without Hp infection found that Streptococcus and Prevotella were relatively abundant (Li et al., 2009). Analysis of the cooccurrence network of gastric microorganisms in chronic gastritis patients showed that there was a significant negative correlation between the abundances of H. pylori and Streptococcus (Parsons et al., 2017). The above studies showed that Streptococcus did not overgrow in the presence of Hp, while gastric acid secretion was inhibited or Streptococcus abundance increased during bactericidal treatment; these findings indicate that Streptococcus was affected by Hp and gastric acid secretion. These studies showed that Streptococcus did not grow in the presence of Hp, while the increase in Streptococcus abundance during the inhibition of gastric acid secretion or bactericidal treatment indicated that Streptococcus abundance was affected by Hp and gastric acid secretion.

The above studies have shown that Streptococcus and Hp are closely correlated in nongastric cancer patients, and some studies have also shown that Streptococcus and Hp are closely correlated in gastric cancer patients. A study in 2016 found that Hp was the most dominant bacterium and that Streptococcus was the second most dominant bacterium in Hp-positive gastric cancer patients (Jo et al., 2016). In the following year, Sohn et al. conducted a study on Hp-negative gastric cancer. According to the overlap analysis of non-Hp urease-producing bacteria and non-Hp nitrate-reducing bacteria, Streptococcus accounted for the largest proportion in Hp-negative gastric cancer at the family level, while Streptococcus pseudopneumoniae, Streptococcus parasanguinis, and Streptococcus oralis accounted for a larger proportion at the species level (Sohn et al., 2017). In the absence of Hp infection, Streptococcus is prominent in gastric cancer and can be considered the pathogenic bacteria underlying gastric cancer. Another study also suggested that Streptococcus and Neisseria may play a role in the development of gastric cancer (Gantuya et al., 2019). Most gastric cancers are Hp-positive gastric cancers, so studies of Streptococcus are affected by Hp. Although Hp was excluded from the analysis of the data, the authenticity and validity of the data were also affected. Although the number of Hp-negative gastric cancer samples was small and few studies were conducted, the influence of Hp could be excluded, which is of great significance for Streptococcus research. Since Hp is a recognized pathogen underlying gastric cancer, it is further speculated that Streptococcus may work together with Hp or play a role in different stages of gastric cancer.

Changes in Streptococcus in the digestive tract during the occurrence and development of gastric cancer

The digestive tract consists of the mouth, pharynx, esophagus, stomach and intestines, and streptococci exist in various parts of the digestive tract. Streptococcus in different parts of the digestive tract has been studied in gastric cancer. Next, we discuss the changes in Streptococcus in the occurrence and development of gastric cancer from the perspective of the oral cavity, stomach and intestinal tract. Studies have shown that Streptococcus exists in the oral cavity of healthy people and is obviously enriched in gastric cancer, but different species of Streptococcus exhibit different changes during gastric cancer. Streptococcus in the stomach also accumulates gradually during the progression from chronic gastritis to atrophic gastritis and finally to gastric cancer and is expected to become a marker for the diagnosis of gastric cancer. The intestinal flora is complex and diverse, and Streptococcus abundance is significantly increased in the intestinal tract of patients with gastric cancer. The difference in Streptococcus in the feces of patients with chronic gastritis and gastric cancer can be used to distinguish them, providing a supplement for noninvasive examination methods for early diagnosis. The changes that occur in the main bacteria of the oral cavity, stomach and intestinal tract of patients with gastric cancer are shown in Figure 2. We will review the changes that occur in Streptococcus in the oral cavity, stomach and intestinal tract during the occurrence and development of gastric cancer.

Changes in oral Streptococcus during the occurrence and development of gastric cancer

The oral cavity is the starting point of the human digestive tract and is home to a variety of bacterial communities, including at least 11 phyla and 70 genera (Ahn et al., 2012). The oral microbiome may affect bacteria in the esophagus, stomach and gut; for example, some lactobacilli found in human feces are heterologous to the gut, originating from the oral cavity (Dal Bello and Hertel, 2006). A large number of bacteria in the stomach are also the dominant bacteria in the oral cavity, which may be microorganisms that are present during swallowing (Andersson et al., 2008). The oral microbiome is the second most complex microbial community in the human body and plays an important role in oral and systemic health. For example, Ndegwa et al. performed a prospective study and found that poor oral health was associated with an increased risk of gastric cancer (Ndegwa et al., 2018).

Studies in healthy volunteers show that streptococci are the dominant bacteria in the oral flora. Mowat and Zilberstein et al. conducted bacterial culture on oral specimens and found that the most common bacteria was α-hemolytic Streptococcus, which appeared most frequently in saliva (Mowat et al., 2000; Zilberstein et al., 2007). Andersson et al. performed 454 pyrosequencing on the highly variable region of 16S rRNA in throat specimens and found that Streptococcus was the dominant genus, followed by Prevotella (Andersson et al., 2008). Tsuda et al. analyzed the saliva of subjects taking PPIs using bacterial culture and high-throughput sequencing methods and found that Streptococcus was the most abundant and that PPI intake did not affect the

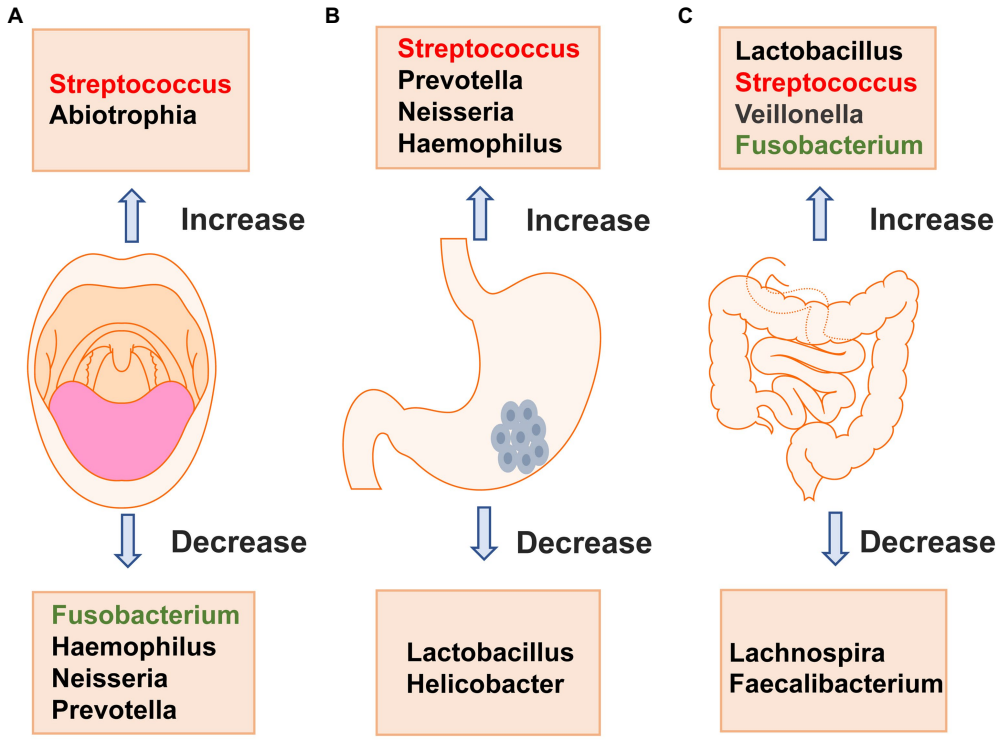

FIGURE 2
Changes in major bacteria in the oral cavity **(A)**, stomach **(B)** and intestinal tract **(C)** in patients with gastric cancer. **A** shows that the changes in oral microbiota of gastric cancer patients, Streptococcus and Abiotrophia are significantly increased compared with healthy people, while Fusobacterium, Haemophilus, Neisseria, and Prevotella are significantly decreased. **B** shows that the changes in major bacteria in stomach of patients with gastric cancer, Streptococcus,Prevotella, Neisseria,and Haemophilus are significantly increased compared with healthy people, while Lactobacillus and Helicobacter are significantly decreased. **C** shows that the changes in major bacteria in intestinal tract of patients with gastric cancer, Lactobacillus, Streptococcus, Veillonella and Fusobacterium are significantly increased compared with healthy people, while Lachnospira and Faecalibacterium are significantly decreased.

results (Tsuda et al., 2015). All the above findings suggest that Streptococcus is dominant in the oral flora and is not affected by PPIs. Since quantities of Streptococcus exist in the oral cavity, they may enter the stomach through the swallowing of food, affecting the flora in the stomach and leading to the occurrence of gastric cancer.

Streptococcus is present in the oral cavity of healthy people, so we wondered whether the Streptococcus in the oral cavity of patients with precancerous lesions and gastric cancer were different. The researchers studied the oral flora of patients with precancerous gastric cancer lesions and detected DNA fragments of Streptococcus mutans in saliva (Salazar et al., 2013). Patients with gastric intestinal metaplasia exhibited an enrichment in the oral species Peptostreptococcus stomatis, whereas Streptococcus mutans, S parasanguinis and S sanguinis abundances were reduced (Wu et al., 2021). These findings suggest that a certain Streptococcus species may play a role in the development of gastric precancerous lesions. At the species level, Streptococcus shows different changes in precancerous lesions. Whether Streptococcus changes again when it develops to gastric cancer is unknown. Wu et al. conducted 16S rRNA sequencing on tongue coating samples from 57 gastric cancer patients in 2018, and the results showed that the relative abundance of Streptococcus was relatively high in gastric cancer patients, indicating that Streptococcus is a common risk factor and has a potential carcinogenic effect (Wu et al., 2018), but the causal relationship between Streptococcus and the occurrence of gastric cancer could not be verified. The following year, Japanese scholars performed 16S rRNA sequencing on saliva samples obtained from 59 patients with digestive tract cancer and 118 controls and found that the relative abundances of Streptococcus sanguinis and Streptococcus parasanguinis in gastric cancer patients were relatively low (Kageyama et al., 2019). Comparing Streptococcus changes during gastric carcinogenesis, Huang et al. used 16S rRNA sequencing to obtain a salivary microbiome map and established a random forest model to classify gastric tissue types in 2021. They found that the abundance of Peptostreptococcus in saliva gradually decreased during the progression from superficial gastritis to atrophic gastritis to gastric cancer, but the abundance of the Streptococcus genus was significantly increased in gastric cancer and was the most representative bacterial genus (Huang et al., 2021). It was proposed for the first time that Streptococcus may be an indicator for the diagnosis of gastric cancer. Studies of different species of Streptococcus have shown that intraoral Streptococcus changes at different stages of gastric disease and may be a predictor of gastric cancer (Table 1). However, whether the changes in Streptococcus

TABLE 1 Changes in Streptococcus in the oral cavity.

Author	Year	Subjects	Region/country	Method	Samples	Main findings	Reference
Wu	2018	57 patients with gastric adenocarcinoma and 80 healthy controls	Jiangsu Province, China	16S rRNA gene sequencing	The tongue coating samples	The relative abundance of streptococcus was higher in gastric cancer patients. The relative abundance of streptococcus was also higher in noncardiac cancer patients than in the controls, however, the effect of streptococcus risk was not significant in cardiac cancer patients.	Wu et al., 2018
Kageyama	2019	59 patients with cancer in any part of the digestive tract (tongue/pharynx, esophagus, stomach, and large intestine), and 118 matched controls	Japan	16S rRNA gene sequencing	Saliva	The relative abundances of Streptococcus sanguinis and Streptococcus parasanguinis in gastric cancer patients were low.	Kageyama et al., 2019
Huang	2021	superficial gastritis (SG), atrophic gastritis (AG), gastric cancer (GC)	Beijing, China	16S rRNA gene sequencing	Saliva	At the genus level, Prevotella, Haemophilus and Streptococcus constitute more than 70% of the salivary flora at each histological stage of GC; Peptostreptococcus abundance gradually decreased from SG→ AG → GC; The abundance of the Streptococcus genus was significantly increased in GC while Peptostreptococcus was enriched in SG.	Huang et al., 2021
Wu	2021	89 patients with intestinal metaplasia and 89 healthy controls	New York	Metagenomics Sequencing	Oral wash samples	The abundance of oral species Peptostreptococcus stomatis was higher in the case group than in the control group, while Streptococcus mutans, S. parasanguinis and S.sanguinis abundances were lower.	Wu et al., 2021

are the cause or the result of gastric cancer is still inconclusive, and the pathogenic mechanism is not clear.

Changes in endogastric Streptococcus during the occurrence and development of gastric cancer

Due to gastric motility, the presence of the mucus layer, low pH, and acid secretion, it was initially thought that no bacteria could survive the hostile gastric environment (Bik et al., 2006; O'Hara and Shanahan, 2006; Nardone and Compare, 2015). After the discovery of Hp, a large number of studies have shown that other bacteria also exist in the stomach and can colonize the stomach instead of just passing through (Li et al., 2009; Khosravi et al., 2014; Spiegelhauer et al., 2020). With the advancement of molecular biotechnology and gene sequencing technology, the mystery of the gastric microbiota has been gradually revealed. The existence of gastric microbiota was also confirmed to promote the occurrence of gastric cancer in an INS-GAS mouse model (Lofgren et al., 2011; Lertpiriyapong et al., 2014; Shen et al., 2022). The study of the relationship between gastric microorganisms and gastric cancer has also become a hot topic in recent years, with much progress in the diagnosis and microbial treatment of early gastric cancer being expected to occur.

Studies have shown that Streptococcus exists in the stomach of healthy people (Sharma et al., 1984; Monstein et al., 2000; Mowat et al., 2000; Kato et al., 2006; Zilberstein et al., 2007) and is the dominant bacterium other than Hp (Seo et al., 2014); in addition, it was also found that Streptococcus abundance was significantly increased in gastric cancer, and Streptococcus remained one of the most abundant genera after subtotal gastrectomy (Tseng et al., 2016). Since the first discovery of DNA fragments of Streptococcus anginosusin gastric cancer tissues in 1998 (Sasaki et al., 1998), subsequent studies have used 16S rRNA sequencing to identify microorganisms to ensure that the analyzed bacteria are live bacteria, which can partially mitigate the impact of upstream oral microorganisms on research results. In 2009, Dicksved et al. found that the gastric cancer flora mainly comprised different species of Streptococcus, Lactobacillus, Veillonella and Prevotella (Dicksved et al., 2009). In 2016, Jo et al. divided the research subjects into four groups: Hp (+) gastric cancer, Hp (−) gastric cancer, Hp (+) nongastric cancer and Hp (−) nongastric cancer. The study found that the abundance of Streptococcus ranked second in all four groups (Jo et al., 2016). Metagenomic analysis of bacteria and extracellular vesicles conducted by Choi et al. the following year found that Helicobacter pylori and Streptococcus were two major bacterial genera, and their abundances increased significantly in gastric cancer patients (Choi et al., 2017). In 2020, Gunathilake et al. conducted a study at the Streptococcus species level and found that Streptococcus CP003667 and Streptococcus vestibularis were enriched in the healthy control group, while Streptococcus NCVM was enriched in the gastric cancer group (Gunathilake

et al., 2020). In 2021, Pimentel-nunes et al. found that the abundance of Dicoccus, especially Streptococcus, was significantly increased in patients with early-stage gastric cancer (Pimentel-Nunes et al., 2021). In the same year, Gunathilake et al. also conducted a study on early gastric cancer and found that the abundances of Streptococcus vestibularis and Peptostreptococcus stomatis decreased significantly in the gastric cancer group (Gunathilake et al., 2021). The above studies showed that the abundance of Streptococcus was significantly increased in gastric cancer at the genus level, but at the species level, the abundances of some Streptococcus species were decreased in gastric cancer.

All the above studies have regarded the whole stomach as a microecological environment, while some researchers believe that different parts of the stomach are different microecological environments, so it is necessary to compare whether the Streptococcus in cancerous gastric tissue and adjacent tissue are different. In 2019, Chen et al. first described the microbial cooccurrence network in the cancerous tissues and adjacent tissues of gastric cancer patients and found that the enriched bacterial groups in cancer tissues were dominated by oral bacteria (such as Peptostreptococcus, Streptococcus, and Fusobacterium), while in adjacent noncancer tissues, lactic acid-producing bacteria (such as Lactococcus lactis and Lactobacillus brevis) were more abundant (Chen et al., 2019). In the same year, Liu et al. found that Helicobacter pylori abundance was significantly reduced in cancer tissue, while Streptococcus anginosus abundance was significantly increased (Liu et al., 2019). Later, Dai et al. found that the abundance of Streptococcus in cancerous tissues increased (Dai et al., 2021). Shao et al. studied the cancerous tissue and adjacent noncancerous tissue of cardia adenocarcinoma patients and found that at the genus level, the relative abundance of Streptococcus in the cancerous tissue was high, and the relative abundance of Helicobacter pylori was low (Shao et al., 2019). The above results indicated that the location of gastric cancer and the microecological environment did not affect the enrichment of Streptococcus.

It is believed that progression from chronic nonatrophic gastritis to chronic atrophic gastritis to intestinal metaplasia to dysplasia to gastric cancer is a common process in the occurrence and development of gastric cancer. Therefore, many researchers began to study the changes in the microbiota from the precancerous lesions of gastric cancer, in the context of achieving early prevention, early diagnosis and early treatment of gastric cancer using an analysis of microorganisms before the occurrence of gastric cancer. A 2018 study found that Streptococcus was most abundant in the microbiota of patients with chronic gastritis (Ferreira et al., 2018). Conti et al. found that Streptococcus was more common in gastritis patients with atrophic gastritis, and Streptococcus was positively correlated with OLGA/OLGIM stages of chronic gastritis (Conti et al., 2021). A 2021 study in New York conducted by Wu et al. found that the abundances of Streptococcus mutans, Streptococcus parahaemolyticus, and Streptococcus sanguinis were lower in the gastric mucosa of patients with intestinal metaplasia than healthy individuals (Wu et al., 2021). In the same year, a study conducted in and around Anhui, China, found that Streptococcus had a high centrality in the progression of gastric precancerous lesions (Liu D. et al., 2021). In 2018, Coker et al. conducted a microbial study on patients with superficial gastritis (SG), atrophic gastritis (AG), intestinal metaplasia (IM) and gastric cancer (GC) in Xi'an, China, and validated the results in Inner Mongolia. The study found that Peptostreptococcus stomatis and Streptococcus anginosus have significant centrality in the gastric cancer ecological network, the area under the curve (AUC) value for distinguishing gastric cancer from superficial gastritis was 0.82, and the AUC obtained in the validation cohort was 0.81 (Coker et al., 2018). In 2021, Pimentel-Nunes et al. conducted a microbial analysis of healthy controls, patients with advanced atrophic gastritis with intestinal metaplasia, and early-stage gastric cancer. The study found that from controls to patients with intestinal metaplasia and then to patients with gastric cancer, Streptococcus abundance increased gradually from 19.3 to 33.7%, and Streptococcus is the predominant bacteria in early-stage gastric cancer (Pimentel-Nunes et al., 2021). The study of intragastric Streptococcus in gastric cancer is shown in Table 2. Streptococcus was enriched during disease progression, and this change was more pronounced and statistically significant when gastric cancer patients were compared with chronic gastritis patients to distinguish the two conditions. Although it is not clear whether the changes in Streptococcus are the cause or effect of gastric cancer, it is significant for the diagnosis of early-stage gastric cancer.

Changes in intestinal Streptococcus during the occurrence and development of gastric cancer

Studies have found that the colonic environment is completely different from the oral and gastric environments in terms of biological and ecological characteristics (Tsuda et al., 2015), and microorganisms in the stomach of healthy people will affect the results of fecal microbiological analysis (Stearns et al., 2011). Several studies have shown that the intestinal flora changes during the occurrence and development of gastric cancer. Therefore, it is uncertain whether the gut, as a downstream organ of the stomach, is affected and causes changes in the intestinal flora, or whether changes in the intestinal flora promote the occurrence and development of gastric cancer. Fecal analysis is mainly used in the study of the intestinal flora because diet and lifestyle are key factors in the formation of gut microbes; thus, studies have shown that lifestyle has a great impact on gastric cancer risk and sex differences in gastric cancer (Zhang et al., 2013). Propensity score matching (PSM) can be used to eliminate the influence of lifestyle on data regarding the reliability and correlation of fecal bacteria and to increase the authenticity of research results. Studies using the INS-GAS mouse model have shown that the gut microbiota promotes the occurrence of gastric cancer (Bik et al., 2006; Maldonado-Contreras et al., 2011; Lertpiriyapong et al., 2014;

TABLE 2 The study of intragastric Streptococcus in gastric cancer.

Author	Year	Subjects	Region/ country	Method	Samples	Main findings	Reference
Sjöstedt	1985	Patients with gastric ulcer, duodenal ulcer, gastritis, gastric cancer, postoperative gastric cancer patients and healthy controls, 10 per condition	Sweden	Bacterial culture	Saliva, esophageal fluid, gastric fluid	Streptococcus was isolated from gastric juice cultures of patients with gastritis and gastric cancer and from those who underwent gastrectomy.	Sjöstedt et al., 1985
Sasaki	1995	43 patients with gastric cancer	Japan	Southern blot analysis and 16S rDNA sequencing	Surgical specimens	DNA fragments of Streptococcus anginosus were found in 9 (20%) surgical specimens	Sasaki et al., 1995
Sasaki	1998	15 esophageal cancer, 43 gastric cancer, 16 lung cancer, 10 cervical cancer, 14 renal cell cancer, 10 colorectal cancer, 19 bladder cancer patients	Japan	Southern blot analysis, the 16S rDNA of streptococcus anginosus was analyzed by PCR	Cancer tissue and adjacent noncancer tissue	DNA fragments of Streptococcus anginosus were found in DNA samples of cancer tissues of esophagus and gastric cancers, but not in adjacent noncancer tissue.	Sasaki et al., 1998
Dicksved	2009	10 patients with gastric cancer, 5 dyspeptic control patients	Sweden	16S rRNA sequencing	Stomach biopsies	The gastric cancer microbiota was instead dominated by different species of the genera Streptococcus, Lactobacillus, Veillonella and Prevotella	Dicksved et al., 2009
Aviles-Jimenez	2014	5 patients with chronic nonatrophic gastritis, 5 patients with intestinal metaplasia, and 5 patients with gastric cancer	Mexico	G3 chip was used to extract DNA for microflora analysis	Stomach biopsies, surgical specimens	Lachnospiraceae and Streptococcaceae representing over 20% of families in patients from all three disease groups.	Aviles-Jimenez et al., 2014
Jo	2016	HP-negative control group ($n = 13$), HP-positive control group ($n = 16$), HP negative cancer group ($n = 19$), and HP-positive cancer group ($n = 15$)	Korea	Barcoded 454 Pyrosequencing of the 16S rRNA Gene	Gastric mucosal (antrum and corpus) biopsies	Streptococcus ranked second in all four groups; in the high intestinal metaplasia group, the proportion of streptococcus increased.	Jo et al., 2016
Sohn	2017	HP-negative control group ($n = 2$), HP-positive control group ($n = 3$), HP negative cancer group ($n = 2$), and HP-positive cancer group ($n = 5$)	Korea	Bar-coded 454 pyrosequencing of the 16S rRNA gene	Antrum and body biopsy	The higher composition of Streptococcus pseudopneumoniae, S. parasanguinis, and S. oralis in Hp (−) cancer groups than the others, only in the body. At the family level, streptococcus accounted for the largest proportion of Hp-negative gastric cancers.	Sohn et al., 2017
Coker	2018	81 patients with superficial gastritis (SG), atrophic gastritis (AG), intestinal metaplasia (IM) and gastric cancer (GC), 126 cases from inner Mongolia, China.	Xi'an and inner Mongolia China,	16S rRNA sequencing	Gastric mucosal samples	Five GC-enriched bacterial taxa whose species identifications correspond to Peptostreptococcus stomatis, Streptococcus anginosus, Parvimonas micra, Slackia exigua and Dialister pneumosintes had significant centralities in the GC ecological network	Coker et al., 2018

(Continued)

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TABLE 2 (Continued)

Author	Year	Subjects	Region/ country	Method	Samples	Main findings	Reference
Hu	2018	6 patients with gastric cancer and 5 patients with superficial gastritis	Beijing, China	Shotgun metagenomic sequencing	Gastric wash samples	The most representative taxa found in gastric cancer are members of known commensal or opportunistic pathogenic bacteria that typically colonize the oral cavity， including species Streptococcus_mitis_ oralis_pneumoniae.	Hu et al., 2018
Chen	2019	62 patients with gastric cancer undergoing subtotal gastrectomy	Shenyang, China	16S rRNA sequencing	Cancer tissue and adjacent noncancer tissue	The genera Streptococcus, Peptostreptococcus were enriched in cancerous tissues.	Chen et al., 2019
Liu	2019	276 patients with gastric cancer who underwent gastrectomy without preoperative chemotherapy	Zhejiang Province, China	16S rRNA gene sequencing	230 were normal, 247 were adjacent noncancer tissue, and 229 were tumor tissue	In the tumor microbial environment, the abundances of Helicobacter pylori and Prevotella significantly decreased, while the abundance of Streptococcus anginosus increased significantly.	Liu et al., 2019
Shao	2019	67 cases of esophageal carcinoma and 36 cases of cardia adenocarcinoma underwent surgical treatment	Henan Province, China	16S rRNA next generation sequencing.	Tumor and adjacent nontumor tissues	At the genus level, the relative abundances of Prevotella, Streptococcus and Veillonella were higher in cardia adenocarcinoma tumor tissue than in nontumor tissue.	Shao et al., 2019
Gunathilake	2020	268 patients with gastric cancer and 288 healthy controls	Korea	16S rRNA gene sequencing	Gastric mucosa tissues	Streptococcus_NCVM species was highly abundant in GC cases	Gunathilake et al., 2020
Dai	2021	37 patients with gastric cancer in Zhejiang Province, China， Validation in 20 gastric cancer patients in Jiangxi Province， China.	Zhejiang Province and Jiangxi Province， China	16S rRNA gene sequencing	Cancerous tissue and gastric antrum mucosa at a distance of 5 cm from the cancerous tissue	Increased abundances of Lactobacillus, Streptococcus, and Prevotella genera in cancerous tissue.	Dai et al., 2021
Gunathilake	2021	268 cases of early gastric cancer and 288 healthy controls	Korea	16S rRNA gene sequencing	Mucosal tissue at 3 cm from the tumor, gastric antrum and gastric corpus mucosa in the control group	The abundances of Streptococcus vestibularis and Peptostreptococcus stomatis decreased significantly in gastric cancer group	Gunathilake et al., 2021
Pimentel-Nunes	2021	Patients with normal stomach (control group, 25), advanced atrophic gastritis with intestinal metaplasia (IM, 18) and early gastric cancer (EGC, 34)	Portugal	16S rRNA next generation sequencing.	Gastric antrum and corpus biopsy specimens	From control to IM, then to EGC, the abundances of two bacteria gradually increased: Gemella from 1.48 to 3.9%; Streptococcus from 19.3 to 33.7%, being the dominant bacteria in EGC. At the species level, even though several streptococcus increased from normal mucosa to cancer, Streptococcus anginosus, Streptococcus oralis and Streptococcus mitis were the more prevalent and frequent in cancer patients	Pimentel-Nunes et al., 2021

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TABLE 3 The study of intestinal Streptococcus in gastric cancer.

Year	Author	Subjects	Region/country	Method	Samples	Main findings	Reference
2019	Qi	116 patients with gastric cancer and 88 healthy controls	Shanxi Province, China	16S rRNA gene sequencing	Feces	12 bacterial genera, including Lactobacillus and Streptococcus, were enriched in GC.	Qi et al., 2019
2020	Wu	134 patients with gastric cancer and 58 matched healthy controls	Jiangsu Province, China	16S rRNA and 18S rRNA gene sequencing	Feces	Streptococcus mitis and Streptococcus salivarius subsp. in stool specimens were associated with the risk of gastric cancer.	Wu et al., 2020
2021	Liu	38 patients with gastric cancer and 35 healthy volunteers	Shandong Province, China	16S rRNA gene sequencing	Feces	The facultative anaerobic (aerotolerant) bacteria were Enterobacteriaceae, Escherichia and Streptococcaceae, and the abundances of all were elevated in the intestine of gastric cancer patients	Liu S. et al., 2021
2021	Yu	49 patients with gastric cancer (C group), 49 healthy control (N group), 26 patients were divided into liver metastasis group (L group) and nonliver metastasis group (M group)(n = 13).	Wuhan, China	16S rRNA gene sequencing	Feces	At the genus level, lactobacillus and streptococcus were enriched in group C. By comparing group L with group M, streptococcus was identified as a microorganism that could predict liver metastasis of gastric cancer.	Yu et al., 2021
2021	Zhang	83 cases of noncardia gastric cancer, 54 cases of chronic atrophic gastritis, 29 cases of colorectal cancer and 61 healthy individuals	Zhejiang Provence, China	16S rRNA gene sequencing	Feces	The abundance of the Streptococcus genus was increased in the intestinal microbiota of gastric cancer patients.	Zhang Y. et al., 2021
2021	Zhang	22 patients with gastric cancer and 30 healthy (Hp negative, no gastrointestinal symptoms)	Qinghai Province, China	16S rDNA gene sequencing	Feces	At the genus level, Prevotella, Streptococcus and Lactobacillus abundances were higher in the gastric cancer group than in the healthy group and the difference was statistically significant.	Zhang Z. et al., 2021

Pinzon-Guzman et al., 2019), indicating that certain bacteria in the gut are associated with the occurrence of gastric cancer.

In recent years, researchers have paid attention to the role of gut microbes in the occurrence and development of gastric cancer. It is generally believed that intestinal Streptococcus is associated with the risk of gastric cancer and can be used as a potential marker for predicting gastric cancer (see Table 3). In 2019, a case–control study was conducted in Shanxi Province, China. Through the analysis of microorganisms in the feces of gastric cancer and healthy control groups, Streptococcus was found to be enriched in gastric cancer patients, and the AUC resulting from the use of Streptococcus to distinguish the gastric cancer from the healthy control group was 0.81, indicating that Streptococcus can be used as a potential marker for predicting gastric cancer (Qi et al., 2019). This is the first study to examine the relationship between intestinal Streptococcus and stomach cancer. The following year, a study in Jiangsu Province, China, found that some common oral community members (such as Streptococcus mitis and Streptococcus salivarius subsp) in stool specimens were associated with the risk of gastric cancer (Wu et al., 2020). Subsequently, researchers from other provinces in China also performed 16S rDNA sequencing and 16S rRNA sequencing on the stool of patients with gastric cancer and found that Streptococcus abundance was increased in the intestinal flora of patients with gastric cancer, and the difference was statistically significant (Zhang Y. et al., 2021; Liu S. et al., 2021; Zhang Z. et al., 2021). Moreover, a Japanese study found that the intestinal microflora after surgery for gastric cancer also changed, with Streptococcus becoming the dominant bacteria (Erawijantari et al., 2020), which was similar to the results of other studies on the changes in gastric microflora observed after surgery. Yu et al. further compared the changes in bacteria in the feces between patients with gastric cancer and healthy controls, as well as patients with liver metastasis and nonliver metastasis, and found that Streptococcus was enriched in the gastric cancer group; Streptococcus was also identified as a microorganism that could predict liver metastasis of gastric cancer by comparing the liver metastasis group (L group) with the nonhepatic metastasis group (M group). However, survival analysis suggested that Streptococcus was not a prognostic factor for gastric cancer (Yu et al., 2021).

Possible carcinogenic mechanism of Streptococcus

Studies have shown that different species of Streptococcus play an important role in cancer, affecting the occurrence and development of tumors through various metabolite changes and regulation of the immune microenvironment (Morita et al., 2003; Narikiyo et al., 2004; Abdulamir et al., 2011; Moritani et al., 2015; Zhou et al., 2017; Sheikh et al., 2020). Streptococcus is enriched in gastric cancer and is the dominant bacteria in gastric cancer flora. Many studies have studied gastric cancer flora as a whole and found that it is associated with changes in various metabolic

pathways and the immune microenvironment. Studies have found that purine metabolic pathways are enriched in gastric cancer, suggesting that the gastric cancer microbiome metabolizes and releases purines in the tumor microenvironment (Coker et al., 2018; Chen et al., 2019) and that purines regulate the immune cell response and cytokine release (Di Virgilio, 2012). The LPS (lipopolysaccharide) biosynthetic pathway is enriched in gastric cancer (Hu et al., 2018), and LPS can promote an inflammatory response in the tumor microenvironment (Rakoff-Nahoum and Medzhitov, 2009; Gagliani et al., 2014), suggesting that the gastric microbiota promotes inflammation. The activation of some pathways that contribute to cell recognition is reduced in gastric cancer, such as bacterial motility and signal transduction pathways (Coker et al., 2018; Chen et al., 2019). In a comparative analysis of gastric cancer and chronic gastritis patients in Portugal and Mexico, Ferreira et al. found that the activities of nitrate reductase and nitrite reductase in gastric cancer flora increased (Ferreira et al., 2018), thereby increasing levels of nitrite, which is the precursor of carcinogen NOC (Correa, 1992). There are also studies showing that the activation of some amino acid metabolic pathways, such as those for isoleucine and valine, is increased in gastric cancer (Jung et al., 2014; Wang H. et al., 2016; Hu et al., 2018; Liu et al., 2019; Gunathilake et al., 2020; Huang et al., 2021). Hp is present in the gastric cancer flora, as well as other bacteria, so the changes in metabolic pathways are not necessarily caused by Streptococcus.

To further determine the carcinogenic mechanism of Streptococcus, researchers separately analyzed the correlation between changes in Streptococcus abundance and metabolites and the regulation of the immune microenvironment to determine the carcinogenic mechanism of Streptococcus. Studies have found that Streptococcus is involved in the formation of NOC (nitroso compounds) in the stomach (Ayanaba and Alexander, 1973; Jo et al., 2016; Sohn et al., 2017), and the formation of NOC increases the risk of gastric cancer (Ayanaba and Alexander, 1973; Mowat et al., 2000; Dicksved et al., 2009; Jo et al., 2016). Streptococcus is associated with a variety of metabolic changes. Wu et al. found that Streptococcus abundance was positively correlated with the levels of serum amino acids (L-alanine, L-threonine, methionine, L-carnitine, guanidinoacetate), heptanal and phenylethylamine by analyzing serum metabolites (Wu et al., 2020). Dai et al. found that Streptococcus abundance was positively associated with glutathione, cysteine, and methionine levels, and the activation of these metabolic pathways was increased in gastric cancer (Dai et al., 2021). In addition to studying metabolites, streptococcal infection may also affect the immune microenvironment in the body. Qi et al. studied the changes in immune cells in peripheral blood and found that the abundance of Streptococcus was positively correlated with the number of CD3+ T cells and negatively correlated with the number of NK cells (Qi et al., 2019). The possible carcinogenic mechanism of Streptococcus is shown in Figure 3. The correlation between Streptococcus and metabolic pathways and the immune microenvironment has only been studied in recent years, and few research results have been achieved; the specific mechanism has not been further explored. Therefore, how Streptococcus affects the occurrence and development of gastric cancer is still unclear.

Summary and prospects

In summary, streptococci are common bacteria in the digestive tract and were first discovered in the stomach. However, due to the immature technology and incomplete understanding at that time, people paid more attention to the pathogenicity of Hp in gastric cancer. With the development of microbial detection technology and progress in scientific knowledge, Streptococcus has been discovered and studied in depth by many researchers. Streptococcus was found to be affected by Hp and gastric acid in the stomach of nongastric cancer patients, while in the absence of Hp, Streptococcus abundance in gastric cancer patients was prominent, indicating that Streptococcus may act together with Hp or play a role in different stages of gastric cancer. Streptococcus changes in different parts of the digestive tract in patients with gastric cancer. We describe the changes in Streptococcus in the oral cavity, stomach and intestinal tract. In the oral cavity, at the genus level, Streptococcus was enriched in gastric cancer patients; at the species level, some species of Streptococcus exhibited reduced abundances. In the stomach, at the genus level, the abundance of Streptococcus was significantly increased in gastric cancer patients; at the species level, the changes in different species of Streptococcus were different. In the gut, at the genus level, Streptococcus abundance in gastric cancer patients increased significantly, which was the same as that in the oral cavity and stomach, while Streptococcus abundance varied at the species level. Streptococcus can affect various metabolic pathways and the immune microenvironment of gastric cancer and play an important role in its occurrence and development. However, the causal relationship between Streptococcus and gastric cancer has not been established, nor has the pathogenesis been determined.

Early-stage gastric cancer has a good prognosis, but most patients already have advanced gastric cancer when they are first diagnosed (Smyth et al., 2020), and the 5-year survival rate is less than 30% (Huang et al., 2021). Gastric cancer is mainly diagnosed by gastroscopy and pathological biopsy. Due to the invasiveness of gastroscopy, it cannot be popularized as an early diagnosis method in the population, and other detection methods that can be widely used and that effectively predict early-stage gastric cancer are needed. The stomach, as an important organ of the digestive tract, has been studied to assess the carcinogenic mechanism of Hp in the early years due to its special environment and the presence of Hp colonization, but the pathogenicity of other microorganisms in gastric cancer has been ignored. Due to the development of molecular biology and gene detection technology, microbial research is no longer limited to traditional bacterial culture, and due to the application of metagenomics to microbial research, scientists have a more systematic and comprehensive understanding of microorganisms, so the study of

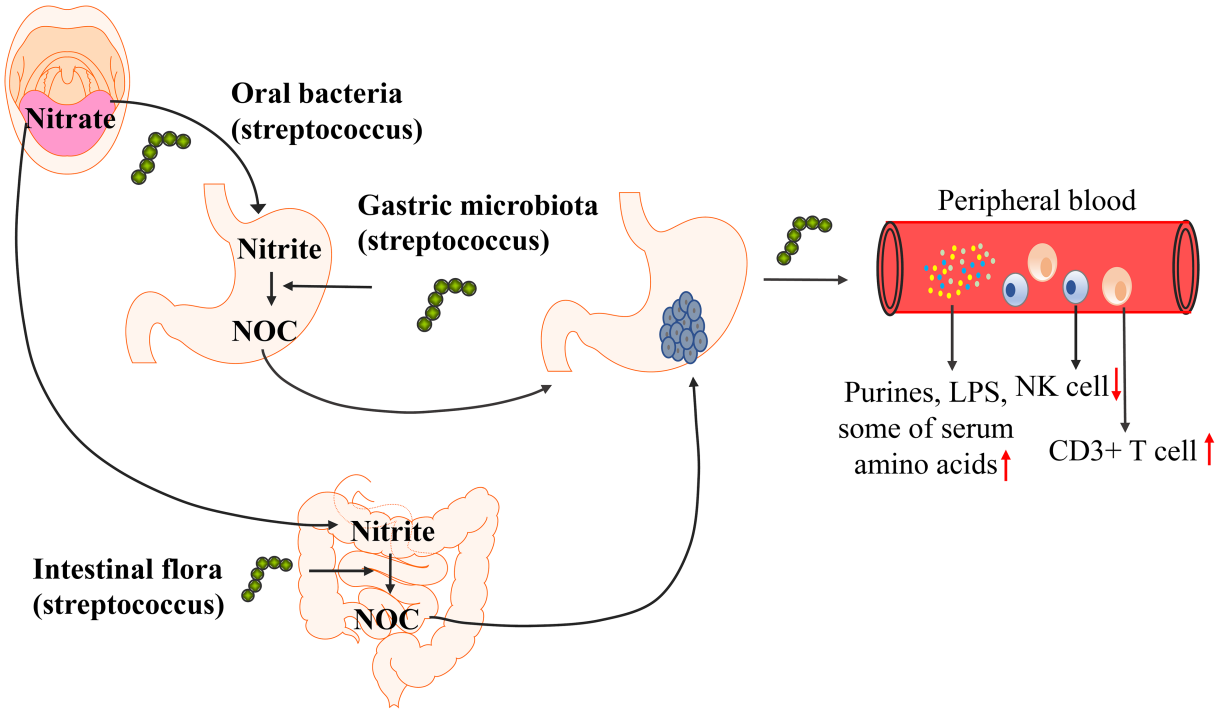

FIGURE 3
Possible carcinogenic mechanism of Streptococcus. The LPS (lipopolysaccharide) biosynthetic pathway and purine metabolic pathways are enriched in gastric cancer. Streptococcus abundance was positively correlated with the levels of serum amino acids (L-alanine, L-threonine, methionine, L-carnitine, guanidinoacetate), glutathione, cysteine, and methionine levels. Streptococcus was positively correlated with the number of CD3+ T cells and negatively correlated with the number of NK cells. Streptococcus is involved in the formation of NOC (nitroso compounds) in the stomach, and the formation of NOC increases the risk of gastric cancer.

the relationship between Streptococcus and tumors has become a hot spot in recent years. Streptococcus has been found to be carcinogenic in esophageal cancer and colorectal cancer (Abdulamir et al., 2011; Wang et al., 2012; Tsai et al., 2016; Liu et al., 2018; Guven et al., 2019; Kawasaki et al., 2021).

The study of Streptococcus in patients with gastric cancer is not as advanced as that of other gastrointestinal tumors, but great progress has been made in recent decades. Streptococcus overgrowth in the oral cavity, stomach and intestine of gastric cancer patients affects metabolites and peripheral immune cells and is a potential biomarker that can be used to assist in the diagnosis of gastric cancer. It is also a possible therapeutic target, providing new ideas for the treatment of gastric cancer. Intragastric Streptococcus affects the gastric microenvironment, but its pathogenic mechanism in gastric cancer remains unclear. Streptococcus in the oral cavity and intestine may be a potential predictor of gastric cancer. The characteristics of easy collection, low cost and noninvasiveness during the acquisition of specimens indicate that the assessment of Streptococcus may become a screening method for early-stage cancer. The current dilemma facing the use of Streptococcus as a treatment target has three components: (1) the pathogenicity and pathogenesis of Streptococcus have not been determined; (2) whether the use of traditional antibiotic treatment will destroy the microecological environment and cause other adverse events due to dysbiosis has not been determined; and (3) the use of probiotics may become a treatment method, but there is no relevant research thus far. Future research can be carried out from two aspects: (1) using animal experiments, an INS-GAS mouse model can be used to clarify the role of Streptococcus in gastric cancer; (2) using clinical studies, including descriptive and cross-sectional studies and function-based studies and prospective studies, studying the effects of Streptococcus and its metabolites in the digestive tract will be helpful for an in-depth understanding of its pathogenesis. However, since the development of gastric cancer takes decades and less than 3% of the H. pylori-infected population eventually develops gastric cancer, longitudinal studies and prospective studies are difficult to achieve. Microbial research on gastric cancer still has far to go, but assessments of Streptococcus, as a noninvasive auxiliary diagnostic method, will usher in a qualitative leap with the efforts of many scientists. If it is successfully applied to the clinic, it will greatly improve the early diagnosis rate and change the future of gastric cancer.

Author contributions

XDC and LY conceptualized the manuscript. MLZ, YQZ, CH, SJZ, and JXC collected the literature, MLZ and YQZ collected the literature, wrote the manuscript and made the figures. XDC and LY

edited and made significant revisions to the manuscript. 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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EDITED BY
Muhammad Shahid Riaz Rajoka,
Tohoku University, Japan

REVIEWED BY
Haiyang Wu,
Tianjin Medical University, China
Ahmad Ud Din,
Sichuan University, China

*CORRESPONDENCE
Ye Chen
yechen@smu.edu.cn
Pu Wang
564035448@qq.com
Qianyun Lin
linqianyun@foxmail.com

†These authors have contributed equally to this work and share first authorship

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Global trends in *Akkermansia muciniphila* research: A bibliometric visualization

Zitong Li[1†], Haoran Ke[2†], Ying Wang[1], Shuze Chen[1], Xiuying Liu[1], Qianyun Lin[3]*, Pu Wang[1]* and Ye Chen[1,4]*

[1]Guangdong Provincial Key Laboratory of Gastroenterology, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou, China, [2]Hepatology Unit, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China, [3]Department of Gastroenterology, Beijing Friendship Hospital, Capital Medical University, Beijing, China, [4]Integrative Microecology Center, Department of Gastroenterology, Shenzhen Hospital, Southern Medical University, Shenzhen, China

Background: *Akkermansia muciniphila* is a member of the gut microbiome, using mucin as sources of carbon, nitrogen, and energy. Since the first discovery of this unique bacterium in 2004, *A. muciniphila* has been extensively studied. It is considered a promising "next-generation beneficial microbe." The purpose of this paper is to sort out the research status and summarize the hotspots through bibliometric analysis of the publications of *A. muciniphila*.

Methods: The publications about *A. muciniphila* from January 2004 to February 2022 were obtained from the Web of Science Core Collection. Visualization analyses were performed using three bibliometric tools and GraphPad Prism.

Results: A total of 1,478 published documents were analyzed. Annual publication number grew from 1 in 2004 to 336 in 2021, with China being the leading producer (33.36%). De Vos, Willem M was the most productive author with the highest H-index (documents=56, H-index=37), followed by Cani, Patrice D (documents=35, H-index=25). And Scientific Reports published the most papers. *PNAS* was the keystone taxa in this field, with high betweenness centrality (0.11) and high frequency. The keywords with high frequency in recent years include: oxidative stress, diet, metformin, fecal microbiota transplantation, short-chain fatty acids, polyphenols, microbiota metabolites and so on. The keyword "oxidative stress" was observed to be increasing in frequency recently.

Conclusion: Over time, the scope of the research on the clinical uses of *A. muciniphila* has gradually increased, and was gradually deepened and developed toward a more precise level. *A. muciniphila* is likely to remain a research hotspot in the foreseeable future and may contribute to human health.

KEYWORDS

***Akkermansia muciniphila*, gut microbiota, bibliometrics, trends, visualization**

Introduction

Akkermansia muciniphila, discovered in 2004, is a Gram-negative, non-motile, ovoid intestinal anaerobe that lacks endospores (Derrien et al., 2004). It belongs to the phylum *Verrucomicrobia* and is the only species of this phylum found in human stools. *A. muciniphila*, which lives in the mucus layer of the intestine, degrades and uses mucin as its sole source of nitrogen, carbon, and energy (Derrien et al., 2004, 2008).

Researchers have investigated "new weapons" at the microbial level to combat disease, and *A. muciniphila* has attracted significant interest in the fields of biological and biomedical research since its discovery. In addition to its relationship with many metabolic diseases (Everard et al., 2013; Depommier et al., 2019; Yan et al., 2021), *A. muciniphila* is negatively associated with numerous conditions including inflammatory bowel disease, amyotrophic lateral sclerosis, autism, epilepsy, and hypertension (Li et al., 2017; Olson et al., 2018; Bárcena et al., 2019; Blacher et al., 2019; Cheng and Xie, 2021; Ke et al., 2021). *A. muciniphila* was implicated in patient responsiveness to programmed cell death protein 1 (PD-1) blockers in cancer immunotherapy studies (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2018). Over the past decade, *A. muciniphila* has attracted significant attention in academic circles due to its "probiotic" effect in many diseases; therefore, it is considered a promising "next-generation beneficial microbe" (Cani and de Vos, 2017). An increasing number of studies revealed that *A. muciniphila* plays important roles in various biological aspects; however, the mechanisms underlying its functions remain unclear.

The global trends and hotspots of *A. muciniphila* research have not been studied systematically on a temporal scale despite intensive research interest in recent years. Journal citations and publications can be tracked with bibliometrics through quantitative and qualitative analyses of scientific production and research status (Chen et al., 2014). Therefore, this study aimed to identify the foci and frontiers in *A. muciniphila* research using bibliometric analyses to facilitate further in-depth research at the clinical and basic research levels.

Materials and methods

Data collection

We obtained bibliometric analysis data from the Web of Science Core Collection database (WoSCC), a popular multidisciplinary database in the field of scientometrics (Kokol and Vošner, 2018; Cheng et al., 2022a,b,c). To avoid bias caused by daily database updates, all WoSCC searches were conducted on February 26, 2022. The search formula used was TS = *Akkermansia muciniphila*. In total, 1,546 publications were retrieved, but only 1,478 publications remained after 68 publications were excluded (meeting abstracts, early access, editorial materials, proceedings papers, book chapters, corrections, news items, letters, and/or non-English literature). A plain-text file was exported with all the full records and cited references for further analysis (Figure 1).

Deduplication of the obtained data was performed using the CiteSpace software (version 5.8. R3). Two researchers independently extracted the publications, countries, institutions, funding agencies, authors, journals, citations, keywords, highly cited references, Hirsch index (H-index; Engqvist and Frommen, 2008), and average citations per item (ACI). To ensure data accuracy and reliability, discrepancies were reconciled *via* discussions and negotiations. The 2021 Journal Citation Report (Clarivate Analytics, Philadelphia, PA, United States) was used to obtain journal information.

Data analyses

The CiteSpace (version 5.8. R3; Chen, 2004; Chen et al., 2014), VOSviewer (van Eck and Waltman, 2010), an online bibliometric platform,[1] and GraphPad Prism (version 8.4.3) were used for bibliometric and visual analyses. The relevant information was summarized in a table using Microsoft Excel (version 16.58). Figure 2 is drawn with Figdraw.[2]

Results

Publication and citation trends

In total, 1,478 papers (1,172 original articles and 306 reviews) were analyzed (Figure 1). Figure 3 shows the upward trend in publications and citations over the past 18 years. The number of publications rose from one to 336 from 2004 to 2021. Approximately 88.30% of the articles were published between 2016 and 2021, and the number of publications in the first 2 months of 2022 exceeded that in all of 2015. The total number of citations was 62,095 (51,188 if excluding self-citations).

Analysis of the countries/regions

Figure 4 shows the distribution of *A. muciniphila*-related publications worldwide. East Asia, North America, Western Europe, and South Europe were the most productive countries/

Abbreviations: ACI, Average citations per item; H-index, The Hirsch Index; IFs, Impact factors; JCR, Journal citation reports; MeSH, Medical subject headings; PNAS, Proceedings of the National Academy of Sciences of the United States of America; TLS, Total link strength; WoSCC, Web of Science Core Collection database.

1 https://bibliometric.com/

2 www.figdraw.com

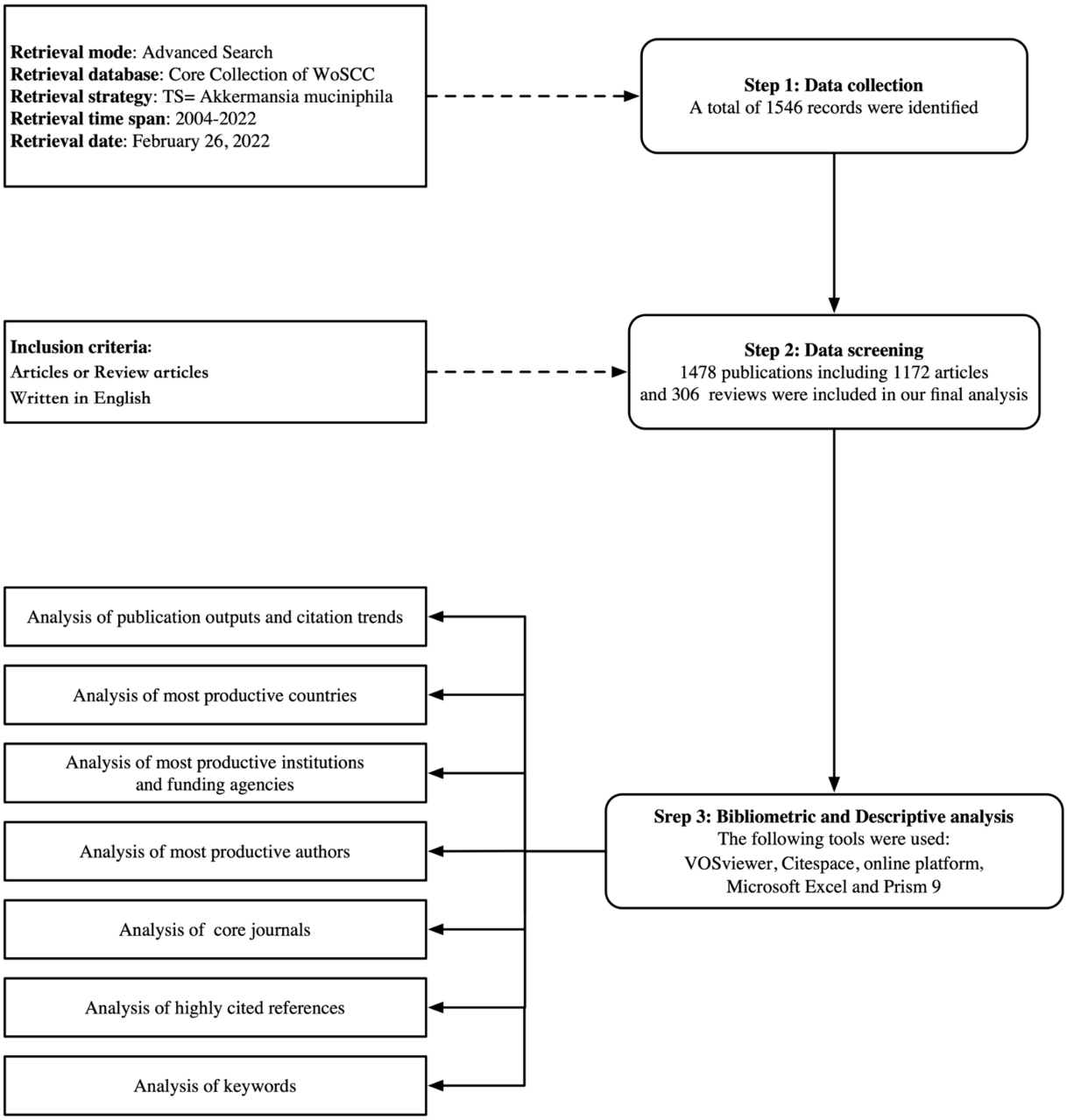

FIGURE 1
Flowchart of literature filtering and data analysis.

regions (Figure 4A). Figure 4B and Figure 4C demonstrate the basic information and trends in annual publication output, respectively, among the top ten countries (2004–2022). Seventy-three countries/regions produced publications on *A. muciniphila*. China ranked first with 493/1,478 publications (33.36%), followed by the United States (387/1,478; 26.18%). The United States had the highest H-index (63), whereas Finland (138.88) and the Netherlands (130.13) had the highest ACI (Figure 4B). In addition, network analysis was used to identify cooperative relationships between countries. As shown in Figure 4D, the closest cooperation occurred between China and the US, followed by that between Finland and the Netherlands.

Analysis of the institutions and funding agencies

Of the top 10 institutions, Wageningen University & Research in the Netherlands had the high H-index and was the most productive institution (H-index = 39,

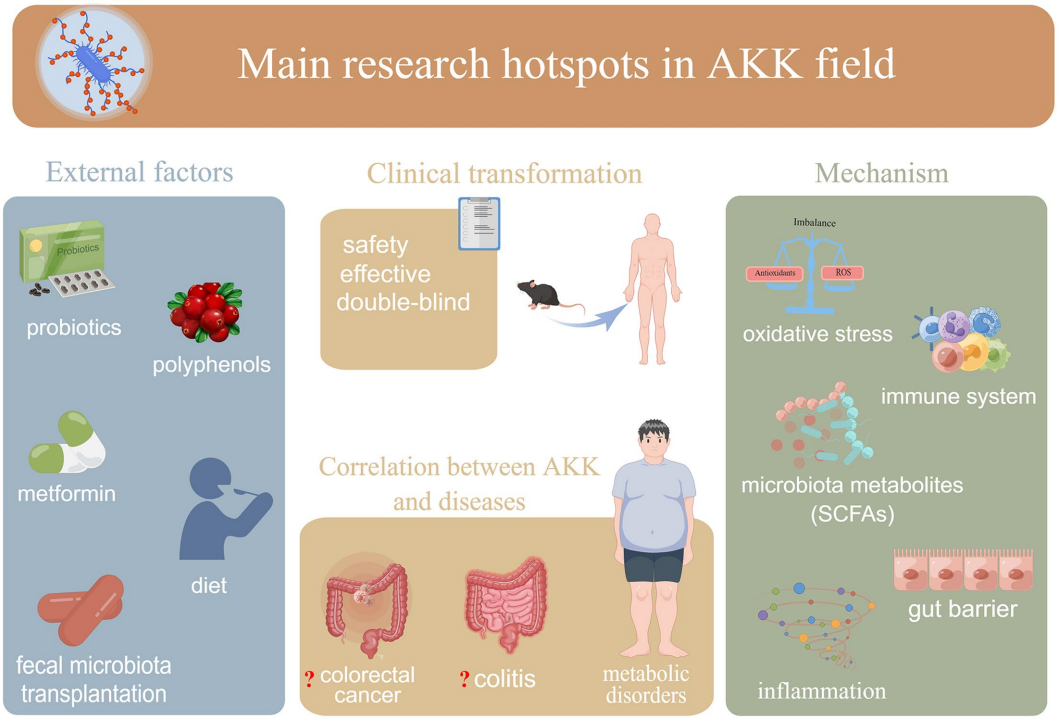

FIGURE 2
Recent hotspot directions of *A. muciniphila* research: (i) external factors affecting *A. muciniphila;* (ii) mechanisms underlying the association between *A. muciniphila* and hosts (including bacteria); (iii) correlations between *A. muciniphila* and different diseases; (iv) safety and efficacy of clinical use of *A. muciniphila*. AKK:*A. muciniphila*.

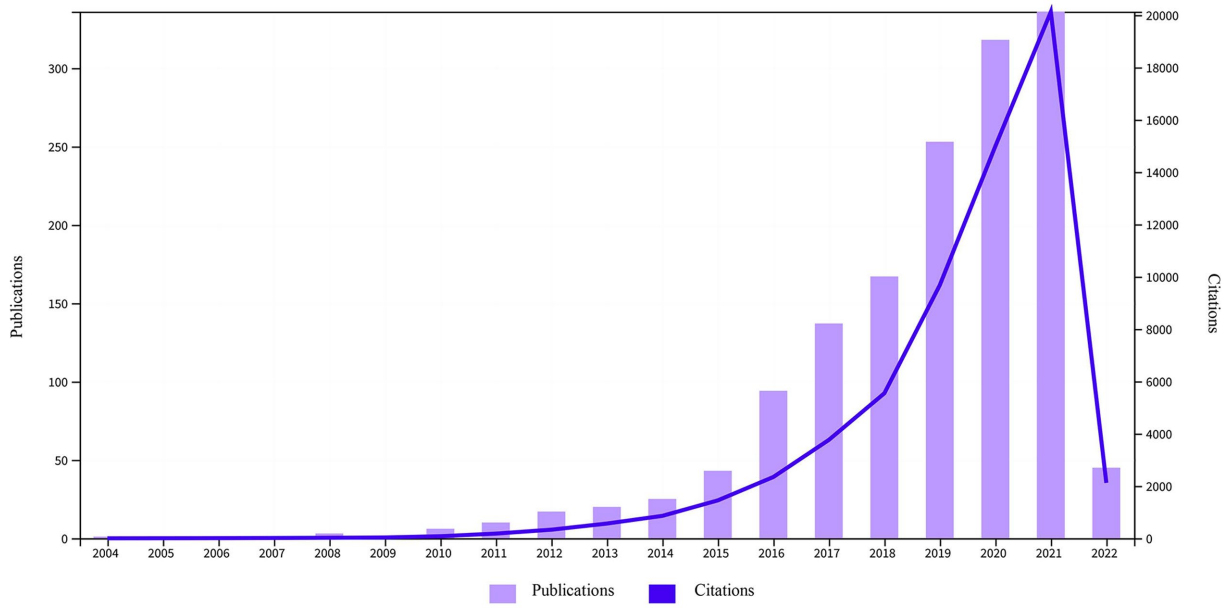

FIGURE 3
Trends in publications and citations regarding *A. muciniphila* (2004–2022).

publications = 69; Figure 5A). It was followed by two institutions each from France, Belgium, and China, and one each from the United States, Finland, and Denmark (Figure 5A). Walloon Excellence in Life Sciences and Biotechnology (WELBIO) had the highest ACI (211.70). Figure 5B illustrates the collaborations between institutions

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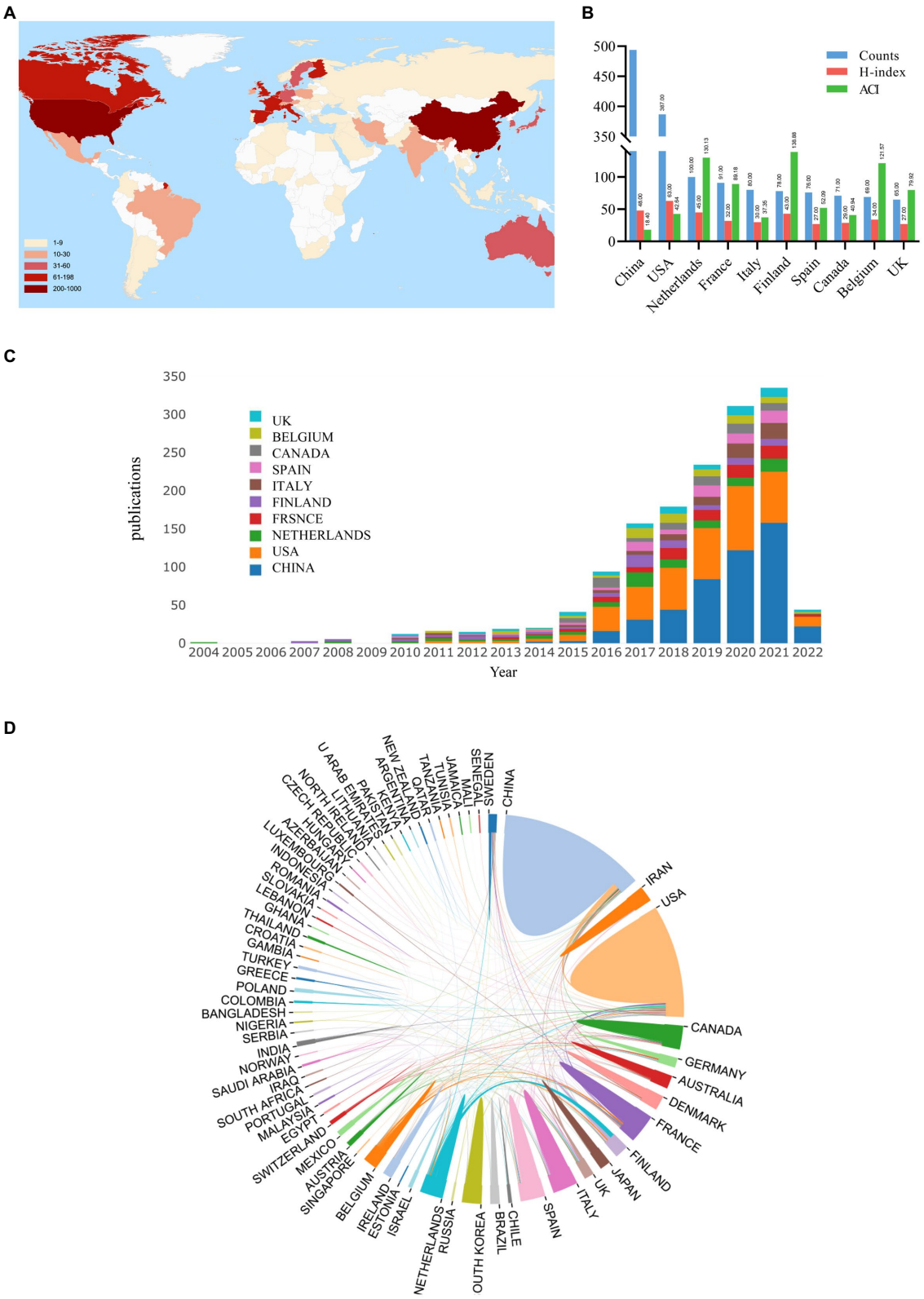

FIGURE 4
Analysis of the contribution of different countries/regions to *A. muciniphila* research. **(A)** Geovisualization of *A. muciniphila* research distribution. Color shades correlate with the number of articles published. England, Northern Ireland, Scotland, and Wales were reclassified together as the United Kingdom; Taiwan was merged into China. **(B)** The publication counts, H-index, and ACI of the top 10 most productive countries/regions. **(C)** Trends in *A. muciniphila* publications from the top 10 countries/regions from 2004 to 2022. The colors represent different countries/regions. **(D)** Cooperation of countries/regions involved in *A. muciniphila* research. The proportion of the area correlates to the number of national publications, and the thickness of the line reflects the strength of cooperation between countries.

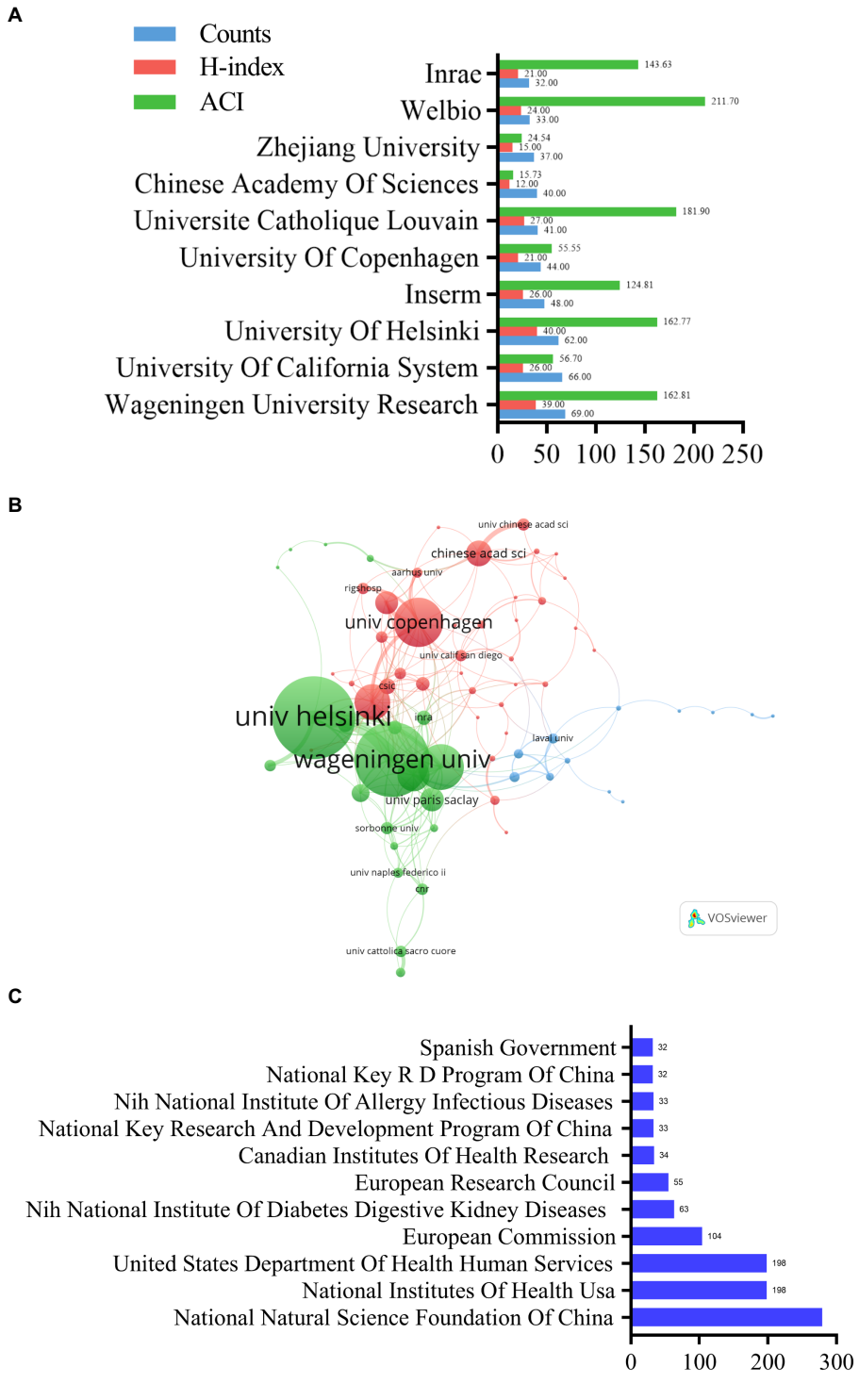

FIGURE 5
Analysis of the contribution of different institutions and funding agencies to *A. muciniphila* research. **(A)** The publication counts, H-index, and ACI of the top 10 most productive institutions. **(B)** Co-authorship analysis of the institutions. Each node represents a different institution. Node size reflects the strength of cooperation. **(C)** Top 10 related funding agencies which support *A. muciniphila* research (Spanish Government and National Key RD Program of China tied for 10th).

with a minimum number of eight published articles (associated institutions only). The node size indicates the degree of cooperation of an institution with other institutions (weighted by the total link strength [TLS]; the higher the TLS value, the stronger the cooperation strength; Li et al., 2022). A total of 201 lines and 70 nodes were present on the

TABLE 1 The most productive authors (those with at least 15 publications) in the field of *A. muciniphila* research.

Rank	Author (Country)	Counts	% of 1,478	Institutions	H-index	ACI[a]
1	De Vos, Willem M (Netherlands; Finland)	56	3.79	Wageningen University University of Helsinki	37	168.20
2	Cani, Patrice D (Belgium)	35	2.37	Universite Catholique de Louvain	25	200.14
3	Belzer, Clara (Netherlands)	32	2.17	Wageningen University & Research	21	165.38
4	Chen, Wei(China)	19	1.29	Jiangnan University	10	20.84
5	Delzenne, Nathalie M (Belgium)	19	1.29	Universite Catholique de Louvain	14	226.16
6	Hansen, Axel Kornerup (Denmark)	18	1.22	University of Copenhagen	11	35.61
7	Everard, Amandine (Belgium)	16	1.08	Universite Catholique de Louvain	13	344.25
8	Marette, Andre(Canada)	16	1.08	Laval University	13	71.31
9	Nielsen, Dennis Sandris (Denmark)	15	1.01	University of Copenhagen	10	42.60
10	Siadat, Seyed Davar (Iran)	15	1.01	Pasteur Institute of Iran	5	6.33
11	Zhang, Hao (China)	15	1.01	Jiangnan University	9	14.80

[a]ACI: average citations per item.

institutional network map, and the University of Helsinki (TLS = 79) and Wageningen University Research (TLS = 72) had the highest TLS (Figure 5B).

The funding agencies' contributions showed similar trends as that of the countries/region (Figure 5C). Four agencies in the US and three in China were included. The National Natural Science Foundation of China was the largest sponsor (279 studies; Figure 5C), followed by the National Institutes of Health (United States) and the United States Department of Health and Human Services.

Analysis of the most productive authors

The most productive authors (those with at least 15 publications) are listed in Table 1. De Vos, Willem M. from the University of Helsinki and Wageningen University was the most prolific author with the highest H-index (publications = 56, H-index = 37), followed by Cani, Patrice D. from the Universite Catholique de Louvain (publications = 35, H-index = 25). Everard, Amandine from the Universite Catholique de Louvain had the highest ACI (344.25). Figure 6A illustrates the collaboration network of authors who had at least six publications (weighted using TLS). The network consisted of 61 nodes and 174 lines (six nodes not shown). De Vos and Willem had the highest TLS (111), followed by Cani and Patrice (100) and Delzenne and Nathalie (77). The co-citation analysis included 101 nodes and 4,999 lines for authors with 70 citations or more. Derrien, Muriel (citations = 1,153), Everard, Amadine (citations = 1,126), and Cani and Patrice (citations = 1,096) were the most-cited authors (Figure 6B).

Analysis of the journals

The top 10 most productive journals in *A. muciniphila* research are listed in Table 2, and accounted for approximately 24.70% of all publications (365/1,478). *Scientific Reports* published the highest number of papers (61/1,478), followed by *Nutrients* (59/1,478) and *Frontiers in Microbiology* (54/1,478). A co-citation analysis was also conducted to investigate the influence of the journals. The top four most-cited journals were *Proceedings of the National Academy of Sciences of the United States of America* (PNAS; 1,181), *PLOS One* (1,129), *Nature* (1,092), and *Gut* (1,092; Figure 7A). Notably, PNAS had a central value of 0.11, indicating a high betweenness centrality.

Figure 7B shows the dual-map overlay depicting the flow from the citing to cited subject categories, mainly including three orange pathways (from "molecular, biology, immunology" to "molecular, biology, genetics," "environmental, toxicology, nutrition," and "health, nursing, medicine"), one green pathway (from "medicine, medical, clinical" to "molecular, biology, genetics"), and one yellow pathway (from "veterinary, animal, science" to "molecular, biology, genetics").

Analysis of highly cited references

Supplementary Table S1 lists the top 10 co-cited articles on *A. muciniphila* research, which are generally viewed as the 'classics' (Li et al., 2022). The most-cited paper was published by Everard et al. (2013) in PNAS, and was cited 319 times in this field. Dao et al. (2016) and Derrien et al. (2017) published the second and third most-cited papers, respectively.

Research hotspots were traced using co-citation analysis of the references. The co-citations were visualized and clustered to analyze the research focus. The modularity value (Q-value) and the mean silhouette value (S-value) were calculated to evaluate the clustering quality, where $Q > 0.3$ and $S > 0.7$ indicate that the clustering structure is significant and convincing (Wu et al., 2021a). Figure 8A illustrates the top 10 largest clusters with good homogeneity (S = 0.9419, Q = 0.7849). Citation bursts were mainly concentrated in cluster #0 (*A. muciniphila*) and cluster #1 (metformin). As shown in Figure 8B, the reference with the

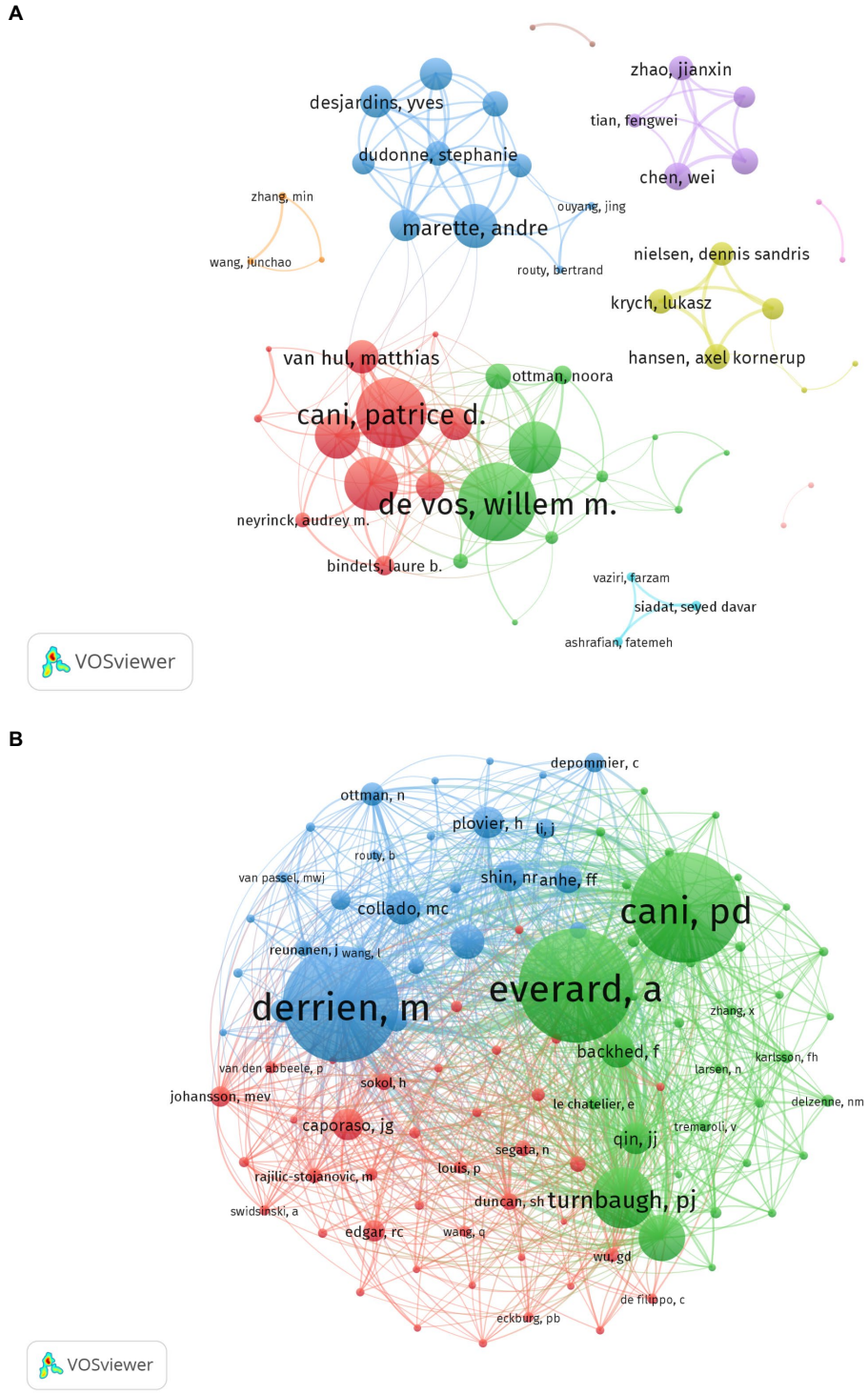

FIGURE 6
Contribution analysis of authorship in *A. muciniphila* research. **(A)** Co-authorship analysis of authors. Each node represents a different author. After the network was generated, cooperation between authors was shown as same-colored clusters. The size of the nodes reflects the strength of their cooperation. **(B)** Co-citation analysis of cited authors generated by VOS viewer. Each node represents a different cited author. The size of the nodes is weighted by citations.

highest citation burst strength was by Everard et al. (2013). Notably, bursts in several studies have been increasing recently (Callahan et al., 2016; Desai et al., 2016; Koh et al., 2016; Cani and de Vos, 2017; Derrien et al., 2017; Plovier et al., 2017; Chelakkot et al., 2018; Grander et al., 2018; Depommier et al., 2019; Zhang et al., 2019).

TABLE 2 Top 10 journals for *A. muciniphila* research.

Rank	Journal	Counts	% of 1,478	IF (2021)	JCR (2021)	H-index	ACI[a]
1	*Scientific Reports*	61	4.13	4.996	Q2	26	36.33
2	*Nutrients*	59	3.99	6.706	Q1	21	22
3	*Frontiers in Microbiology*	54	3.65	6.064	Q1	26	46.46
4	*Food Function*	33	2.23	6.317	Q1	15	16.73
5	*Gut Microbes*	33	2.23	9.434	Q1	13	34.45
6	*Plos One*	30	2.03	3.752	Q2	19	75.17
7	*Molecular Nutrition Food Research*	26	1.76	6.575	Q1	14	26.27
8	*Microorganisms*	25	1.69	4.926	Q2	10	35.56
9	*Gut*	24	1.62	31.793	Q1	22	234.58
10	*Frontiers in Cellular and Infection Microbiology*	20	1.35	6.073	Q1	7	17.3

[a]ACI: average citations per item.

Analysis of the keyword research knowledge

After synonym merging, 2,389 author keywords were obtained from 1,478 articles. A heatmap was generated for author keywords that occurred at least 30 times from 2004 to 2022 in the *A. muciniphila* research field (Figure 9A). The heatmap is colored and sized according to the frequency of the keywords. The top 10 keywords were "*A. muciniphila*," "gut microbiota," "obesity," "inflammation," "prebiotics," "chain fatty acids," "diet," "insulin resistance," "metabolism," and "diet-induced obesity." Additionally, Figure 9B color-codes the keywords based on the year in which they appeared. The keywords with an average appearance year after 2019 (more recent appearance) include "microbiota metabolites," "metformin," "fecal microbiota transplantation," "oxidative stress," "immune system," "short-chain fatty acids," "diet," "colorectal cancer," "diabetes," "gut barrier function," "double-blind," "polyphenols," etc. (Figure 9B). Furthermore, among the top 25 keywords with the strongest citation bursts, "oxidative stress" was observed to be increasing in frequency recently (Figure 9C). There may be a continued focus on these emerging keywords in the future.

Discussion

The frequency of *A. muciniphila* publications has shown exponential growth curve over the last 18 years, possibly due to growing treatment needs and advances in microbiome technology (e.g., 16S rRNA sequencing, metagenomics, metabolomics, etc.). The remarkable efficacy of *A. muciniphila* on obesity and diabetes has promoted its exploration in various fields. The cliff-like shape of the citation curve shown in Figure 3 indicates that this field is a research hotspot, and its popularity will continuously increase and become a hot research topic in the future.

The most productive countries were China and the United States. Initially, the United States was the topmost productive country; however, with increasing interest in the field among Chinese researchers, this gap gradually narrowed as publications from China increased in frequency. Combined with the institutional and funding agencies analyses, the high output of China and the United States is likely related to human investments and financial resources. The H-index is a crucial parameter for assessing the publication quality and academic influence of countries, institutions, journals, or researchers (Engqvist and Frommen, 2008). As with the H-index, the ACI can also represent the scientific output and academic status of publications. Based on the ACI, Finland played a crucial role in this field. Although China had the highest number of publications, the two institutions from China had the lowest average citation rate and H-index among the top ten institutions. Thus, the quality of the publications requires improvement. Cooperation between countries is essential, as strong cooperative relationships were observed among the countries with the most publications and highest ACI. Many countries/institutions have low influence levels, and inter-agency cooperation should be prioritized.

Analysis of the cooperative relationships between authors revealed inter-author connection networks. De Vos and Willem was the most productive author with the highest H-index. The author and the institutions and/or country the author belongs to can exert a significant influence on the emerging *A. muciniphila* research field. Furthermore, De Vos and Willem is a leading expert in gut microbiota research and is at the forefront of exploring microorganisms through molecular (meta-) genomics and systems approaches, focusing on the human gut (Belzer and de Vos, 2012). Another highly influential author is Cani and Patrice. Their research interests include interactions among gut microbes, the host, and specific biological systems, such as the endocannabinoid and the innate immune system, and their associations with metabolic disorders. Interestingly, De Vos and Willem and Patrice and Cani are co-founders of A-Mansia Biotech SA, the *Akkermansia* company.[3] They facilitate the transformation of basic research into clinical applications (Depommier et al., 2019). It is evident that their academic collaboration has contributed to their

3 https://www.a-mansia.com/

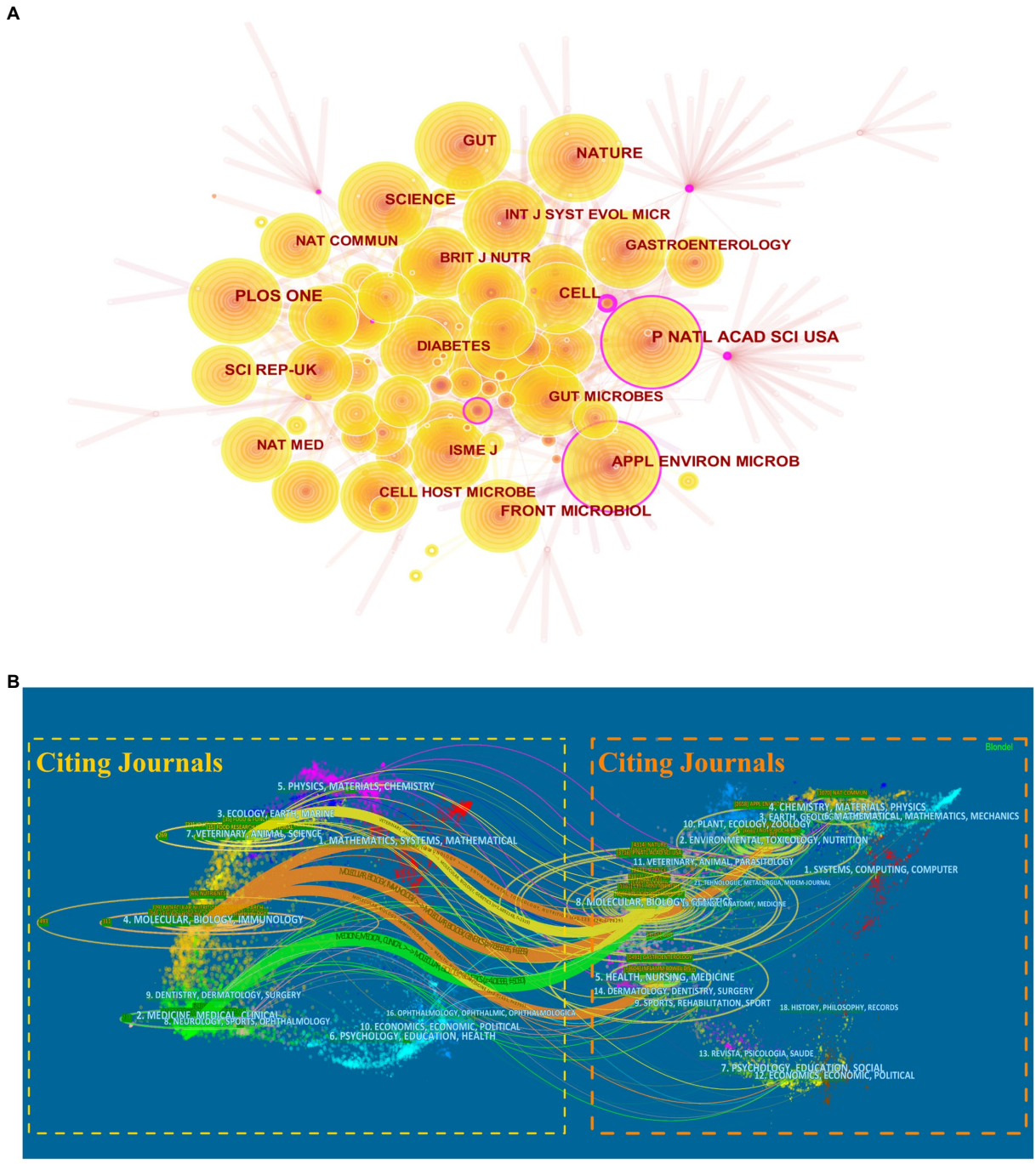

FIGURE 7
Analysis of core journals of *A. muciniphila* research. **(A)** Co-citation analysis of journals. Each node represents a different journal. The size of the nodes is weighted by the number of citations. The purple outer circle highlights nodes with intermediary centrality greater than 0.1. **(B)** Dual-map overlay of the journals publishing *A. muciniphila* articles generated by CiteSpace. Each label indicates a separate research subject covered by the journal. On the map, the left side represents the citing journals, while the right side represents the cited journals. There are different colored lines for the different reference paths, which begin with the citing map and end at the cited map.

current success in this field. Both of these pioneering researchers make significant contributions to this field, and many highly cited references and citation burst references were published by their teams (Everard et al., 2011, 2013; Belzer and de Vos, 2012; Schneeberger et al., 2015; Cani and de Vos, 2017; Derrien et al., 2017; Plovier et al., 2017; Depommier et al., 2019).

Journal statistics help researchers select suitable journals for publishing their research. *Scientific Reports*, *Nutrients*, and *Frontiers in Microbiology* were the major journals that

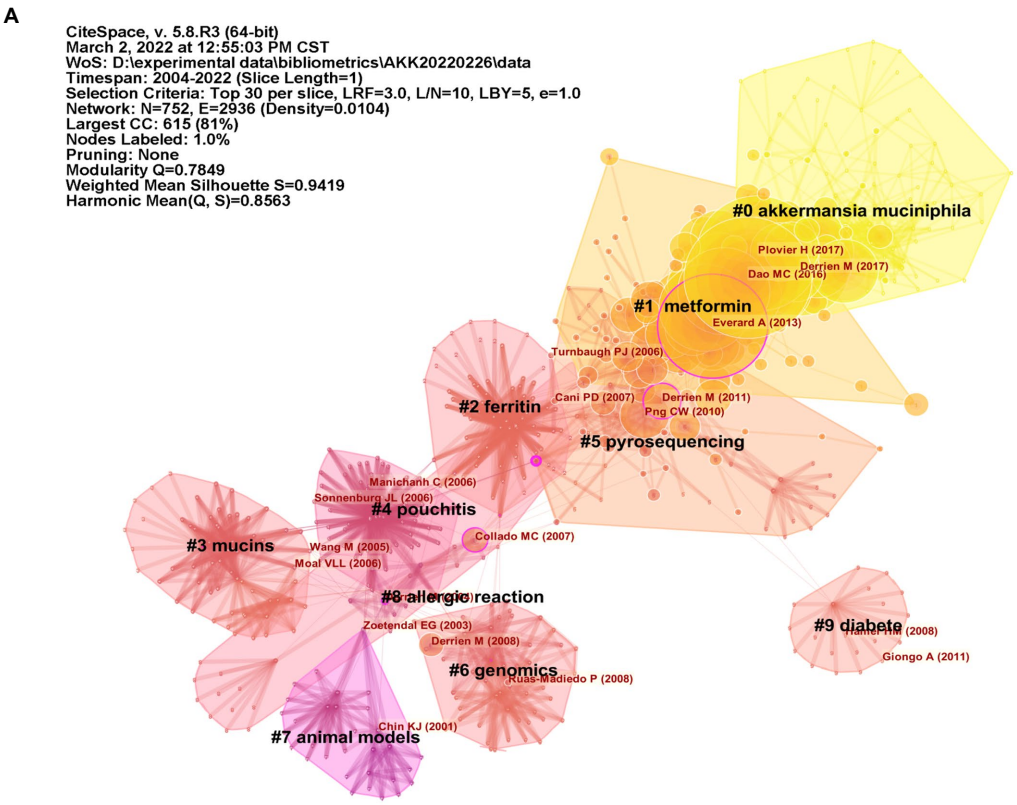

FIGURE 8
Analysis of references in *A. muciniphila* research. **(A)** The network map of co-cited references. Each node represents a different reference. The cited references form several natural clusters, which are closely related. The purple outer circle highlights nodes with intermediary centrality greater than 0.1. **(B)** Top 25 *A. muciniphila*-related references with the strongest citation bursts (2004–2022).

published *A. muciniphila*-related articles. The betweenness centrality of nodes in a network is a vital centrality indicator (Wu et al., 2021b), indicating co-citation relationships between multiple nodes and which journals are "transportation hubs." *PNAS* had high betweenness centrality and frequency, and is considered a keystone taxon in this field. As article carriers,

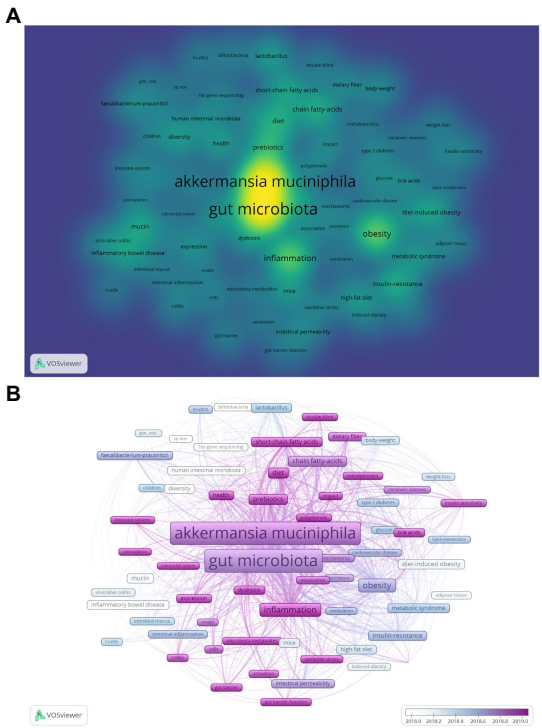

FIGURE 9
Author keywords analysis. **(A)** Keywords co-occurrence heatmap. The heatmap color and size reflect the frequency of keywords. **(B)** Overlay visualization of *A. muciniphila*-related keywords. Each node represents a different author keyword. The color of the nodes corresponds to the average year in which the keyword appeared according to the color gradient shown at the bottom right. **(C)** Top 25 *A. muciniphila*-related keywords with the strongest citation bursts (2004–2022).

journals reflect the position of published articles in the field. The key position of *PNAS* is the result of the articles published in the field, such as the study published by Everard et al. (Everard et al., 2013), which is discussed in the next paragraph. These journals are predicted to publish more high-quality research. A dual-journal overlay shows how topics and journals are interrelated (Li et al., 2022). Although this research draws on the distribution of the cited articles, citing articles on *A. muciniphila* were more active in the "veterinary, animal, science," "molecular, biological, immunology," and "medicine, medical, genetics" fields.

Within *A. muciniphila* research, highly cited references or references with strong citation bursts are important nodes through which *A. muciniphila* has distinguished itself from other probiotics and has become a "next generation probiotic." Citations are a simple and effective indicator of the impact and quality of research. The article published in *PNAS* by Everard et al. (2013) has the highest citation frequency as well as a high mediation centrality, indicating that its content provides information on a currently relevant topic. This article proposes that live *A. muciniphila* can reverse high fat diet-induced metabolic disorder in mice by restoring mucus secretion and improving intestinal permeability, while heat-killed *A. muciniphila* lacks this effect. The article details an important milestone in our knowledge on the interaction between microbiota and the intestinal epithelium. The second most-cited article is a clinical research study published by Dao et al. (2016). The authors described the relationship between *A. muciniphila* and metabolism in overweight/obese adults after clinical dietary intervention. The article stated that *A. muciniphila* may be a potential prognostic tool for predicting the success of dietary interventions. The third most-cited paper was published by Plovier et al. (2017). This study identified a component of the *A. muciniphila* outer membrane protein-AMUC_1100 (which interacts with TLR2) that plays a role in reducing fat development, insulin resistance, and dyslipidemia in mice after pasteurization. This study proposed a solution for the unknown safety of *A. muciniphila* growth medium substances when ingested by humans. The most logical explanation for a sudden increase in the citation frequency of an article is that it addresses a specific lack of information in currently available literature (2003). The article with the strongest citation burst was the article published by Everard et al. (2013). The second-strongest citation burst was the randomized double-blind controlled study published by Depommier et al. (2019), which detailed the first human experimental results for *A. muciniphila* supplementation. This study indicated that *A. muciniphila* is safe and well-tolerated by patients; it also suggested that dead *A. muciniphila* bacteria may be more beneficial than live bacteria. The third strongest citation burst was that of an article published by Shin et al. (2014), which determined whether the antidiabetic effects of metformin were associated with changes in gut microbiota composition. It is evident from the changes in citation burst trends that the research hotspot had transitioned from the correlation between *A. muciniphila* and disease to the causal relationships between them, and from animal experiments to human studies of safety and efficacy.

Since keywords represent a publication's core content, keyword co-occurrence analysis (a method developed through bibliometric research and data visualization) can be applied to identify popular research topics in a particular field at a certain time. Our results showed the following four main research directions in the *A. muciniphila* field (Figure 2):

i. External factors affecting *A. muciniphila* (e.g., "diet," "polyphenols," "metformin," and "fecal microbiota transplantation"). In recent years, dietary strategies for improving gut *A. muciniphila* abundance have attracted research and development interest (Zhou, 2017). Although these promotion strategies are not necessarily applicable to the general population, these results strongly suggest the potential efficacy of certain foods or supplements for increasing intestinal *A. muciniphila* levels.

ii. The correlation between *A. muciniphila* and different diseases. The associations between *A. muciniphila* and metabolic disorders, including obesity, type 2 diabetes, nonalcoholic fatty liver disease, and cardiovascular diseases, are key to explaining many existing questions in *A. muciniphila* research. Highly cited references or references with strong citation bursts with respect to *A. muciniphila* research have all been related to metabolic disorders. For specific developmental milestones in research on *A. muciniphila* and metabolic disorders, please refer to the earlier discussion on references. In recent years, colitis and colorectal cancers have also attracted much attention. This could be attributed to the still-unclear relationship between *A. muciniphila* and colitis or colorectal cancers. Ring et al. (2019) demonstrated that *A. muciniphila* colonization does not affect colitis. This is somewhat different from the conclusions of an earlier study by Seregin et al., which reported that *A. muciniphila* can promote the occurrence of colitis in mouse models. Some studies have found that the abundance of *A. muciniphila* is increased in patients with colorectal cancer (Sanapareddy et al., 2012; Weir et al., 2013; Zackular et al., 2013; Dingemanse et al., 2015; Wang et al., 2022), whereas others have suggested that *A. muciniphila* is unrelated to colon tumors (Lopez-Siles et al., 2018) or even prevents colitis-associated colorectal cancer (Wang et al., 2020). Thus, the relationship between *A. muciniphila* and both diseases remains controversial. However, it is worth noting that most of the current studies have not distinguished between these microorganisms to the species level. To clarify the relationships between *A. muciniphila* and various diseases, this nuance should not be ignored in future clinical studies.

iii. Mechanisms underlying *A. muciniphila*–host (including bacteria) associations. Basic research focuses more on the biological mechanisms and potential therapeutic targets of *A. muciniphila*. The active components of *A. muciniphila* are still being clarified. Interestingly however, pasteurized *A. muciniphila*, live *A. muciniphila*, and even secreted proteins (e.g., Amuc_1100) and extracellular vesicles can regulate gut barrier function and/or the immune system by acting on different molecules. Regarding the effects of *A. muciniphila* on human health (e.g., "microbiota metabolites," "immune system," and "gut barrier function"), interested readers may wish to refer to previous detailed explorations of such topics (Yan et al., 2021; Rodrigues et al., 2022). The molecular mechanisms underlying these effects are an ongoing hot topic in the field. Recently, Bae et al. (2022) revealed that *A. muciniphila* induces immune cells to secrete specific cytokines *via* cell membrane phospholipids and resetting the activation threshold of dendritic cells, clarifying the molecular mechanism underlying *A. muciniphila*-mediated immune regulation *in vitro*. However, the molecular mechanisms underlying *A. muciniphila*–host interactions still require further research.

iv. The safety and efficacy of the clinical use of *A. muciniphila* (e.g., "double-blind") (Depommier et al., 2019).

Among the top 25 keywords with the strongest citation bursts, "oxidative stress" showed increasing strength. Oxidative stress is caused by imbalances between intracellular reactive oxygen species and antioxidant defense systems (Papadia et al., 2008), and is considered an important risk factor for cardiovascular diseases, diabetes, and other diseases. Research on oxidative stress mainly focuses on three aspects: promoting oxidative stress, fighting oxidative stress, and balancing the oxidative and antioxidant systems. Several studies suggested that *A. muciniphila* may be associated with oxidative stress regulation (Yassour et al., 2016; Roshanravan et al., 2017; Mitsou et al., 2019; Wu et al., 2020; Zhang et al., 2020; Deng et al., 2021; Mesnage et al., 2021; Chen et al., 2022) and may promote oxidative stress resistance in various diseases (Cerro et al., 2022; Qian et al., 2022; Xia et al., 2022). Polyphenols decrease intestinal oxidative stress by inducing *A. muciniphila* growth (Anhê et al., 2015). The relationship between oxidative stress and *A. muciniphila* is an important topic for future research.

Our study has some limitations. Firstly, since it takes time for an article to achieve a certain number of citations, recent high-quality articles may not have been included, causing biased results. Secondly, there may be a time delay when exploring the research frontier. Finally, our analysis can only show the influence of the research content in the *A. muciniphila* research field, and cannot represent influences outside this field.

Conclusion

We evaluated and quantified articles on *A. muciniphila* and visualized the hotspots and global research trends in this field. Over the past 19 years, publications on *A. muciniphila* have increased significantly in frequency, with China having the highest number of publications. De Vos and Willem was the most productive author and had the highest H-index, followed by Cani and Patrice. "Oxidative stress," "diet," "metformin," "fecal microbiota transplantation," "short-chain fatty acids," "polyphenols," and "microbiota metabolites" are some of the frequently used keywords in recent years. These keywords are potential hotspots for future research and require further exploration. Although studies consider

A. muciniphila to be a beneficial probiotic and has potential in the treatment of many diseases, providing an in-depth analysis of the mechanisms underlying its role in promoting human health with respect to high-frequency diseases may improve the research status of *A. muciniphila*.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

YC, ZL, QL, and PW: conceptualization. HK, YW, XL, and SC: data curation. HK and ZL: writing—original draft preparation. ZL, HK, YW, SC, and YC: writing—review and editing. YC, QL, and PW: supervision. YC: funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1037708/full#supplementary-material

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EDITED BY
Karolina Skonieczna-Żydecka,
Pomeranian Medical University,
Poland

REVIEWED BY
Tarique Hussain,
Nuclear Institute for Agriculture and Biology, Pakistan
Dafei Yin,
Shenyang Agricultural University,
China

*CORRESPONDENCE
Katarzyna Stadnicka
katarzyna.stadnicka@cm.umk.pl

†These authors have contributed equally to this work and share first authorship

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How to employ metabolomic analysis to research on functions of prebiotics and probiotics in poultry gut health?

Mengjun Wu[1,2†], Sanling Zuo[1†], Giuseppe Maiorano[2], Przemysław Kosobucki[3] and Katarzyna Stadnicka[1,4*]

[1]Department of Animal Biotechnology and Genetics, Faculty of Animal Breeding and Biology, Bydgoszcz University of Science and Technology, Bydgoszcz, Poland, [2]Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy, [3]Department of Food Analysis and Environmental Protection, Faculty of Chemical Technology and Engineering, Bydgoszcz University of Science and Technology, Bydgoszcz, Poland, [4]Department of Geriatrics, Ludwik Rydygier Collegium Medicum in Bydgoszcz Nicolaus Copernicus University, Torun, Poland

Gut health can be considered one of the major, manageable constituents of the animal immunity and performance. The fast spread of intestinal diseases, and increase of antimicrobial resistance have been observed, therefore the intestinal health has become not only economically relevant, but also highly important subject addressing the interest of public health. It is expected, that the strategies to control infections should be based on development of natural immunity in animals and producing resilient flocks using natural solutions, whilst eliminating antibiotics and veterinary medicinal products from action. Probiotics and prebiotics have been favored, because they have potential to directly or indirectly optimize intestinal health by manipulating the metabolism of the intestinal tract, including the microbiota. Studying the metabolome of probiotics and gut environment, both *in vivo*, or using the *in vitro* models, is required to attain the scientific understanding about the functions of bioactive compounds in development of gut health and life lasting immunity. There is a practical need to identify new metabolites being the key bioactive agents regulating biochemical pathways of systems associated with gut (gut-associated axes). Technological advancement in metabolomics studies, and increasing access to the powerful analytical platforms have paved a way to implement metabolomics in exploration of the effects of prebiotics and probiotics on the intestinal health of poultry. In this article, the basic principles of metabolomics in research involving probiotics and probiotics are introduced, together with the overview of existing strategies and suggestions of their use to study metabolome in poultry.

KEYWORDS

metabolomics, prebiotics, probiotics, gut health, poultry

Introduction

Intestinal health of poultry

It has been projected that the intensive animal production will grow continuously. By 2030, the consumption of poultry proteins is expected to increase by 15% rate in low income countries and by 25% in lower-middle income countries (OECD, FAO (2022)). Within this trend, 47% of the protein consumed from meat sources is expected to originate from poultry products. The immunity in poultry is tightly bound with optimal function of the gut and other systems within the organism that are biochemically connected with the intestine and it's microbiome. Most of the biological systems have a defined, conceptual bidirectional networks referred to as gut axes (gut-brain axis, microbiota-immune axis, neuro- immune axis, etc.). The spread of intestinal diseases and many other pathological conditions in animals, have their beginning in dysbiosis.

In recent years, the demand for poultry products as a high-quality and affordable protein source for most people has increased year by year. According to the latest data on Meat consumption, the consumption of Poultry meat is 33.0 Kilograms/capita (OECD Meat consumption, 2022).[1] However, the spread of enteric diseases has taken a financial toll on the global poultry industry. According to agricultural statistics in the early twenty-first century, broiler companies invested an average of $0.197 per broiler during the breeding process, but when payments to growers were included, they paid $1.15 per broiler (Clark et al., 2002). For example, the global economic loss caused by necrotizing enteritis has increased from 2 billion US dollars to 6 billion US dollars in 2015 (Zahoor et al., 2018). Meanwhile, food-borne diseases caused by Salmonella serovars and Campylobacter spp. can lead to food safety risks of zoonotic intestinal infections and increase economic losses (Hafez and Attia, 2020). It is associated with the known infectious agents (like *Salmonella*), but also with the emerging opportunistic pathogens including the isolates of enterococci, e.g., *Escherichia coli, Enterococcus cecorum, Enterococcus faecium*. Many of those species have potential to become vectors of antimicrobial resistance and potential threats to human and environment. An emerging danger and today challenges, had been accurately foreseen, over a decade earlier, at a time of implementing the regulations that put a ban on use of antimicrobial growth promoters (Yegani and Korver, 2008). Therefore, the challenges to identify and apply efficient strategies to naturally modulate the gut health and immunity are increasingly meaningful. Public investments and social demands for those challenges are being part of the European One Health Action Plan and the Farm to Fork Strategy, along with the regulation on the maximally restricted applications of the medicinal veterinary products and medical feed, which did come into force on 28th of January 2022 (European Parliament and of the Council (2019)).

Prebiotics (natural, indigestible dietary compounds that promote growth of probiotics) and probiotics (beneficial bacteria applied to the host animal and colonizing it's gut), play significant role in strategies to optimize the poultry intestinal health, especially in the intensive animal production. Prebiotics, probiotics, and the metabolites of their activity, including postbiotics, are applied to the animals at different developmental stages, in feed and in water, with an aim to modulate and improve the host immunity and maintain the health of intestinal tract (Jha et al., 2020).

Role of metabolomics in studying poultry gut health

Metabolomics has become an accessible and intensively used scientific study, and reveal metabolic composition and changes by examining small metabolites in various samples (Chung et al., 2018). The metabolome is a small-molecule intermediate in the metabolic process of biological systems, which has complex biologically meaningful regulation. For example, metabolomics can play a role in dietary assessment and identification of novel biomarkers of dietary intake (O'Sullivan et al., 2011), and studies of related metabolic profiles can be found in There is a lot of hypothetical role in future dietary assessments. While the metabolome reflects events downstream of gene expression, it is thought to be closer to the actual phenotype than proteomics or genomics. Słowińska et al. (2018) first applied metabolomics to identify metabolites that differentiate white and yellow turkey seminal plasma, differentially expressed metabolites involved in molecules and cells important for sperm physiology Function. Researchers can analyze the changes of related metabolic pathways from differences in metabolic profiles, such as those related to lipid, energy, and amino acid metabolic pathways, providing a line for the host's physiological and metabolic transitions (Afrouziyeh et al., 2022). Therefore, analysis of metabolites in body fluids (e.g., urine, serum), feces and intestinal tissues after taking probiotics can improve the understanding as to how the gut microbiota and gut metabolome change. The composition and changes of these metabolites could reflect the host's metabolic conditions and patterns, which help discover or interpret potential biological mechanisms (Cevallos-Cevallos et al., 2009; Mozzi et al., 2013).

Genomic (Zhang et al., 2014), transcriptomic (Xue et al., 2017) and proteomic (Simon et al., 2019) data of chicken have already been reported. However, only few detailed analyses of the chicken metabolome have been provided so far, especially in the gut stimulated by prebiotics and probiotics. In this review, basic principles and strategies in metabolomics of prebiotics and probiotics are presented, including nuclear magnetic resonance (NMR) and multiple MS-based analytical platforms for metabolomics. The review mainly focuses on the application of metabolomics approaches for the analysis of prebiotics and probiotics functions in poultry gut health.

1 https://data.oecd.org/agroutput/meat-consumption.htm

Main strategies and analytical techniques applied in metabolomics of prebiotics and probiotics

Non-targeted and targeted metabolomics

The two main metabolomic strategies include hypothesis-generating and hypothesis-testing metabolomics, which are also named "non-targeted-discovery-global" and "targeted-verification-tandem" metabolomics (Nalbantoglu, 2019; Figure 1).

Non-targeted strategy is a global metabolite screening method, that allows comprehensive scanning and pattern recognition of the metabolome. It is based on the exploratory, qualitative or semi-quantitative analysis, during which the unknown metabolic identities are screened as widely as possible, with no prior knowledge of these characteristics. The main purpose of this method is to obtain an overall overview of different types of metabolites and to determine the qualitative difference between the two sets of samples (Aszyk et al., 2018). It requires development of a protocol specific for the sample, and allows to obtain a high metabolome coverage, with the number of metabolites determined. In order to systematically identify and quantify metabolites from biological samples and achieve a comprehensive characterization of biomarker targets, this analysis may cover both endometabolome (intracellular) and exometabolome (extracellular). Metabolomic fingerprinting examines the global snapshot of the intracellular metabolome to determine a general profile and classify the ingested or produced metabolites, while the metabolomic footprint analysis explores the global snapshot of the extracellular fluid metabolome (changes in cell secretions or metabolites consumed by the outer metabolome). Fingerprint and footprint analysis involve rapid analysis and usually does not require any quantification of metabolites (Villas-Bôas et al., 2005). However, due to a lack of standards, the absolute concentrations of the analytes cannot be provided, which may lead to unreliable quantification and poor repeatability.

On the contrary, a targeted metabolomics, also known as "biased or directed metabolomics" or "metabolic analysis" is referred to known standards and focused on quantitative (concentration determination) or semi-quantitative (relative intensity value evaluation) analysis of specific, acknowledged molecules/metabolites or a subset of annotated metabolic pathways (Wang et al., 2010; Klassen et al., 2017). Therefore, the targeted analysis does not necessarily require additional, extensive work for data processing but the focus on specific metabolites (Zhou and Yin, 2016). Hypothesis testing strategy of targeted metabolomics is also used to validate the results from non-targeted analysis in practice (Zhang et al., 2016). One of the types of targeted analysis is metabolome profiling, which aims to analyze a small number of metabolites in order to study biological pathways. The use of stable isotope labels ensures accurate and reliable quantification of metabolites by compensating for ion suppression effects and controlling loss of the analyte. Main drawbacks of targeted analysis are inability to identify unknown

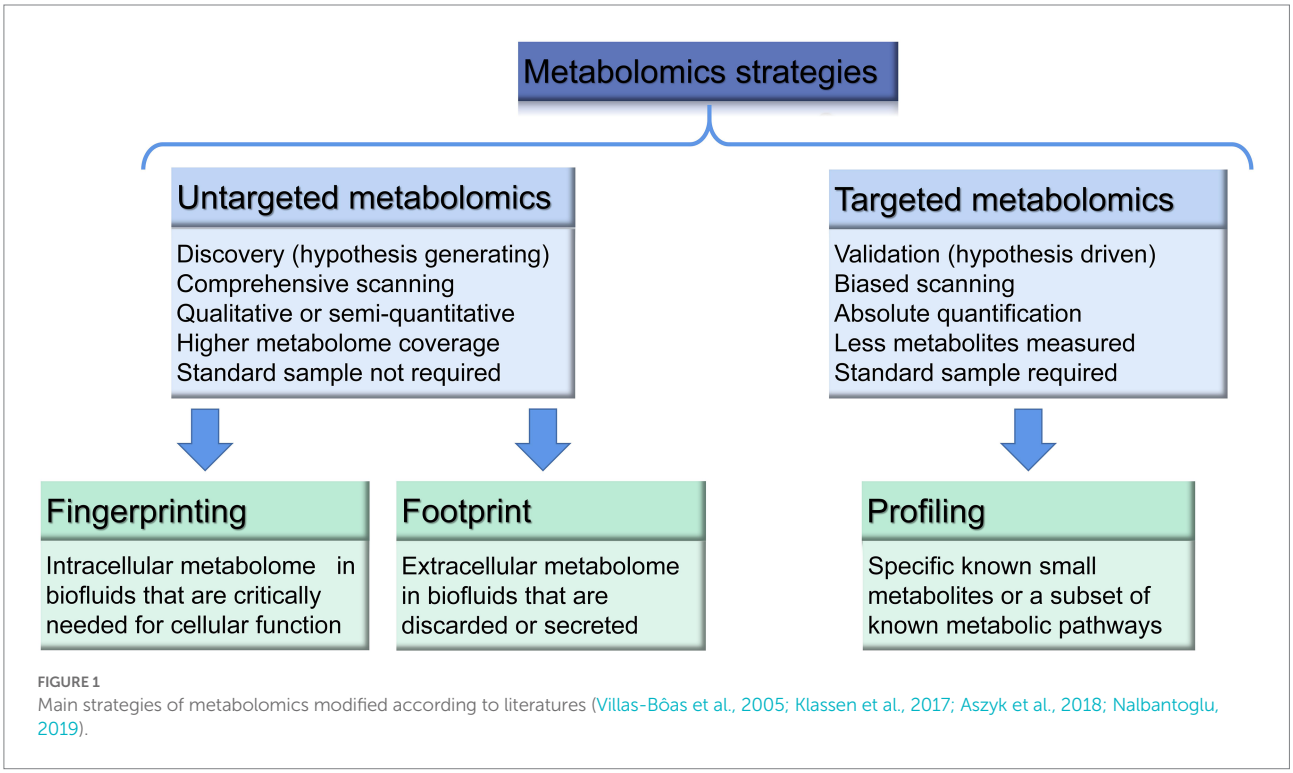

FIGURE 1
Main strategies of metabolomics modified according to literatures (Villas-Bôas et al., 2005; Klassen et al., 2017; Aszyk et al., 2018; Nalbantoglu, 2019).

metabolites, narrow range of stable isotope labeling and the high cost (Klassen et al., 2017).

Which metabolomic strategy to choose?

Non-targeted metabolomics to study function of probiotics and prebiotics

Recently, The International Scientific Association for Probiotics and Prebiotics has expanded the concept of prebiotics to include other types of compounds besides non-digestible carbohydrates, such as non-carbohydrate substances, polyphenols and certain fatty acids (e.g., polyunsaturated fatty acids), which has led to more attention put to non-targeted metabolome analysis (Bindels et al., 2015; Gibson et al., 2017; Spacova et al., 2020). There are reports on exercising non-targeted methods to obtain a comprehensive overview of altered metabolites, due to specific bioactivity of prebiotics or probiotics. E.g., production of specific bioactive metabolites was described in host organisms that utilized seaweed components as putative prebiotics (Cherry et al., 2019). In another study, the untargeted metabolomics was applied to explore probiotic survival and functionality of the bio accessible compounds in fermented camel and bovine milk after *in vitro* digestion (Ayyash et al., 2021). This method shows a discovery potential, but has also several shortcomings. Due to the large dynamic range of metabolites up to 7–9 orders of magnitude (Zhang and Powers, 2012) and sensitivity limitations, the simultaneous quantification of a large number of metabolites using MS is still challenging. If the sample contains numerous ion fragments with the same quality characteristics, unambiguous identification of bacterial metabolites may also pose a challenge. Although broad-spectrum metabolomics has a potential to reveal metabolites from the gut microbiota with an unprecedented resolution, compound quantification is extremely time-consuming, and left aside in some metabolomics programs (Klemashevich et al., 2014).

Feasibility of targeted metabolomics

On a contrary to drug mode of action, dietary interventions rarely have a potential to instantly block or "close" biochemical pathways or metabolic activities. Instead, they may modulate the rate of metabolite production. An accurate quantification is particularly important if subtle changes in metabolite levels are the aim of analysis. Advances in mass spectrometry (MS) instruments and methods have made the development of "targeted metabolomics" methods more accessible (Verbeke et al., 2015). With a pre-determined set of targets, it is possible to tailor extraction protocols and MS operating parameters for specific classes of metabolites to increase analytical sensitivity. E.g., phytase is one of the most common postbiotics applied in animal production, and by employing targeted metabolomics analysis, it was found as to how phytase affects specific metabolic pathways in broilers (Gonzalez-Uarquin et al., 2020). Recently, the metabolic profiling was applied in an interesting study of rapid differentiation of closely related *Lactobacilli* species. A triple quadrupole mass spectrometry (MS) was applied in combination with a linear ion trap-Orbitrap hybrid MS. The study is a good example of complementary capabilities of targeted and non-targeted metabolomics for compounds detection and their quantification in research involving closely related probiotic candidates (Yang K. et al., 2018).

Summarizing, adoption of a specific strategy, whether untargeted or targeted, depends on the scientific problem to be solved and the type of information that the researcher intends to obtain. The general workflow of metabolomics for prebiotics and probiotics applications in poultry is presented in Figure 2.

Analytical platforms employed in metabolomics of prebiotics and probiotics

Various analytical instruments have been successfully adopted to metabolomics (Table 1). Nuclear magnetic resonance (NMR) is one of the most commonly used analytical platforms in metabolomics in the past decades, due to its reliability and practicality in absolute quantification (Wishart, 2009). However, NMR is relatively insensitive and the measurement range is limited to micromolar- millimolar magnitude (μM-mM). Recent advancement in development of mass spectrometry platforms including Liquid chromatography coupled to Mass spectrometry (LC–MS), Gas chromatography coupled to Mass spectrometry (GC–MS), Capillary electrophoresis–mass spectrometry (CE-MS) and Ion-mobility spectrometry–mass spectrometry (IMS-MS) provide the possibility to detect metabolites from nanomolar (nM) to picomolar (pM) concentrations, greatly improving the metabolome characterization (Goldansaz et al., 2017). Due to complexity of gut health research, the mentioned platforms can be considered complementary, providing sensitivities applicable to different molecular classes.

NMR

Nuclear magnetic resonance is a quantitative, robust and reliable technique that can be used to analyze molecular structures in biological samples, which requires minimal sample preparation, therefore relatively high-throughput analysis can be performed with this technique (Rzeznik et al., 2017). This is important for metabolomic analysis of large cohorts in animal studies. Another advantage of using NMR-based methods is that the technique is non-destructive, thus biological fluids can be preserved and allow further analysis. NMR-based metabolomics provides both structural and quantitative information, which is of great help for identifying unknown metabolites, the main bottleneck of metabolomics. NMR can simultaneously identify and quantify from dozens to hundreds of metabolites, with a detection limit of 1 μM, and has been used to characterize biological fluids in the past few decades (Emwas et al., 2019).

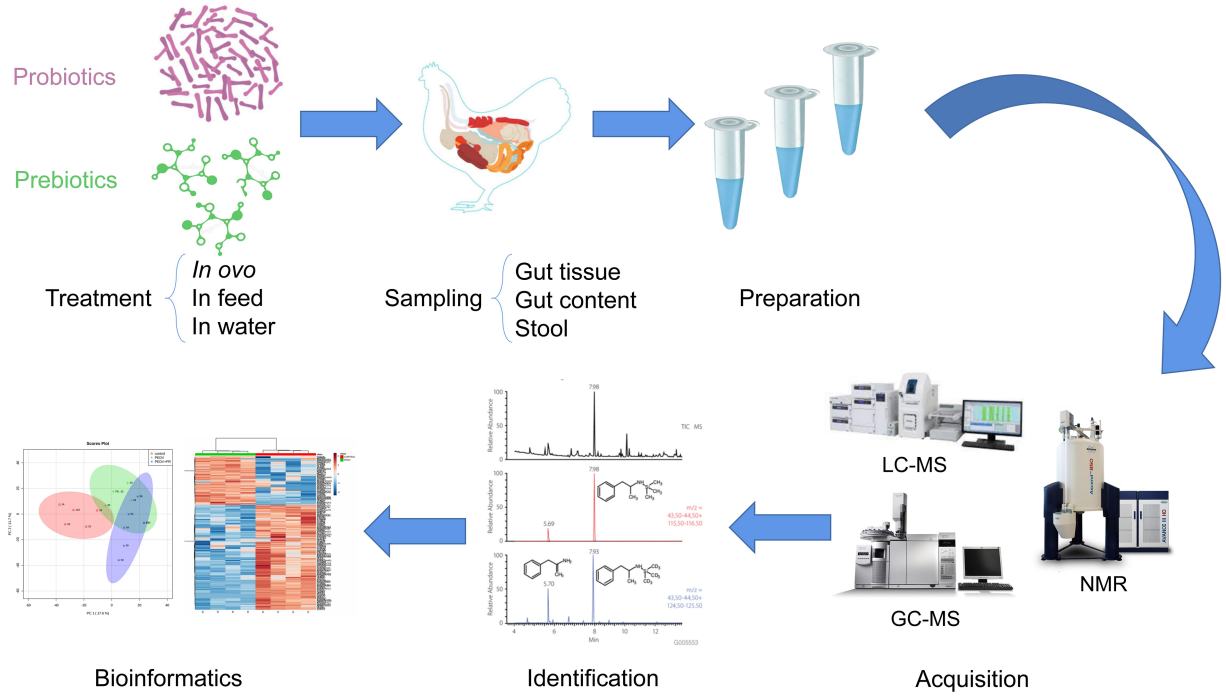

FIGURE 2
The general workflow of metabolomics analyses to study the effects of treatments with prebiotics and probiotics, in poultry.

Despite these advantages, it must be acknowledged that NMR-based metabolomics has many limitations. Compared with mass spectrometry-based methods, the most critical limitation is the low sensitivity of this method. Despite recent advances in instrumentation, the sensitivity is still lower than that of mass spectrometry-based methods (Brennan, 2014). NMR-based metabolomics has obvious advantages in tissue metabolomics because ^{1}H high-resolution magic angle rotation (HRMAS) can be used for direct sample analysis (Beckonert et al., 2010).

In monogastric animals, a study employed ^{1}H NMR spectroscopy (HRMAS) to assess the effects of mouse supplementation with *Lactobacillus paracasei*, demonstrating the importance of the transgenomic, metabolic interactions between *L. paracasei* and the host to modulate the gut function, including amino-acid metabolism, methylamines and SCFAs (Martin et al., 2007, 2008). In another study, researchers used ^{1}H NMR spectroscopy to characterize various tissues (including the intestine) of chicken followed by metabolite identification. In this work, around 80 metabolites were identified and utilized to develop the first chicken metabolome atlas among which only eight metabolites were found to be common for all tissue samples (Le Roy et al., 2016).

LC–MS

The great ability of LC to separate different compounds, from highly polar to extremely non-polar compounds, is attributed to many chromatography columns with a variety of available stationary phases (Kuehnbaum and Britz-Mckibbin, 2013). Reversed-phase chromatography and normal-phase liquid chromatography (NPLC) are traditional standard tools for the separation of non-polar, medium- polar and polar-analytes, respectively (Bieber et al., 2016; Grün and Besseau, 2016). The samples from animal like poultry contain highly polar compounds (amino acids) as well as highly hydrophobic compounds (phospholipids). Therefore, if the strategy of the research is set up for targeted metabolomics, the stationary phase can be selected according to the type of compound of interest. However, in non-targeted metabolism research, a persistent and difficult problem is that none of the current methods can comprehensively analyze all of the metabolites with different structures in a single separation. Recently, some newly developed methods like Ultra Performance Liquid Chromatography (UPLC) and hydrophilic interaction chromatography (HILIC), improved productivity and metabolome coverage (Lopes et al., 2017; Gika et al., 2019). However, if the goal is to obtain as much information as possible, more than a single type of column may be required (Rainville et al., 2014).

To meet requirements of a high resolution, rapid data acquisition and high accuracy (typically <5 ppm), the quadrupole-time-of-flight (Q-TOF) mass spectrometer, Linear trap quadrupole-Orbitrap (LTQ-Orbitrap) and Fourier transform ion cyclotron resonance (FT-ICR; Park et al., 2020) have been developed and are the most commonly employed platforms in non-targeted analysis. Instead, other mass analyzers characterized by high sensitivity and selectiveness, such as triple quadrupole (QQQ) or triple quadrupole-linear ion trap [QqQ (LIT)] mass spectrometers, are primarily dedicated to targeted analyses (Nagana Gowda and Djukovic, 2014). Due to a wide range of

TABLE 1 Brief comparison of different metabolomic technologies.

Technology	Advantages	Disadvantages
NMR	Requires minimal sample preparation	Low sensitivity
	High-throughput analysis	Few numbers of metabolites
	Robust, reliable and no discriminating	Quantification challenging
	Non-destructive and direct sample analysis	
GC–MS	Mature technology	Requires sample derivatization
	Cost friendly	Long time for sample acquisition
	High reproducibility	Unable to produce parent ions
	Suitable for the detection of volatile compounds with universal databases	Difficult to identify novel compound
LC–MS	High sensitivity	Comparatively expensive
	Simple sample pretreatment	Lower reproducibility
	Wide coverage of metabolite detection	Not compatible with volatiles
	Relatively short time for sample analysis with sub-2 mm stationary phase particles	Novel compound identification is difficult

metabolites detectable at high resolution with R(U)PLC–MS, it has been employed for the non-targeted metabolomics of poultry intestines. Recently, the effect of dietary supplementation with *Bacillus subtilis* direct-fed microbials on chicken intestinal metabolite levels was described based on UPLC-MS global metabolomic profiling (Park et al., 2020).

GC–MS

Gas chromatography–mass spectrometry (GC–MS) is one of the widely used metabolomics platforms, covering both untargeted and targeted analysis. The basis of GC consists to separate volatile metabolites (or with increased volatility due to chemical derivatization), and thermally stable metabolites. GC–MS is less sensitive than LC–MS, but is generally more robust and more reproducible. Therefore, GC–MS has the potential to identify and quantify the metabolome with a higher precision and reproducibility than LC–MS (Goldansaz et al., 2017). However, unlike LC, GC typically requires chemical derivatization of the metabolic species prior to the GC–MS analysis (Nagana Gowda and Djukovic, 2014).

GC–MS is capable of analyzing less polar biomolecules involving alkyl silyl derivatives, essential oils, esters, terpenes, volatiles, carotenoids, flavonoids, and lipids, etc. Among these molecules, Volatile organic compounds (VOCs) such as fatty acids and organic acids which are important biomarker candidates in biological samples can be successfully identified by GC–MS (Nalbantoglu, 2019).

GC-TOF-MS was also commonly used to study poultry intestinal fecal metabolomics. E.g., researchers employed caecal metabolomic profiling to explore the effect of early inoculation of caecal fermentation broth, on small intestine of broilers (Gong et al., 2020). Another work adopted metabolomic analysis to study the effect of *Pediococcus acidilactici* BCC-1 and xylan oligosaccharides, in broiler chickens (Wu et al., 2021a).

Metabolome databases and analytical pipelines with relevance to poultry species

Metabolites are identified through in-house developed, or commercial databases, such as Fiehn RTL library, MassBank, HMDB, Metlin, NIST, XCMS, Metaboanalyst, Progenesis, MetaCore, and 3Omics, etc., which are summarized in Table 2. For example, the advantage of the Fiehn library is that it contains retention index and information on retention time of the solutes, which can be compared with experiments performed according to the same analysis method (Kind et al., 2009). However, The NIST database does not contain information provided by the TOF analyzers, the high-resolution mass spectrometry; therefore, more verification steps need to be taken in data processing (Peralbo-Molina et al., 2015). Identification of metabolites also can be used in vendor software: XCalibur, MassLynx, Analyst, MassHunter, Chemstation, or Compass (Vinaixa et al., 2016).

In addition, free available software bioinformatics analysis tools available on the market can automatically perform peak selection, evaluation, and relative quantification processing, and connect the results to the metabolite database. Subsequently, data preparation workflow includes data integrity checking, data standardization, and compound name recognition (Cambiaghi et al., 2017), and further, function interpretation, enrichment analysis, pathway analysis, and metabolite pathway network diagram (KEGG, REACTOME, IPA, etc.).

General data processing and bioinformatics analysis in metabolomics

Data preprocessing

Common data analysis methods in metabolomics are illustrated in Figure 3, which also includes some popular and widely used bioinformatics analysis platforms. The first step of data analysis is data preprocessing. For example, on one of the most popular analytics platforms, MetaboAnalyst[2], data integrity

2 https://www.metaboanalyst.ca/

TABLE 2 Comparison of commonly used metabolome databases.

Database	No. Records	Spectra	Metabolic pathway	Structural information	Free access	Website
NIST chemistry WebBook	31,000 compounds	MS	×	√	√	http://webbook.nist.gov/chemistry/
Golm metabolome database (GMD)	2,222 metabolites	MS	×	√	×	http://gmd.mpimp-golm.mpg.de/
Human metabolome database (HMDB)	217,920 metabolites	MS, NMR	√	√	×	http://www.hmdb.ca/
Kyoto encyclopedia of genes and genomes (KEGG)	18,920 metabolites and other small molecules	×	√	√	×	http://www.genome.jp/kegg/
Metabolite and tandem MS database (METLIN)	960,000 compounds	MS	×	√	√	http://metlin.scripps.edu/index.php
Small molecule pathway database	30,000 small molecule pathways	×	√	√	×	https://www.smpdb.ca/
Chemical entities of biological interest (ChEBI)	60,094 compounds	×	√	√	×	http://www.ebi.ac.uk/chebi/
Spectral data base (SDBS)	34,600 compounds	MS, NMR	×	√	×	http://sdbs.db.aist.go.jp/
BioCyc	20,005 pathways	×	√	√	√	http://biocyc.org
Reactome	11,291 proteins	×	√	√	×	http://www.reactome.org/
Livestock metabolome database (LMDB)	1,202 metabolites	MS	×	√	×	https://lmdb.ca/

checking includes data checking and data filtering. Data check mainly checks whether the data format is correct, whether the classification labels are correct (at least three biological replicates are required for each group), whether it contains non-numeric data, and whether it contains missing values and indicators that are always 0. Based on this information, the basic situation of the data can be obtained, and the missing value will be replaced by a smaller value by default. Of course, MetaboAnalyst also provides more advanced programs/algorithms to deal with missing values (Xia and Wishart, 2016).

In metabolome or proteome datasets, some of the variables are caused by baseline noise and are not available in data modeling and analysis. Generally speaking, they have the following characteristics: (1) Minimal values (values near the baseline or detection limit); (2) constant values (values that do not vary with experimental conditions); (3) variables with poor reproducibility. This part of the data can be removed through data filtering functions (Chong et al., 2019).

Commonly, sample normalization can highlight the characteristics of the sample. Data conversion and data normalization mainly limit the data to a certain range, which makes subsequent analysis more convenient, and the convergence of program operation is accelerated or obeys the distribution of certain characteristic functions, so as to analyze the characteristics of the model (Pang et al., 2022). Log transformations are often employed in the metabolome and proteome (Klein, 2021).

Statistical analysis

Normally, statistical analysis in metabolomics of poultry usually includes unary analysis, multivariate analysis, clustering and classification analysis, variable selection, and feature selection. However, the latter two methods are less used and not discussed here.

Among unary analysis, fold change analysis and volcano plot, are typically used, to intuitively shows the difference effect (Ma et al., 2022). ANOVA and correlation analysis are also common used in analysis. Generally, differentially expressed metabolites, the possible candidate of biomarkers, are found at this step. On the other hand, more complicated multivariate analysis, regularly contains principal component analysis (PCA), partial least squares - discriminant analysis (PLS-DA), sparse partial least squares - discriminant analysis (sPLS-DA), orthogonal partial least squares - discriminant analysis (orthoPLS-DA). Unsupervised learning PCA is primarily used to discern whether there are inherent similarities and to identify possible outliers in a dataset (Saha et al., 2016). PLS-DA works well with a larger number of features than objects. For instance, an article explore changes in the metabolites of broilers supplemented with butyrate glycerides in the diet (Yang X. et al., 2018). Benefiting from the advantages of

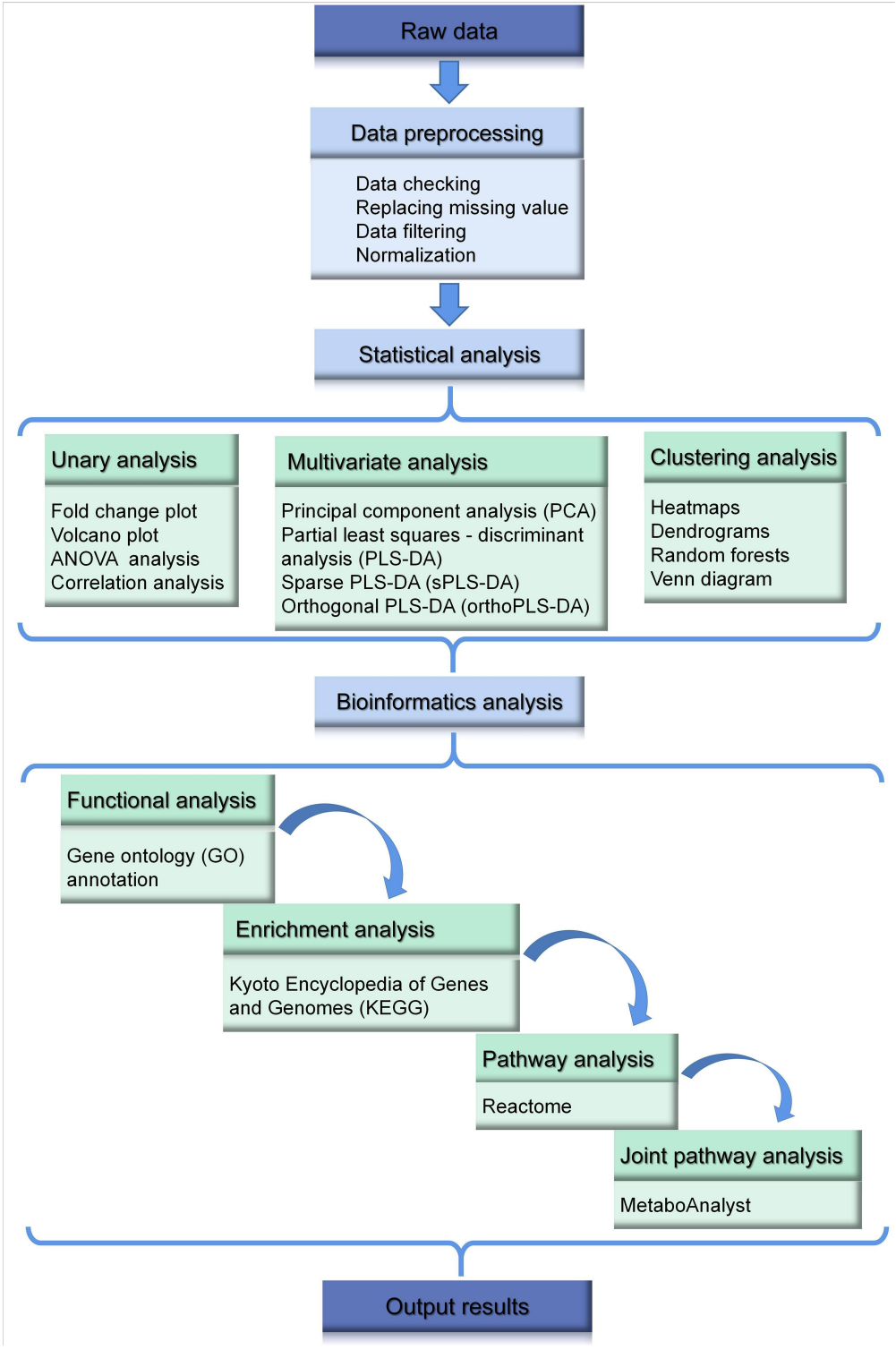

FIGURE 3
General data processing and bioinformatics analysis in metabolomics.

supervised learning, PLS-DA and orthoPLS-DA show a more pronounced difference than PCA. Nevertheless, even PLS are prone to fall into overfitting if the number of PLS components included in the model is larger than necessary (Liu et al., 2019).

Heatmaps and dendrograms are mostly performed during hierarchical clustering, to show the distinction of samples or/and the trend of quantities of metabolites between different samples (Wu et al., 2020). For example, a heat map was utilized to visualize

the abundance of the differential metabolites in poultry treated with Galacto-oligosaccharides and xylo-oligosaccharides (Yang et al., 2022). Random forest analysis (RFA), as a supervised classification, was performed to identify metabolite signatures and the biochemical significance of the most notably altered metabolites (Park et al., 2020). The method could make biochemicals listed from bottom to top in increasing order of importance for contributing to the biochemical signatures separating the two treatment groups. In addition, venn diagrams of metabolites reveal metabolites that are commonly or uniquely regulated across groups under different conditions (Jia et al., 2020).

Bioinformatics analysis

Gene Ontology (GO, Gene ontology)[3] and Kyoto Encyclopedia of Genes and Genomes Database (KEGG, Kyoto Encyclopedia of Genes and Genomes Pathways)[4] are two classic and commonly used biomedical resource databases that provide biological function, location and pathway information of genes in multiple species. Enrichment analysis can characterize the most significantly involved metabolic terms. In addition, some other biological pathway databases such as Reactome are also an important part of the biological information database and could help to identify top-altered pathways. Recently, the research module of bioinformatics also includes joint pathway analysis, which combines data mining and biomedical research, finally could predict candidate key genes (Maity et al., 2021).

Application of metabolomics to explain function of bioactive substances

Metabolome characteristics under stressful conditions

In the commercial farming model, the intestinal health of poultry is very important, and many diseases can lead to the imbalance of intestinal homeostasis and thus affect the health and performance of chickens. In poultry farming, immunosuppressive diseases are caused by different diseases of the body's immune response, affecting abnormal daily feed intake, feed conversion ratio, body weight gain, poor egg production and mortality (Li et al., 2022). *Salmonella enterica* serotype *Salmonella enteritidis* is a typical representative of non-host-specific Salmonella found in poultry, mainly through the fecal-oral route, can cause intestinal inflammation and barrier dysfunction in chickens, and has a significant impact on the poultry industry. When the feeding conditions are not good, under high temperature conditions, birds alter their behavior and physiological homeostasis to seek thermoregulation, thereby lowering their body temperature. Heat stress alters neuronal secretion profiles in birds by reducing feed intake and activating the HPA axis, thereby impairing overall poultry and egg production. Differences in the metabolome and changes in associated metabolic pathways under disease compared to normal are shown in Table 3. The latter described prebiotics and probiotics have a positive feedback on poultry metabolism.

Metabolites of probiotics

Probiotics are externally delivered microorganisms that colonize the intestines and exert positive health effects in the host organism, through changes in genes expression, modulating the function of immune system, and increasing resilience against environmental stressors. The beneficial functions of metabolites produced by probiotics in poultry gut were summarized in Figure 4. The examples of probiotics are: *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Bifidobacterium*, *Enterococcus faecalis* and *Clostridium butyricum*. There are numerous evidences for the key roles of metabolites produced by probiotics in contact with intestine cells, referred to as postbiotics, metabiotics (Nicholson et al., 2012). The biochemical mechanisms at the bottom of the improved gut (health) include various effects of probiotic activities leading to lower pH value in the intestines, improved absorption of calcium, iron, and vitamin D, and enhanced synthesis and absorption of multiple vitamins in the body. Some probiotics support the production of host short-chain fatty acids, cholic acids, phenols and many other metabolites, all of which are closely related to the normal or improved intestinal function, permeability and immunocompetence (Table 4). Complex carbohydrates are fermented by microorganisms in the colon into short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate, which belong to the most important products of microbial metabolism. Choline is an essential dietary nutrient, metabolized mainly in liver. Intestinal microbial enzymes can catalyze the conversion of choline into trimethylamine, which is further oxidized in the liver to produce trimethylamine N-oxide, a marker metabolite related to liver and cardiovascular diseases (Schugar and Brown, 2015). Secondary bile acids can control specific host metabolic pathways, participate in intestinal immune regulation and metabolic regulation through G protein-coupled receptors, and affect the composition of the microbial community (Vavassori et al., 2009).

For example, researchers using the online software MetaboAnalyst (version 4.0)[5] to study the effect of supplemented diets with *Bacillus subtilis* in broilers and found that it altered overall gut metabolite levels. Among these metabolites, 25 compounds significantly increased and 58 compounds significantly increased ($p < 0.05$). Pathway analysis were based on significantly different metabolites. From amino acid metabolite analysis, leucine was significantly increased, allyl alcohol (Ala-Leu), glutamyl leucine (Gln-Leu), valine (Val-Leu) and glycyl leucine (Gly-Ile) levels have

3 http://www.geneontology.org
4 http://www.genome.ad.jp/kegg
5 http://www.metaboanalyst.ca/

TABLE 3 Metabolome characteristics under diseases.

Disease	Different stages	Samples	Significantly different metabolites compared to normal	Related metabolic pathways	References
Immunosuppression	Broilers	Cecal contents	2-Ketoglutaric acid		Li et al. (2022)
			Beta-glutamic acid	Cyanoamino acid metabolism	
			4-Hydroxyphenylacetic acid	Cystenie and methionine metabolism	
			Fructofuranose	Strach and sucrose metabolism	
			Gluconic acid	Glycerolipid metabolism	
			Glycyl-leucine	Aminoacyl-tRNA biosynthesis	
Salmonellosis Enteritidis	Neonatal chickens	Cecal contents	LysoPE(0:0/16:0)		Mei et al. (2021)
			3-Oxohexadecanoic acid		
			Methamphetamine	Arginine metabolism	
			Anandamide	Proline metabolism	
			Phosphocholine	Lysine biosynthesis	
			Deoxycholic acid	Lysine degradation	
			Lithocholic acid	D-Glutamate metabolism	
			L-Arabito		
Heat stress (HS)	Broilers	Plasma		Glyoxylate and dicarboxylate metabolism	Sutton (2021)
			Fumaric acid	Aspartic acid and glutamate metabolism	
			Ribitol	D-glutamine and D-glutamate metabolism	
			Succinic acid	Glycine, serine and threonine metabolism	
			Uric acid	Phenylalanine, tyrosine, and tryptophan biosynthesis	
			Mucic acid	Starch and sucrose metabolism	
			Alpha-ketoplutaric	Linoleic acid metabolism	
			2-hydroxyvaleric		
Immune stress	Broilers	Plasma	Phenyllactic acid		Bi et al. (2022a)
			3-Phenylpropanoic acid	mTOR signaling pathway	
			4-Hydroxycinnamic acid (L-phenylalanine methyl ester) amide	Apoptosis	
			Alpha-ketoglutarate	Valine, leucine, and isoleucine biosynthesis	
			N-Acetylmannosamine	Valine, leucine, and isoleucine degradation	
			Glutaric acid	Pantothenate and CoA biosynthesis	
			Alpha-ketoglutarate	Aminoacyl-tRNA biosynthesis	

(Continued)

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TABLE 3 (Continued)

Disease	Different stages	Samples	Significantly different metabolites compared to normal	Related metabolic pathways	References
Fatty liver hemorrhagic syndrome (FLHS)	Laying hens	Liver	Cytidine	Glycerophospholipid metabolism	Meng et al. (2021)
			Isomaltose	Tryptophan metabolism	
			Lysophosphatidylcholine (LysoPC) (14:0)	ARA metabolism	
			1-palmitoylglycerol	Tyrosine metabolism	
			Glutathione	Galactose metabolism	
			Lactate	Starch and sucrose metabolism	
			Glutaric acid	Biosynthesis of unsaturated fatty acids	
			Pyruvaldehyde	Phenylalanine, tyrosine and tryptophan biosynthesis	
			Tyrosine	linoleic acid metabolism	
			Uric acid	Pyruvate metabolism and glutathione metabolism	
			Arachidonic acid		
Immune stress	Broilers	Liver	5-Methylcytidine	Amino acid metabolism (valine, leucine and isoleucine biosynthesis, biosynthesis of amino acids, histidine metabolism, glycine, serine and threonine metabolism)	Bi et al. (2022b)
			(R)-3-Hydroxybutyric acid	glycerophospholipid metabolism	
			Carbofuran		
			Glycerophsphocholine		
			AICAR		
			But-2-enoic acid	Glycan metabolism (mucin type O-glycan biosynthesis, mannose type O-glycan biosynthesis)	
			Methylsuccinic acid		
			Citicoline		
			PC(18:1/14:0)	Intestinal immune network for IgA production	
			2,6-Dimethylpyrazine	Apoptosis	
			Pryruvic acid	Mannose type O-glycan biosynthesis	

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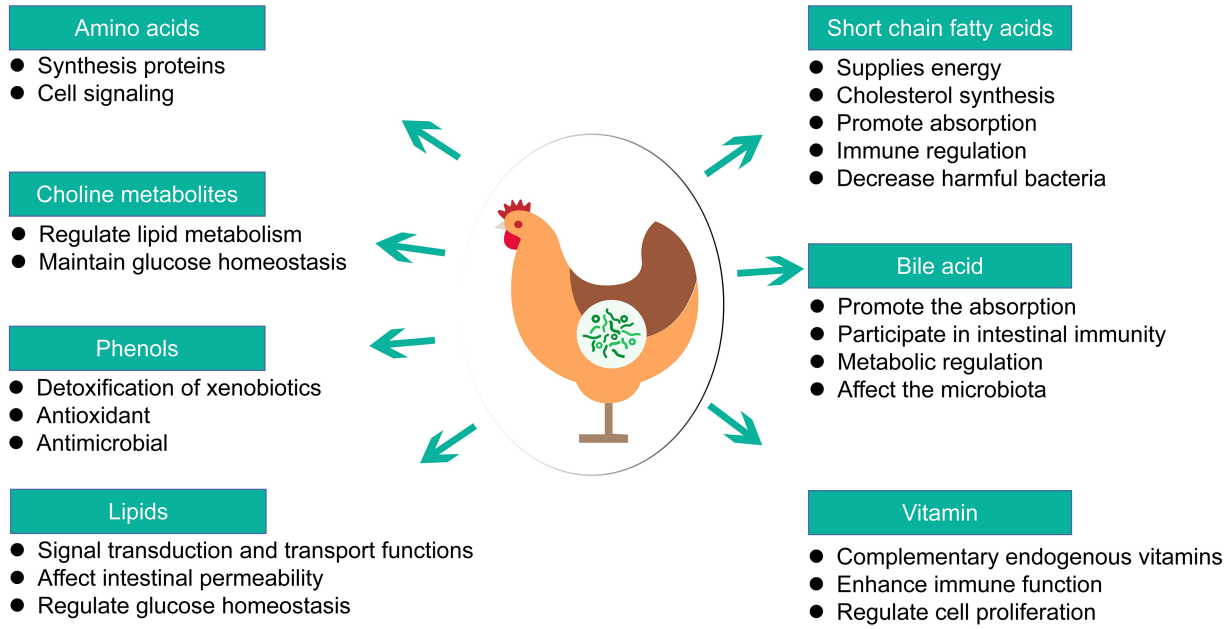

FIGURE 4
Overview of beneficial functions of metabolites produced by probiotics in poultry gut.

roughly doubled. From the carbohydrate metabolism analysis, fructose levels increased and lactate levels decreased. Among them, glutamic acid and glutamine are mainly involved in the metabolism of carbohydrates and amino acids. As important synthetic precursors, they can promote the proliferation and maintenance of immune cells such as lymphocytes, and have an important immunomodulatory effect. Significant differences in lipid metabolism include fatty acids such as sebacate, valerylglycine, linoleoylcholine, and others. Lipid metabolites are sensed by G protein-coupled receptors (GPRs), which are present on epithelial cells and macrophages, and associated with cytokines and tight junction proteins, suggesting a role in the regulation of inflammation in the gut and the epithelium Cells are stable. The above shows that Bacillus subtilis, a probiotic, alters significantly differential metabolites in the gut, affecting amino acid, carbohydrate and fatty acid metabolism, which can be used to maintain the stability of intestinal epithelial cells and immune cells (Park et al., 2020).

Metabolites of prebiotics' bioactivity

Prebiotics refer to as non-digestible food components, most of which cannot be digested when passing through the digestive tract, and are used as substrates by the normal intestinal flora. Prebiotics can selectively stimulate the growth and activity of one or several specific intestinal bacteria. The most important thing is that it only stimulates the growth of beneficial bacteria, not harmful bacteria with potential pathogenicity or spoilage activity. Prebiotics increase the number of beneficial bacteria in the intestinal tract and prevent the inflammatory reaction caused by the invasion and colonization of the intestinal mucosa by *aerobic Enterobacteriaceae,* which are emerging opportunistic pathogens.

The common oligosaccharides of proven prebiotic functions, are inulin, fructooligosaccharides, galacto-oligosaccharides, isomalto-oligosaccharides and lactulose. Among them, functional oligosaccharides are the most important and the most studied type of prebiotics. Prebiotics can stimulate the growth and activity of sugar-utilizing bacteria (including *Bifidobacteriaceae* and *lactic acid bacteria*) and promote the release of organic acids. These organic acids create an antibacterial environment and inhibit the growth of intestinal pathogens. Organic acids, such as short-chain fatty acids such as acetic acid, lactic acid, propionic acid, isobutyric acid, and butyric acid, help to increase the utilization of calcium, phosphorus, and iron, promote the absorption of iron and vitamin D, acidify the intestine and reduce the abnormal fermentation caused by harmful bacteria make it difficult for the growth of pathogenic and deteriorating bacteria and consequently reduce the production of toxic compounds such as ammonia, hydrogen sulfide, indole and skatole in the metabolites of spoilage bacteria (Markowiak-Kopeć and Śliżewska, 2020).

Prebiotics selectively stimulate beneficial bacteria in the intestines and release volatile short-chain fatty acids, which lowers the pH of the intestines, making it more difficult for harmful bacteria to survive. Such changes in intestinal flora can reduce the release or expression of inflammatory transmitters, reduce disease activity index and improve intestinal mucosal damage caused by intestinal inflammation. Moreover, prebiotics can regulate the immune system of the intestine through the release or formation of organic acids, and the bacterial cell wall or cytoplasm that interacts with immune cells. The intake of oligosaccharides

TABLE 4 Metabolites produced by probiotics.

Type	Metabolites	Potential biological function	Related probiotics	References
SCFA	Acetate, propionate,butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate, hexanoate	It supplies energy for epithelial cells, participates in cholesterol synthesis, regulates the absorption of water and sodium, participates in microbe-brain-gut axis, and immune regulation.	*Bacillus subtilis; Faecalibacterium, Campylobacter jejuni*	Park et al. (2020) Samuel et al. (2008) Nothaft et al. (2017)
Lipids	Conjugated fatty acids, LPS, peptidoglycan, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides	Affect intestinal permeability, activate the brain-hepatic nerve axis in the intestine to regulate glucose homeostasis; lipopolysaccharide induces chronic systemic inflammation.	*Bifidobacterium, Lactobacillus, Enterococcus faecalis*	Nicholson et al. (2012) Serino et al. (2012) Zhang et al. (2019)
Phenolic, benzoyi and phenyl derivatives	Benzoic acid, hippuric acid, 2-hydroxyhippuric acid, 2-hydroxybenzoic acid, 3-hydroxyhippuric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylpropionate, 4-hydroxyphenylpropionate, 3-hydroxycinnamate, 4-methylphenol, tyrosine, phenylalanine, 4-cresol, 4-cresyl sulfate, 4-cresylglucuronide, 4-hydroxyphenylacetate, 3,4-dihydroxyphenylacetate, phenylacetylglycine, phenylacetylglutamine, phenylacetylglycine, phenylacetate, phenylpropionate, phenylpropionylglycine, cinnamoylglycine	The detoxification of xenobiotics indicates the composition and activity of intestinal microbes, using polyphenols.	*Bifidobacterium, Lactobacillus*	Zheng et al. (2011)
Bile acid	Cholate,hyocholate, deoxycholate, chenodeoxycholate, a-muricholate, b-muricholate, w-muricholate, taurocholate, glycocholate, taurochenoxycholate, glycochenodeoxycholate, taurocholate, Tauro-a-muricholate, tauro-b-muricholate, lithocholate, ursodeoxycholate, hyodeoxycholate, glycodeoxylcholate, taurohyocholate, taurodeoxylcholate	Promote the absorption of lipids and fat-soluble vitamins, participate in intestinal immunity and metabolic regulation, and affect the composition of the microbial community.	*Lactobacillus, Bifidobacteria, Enterobacter, Bacteroides, Clostridium*	Nicholson et al. (2012)
Choline metabolites	Methylamine, dimethylamine, trimethylamine, trimethylamine-N-oxide, dimethylglycine, betaine	Regulate lipid metabolism and glucose homeostasis.	*Faecalibacterium prausnitzii, Bifidobacterium*	Wang et al. (2011) Martin et al. (2010)
Vitamin	Vitamin K, vitamin B12, biotin, folate, thiamine, riboflavin, pyridoxine	Provide complementary sources of endogenous vitamins, enhance immune function, and exert epigenetic effects to regulate cell proliferation.	*Bifidobacterium*	Said (2011)

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TABLE 5 Intestinal microbial metabolites with prebiotics.

Type	Metabolites	Potential biological function	Related prebiotics	References
SCFA	Acetate, propionate, butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate, hexanoate	Make the intestinal pH drop, more harmful bacteria difficult to survive.	Dietary fibers, Inulin, fructo-oligosaccharide, galactooligosaccharide	Dunkley et al. (2007) Lei et al. (2012) Ma et al. (2022)
Organic acids and derivatives	L-lysine, L-arginine, L-methionine, L-phenylalanine, L-histidine, L-proline, L-valine and L-citrulline	Amino acids are not only precursors of metabolic proteins, but also involved in cell signaling	Fructo-oligosaccharide	Said (2011)
Lipids	Glycerophospholipids, stearidonic acid, montecristin, cohibin C, cohibin B, DG (18:0/18:4/0:0), DG (18:3/18:3/0:0), l-hexanoylcarnitine, arachidyl carnitine, prenol lipid	PC and PE are the most abundant phospholipids in cell membrane. Glucophospholipid has a wide range of signal transduction and transport functions. Glycerophospholipids are precursors of lipid mediators in signal transduction.	Fructo-oligosaccharide	Said (2011)
Phenylpropanoids and polyketides	Gerberinol and dicoumaroylspermidine, biochanin A and daidzein, dihydroxybenzoate	Phenylpropanoids and polyketides have a variety of effects, including antioxidant (Jia et al., 2020), antimicrobial (Kępa et al., 2018) and anti-inflammatory (Doss et al.,2016).	Fructo-oligosaccharide	Said (2011)

increases the relative abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while the relative abundance of harmful bacteria such as *Escherichia* is reduced.

Therefore, most of the current prebiotic studies have focused on determination of the concentration of short-chain fatty acids as an important targeted metabolic test (Dunkley et al., 2007; Lei et al., 2012). The intestinal microbial metabolites involved in prebiotics which commonly used are summarized in Table 5. Fang Ma et al. found that the chicks fed with fructo-oligosaccharide (FOS) added to the feed had 93 significantly different metabolites compared with no addition, and classified them into the following 8 categories: organic heterocyclic compounds, nucleosides, nucleotides and analogs, phenylpropionic acid and polyketides, benzenes, organic oxygen compounds, organic acids and their derivatives, lipids and lipid molecules, organic nitrogen compounds (Ma et al., 2022). Differential metabolites were analyzed using one-way ANOVA and Tukey's test. Each differential metabolite was cross-linked to a pathway in KEGG[6], and using scipy.stats (a Python package)[7] and use a metabolic profiler to identify the most important altered pathways and finally build. Studies have found that fructooligosaccharides(FOS) have a significant effect on the expression levels of organic matter and its derivatives, such as L-lysine, L-methionine, L-valine, L-histidine and so on. Most of these metabolites are enriched in the biosynthesis of amino groups. Amino acids are not only precursors in protein metabolism, but also intermediates in cell signaling. Protein digestion and absorption and related amino acid metabolism affect host growth. It also has the effect on the metabolism of lipids and lipid-like molecules, include 10 glycerophospholipids, 8 fatty acyl groups, 1 primary alcohol lipid, sterol and steroid derivatives. Among them, PC and PE are the most abundant phospholipids on cell membranes and play an important role in lipid metabolism and health. Glycerophospholipids are structural components of cell membranes and precursors of lipid mediators in signal transduction, suggesting that FOS is involved in the gut signal transduction and transport functions. And the arachidonic acid metabolism is an important mediator in the formation of inflammation. The down-regulated expression of coumarin and its derivatives, two isoflavones, and dihydroxybenzoates among differential metabolites indicated reduced accumulation of phenylpropionic acid and polyketides in the ileum. Phenylpropane and polyketides have multiple effects, including antioxidants, antibacterials, and anti-inflammatory agents, which may indicate reduced ileal inflammation and enhanced immune function in chickens. From the positive regulatory effect of the above FOS on the intestinal metabolism of chickens, it shows how it can improve the production, metabolism and immunity of poultry.

Metabolites of synbiotics

Synbiotics refer to the mixed products of probiotics and prebiotics, or add vitamins and trace elements. It can not only exert the physiological bacterial activity of probiotics, but also can

6 http://www.genome.jp/kegg/

7 https://docs.scipy.org/doc/scipy/

selectively increase the number of the bacteria, so that the probiotics effect is more significant and lasting. A study analyzed the cecal metabolome of broilers, fed diet supplemented with vitamin B2, found two significant different metabolites of interest, namely short-chain fatty acids (acetic acid, propionic acid, lactic acid, lactic acid, succinic acid and butyl Acid) and metabolites related to energy metabolism (aspartic acid, glutamic acid, niacin, formic acid and pyruvate; Biagi et al., 2020).

Therefore, synbiotics has the metabolite characteristics of both probiotics and prebiotics, and is also related to energy metabolism. However, there are few relevant studies at present, and further exploration is urgently needed.

Metabolomic study in chicken at different developmental stages

In ovo stimulation of microbiome and gut development

Prenatal nutrition is essential for embryonic development and newborn growth, and one of the major epigenetic determinants of lifelong health. Delivery of bioactive compounds *in ovo* is an excellent model to study the embryonic development and gut health. The compounds (e.g., probiotics and prebiotics) can be optimally injected to air chamber on day 12 of egg incubation or between 18-19th days, without affecting the hatchability. The last several days prior to hatching and the first week after the hatch, are the most critical period for development of chick intestine and immunity. One investigation supplemented the eggs with chitooligosaccharide (COS) and chlorella polysaccharide (CPS) on the 12.5th day of incubation and injected them into the amniotic sac of the eggs. In the collected cecal digests, short-chain fatty acids were determined by gas chromatography (Zhang et al., 2020). The metabolic pathways of microorganisms and the changes of SCFA were explored. The SCFA in the cecum were composed of acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid. COS were found to enrich the pathways of gluconeogenesis, anaerobic energy metabolism, L-isoleucine degradation, L-histidine biosynthesis and fatty acid biosynthesis. CPS enriched biosynthesis of isoprene, affected the mevalonate and fructan biosynthesis pathways, allantoin degradation and formaldehyde assimilation.

A study used a layered chick model, *in-ovo* feeding (IOF) L-arginine (Arg), and analyzed its induced metabolite changes based on LC–MS/MS metabolomics. 81 different metabolites were selected, out of which 24 different metabolites were found after the *in ovo* stimulation: 4 metabolites involved in carbohydrate metabolism, 6 related to lipid metabolism, and 4 involved in biosynthesis of primary bile acid (Dai et al., 2020).

Some researchers supplemented one-day-old male Arbor Acres plus chicks with xylan oligosaccharides (XOS) and *Pediococcus acidilactic* BCC-1. A significant increase in the content of butyric acid in the cecal chyle was observed. Differences in 32 metabolites were found, with increased concentrations of allo-inositol and 4-hydroxyphenylpyruvate. The major enriched pathways were those involved in terpene quinone-quinone biosynthesis, including ubiquinone, propionate metabolism, citrate cycle, alanine, aspartic acid and glutamate metabolism, tyrosine metabolism, arginine and proline metabolism. Microbiota and metabolome analysis has lead to assumption, that the combined supplementation of XOS and BBC-1 may have acted synergistically to reduce pathogenic bacteria, increase butyrate bacteria and promote carbohydrate fermentation (Wu et al., 2021a).

An animal experiment was conducted to study the effects of feeding comb Leghorn hens with high-fiber and non-starch polysaccharides, then the concentration of SCFA in cecum content was determined with gas–liquid chromatography (Dunkley et al., 2007). Researchers found the increased production of acetic acid was found, while the amounts of detected propionic acid and butyric acid were relatively lower. The study showed that dietary fiber components could be fermented by cecal microorganisms to form final products, such as SCFA, ammonia, CO2 and methane.

Based on non-targeted HPLC/MS metabolomics, A study explored the metabolic changes in male Ross 308 broilers, after supplementation with lauric acid (LA), a major medium-chain fatty acid (MCFA). 24 differentially produced metabolites were identified. It was found that LA significantly changed the level of the lipid compounds by down-regulating the abundance of phosphatidylcholine (PC), and increasing lysophosphatidylcholine and lysophosphatidylethylamine. Most compounds belonged to lipid and amino acid metabolism pathways, out of which the sphingolipid metabolism is the main pathway, followed by cysteine and methionine metabolism, phenylalanine metabolism, tryptophan and β-alanine metabolism. Moreover, LA also inhibits the growth of harmful bacteria to alter the host gut microbiota. So a reduction in the gut microbiota resulted in reduced levels of acetic acid, propionic acid, butyric acid, isobutyric acid and valeric acid. LA mainly modulates lipid metabolism in broilers and alters the immune-enhancing microbiota. (Wu et al., 2021b).

In another study, Ross 708 broilers were supplemented with *Bacillus subtilis*, and the metabolomics analysis was performed in ileum content. There were 30 significantly changed metabolite indicators found, among which the amino acids, peptides, lipids, vitamins, cofactors and nucleoside metabolites had the highest concentration (Park et al., 2020). Those altered metabolites were expected to maintain intestinal homeostasis in epithelial or immune cells, which may be the reason for their impact on overall intestinal health.

At present, prebiotics and probiotics are injected to the incubated eggs, and the microbiome of chickens after probiotic supplementation has been increasingly explored (Pourabedin and Zhao, 2015). It is expected that IOF has a high applicative potential to induce large-scale and life-lasting changes in structure and composition of microbial community. The knowledge about the potential of metabolic molecules driving the change of the microbiota from the perspective of metabolome, has to be established.

Characteristics of metabolites in the gut of other avian species

Goose is often used as an animal model to study the effect of fatty liver. Metabolites in the ileum and cecum, are important players in the formation of goose fatty liver by affecting various metabolic pathways, such as glucose and fatty acid metabolism, oxidative stress and inflammation. Those pathways involve short-chain fatty acids, branched-chain amino acids and sterols, especially glycerol 3-phosphate, sphingosine, inositol, taurine, adipate, palmitic acid and cholesterol (Zhao et al., 2020).

In quails, an experiment used the UHPLC-Q-TOF/MS untargeted method to analyze blood and stool samples after supplementation of chicory. The principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) allowed for pattern recognition and identification of characteristic metabolites. The chicory supplementation showed the effect of regulating lipolysis in fat cells. Pathway enrichment analysis showed that chicory had a strong effect on quail's glycosylphosphatidylinositol (GPI-) anchoring biosynthesis, inositol phosphate metabolism, glycerophospholipid metabolism and steroid hormone biosynthesis (Bian et al., 2018).

By supplementing *Bacillus subtilis* to turkey by direct-fed microbial (DFM), a reduced concentration of ammonia in turkey feces was found, related with high levels of branched-chain fatty acids and microbiota fermentation activity products (Tellez et al., 2020).

In another study, commercial turkeys were fed bacitracin methylene disalicylate (BMD) to commercial turkeys. Global metabolomics showed that there are more than 700 metabolites in turkey ceca (Johnson et al., 2019). The largest categories of metabolites identified were amino acid metabolites, such as tryptophan, tyramine and valine. Tryptophan is the precursor of a large number of microorganisms and host metabolites, many of which are endogenous ligands of aromatic hydrocarbon receptors (AhR), which regulate immune response and homeostasis at the intestinal epithelial level.

The Himalayan Griffin is an important reservoir of *Clostridium perfringen*s. One recent study analyzed the gut microbiome and metabolome of this bird scavenger by means of LC–MS metabolomics. 4,490 metabolites were detected in stool samples, and 154 metabolites were identified. Among them, several metabolic compounds with important physiological functions were identified, such as 2-methylbutyrylcarnitine, 3-(phosphoacetylamino)-L-alanine, adenine, cucurbitacin B, cholic acid and N-acetyl-L-aspartic acid. The main functional categories of the meatbolites were related to carbohydrate and amino acid metabolism, replication and repair, and membrane transport (Wang et al., 2021).

Within a protection program of the wild Chinese monal (*Lophophorus lhuysii*), Jiang et al. (2020) performed non-targeted metabolomics analysis in collected stool samples and identified 58 important metabolites. These metabolites were fatty acids, bile acid derivatives, sugars and indole derivatives. Their metabolic pathways are mainly related to galactose metabolism, starch and sucrose metabolism, fatty acid biosynthesis, bile acid biosynthesis and bile secretion. A significant correlation between the fecal microbiota and metabolites was found. Major highlights of metabolomic studies performed in birds supplemented with prebiotics and probiotics in different periods of development, are presented in Table 6. Relatively little analysis of changes in the intestinal microbiome in relation to metabolomics has been performed so far, whilst the composition of microbiota is highly correlated with the composition of the metabolome.

Using metabonomics methods to detect metabolites of microbiota community in blood, feces or intestinal contents, is a way to understand mechanisms of microbiome modulation and interaction in biological systems of the host (Zhao et al., 2017).

Over a half of the published articles regarding intestinal metabolomics in poultry, focused on the chicken, followed by turkey (Table 7). Among the analyzed studies, ≤40 animals or samples per study were used to conduct the metabolomics analysis. It is worth noting that sample size did not always reflect the total number of animals used in the study. For instance, multiple samples of the same animals, but at different ages were collected and subject to analysis in some of the studies (Hansen et al., 2019; Johnson et al., 2019).

The most commonly used tissue types in livestock metabolomics include cecal contents, ileum contents, and feces. The cecum is the most critical segment of the poultry intestine, where the microorganisms can hydrolyze polysaccharides, oligosaccharides and disaccharides into monosaccharides, and then further ferment them into short-chain fatty acids (SCFA). As well as gut-derived metabolites, some other biological fluids (eg blood, bile acids, not shown in table) are also used for analysis. Changes in these metabolites in the biofluids (Possible disease biomarkers) can aid to understand how functional prebiotics and probiotics affect host homeostasis in chickens (Chen et al., 2020; Zou et al., 2022). The advantage of blood samples is more fast and easy, compare to that intestinal contents or tissue. However, in terms of chicken gut study, the number of metabolites in the blood is limited and therefore the information provided is less. Biological fluids samples could be an alternative in some situations, especially when researchers focus on a certain metabolic pathway. For example, a study was performed to evaluate the effect of bile salt hydrolase inhibitors for modulating host bile profile and physiology using a chicken model system. The metabolomic analysis found that the inhibitors led to significant alterations in both circulating and intestinal bile acid signatures (Geng et al., 2020). In short, the main effects of prebiotics/probiotics intake involve increased bacterial saccharolytic activity and SCFA generation in the distal gut, It is more recommended to directly use cecal contents to study the effects of prebiotics and probiotics on gut metabolomics in poultry. Although, we still encourage researchers to use more various kinds of samples to explore thus may provide new insights into explaining the roles of prebiotics/probiotics.

TABLE 6 Metabolomic study in poultry species, that were supplemented with prebiotics and probiotics at various developmental timepoints.

Species	Supplementation	Important metabolites	References
In ovo feeding (Cobb 500)	Chitooligosaccharide (COS) and chlorella polysaccharide (CPS)	Short-chain fatty acids	Zhang et al. (2020)
In ovo feeding (Jinghong layers)	L-arginine	Galactose, taurine-conjugated bile acids and lipids	Dai et al. (2020)
Ross 708	*Bacillus subtilis*	Dipeptides, nucleosides, fatty acids, and carbohydrates	Park et al. (2020)
Ross 308	*Lactobacillus reuteri CSF8*	N/A	Nothaft et al. (2017)
Single comb Leghorn hens	Alfalfa crumbles	Short-chain fatty acids	Dunkley et al. (2007)
Hubbard	N/A	Volatile Fatty Acids	Lei et al. (2012)
Taiping chickens	Fructo-oligosaccharide	Organic acids and derivatives	Ma et al. (2022)
Arbor Acres plus chicks	Xylan oligosaccharides (XOS), Pediococcus acidilactic BCC-1	Sorbitol, pyridoxine, hydroxyphenyl derivatives 4-hydroxyphenylpyruvate 1 and 3-(3-hydroxyphenyl) propionic acid	Wu et al. (2021a)
Ross 308 broilers	Lauric acid (LA)	Acetic acid, propionic acid, butyric acid, isobutyric acid, valerate acid, and isovaleric acid	Wu et al. (2021b)
Chinese monal	N/A	Galactose, starch and sucrose metabolism, fatty acid, bile acid biosynthesis and bile secretion	Jiang et al. (2020)
Landes geese	N/A	Short-chain fatty acids, branched-chain amino acids, and cortisol	Zhao et al. (2020)
Nicolas turkey poults	Bacitracin methylene disalicylate	Amino acids, carbohydrates, nucleotides, peptides, and lipids	Johnson et al. (2019)
Nicholas turkey poults	Bacitracin methylene disalicylate	Indole-3-carboxylic acid, thymine,equol, 1-myristoylglycerol and pentadecanoate	Hansen et al. (2019)
Turkey	*Bacillus*	3-methylindole, p-cresol, phenol and ammonia	Tellez et al. (2020)
Japanese rock ptarmigans	N/A	Nucleic acid, free amino acids	Kobayashi et al. (2020)
Quails	Chicory	luteolin, lactucopicrin, cyanidin, taraxasterol, and β-sitosterol	Bian et al. (2018)
Shaoxing ducks	Compound probiotics	Pyridoxal (Vitamin B6), L-Arginine, and Betaine aldehyde, 7-oxocholesterol, 3-hydroxy-L-kynurenine, and N-acetyl-d-glucosamine	Sun et al. (2022)

Challenges and future perspectives in studying footprint and fingerprint of probiotics activity

The majority of poultry intestinal metabolomics publications have been employing untargeted methods while fewer published studies has employed targeted strategies. This is because most of the current articles are hypothesis-generating research, the purpose is to obtain or explore as much metabolite information as possible, rather than verifying a few special metabolite information. In addition, more scientific researchers use non-targeted strategies to discover new metabolites, which will provide material for the construction of an authoritative poultry metabolome database in the future.

According to our investigation, mass spectrometry-based platforms account for most of the poultry intestinal metabolomics research. As mentioned earlier, although NMR has high reliability and practicability, in recent years, in order to detect lower concentrations of metabolites, high-resolution mass spectrometry has provided indispensable help. The number of articles using GC–MS and LC–MS to study poultry intestinal metabolomics is almost the same, showing the respective advantages of these two platforms. In addition, other non-traditional or more special mass spectrometry-based poultry intestinal metabolomics methods are also constantly being developed. These platforms include but are not limited to matrix-assisted laser desorption/ionization mass spectrometry (MALDI)-MS (Hansen et al., 2019) and Fourier transform ion cyclotron resonance (FTICR) MS (Park et al., 2020).

Another issue limiting the poultry intestinal metabolomics is the incomplete reporting of relevant background data for the metabolites identities, which are approximately from 6 to 2000, depending on the strategy used: targeted or untargeted. If possible, a good metabolomics study should include various orthogonal analytical platforms, to expand the coverage of metabolites and cross-validate the results. In most of the cases in poultry intestinal metabolomics, single analytical platforms are used, which may be due to an overlook in the experimental design, or the limited research funding. Another gap found in the analysis of the literature is the general lack of integration of the other omics analyses (proteomics, transcriptomics, and microbiome) with the metabonomics. In a light of the growing trend to study systems biology and multi-omics research, the lack of complex data integration may be considered a gap in a gut health metabolomics research.

The knowledge on the interactions of poultry intestinal cells with probiotic bacteria and metabolites their of, requires a continuous development and filling the gaps of information. The use of *in ovo* model is a very good tool to study the

TABLE 7 Studies involving intestinal metabolomics in poultry.

Specie	Sample size	Sample	Strategy	Instrument	Number of metabolites	References
In ovo feeding (Cobb 500)	36	Cecal digesta	Targeted	GC–MS	6	Zhang et al. (2020)
In ovo feeding (Jinghong layers)	2	N/A	Untargeted	UPLC-MS	N/A	Dai et al. (2020)
Ross 708	32	Ileal contents	Untargeted	FTICR- MS	674	Park et al. (2020)
Ross 308	40	Cecal contents	Untargeted	NMR	20	Nothaft et al. (2017)
Single comb Leghorn hens	15	Cecal contents	Targeted	GC–MS	6	Dunkley et al. (2007)
Hubbard	6	Cecal contents	Targeted	GC–MS	N/A	Lei et al. (2012)
Taiping chickens	12	Ileum sample	Untargeted	LC–MS	435	Ma et al. (2022)
Arbor Acres plus chicks	40	Cecal Chyme	Untargeted	GC–MS	498	Wu et al. (2021a)
Ross 308 broilers	32	Cecal Chyme	Untargeted	GC	6	Wu et al. (2021b)
Chinese monal	9	Fecal samples	Untargeted	UHPLC–MS	323	Jiang et al. (2020)
Landes geese	24	Jejunum, ileum and cecum content	Untargeted	GC–MS	530, 589, and 657	Zhao et al. (2020)
Nicolas turkey poults	20	Cecal contents	Untargeted	UPLC–MS	712	Johnson et al., 2019
Nicholas turkey poults	30	Cecal contents	Untargeted	MALDI LTQ-Orbitrap	2000	Hansen et al. (2019)
Japanese rock ptarmigans	8	Cecal feces	Untargeted	LC–MS	116	Kobayashi et al. (2020)
Quails	32	Stool	Untargeted	LC–MS	148	Bian et al. (2018)
Shaoxing ducks	16	Cecal feces	Untargeted	LC–MS	484	Sun et al. (2022)
Himalayan Griffons	12	Stool	Untargeted	LC-MS	154	Wang et al. (2021)

metabolomics of gut health in poultry. The previously established procedure allows to verify the optimal probiotic and prebiotic compounds *in vitro*, deliver them to the embryonic environment *in ovo* and track the phenotypic and genetic effects through life span of the animal (Dunislawska et al., 2017; Maiorano et al., 2017; Sobolewska et al., 2017). Therefore, the gut microbiome development is stimulated by the precise injection of probiotics and prebiotics to the air chamber, or the amnion, prior to hatch. The specific markers of the metabolic activity of probiotics can be identified *in vitro,* as so called metabolic footprints of probiotics activity, in culture medium supplemented with prebiotics. These metabolic footprints can be further explored in metabolome of a host chicken gut content/tissue, after *in ovo* injecting the selected, simple synbiotic combinations. It is proposed, that the complex picture of function of probiotics and their metabolites *in vivo* (*in ovo* model) can be complemented with tracking the metabolic footprints and fingerprints by employing new *in vitro* chicken intestine models, e.g., the Chick8E11 cell line (Khan et al., 2021), and using validated, referential intestinal *in vitro* models like the Caco-2 cell line.

The further knowledge about the function of probiotics and prebiotics in the host organism by means of metabolomic activities, is necessary to develop safe and efficient early life strategies for the pre-matured animals.

Author contributions

MW and SZ conceived and wrote the manuscript and produced tables and figures. GM, PK, and KS conceived, supervised, and reviewed the manuscript. 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.

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EDITED BY
Karolina Skonieczna-Żydecka,
Pomeranian Medical University, Poland

REVIEWED BY
Mariusz Kaczmarczyk,
Pomeranian Medical University, Poland
Anna Wierzbicka-Wos,
Sanprobi, Poland

*CORRESPONDENCE
Vladimira Vuletić
vladimira.vuletic@uniri.hr

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The effects of microbiota abundance on symptom severity in Parkinson's disease: A systematic review

Eliša Papić[1,2], Valentino Rački[1,2], Mario Hero[1,2], Zoran Tomić[2], Nada Starčević-Čižmarević[3], Anja Kovanda[4], Miljenko Kapović[3], Goran Hauser[5], Borut Peterlin[4] and Vladimira Vuletić[1,2]*

[1]Department of Neurology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Clinic of Neurology, Clinical Hospital Center Rijeka, Rijeka, Croatia, [3]Department of Medical Genetics and Biology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [4]Clinical Institute of Genomic Medicine, Ljubljana University Medical Center, Ljubljana, Slovenia, [5]Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Introduction: Parkinson's disease (PD) is neurodegenerative disease with a multifactorial etiopathogenesis with accumulating evidence identifying microbiota as a potential factor in the earliest, prodromal phases of the disease. Previous research has already shown a significant difference between gut microbiota composition in PD patients as opposed to healthy controls, with a growing number of studies correlating gut microbiota changes with the clinical presentation of the disease in later stages, through various motor and non-motor symptoms. Our aim in this systematic review is to compose and assess current knowledge in the field and determine if the findings could influence future clinical practice as well as therapy in PD.

Methods: We have conducted a systematic review according to PRISMA guidelines through MEDLINE and Embase databases, with studies being selected for inclusion via a set inclusion and exclusion criteria.

Results: 20 studies were included in this systematic review according to the selected inclusion and exclusion criteria. The search yielded 18 case control studies, 1 case study, and 1 prospective case study with no controls. The total number of PD patients encompassed in the studies cited in this review is 1,511.

Conclusion: The link between gut microbiota and neurodegeneration is a complex one and it depends on various factors. The relative abundance of various microbiota taxa in the gut has been consistently shown to have a correlation with motor and non-motor symptom severity. The answer

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could lie in the products of gut microbiota metabolism which have also been linked to PD. Further research is thus warranted in the field, with a focus on the metabolic function of gut microbiota in relation to motor and non-motor symptoms.

KEYWORDS

microbiota, Parkinson's disease, motor symptoms, systematic review, relative abundance

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world (Van Den Eeden et al., 2003). It mainly presents with a triad of symptoms including rigor, bradykinesia, and tremor (Kouli et al., 2018), along with a variety of other motor and non-motor symptoms (Poewe et al., 2017).

It is postulated that the main pathophysiological mechanism lies in the accumulation of α–synuclein in the brain, primarily in the substantia nigra, which leads to the loss of dopaminergic neurons and the typical symptoms of PD. As the disease progresses, said changes spread to other regions of the brain, causing neurodegeneration, slowly leading to severe motor and cognitive impairment (Shulman et al., 2011). PD is a multifactorial condition, with causes ranging from exposure to various environmental factors, such as pesticides (Chen and Ritz, 2018), traumatic brain injury (Delic et al., 2020), gene mutations (Klein and Westenberger, 2012), and more recently, microbiota (Fitzgerald et al., 2019).

Novel research has identified microbiota as a potential factor in the earliest prodromal phases of the disease (Shen et al., 2021). The mechanism behind this is thought to lie in the gut-brain axis, a complex bidirectional system of communication between the intestines and the brain (Carabotti et al., 2015), with various potential pathways described with the vagal nerve (Breit et al., 2018) and the proven transneuronal propagation of α–synuclein from the gut to the brain being the most promising in the research of gut microbiota influence on the brain (Kim et al., 2019). It has been shown in previous studies that the underlying changes in the gut that could potentially lead to this pathological retro-axonal transport include microbiota composition, with the composition greatly differing in PD patients when compared to the healthy controls (Pereira et al., 2017), as well as microbiota metabolic function, mainly through the secretion of various SCFA (Shen et al., 2021). Alterations in the microbiome could potentially lead to prodromal symptoms such as hyposmia and GI dysfunction (Pereira et al., 2017), as well as modulating motor symptoms in the later stages of the disease (Sampson et al., 2016).

The changes in the gut microbiome have also been linked with the response to PD therapy, especially levodopa (Keshavarzian et al., 2020). It is thus clear that the potential effects of dysbiosis could play a part in the prodromal, but also in the latter stages of the disease and as such should be carefully studied further (Fitzgerald et al., 2019). Studies have already shown that there are certain potential therapeutic approaches that could be taken to prevent or reverse the changes in the microbiome and consequently modulate the disease course and severity. For example, potential beneficial effects have been proposed in the application of antibiotics (Li et al., 2004; González-Lizárraga et al., 2017; Pu et al., 2019), probiotics (Cassani et al., 2011; Surwase and Jadhav, 2011; Srivastav et al., 2019), prebiotics (Cantu-Jungles et al., 2019; Qiao et al., 2020), dietary intervention (Watson et al., 2018; Hegelmaier et al., 2020), and fecal microbiota transplant (FMT) (Xue et al., 2020). The field of research is still growing, and further studies could reveal additional therapeutic approaches targeting the microbiome.

Our aim in this systematic review is to compose and assess current knowledge in the field and determine if the findings could influence future clinical practice as well as therapy in PD.

Methods

Search strategy

We have conducted a systematic review according to PRISMA guidelines (Page et al., 2021). Our search was focused on the MEDLINE and Embase databases. The search was done on articles published from January 1st of 2012 up to June 1st of 2022. We used the following keywords on all fields and MeSH terms: "PD," "microbiota," "microbiome," along with Boolean terms "AND" and "OR." The search rendered 692 records after we applied appropriate filters. The studies were then selected based upon the following inclusion and exclusion criteria (**Figure 1**). Articles were first screened by title and abstract, followed by full-text checking for their eligibility. The selection of articles was done independently by 5 authors (EP, VR, MH, ZT, AK), and final inclusion was done by agreement.

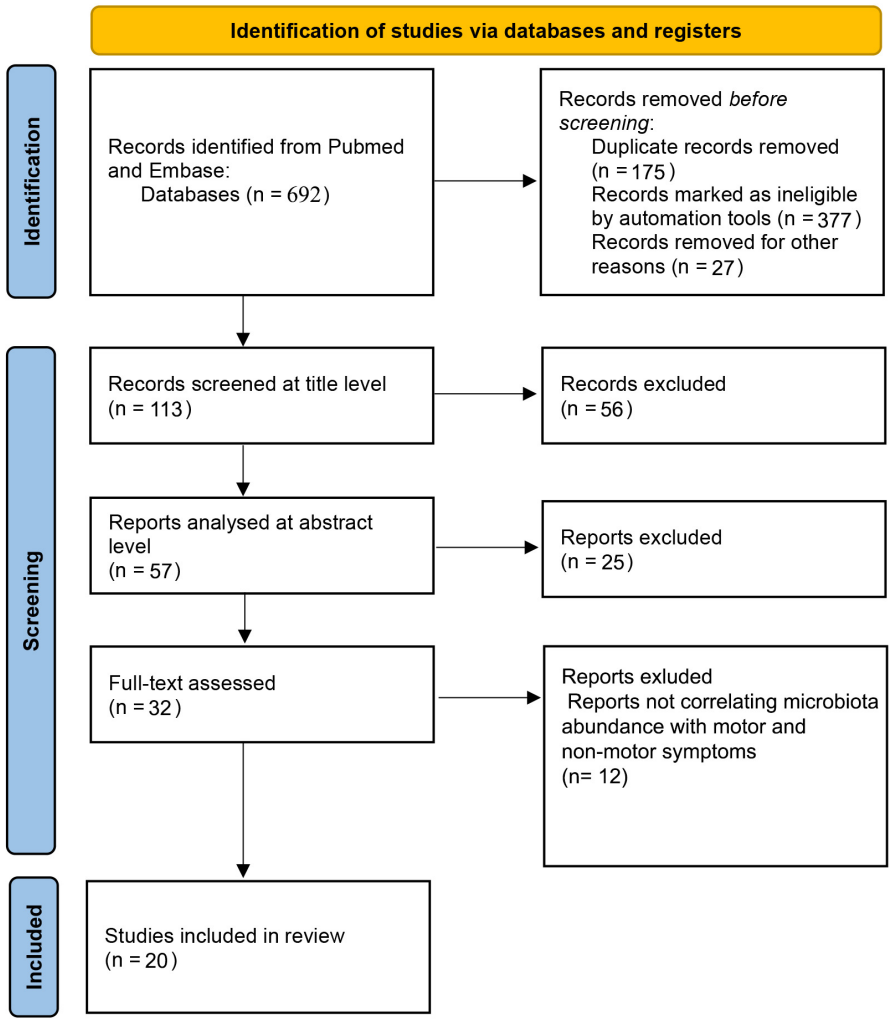

FIGURE 1
PRISMA flow diagram.

Inclusion and exclusion criteria

Studies accepted for inclusion were: (a) studies with patients diagnosed with PD; (b) studies published from January 1st of 2012 up to June 1st of 2022; (c) studies published in the English language; (d) studies published in indexed and peer-reviewed journals; (e) studies that evaluated motor symptoms through the Unified PD Rating Scale Part III (UPDRS III) and/or Hoehn and Yahr disease stage progression in correlation with gut microbiota abundance (f) studies that evaluated non-motor symptoms through the Unified PD Rating Scale Part I (UPDRS I), Non-Motor Symptoms Scale (NMSS), Non-Motor Symptoms Questionnaire (NMSQ), Mini Mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA) in correlation with gut microbiota abundance.

Exclusion criteria include: (a) studies published in regional languages other than English, (b) studies not correlating gut microbiota abundance with motor and/or non-motor symptoms through verified clinical scales. Study design was evaluated for type, and 20 studies were finally included in the review (Table 1).

Clinical scales for the evaluation of motor and non-motor symptoms in Parkinson's disease used in the selected studies

Unified PD Rating Scale is a widely used clinical scale for the evaluation of both motor and non-motor symptoms in PD and it is split into four parts. Part I concerns non- motor experiences of daily living and covers areas such as sleep and mood, Part II covers motor experiences of daily living such as hygiene, clothing and other daily activities requiring healthy motor skills,

TABLE 1 Cited studies listed by study design.

References	Study design	Population	Composition and correlation
Scheperjans et al. (2015)	Case-control study	72 patients and 72 controls	Family *Prevotellaceae*: relative abundance negatively correlates with UPDRS III
Aho et al. (2019)	Case-control study	64 PD patients, controls	Family *Prevotellaceae* less abundant in progressed PD (H&Y) Genus *Prevotella* more abundant in stable subjects
Lin et al. (2019)	Case-control study	80 PD patients and 77 controls	Genus *Bacteroides:* relative abundance positively correlated with motor symptom severity
Heintz-Buschart et al. (2018)	Case—control study	76 PD patients, 21 idiopathic REM sleep behavior disorder patients, and 7 controls	Genus *Akkermansia* more abundant in PD patients—related to non-motor symptoms. The relative abundances of *Anaerotruncus* spp., *Clostridium XIVa*, and *Lachnospiraceae* family and genus *Akkermansia* positively correlated with UPDRS III *Anaerotruncus* species related to depression in Parkinson's disease
Li et al. (2022)	Case-control study	91 PD patients and 91 healthy controls (HC)	*Clostridia, Clostridiales, and Ruminococcaceae* negatively correlated with MMSE
Barichella et al. (2019)	Prospective observational case-control study	350 patients (193 PD, PSP 22, MSA 22; HC 113)	Higher abundance of *Christensenellaceae* linked with worse non-motor symptoms *Lactobacillaceae* positive correlation with UPDRS III. *Lachnospiraceae* negative correlation with UPDRS III.
Zhang et al. (2020)	Case-control study	63 PD patients, 63 healthy spouses (HS) and 74 healthy people (HP)	*Parabacteroides, Akkermansia, Coprococcus, Bilophila, Collinsella, Methano-brevibacter, Eggerthella, Adlercreutzia* associated with PD progression and symptom severity.
Li et al. (2019)	Case-control study	51 PD patients and 48 healthy controls	Relative abundances of families *Acidaminococcaceae, Erysipelotrichaceae,* genera *Phascolarctobacterium, Coprococcus, Tyzzerella,* and species *Ruminococcus_torques* showed a positive correlation with UPDRS III Relative abundances of families *Acidaminococcaceae, Erysipelotrichaceae,* genera *Phascolarctobacterium, Akkermansia, Coprococcus, Tyzzerella,* and species *Ruminococcus_torques* showed a positive correlation with non-motor symptoms (NMSQ and SCOPA) Relative abundances of order *Bacillales* and species *Pseudomonas_veronii* showed a negative correlation with UPDRS III Relative abundance of order *Lactobacillales* is negatively correlated with NMSQ
Murros et al. (2021)	Case-control study	20 PD patients and 20 healthy controls.	Eleven patients with a more severe disability of PD had a significantly higher amount of *DSV* bacteria than the nine patients that were classified below 2.0 points under the Hoehn-Yahr system; *DSV* bacteria were significantly more abundant in patients with hyposmia
Rosario et al. (2021)	Case-control study	26 drug naive PD and 25 controls, [11 healthy controls (COs), 14 diseased controls (DCs) with cardiovascular risk factors]	The abundance of the genus *Erysipelatoclostridium,* the species *E. coli* and the species *Victivallis vadensis* was positively correlated with UPDRS III and age in people with PD + *E. coli* and *V. vadensis* also linked to GI dysfunction
Li et al. (2017)	Case-control study	24 PD patients; 14 controls	*Genera:* The relative abundance of *Faecalibacterium* decrease in severe Parkinson compared to mild Parkinson and control (H&Y and UPDRS III) *Megasphaera* relative abundance increased in severe Parkinson compared to mild Parkinson (H&Y and UPDRS III) and control UPDRS III scores positively correlated with *Enterococcus, Proteus,* and *Escherichia-Shigella* UPDRS III negatively correlated with *Blautia Faecalibacterium, Ruminococcus, Haemophilus,* and *Odoribacter*
Qian et al. (2018)	Case-control study	45 PD patients, 45 healthy controls	*Butyricicoccus* and *Clostridium XlVb* positively associated with MMSE scores

(Continued)

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TABLE 1 (Continued)

References	Study design	Population	Composition and correlation
Pietrucci et al. (2019)	Case-control study	80 PD and 72 controls	Lower levels of *Lachnospiraceae* and higher levels of *Enterobacteriaceae* families correlated with increased disease severity and motor impairment
Takahashi et al. (2022)	Case study	223 PD patients	*Blautia* significantly decreased and *Lactobacillus* significantly increased in PD patients with motor complications
Mertsalmi et al. (2017)	Case-control study	74 PD patients with 75 controls	Lower abundance of the family *Prevotellaceae* as well as the genera *Prevotella* and *Bacteroides* in IBS like symptoms in PD patients
Baldini et al. (2020)	Case-control study	147 typical PD cases, 162 controls	Relative abundances of genera *Peptococcus* and *Flavonifractor* are positively correlated with UPDRS III Relative abundance of genus *Bilophila* is positively correlated with Hoehn and Yahr Relative abundance of genus *Paraprevotella* is negatively correlated with UPDRS III and Hoehn and Yahr Genus *Bifidobacterium* is positively correlated with constipation
Minato et al. (2017)	Prospective study	36 PD patients divided into deteriorated and stable groups	Low count of *Bifidobacterium* at year 0 was associated with worsening of UPDRS I (hallucinations) no individual bacterial groups/genera/species at year 0 were correlated with worsening of total UPDRS scores in 2 years
Ren et al. (2020)	Case-control study	13 PD patients PD-MCI/14 PD –NC (normal cognition) and 13 healthy controls	Relative abundances of genera *Ruminococcus, Bilophila, Desulfovibrio, Barnesiella, Butyricimonas, Acidaminococcus, Pyramidobacter*, and *Oxalobacter* were negatively associated with the MMSE scores; Relative abundances of genera *Alistipes, Sutterella, Odoribacter, Butyricimonas, Hungatella, Helicobacter, Solobacterium, Oscillospira*, and *Hydrogenoanaerobacterium* were negatively associated with the MoCA scores
Cosma-Grigorov et al. (2020)	Case-control multivariate study	71 PD patients and 30 healthy	Significant positive correlation of *Parabacteroides* and *Turicibacter* with disease duration and UPDRS III
Weis et al. (2019)	Case-control study	34 PD and 25 controls	H and Y 1–2.5: significantly increased relative abundance of *Peptoniphilus* and F*aecalibacterium* compared to controls H and Y 3–4: significant increase in the relative abundance of *Peptoniphilus*

PD, Parkinson's disease; HC, healthy controls; PSP, progressive supranuclear palsy; MSA, multiple system atrophy.

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Part III measures the severity for motor symptoms such as rigor, tremor, bradykinesia, and others, while the Part IV is used to describe eventual motor complications (i.e., dyskinesia) (Goetz et al., 2008). In the studies collected for this systematic review, UPDRS III was used in correlation between motor symptom severity and microbiota relative abundance, while UPDRS I was used in some studies to express non-motor symptom severity.

The Hoehn and Yahr (H&Y) system is used to grade the severity of PD symptoms and is expressed as a scale of 1–5. The stages 1–3 represent patients who are considered to be minimally disabled, while stages 4 and 5 represent patients who are considered to be severely disabled. Besides representing the motor symptom severity linked with disease progression, the H&Y scale has also been positively correlated with cognitive decline and dementia in PD patients (Modestino et al., 2018).

The NMSQ is a patient-based screening tool for the presence of non-motor symptoms ranging from hyposmia, incontinence, sexual performance to hallucinations and diplopia. It does not evaluate the severity of the symptoms (Chaudhuri et al., 2006). On the other hand the NMSS is used for evaluation of the severity of non-motor symptoms and uses a 30-item rater-based scale to cover a wide array of non-motor symptoms, rating their severity on the scale of 0–3 as well as their frequency on the scale of 1–4 (Chaudhuri et al., 2007).

The MoCA is a screening tool used for the evaluation of cognitive impairment and covers various cognitive domains such as visuospatial abilities, executive functions, short-term memory recall, language, abstract reasoning, orientation and more. It contains 30 points, with a score of 26 or over is considered to be normal (Nasreddine et al., 2005).

Similarly to MoCA, the Mini-Mental State Examination (MMSE) is also a 30-point questionnaire used for the measurement of cognitive impairment. It requires less time for administration, and can help differentiate between different types of dementia such as Alzheimer's disease or PD dementia (Pangman et al., 2000). It should be noted that both MoCA and MMSE are used mostly as screening tools, and are by no means specific enough without follow-up imaging and additional diagnostic procedures to provide a final diagnosis.

Methods for microbiota analysis implemented in the selected papers

The papers analyzed in this reviewed have used a wide array of different methods for microbiota isolation and sequencing, taxonomic assignment and clustering through Operational Taxonomic Units (OTU) and compositional and statistical analysis. The information has been included in **Table 2** and further covered and discussed in the Discussion section.

Results

The primary search yielded a total of 692 studies using the described method and search parameters. 113 studies remained after excluding duplicate records and filtering them out with automation tools. These were screened on the title level and 56 studies were excluded, leaving 57 studies that were analyzed on the abstract level, where additional 25 studies were excluded. The full text was analyzed for 32 studies, and additional 12 studies were excluded (not correlating microbiota abundance with motor and/or non-motor symptoms; n = 12). Therefore, 20 studies were included in this systematic review according to the selected criteria. The complete PRISM flow chart for this systematic review is given in **Figure 1**. When looking at study designs, the search yielded 18 case control studies, 1 case study, and 1 prospective case study with no controls. The total number of PD patients encompassed in the studies cited in this review is 1,511.

Microbiota abundance in relation to motor and non-motor symptoms

The link between the composition of the gut microbiota and PD symptoms has been scarcely researched so far. Most of the studies included in this systematic review analyzed the abundance of gut microbiota from the feces through amplification and sequencing methods of the different regions of the bacterial 16s ribosomal gene. They were then correlated with either UPDRS and the modified Hoehn and Yahr scale or through non-motor symptom scales and questionnaires such as NMSS and NMSQ, as well as through cognitive tests, more specifically MoCA and MMSE.

Impact of microbiota on motor symptoms in Parkinson's disease

Most papers analyzed in this systematic review suggest a positive correlation of gut microbiota abundance with motor symptoms and disease severity in PD (**Table 3**).

Phylum *Bacillota* (formerly *Firmicutes*)

Bacteria belonging to the *Bacillota* phylum have shown a mostly positive correlation with UPDRS III scores. For instance, the relative abundances of the genera *Peptococcus* and *Flavonifractor*, which belong to the *Clostridiales* order, have shown a positive correlation with UPDRS III scores (Baldini et al., 2020). This has also been shown in the case of the orders *Bacillales* and *Accidaminococcales,* more specifically its family *Acidaminococcacea,* as well as the genus *Phascolarctobacterium* (Li et al., 2019).

Regarding the *Eubacteriales* order, the relative abundance of the family *Lachnospiraceae* has been shown to have a negative correlation with UPDRS III (Barichella et al., 2019;

TABLE 2 Methods for microbiota isolation, sequencing, taxonomic assignment, data, and statistical analysis.

References	Methods for microbiota isolation and sequencing	Taxonomic assignment of operational taxonomic unit (OTU)	Microbiota data analysis methods and statistical methods
Scheperjans et al. (2015)	Stool samples. Pyrosequencing of V1–V3 regions of 16SRNA	Mothur's Standard Operating Procedure (SOP) for MiSeq	*t*-test, Mann-Whitney *U*-test, Fisher's exact test, Metastats. Generalized linear model (GLM) for the distribution of bacterial abundances, Spearman correlation coefficient for correlations between rel. abundances and clinical factors; other methods
Aho et al. (2019)	Stool samples. V3–V4 regions of 16S RNA PCR amplification.	Mothur's Standard Operating Procedure (SOP) for MiSeq	*t*-test, Wilcoxon signed rank test, Fisher's exact test, False discovery rate (FDR), phyloseq. ANCOM, DESeq2, random forests for the distribution of abundances; Spearman correlation coefficient; other methods
Lin et al. (2019)	Stool samples. V4 16s RNA PCR amplification	Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1; classification based on the Greengenes gg_13_8 database (Miseq)	*t*-test, chi-square test, ANOVA, Levene's test, Mann-Whitney *U*-test, Kruskal-Wallis test; ANCOM, Linear discriminant analysis (LDA) effect size (LEFSe), FDR; other methods
Heintz-Buschart et al. (2018)	Stool samples. V4 region of 16S RNA and 18S RNA PCR amplification. Shotgun sequencing	LotuS R (R Foundation for Statistical Computing, Vienna, Austria) HiSeq	Permutational multivariate analysis of variance, Fisher's exact test, Kruskal-Wallis test, phyloseq, Mann-Whitney *U*-test, DESeq2, ANCOM, FDR; other methods
Li et al. (2022)	Stool samples. V4–V5 regions of 16S RNA amplification and sequencing	Uparse software with Mothur algorithm, MUSCLE software	*t*-test, Wilcoxon's rank-sum test, (LDA) effect size (LEFSe), random forests, Spearman correlation coefficient, GLM for elimination of confounding factors; other methods
Barichella et al. (2019)	Stool samples. V3–V4 regions of 16S RNA amplification and sequencing	QIIME pipeline; data clustered and taxonomically assigned via Ribosomal Database Project (RDP) classifier against a Greengenes database. MiSeq	R package "vegan," multivariate GLM (negative binomial distribution with log link), regression analysis, P-MANOVA, Spearman correlation coefficient and others
Zhang et al. (2020)	Stool samples. V4 region of 16S RNA amplification and sequencing	QIIME2 pipeline, DADA2. Clustering via VSEARCH against Greengenes 13_7 HiSeq	R, Fisher's exact test, Kruskal-Wallis, LDA effect size (LEfSe), Spearman correlation coefficient, and others
Li et al. (2019)	Stool samples. V4 region of 16S RNA amplification and sequencing	Uparse v7.0.1001. Mothur. SILVA SSU rRNA database.	R, *t*-test, Wilcoxon rank-sum test, Linear discriminant analysis. (LDA) effect size (LEfSe) analysis, FDR, Spearman correlation coefficient, and others
Murros et al. (2021)	Stool samples. Primers for 16S rRNA—for specific detection of *Desulfovibrio* genus and subspecies. PCR amplification.	Not applicable	Multiple statistical tests (Fisher's exact test; strength of association tested by Phi and Cramer's *V*-test; Mann Whitney *U*-test for comparison of DSV in PD vs. controls and patients with high vs. low levels of disease progression) and others
Rosario et al. (2021)	Shotgun metagenomic data from a German PD Cohort	DirichletMultinomial	Wilcoxon signed-rank test, FDR, Spearman correlation coefficient, Cytoscape (integrative correlation network), and others
Li et al. (2017)	Stool samples. V3–V5 region of 16S RNA amplification and sequencing	Mothur and USEARCH (v8.0) SILVA 16S rRNA database v119. Mothur SOP for MiSeq.	Metastats method for abundance features; R, UniFrac distance metrics analysis, Spearmen's rank correlation. Kruskal-Wallis-test, t-test, Chi-squared test)

(Continued)

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TABLE 2 (Continued)

References	Methods for microbiota isolation and sequencing	Taxonomic assignment of operational taxonomic unit (OTU)	Microbiota data analysis methods and statistical methods
Qian et al. (2018)	Stool samples. V3–V4 region of 16S RNA amplification and sequencing	QIIME OTU assigned using UPARSE. Reference database - Ribosomal Database Project (RDP).	R software, statistical tests (*t*-test, Pearson's Chi-square test), GLM, RF, Spearmen's correlation analysis, LASSO (least absolute shrinkage and selection operator), and others
Pietrucci et al. (2019)	Stool samples. V3–V4 regions of 16S RNA amplification and sequencing	QIIME 1.9.1. USEARCH 6.1 and GreenGenes 13.8	GLM, Willcoxon-Mann-Whitney. DESeq2, PERMANOVA test, regression analysis, and others
Takahashi et al. (2022)	Stool samples. V3–V4 regions of 16S RNA amplification and sequencing	QIIME2 DADA2 SILVA taxonomy database release 132 (60)	Wilcoxon rank-sum test, ANCOVA, GLM, Bonferroni correction, and others
Mertsalmi et al. (2017)	Stool samples. V1–V3 region of 16S RNA—amplification and pyrosequencing	Mothur's Standard Operating Procedure (SOP) for MiSeq	*T*-test; Mann—Whitney test; Fisher's two sided exact test. Microbiome data: Phyloseq, DESeq2 package 14 (based on binomial generalized linear models); FDR
Baldini et al. (2020)	Stool samples. V3–V4 regions of 16S RNA amplification and sequencing	SPINGO (SPecies level IdentificatioN of metaGenOmic amplicons) classifier	Genome scale metabolic reconstructions; flux balance analysis (FBA), community metabolic modeling; fractional regression (family of GLM), FDR correction
Minato et al. (2017)	Stool samples. PCR of 16S or 23S RNA	Composition of gut microbiota was analyzed using the Yakult intestinal Flora-SCAN (YIF-SCAN), which exploited qRT-PCR of bacterial 16S or 23S rRNA using SYBR Green I. 19 bacterial taxa were preselected based on high prevalence in the human intestines, frequently observed pathogens, and preference of the Yakult company that merchandises *Lactocbacillus*-containing yoghurt. Other data was not included.	Wilcoxon signed ranked test; Fisher's exact test; Pearson correlation; FDR
Ren et al. (2020)	Stool samples. V3–V4 regions of 16S RNA amplification and sequencing. Gas Chromatography and Mass Spectrometry (GC-MS)	Mothur, UPARSE and R. UPARSE pipeline used for OTU clustering. Silva 128 database used for assignment of representative OTU sequences.	Shapiro-Wilk test; Pearson chi-square; Bonferroni adjustment; *t*-test; LDA effect size (LEfSe), GLM, DESeq; predictions via KEGG orthologs; Kruskal-Wallis test
Cosma-Grigorov et al. (2020)	Stool sample. V3–V4 regions of 16S RNA amplification and sequencing	Usearch and greengenes 16S rRNA database v13.5	Kolmogorov-Smirnov test, *t*-test, Mann-Whitney *U*-test, Fisher's exact test, *z*-test with Bonferroni correction; abundance—Kruskal Wallis, Wilcoxon signed test. Sparce correlation (SparCC) in MicrobiomeAnalyst and others
Weis et al. (2019)	Stool sample. V4–V5 region of 16S RNA amplification and sequencing	QIIME 1.9.1. SILVA database for taxonomy assignment	ANOVA, Wilcoxon-Mann-Whitney test; FDR, Spearman correlation analysis, and others

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TABLE 3 Relative abundances of bacterial taxa in correlation with motor symptoms.

Kingdom	Phylum	Order	Family	Genus
Bacteria	*Bacillota*—positive correlation with UPDRS III	*Eubacteriales*	*Oscillospiraceae*	*Ruminococcus*—negative correlation with UPDRS III
				*Mediterraneibacter** species *Ruminococcus_torques*—positive correlation with UPDRS III
				Anaerotruncus—positive correlation with UPDRS III
				Faecalibacterium—negative correlation with UPDRS III/H&Y
			Lachnospiraceae—negative correlation with UPDRS III	*Coprococcus*—positive correlation with UPDRS III
				Tyzzerella—positive correlation with UPDRS III
				Blautia—negative correlation with motor complications
				Clostridium cluster XIVa—positive correlation with UPDRS III
			Peptoniphillaceae	*Peptoniphilus*—positive correlation with H&Y
		Clostridiales	*Peptococcaceae*	*Peptococcus*—positive correlation with UPDRS III
				Flavonifractor—positive correlation with UPDRS III
		Bacillales—negative correlation with UPDRS III		
		Lactobacillales	*Lactobacillaceae*	*Lactobacillus*—positive correlation with motor complications
			Enterococcaceae	*Enterococcus*—positive correlation with UPDRS III
		Erysipelotrichales	*Turicibacteraceae*	*Turicibacter*—positive correlation with UPDRS III
			Erysipelotrichaceae—positive correlation with UPDRS III	*Erysipelatoclostridium*—positive correlation with UPDRS III
		Acidaminococcales	*Acidaminococcaceae* positive correlation with UPDRS III	*Phascolarctobacterium*—positive correlation with UPDRS III
		Selemonadales	*Veillonellaceae*	*Megasphaera*—positive correlation with UPDRS III
	Bacteroidota	*Bacteroidales*	*Porphyromonadaceae*	*Parabacteroides*—positive correlation with UPDRS III
			Bacteroidaceae	*Bacteroides*—positive correlation with UPDRS III
			Prevotellaceae—negative correlation with UPDRS III	*Prevotella*—negative correlation with symptom severity
				Paraprevotella—negative correlation with UPDRS III/H&Y
	Pseudomonadota	*Enterobacteriales*	*Enterobacteriaceae*—positive correlation with UPDRS III	*Escherichia*—positive correlation with UPDRS III * species *E. coli*—positive correlation with UPDRS III
				Shigella—positive correlation with UPDRS III
				Proteus—positive correlation with UPDRS III
		Pasteurellales	*Pasteurellaceae*	*Haemophilus*—negative correlation with UPDRS III
		Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas** species *Pseudomonas_veronii*—negative correlation with UPDRS III
	Verrucomicrobiota	*Verrucomicrobiales*	*Akkermansiaceae*	*Akkermansia*—positive correlation with UPDRS III
	Thermodesulfo-bacteriota	*Desulfovibrionales*	*Desulfovibrionaceae*	*Desulfovibrio*—positive correlation with H&Y

(Continued)

TABLE 3 (Continued)

Kingdom	Phylum	Order	Family	Genus
				Bilophila—positive correlation with H&Y
	Actinomycetota	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*—positive correlation with H&Y
		Eggerthellales	*Eggerthellaceae*	*Eggerthella*—positive correlation with H&Y
				Adlercreutzia—positive correlation with H&Y
	Euryarchaeota	*Methanobacteriales*	*Methanobacteriaceae*	*Methanobrevibacter* –positive correlation with H&Y
	Lentisphaerota	*Victivallales*	*Victivallaceae*	*Victivalis** species *Victivalis_vividensis*—positive correlation with UPDRS III

*Species denotes a subcategory of genus which is species.

Pietrucci et al., 2019). The relative abundance of the genus *Coprococcus* has shown a positive correlation with PD progression and symptom severity (Li et al., 2019; Zhang et al., 2020). The relative abundance of another member of this family, the genus *Tyzzerella,* has shown a positive correlation with UPDRS III (Li et al., 2019). Same has been shown with *Clostridium* cluster XIVa (Heintz-Buschart et al., 2018). The relative abundance of the genus *Blautia* has been shown, on the other hand, to have a negative correlation with motor complications in PD patients (Takahashi et al., 2022). Furthermore, genera from the *Oscillospiraceae* family have also shown a link to motor symptoms. In one study, the relative abundance of the genus *Anaerotruncus* has been shown to have a positive correlation with UPDRS III scores (Heintz-Buschart et al., 2018), while in the case of the genus *Faecalibacterium,* the relative abundance demonstrated a negative correlation with UPDRS III (Li et al., 2017) as well as with H&Y scales (Li et al., 2017; Weis et al., 2019). Similarly, in the case of the genus *Ruminococcus,* its relative abundance has also shown a negative correlation with UPDRS III scores (Li et al., 2017). In a different study, the relative abundance of the species *Ruminococcus_torques,* which is taxonomically counted as part of the *Mediterraneibacter* genus, has shown a positive correlation with UPDRS III (Li et al., 2019). Similarly, the relative abundance of the genus *Peptoniphilius,* which is a member of the *Peptoniphilaceae* family, has shown a positive correlation with H&Y scales (Weis et al., 2019).

Regarding the *Lactobacillales* order, the relative abundances of the family *Lactobaccilaceae* as well as the genus *Enterococcus* from the *Enterococcaceae* family, have shown a positive correlation with UPDRS III scores (Barichella et al., 2019). In a different study, the relative abundance of the genus *Lactobacillus* has shown a significant increase in patients with motor complications (Takahashi et al., 2022). Furthermore, the relative abundance of the genus *Turicibacter*, a genus belonging to the *Erysipelotrichia* class, has shown a positive correlation with UPDRS III scores (Cosma-Grigorov et al., 2020). Same has been shown in the case of the family *Erysipelotrichaceae* (Li et al., 2019) and its genus *Erysipelatoclostridium* (Rosario et al., 2021). In another study, the relative abundance of the genus *Megasphaera* has been shown to correlate positively with motor symptom severity, as demonstrated through H&Y scores (Li et al., 2017).

Phylum *Bacteroidota* (formerly *Bacteroidetes*)

In the *Bacteroidota* phylum the link between relative abundances and motor symptoms has been shown to be varied. A positive correlation with UPDRS III scores has been shown in families *Porphyromonadaceeae*, more specifically its genus *Parabacteroides* (Cosma-Grigorov et al., 2020; Zhang et al., 2020) and *Bacteroidaceae*, more specifically its genus *Bacteroides* (Lin et al., 2019). On the other hand, the relative abundance of the *Prevotellaceae* family has been shown to have a negative correlation with UPDRS III scores (Scheperjans et al., 2015; Aho et al., 2019). More specificially, a lower relative abundance of the genus *Prevotella* has been shown to be linked with earlier age of onset with a correlation in symptom severity (Aho et al., 2019). The abundance of the genus *Paraprevotella*, besides having a negative correlation with UPDRS III scores has also been shown to have a negative correlation with H&Y scales (Baldini et al., 2020).

Phylum *Pseudomonadota* (formerly *Proteobacteria*)

Like other bacteria in this review, members of the *Pseudomonadota* phylum have also shown a correlation to motor symptoms in PD. For instance, the relative abundance of the family *Enterobacteriaceae*, which is a part of the *Gammaproteobacteria* class, has shown a positive correlation both with motor symptoms and disease severity (Pietrucci et al., 2019). Moreover, the genera *Proteus, Escherichia,* and *Shigella*, which are a part of this family, have also individually shown a positive correlation between their relative abundances and UPDRS III (Li et al., 2017). Furthermore, the relative abundance of the species *E. coli*, a species belonging to the *Escherichia* genus, has been positively correlated with disease severity (Rosario et al., 2021). On the other hand, the relative abundance of the genus *Haemophilus*, which is a part of the *Pasteurellaceae* family, has shown a negative correlation with UPDRS scores (Li et al., 2017). This has also been shown with the species

Pseudomonas_veronii, which belongs to the *Pseudomonaceae* family (Li et al., 2019).

Other

There have been studies that have reported representatives with correlation to PD from other phyli too. For instance, the relative abundance of the genus *Akkermansia,* from the phylum *Verrucomicrobiota,* has been shown to have positive correlation with UPDRS III scores (Heintz-Buschart et al., 2018). The relative abundance of the genus *Bilophila,* which is a part of the phylum *Thermodesulfobateriota*, has been shown to have a positive correlation with H&Y scales (Baldini et al., 2020) as well as disease progression and symptom severity (Zhang et al., 2020). The relative abundance of the *Desulfovibrio* genus has been shown to be positively correlated with higher scores on the H&Y scale (Murros et al., 2021). The relative abundance of genera *Colinsella, Eggerthella,* and *Adlercreutzia,* all a part of the *Actinomycetota* phylum, have also been shown to have a positive correlation with PD progression and symptom severity (Zhang et al., 2020), something which has also been demonstrated in the case of *Methanobrevibacter* genus, which is a part of the *Euryachaeota* phylum (Zhang et al., 2020; Rosario et al., 2021).

In the earlier mentioned study conducted by Rosario et al. (2021), the relative abundance of the species *Victivalis vadensis*, which belongs to the *Lentisphaerota* phylum, has also shown a positive correlation with UPDRS III scores in the same study.

Impact of microbiota on non-motor symptoms in Parkinson's disease

Similarly to motor symptoms, research has shown a mostly positive correlation between the abundances of certain microbiota taxa and non-motor symptoms in PD (**Table 4**).

Phylum *Bacillota*

Besides having a connection to motor symptoms, bacteria from this phylum have been shown to have a link to MoCA and MMSE scores, as well as other non-motor symptoms.

For instance, the relative abundance of the class *Clostridia* has shown a negative correlation with MMSE (Li et al., 2022). This can also be seen at lower taxonomic levels, with the abundance of the genus *Hydrogenoanaerobacterium* showing a negative association with MoCA scores and the relative abundance of the genus *Ruminococcus* showing a negative association with MMSE (Ren et al., 2020).

In regards to the *Eubacteriales* order, the relative abundances of the genera *Coprococcus* and *Tyzzerella*, which are a part of the family *Lachnospiraceae,* have demonstrated a positive correlation with non-motor symptoms (Li et al., 2019). A higher relative abundance of the family *Christensenellaceae* has also been positively correlated with non-motor symptoms (Barichella et al., 2019). The relative abundance of the genus *Oscillospira*, which is a part of the *Oscillospiraceae* family, has shown a negative association with MoCA scores (Ren et al., 2020). Furthermore, changes in the relative abundance of the genus *Anaerotruncus* have been linked to depression (Heintz-Buschart et al., 2018). Moreover, the relative abundance of the earlier mentioned species *Ruminococcus_torques* has shown a positive correlation with NMSQ (Li et al., 2019). On the other hand, the relative abundances of *Clostridium XIVb* and the genus *Butyriicoccus,* which are a part of the family *Clostridiaceae,* have shown a positive association with MMSE (Qian et al., 2018).

The *Accidaminococcales* order also showed a link to non-motor symptoms, with the relative abundance of the family *Acidaminococcaceae,* demonstrating a positive correlation with NMSQ scores (Li et al., 2019). In a different study, the relative abundance of its genus *Acidaminococcus* was negatively associated with MMSE scores (Ren et al., 2020).

Regarding the *Erysipelotrichiales* order, the relative abundance of the genus *Solobacterium*, which is a part of the family *Erysipelotrichidae,* has shown a negative association with MoCA scores (Ren et al., 2020). In the study conducted by Li et al. (2019), the family *Erysipelotrichaceae* has shown a similar connection to non-motor symptoms, with its relative abundance positively correlating with NMSQ scores. On the other hand, in the same study, the relative abundance of the order *Lactobacillales* was negatively correlated with NMSQ (Li et al., 2019).

Phyli *Bacteroidota* and *Pseudomonadota*

A study conducted by Ren et al. (2020) reveals several genera from both phyla which have been shown to have a negative correlation between their relative abundance and MMSE/MoCA. In the phylum *Bacteroidota*, this has been shown with the family *Odoribacteraceae.* The relative abundances of the genera *Odoribacter* and *Butyricimonas* have been negatively correlated with MMSE. Same has been shown for the genus *Barnesiella*, which is a member of the *Barnesiellaceae* family. Furthermore, the relative abundance of genus *Alistipes,* a part of the *Rikenellaceae* family, has shown a negative association with MoCA scores. The same connection was established for members of the phylum *Pseudomonadota*. In the *Betaproteobacteria* class, the relative abundance of the genus *Oxalobacter* has shown a negative association with MMSE, while the abundance of the genus *Sutterella* has shown a negative association with MoCA (Ren et al., 2020).

The link to GI dysfunction has also been explored. In one study, representatives of the *Bacteroidota* phylum, more specifically the family *Prevotellaceae* as well as its genus *Prevotella*, have shown a lowered abundance in PD patients with irritable bowel syndrome-like symptoms (Mertsalmi et al., 2017). In a different study, the relative abundance of the species *E. coli*, which belongs to *Gamaproteobacteria*, has shown a positive correlation with gastrointestinal (GI) dysfunction (Rosario et al., 2021). In the same study, the earlier mentioned *Victivallis vadensis* has shown a similar connection.

TABLE 4 Relative abundances of bacterial taxa in correlation with non-motor symptoms.

Kingdom	Phylum	Order	Family	Genus
Bacteria	*Bacillota*	*Eubacteriales*	*Oscillospiraceae*	*Ruminococcus*—positive correlation with NMSQ
				Hydrogenoanaerobacterium—negative correlation with MoCA
				Oscillospira—negative correlation with MoCA
				Anaerotruncus—positive correlation with depression
				Mediterraneibacter * species *Ruminococcus_torques*—positive correlation with NMSQ
			Lachnospiraceae	*Coprococccus*—positive correlation with NMSQ
				Tyzzerella—positive correlation with NMSQ
			Clostridiaceae	*Clostridium XIVb*—positive correlation with MMSE
				Butyriicococcus—positive correlation with MMSE
			Christensenellaceae—positive correlation with non-motor symptoms	
		Acidaminococcales	*Acidaminococcaceae*—positive correlation with NMSQ	*Acidaminococcus*—negative correlation with MMSE
		Erysipelotrichales	*Erysipelotrichaceae*—positive correlation with NMSQ	*Solobacterium*—negative correlation with MoCA
		Lactobacillales—negative correlation with NMSQ		
	Bacteroidota	*Bacteroidales*	*Odoribacteraceae*	*Odoribacter*—negative correlation with MMSE
				Butyricimonas—negative correlation with MMSE
			Barnesiellaceae	*Barnesiella*—negative correlation with MMSE
			Rikenellaceae	*Alistipes*—negative correlation with MoCA
			Prevotellaceae—negative correlation with IBS-like symptoms	*Prevotella*—negative correlation with IBS-like symptoms
	Pseudomonadota	*Burkholderiales*	*Oxalobacteraceae*	*Oxalobacter*—negative correlation with MoCA
			Sutterellaceae	*Sutterella*—negative correlation with MoCA
		Enterobacteriales	*Enterobacteriaceae*	*Escherichia*—positive association with GI dysfunction
	Verrucomicrobiota	*Verrucomicrobiales*	*Akkermansiaceae*	*Akkermansia*—positive correlation with NMSQ
	Thermodesulfo-bacteriota	*Desulfovibrionales*	*Desulfovibrionaceae*	*Desulfovibrio*—negative correlation with MMSE; linked to hyposmia
				Bilophila—negative correlation with MMSE
	Actinomycetota	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*—positive correlation with UPDRS I and constipation
	Campylobacterota	*Campylobacterales*	*Helicobacteraceae*	*Helicobacter*—negative correlation with MoCA
	Synergistota	*Synergistales*	*Synergistaceae*	*Pyramidobacter*—negative correlation with MoCA

*Species denotes a subcategory of genus which is species.

Other

There have been additional genera reported to influence non-motor symptoms in PD. In one study, the relative abundance of the earlier mentioned *Akkermansia,* besides being connected to motor symptoms, demonstrated a positive correlation between its relative abundance and NMSQ (Li et al., 2019). The relative abundances of *Bilophila* and *Desulfovibrio,* two genera from the family of *Desulfovibrionaceae,* which is a part of the *Thermodesulfobacteriota* phylum, have been found to have a negative correlation between relative abundance and MMSE (Ren et al., 2020). Also, the genus *Desulfovibrio* has been found to be more abundant in patients with hyposmia (Murros et al., 2021).

In one study, the relative abundance of the genus *Bifidobacterium*, which is a part of the *Actinomycetota* phylum, has been associated with constipation (Baldini et al., 2020) and its relative abundance has also been found to have a positive

correlation with UPDRS I scores, mainly through its link with hallucinations (Minato et al., 2017).

In the earlier mentioned study by Ren et al. (2020), two more genera have been linked to lower performances on MoCA and MMSE scores. For instance, the relative abundance of the genus *Helicobacter*, which belongs to the *Campylobacterota* phylum, has shown a negative association with MoCA scores, while on the other hand, the relative abundance of the genus *Pyramidobacter*, which is a part of the *Synergistotta* phylum, has shown a negative association with MMSE (Ren et al., 2020).

Discussion

In the last decade, more studies have tackled the effect of gut microbiome alteration on the emergence and development of neurodegenerative diseases, with PD being an especially interesting target for research due to the wide range of different motor and non-motor symptoms.

The studies collected in this systematic review have mostly correlated the relative abundance of various gut microbiota taxa with UPDRS III scores and H&Y scale, used to express motor symptom severity and disease severity, while the non-motor symptoms were tested mainly through MoCA, MMSE, and NMSS. Oral and nasal microbiota was not considered for this review, due to a limited number of research. Multiple confounders such as diet, therapy and comorbidities were partially considered in the research conducted by the authors. For instance, in a study by Li et al. (2019), several correlations were found between certain microbiota taxa and clinical scales when analyzed both on the PD patients and the healthy controls together. However, after analyzing them individually, no significant correlations were found in either the PD group or the controls (Li et al., 2019). This difference could reflect the impact of the disease as a general state, rather than present as a connection between specific microbiota and individual symptoms and clinical scales showing that the confounding factors should be carefully considered when conducting microbiome research regardless of a potential link between the relative abundance of specific microbiota taxa and s clinical scales that might present itself in the initial results. It should also be noted that the studies in this review have demonstrated a variability in the number of participants, with numbers ranging from 20 to 350, with some research not including healthy controls.

Another problem could arise in the varied methodology used for the analysis of microbiota composition (**Table 2**). While most of the studies used the V3–V4 regions of 16S RNA for amplification and sequencing (Qian et al., 2018; Aho et al., 2019; Barichella et al., 2019; Pietrucci et al., 2019; Baldini et al., 2020; Cosma-Grigorov et al., 2020; Ren et al., 2020; Takahashi et al., 2022) some of the studies used other regions such as V1–V3 regions (Scheperjans et al., 2015; Mertsalmi et al., 2017), purely V4 regions of 16S RNA (Heintz-Buschart et al., 2018; Li et al., 2019; Lin et al., 2019; Zhang et al., 2020), V3–V5 (Li et al., 2017), V4–V5 regions (Weis et al., 2019; Li et al., 2022). A study by Heintz-Buschart et al. (2018), besides using 16S RNA also used the 18S RNA. In a study conducted by Minato et al. (2017), besides analyzing the 16S RNA, the 23S RNA was also included, and a special protocol was used, called SYBR Green 1, with a selected number of only 19 bacterial taxa used. A study by Rosario et al. (2021) used previously acquired metagenomic data from a German PD Cohort conducted earlier and did not specify the methods of amplification and sequencing. In a different study, specific primers for the 16S rRNA were used for detection of only one genus and its subspecies, the genus being *Desulfovibrio* (Murros et al., 2021). It is apparent that through the inclusion of, not only different regions of 16S RNA, but also different RNA-s altogether, the results could significantly vary. The methods of OTU (Operational Taxonomic Unit) designation were also varied, including Mothur, QIIME pipeline, QIIME2 pipeline, USEARCH, UPARSE, and databases such as GreenGenes, SILVA, among others (**Table 2**). The biggest concern, however, is the extremely variable microbiota data and statistical analysis methodology, which in turn could lead to potentially spurious correlations between clinical scales and relative abundances acquired and analyzed through these various methods. Due to microbiome data being compositional in nature, compositionality-aware methods for correlation and differential abundance should be used, such as SparCC and Spearman's rank correlation coefficient, when it comes to correlation and ALDEx2/ANCOM for differential abundance (Gloor et al., 2017). A number of studies represented in this review did in fact use some form of compositional analysis such as Spearman's rank correlation coefficient or SparCC to calculate and present the correlations between relative abundances of gut microbiota and symptoms of PD (**Table 2**). However, methods like ANCOM were used to a lesser extent (Heintz-Buschart et al., 2018; Aho et al., 2019; Lin et al., 2019). A model for a compositional approach as opposed to a standard one was proposed in a paper by Gloor et al. (2017), and could help in developing a standardized approach for microbiota analysis in the future.

With all of this in mind, the results of this review and the studies encompassed should be interpreted cautiously.

When it comes to motor symptoms, the relative abundance of gut microbiota taxa has been often shown to positively correlate with UPDRS III scores and H&Y scale. The exception to this was microbiota belonging to the order *Bacillales* (Li et al., 2019), families *Prevotellaceae* (Scheperjans et al., 2015; Aho et al., 2019), and *Lachnospiraceae* (Barichella et al., 2019; Pietrucci et al., 2019), while on a genus level, this has been shown with genera *Ruminococcus*, *Haemophilus* (Li et al., 2017), *Prevotella* (Aho et al., 2019), *Flavonifractor, Paraprevotella* (Baldini et al., 2020), *Blautia* (Takahashi et al., 2022), *Faecalibacterium* (Li et al., 2017; Weis et al., 2019) and

Prevotella (Aho et al., 2019), and species *Pseudomonas_veronii* (Li et al., 2019). It can be thus hypothesized that the increase in relative abundance of various microbiota taxa could lead to more expressed motor symptoms in PD patients. It should be noted, however, that the results focused on higher taxonomic instances should be interpreted more carefully, especially with regards to the microbiota families and classes, since sometimes there are members of said groups that have an entirely opposite correlation when observed on a genus level. This is the case of *Coprococcus* (Li et al., 2019; Zhang et al., 2020), which has been shown to have a positive correlation with UPDRS III scores and motor symptom severity, in contrast to *Lachnospiraceae* family of which it is a part of. Another factor is the correlation between relative abundances of different microbiota taxa in the same sample, whereas the increase of relative abundances on certain taxonomic levels, such in the case of *Ruminococcaceae,* has been shown to compensate the lower levels of *Prevotellaceae* (Scheperjans et al., 2015).

Considering non-motor symptoms, the microbiota taxa identified in this review has been found to have a mostly negative correlation with non-motor symptoms, apart from the order *Lactobacillales* which showed a negative correlation with NMSQ scores (Li et al., 2019) as well as the genera *Clostridium XIVb* and *Butyriciococcus* (Qian et al., 2018) which demonstrated positive correlations to MoCA/MMSE scores. The link between microbiota and cognitive decline is being researched regarding Alzheimer's disease (Khedr et al., 2022) and the etiopathogenesis behind changes leading to cognitive deterioration potentially modulated by microbiome alterations are yet to be discovered in both diseases, with the possibility of shared mechanisms. It should also be noted that direct correlation between microbiota and non-motor symptoms is still under question, since both cognitive decline (Fang et al., 2020) and changes in microbiome (Li et al., 2017; Weis et al., 2019; Cosma-Grigorov et al., 2020; Murros et al., 2021; Rosario et al., 2021) have been shown as an intrinsic part of later disease stages. A negative connection to specific symptoms has been found in the case of the species *E. coli,* which is linked to GI dysfunction in PD patients (Rosario et al., 2021) and the genus *Desulfovibrio*, which has been linked to hyposmia (Murros et al., 2021). GI dysfunction is a staple of PD (Lubomski et al., 2020), but it poses the question of whether the microbiome changes are behind GI dysfunction or a cause for it. The link to hyposmia, which is thought to be caused by the early deposition of Lewy pathology in the olfactory bulb (Fullard et al., 2017), and microbiota could play a part in this through the earlier mentioned gut-brain hypothesis.

The mechanistic answer for these changes could lie in the metabolites of microbiota. One of the most prominently researched ones are the short-chain fatty acids (SCFA). In one study, the relative abundances of the species *Ruminococcus* sp. *AM07 15* and *Clostridiales bacterium NK3B98* have shown a correlation with the plasma and fecal levels of SCFA, most notably propionic acid. Furthermore, the same study showed that the decreased fecal levels and increased plasma levels of SCFA, most notably propionic acid, had a positive correlation to UPDRS III scores (Chen et al., 2022). This has also been shown in a different case-control study where serum level of propionic acid was correlated with UPDRS III scores, MMSE and Hamilton Depression Scale (HAM-D) (Wu et al., 2022). In a different study conducted by Aho et al. (2019), the genus *Prevotella* has been linked with a higher butyric acid production, which has been shown to postpone the age of disease onset in PD patients. The species *Akkermansia municiphila*, which belongs to the *Verrucomicrobiota* phylum, has shown a role in taurine metabolism, mainly through lowering plasma taurine levels, which in turn has a negative effect on UPDRS III scores. Same has been shown in the case of *Bilophila wadsworthia*, part of the *Thermodesulfobacteriota* phylum (Hertel et al., 2019). Another member of this phylum, the genus *Desulfovibriobacteria* has been proposed to produce magnetite as well as hydrogen sulfite which could accelerate alpha-synuclein aggregation (Murros et al., 2021).

In general, the limitation of the field is a scarce quantity of studies connecting microbiota abundance and metabolism with PD symptoms, and more research is needed to confirm the causal link between the two. When looking at the study design, clearly there is a lack of randomized controlled studies, case control studies with *de novo* patients and longitudinal studies. These are required to confirm the correlations mentioned in this systematic review, but also to highlight whether these changes are intrinsic to the disease or are perhaps a consequence of therapy as well. We are currently conducting a longitudinal study with *de novo* patients (Clinicaltrials.gov, NCT05008094) and are looking to add to the current knowledge of both symptom and abundance correlation, and the effects of therapy on the composition of microbiota.

All of this is important when looking at potential future therapeutic options, since gut microbiota can be altered by various intrinsic and extrinsic factors, which could in turn potentially influence the severity of symptoms of PD and other neurodegenerative diseases. For instance, antibiotics have been shown a potential benefit in a study conducted by Pu et al. (2019), where an antibiotic cocktail (ampicillin, neomycin sulfate, metronidazole) was applied in a MPTP rodent model. This caused changes in microbiome composition compared to the control group and countered the neurotoxic effects of MPTP (Pu et al., 2019). In a different study, the antibiotic rifampicin has been shown to inhibit α-synuclein fibrillation, a pathological mechanism behind PD (Li et al., 2004). Probiotics have also been explored. In one study, probiotic mixtures of different bacteria have been shown in one study to reduce dopaminergic neuron loss as well as increase dopamine levels (Srivastav et al., 2019). The Mediterranean diet has shown a beneficial effect in Alzheimer's disease, but also in PD. The diet has been shown to be rich in *Lactobacilli,* and the adherence to the diet lowered the odds for both Alzheimer's disease and PD (Alcalay et al., 2012). Another interesting study researched the potential for enema application as a modulation of microbiota

composition. The UPDRS III scores improved after enema, with lowered abundances of the family *Ruminococcaceae* and the genus *Clostridium* (Hegelmaier et al., 2020). Another method that has been explored is FMT. In one study, colonic FMT has been shown to decrease UPDRS III, NMSQ, PDQ-39, HAM-D, and Hamilton Anxiety Scale (HAM-A) scores in a small group of 10 PD patients with only a minor number of mild self-limiting side effects, mostly pertaining to the GI tract (Xue et al., 2020). It is apparent that therapeutic intervention on the microbiome level, be it on a medical or a dietary, could increase the overall therapeutic yield and response to medication, or even potentially postpone the initial symptoms of the disease, something which should be further explored.

Conclusion

It is apparent that the link between the microbiome and the neurodegeneration that could lead to motor and non-motor symptoms of PD complex and multifactorial. The relative abundance of specific microbiota taxa has been consistently shown to be correlated with symptom severity, either positively or negatively, but the causal link is still in question. The mechanistic answer could lie in the products of microbiota metabolism, which have also been linked to symptom severity through intricate metabolic pathways that are under influence of various confounding factors, with PD being just one part of the bigger picture. In a clinical setting, therapeutic interventions have already been explored regarding microbiome manipulation, showing promising results, be it through the use of antibiotics, probiotics, diet changes, or more specific methods such as enema application and FMT. Combined with established PD treatment, these methods could enhance the overall therapeutic success and provide a more personalized approach to each patient. Further research is thus warranted in the field, with a focus on both abundance and metabolic function of microbiota in relation to motor and non–motor symptoms, along with studies greater in quality, such as randomized controlled studies and case control studies with *de novo* patients and longitudinal studies, as well as more standardized methods for isolation and compositional data analysis.

Author contributions

EP and VV conceptualized the systematic review. EP, VR, MH, ZT, NS-Č, AK, MK, GH, BP, and VV developed and consulted on the search strategy and methodology. EP, VR, MH, and AK assisted with screening article. EP, VR, and MH abstracted data from the article. EP drafted the manuscript. All authors reviewed, edited, assisted with writing subsequent drafts of the manuscript, and approved the final version of the manuscript.

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Conflict of interest

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EDITED BY
Muhammad Shahid Riaz Rajoka,
Tohoku University, Japan

REVIEWED BY
Jadoon Khan,
COMSATS University Islamabad, Pakistan
Shakeel Ahmad,
Bahauddin Zakariya University, Pakistan

*CORRESPONDENCE
Tatsuya Unno
✉ tatsu@jejunu.ac.kr
Jae-Ho Shin
✉ jhshin@knu.ac.kr

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Butyrate producers, "The Sentinel of Gut": Their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics

Vineet Singh[1], GyuDae Lee[1], HyunWoo Son[1], Hong Koh[2], Eun Soo Kim[3], Tatsuya Unno[4]* and Jae-Ho Shin[1,5]*

[1]Department of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea, [2]Department of Pediatrics, Severance Fecal Microbiota Transplantation Center, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea, [3]Department of Internal Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [4]Faculty of Biotechnology, School of Life Sciences, SARI, Jeju National University, Jeju, Republic of Korea, [5]Department of Integrative Biotechnology, Kyungpook National University, Daegu, Republic of Korea

Gut-microbial butyrate is a short-chain fatty acid (SCFA) of significant physiological importance than the other major SCFAs (acetate and propionate). Most butyrate producers belong to the Clostridium cluster of the phylum Firmicutes, such as *Faecalibacterium*, *Roseburia*, *Eubacterium*, *Anaerostipes*, *Coprococcus*, *Subdoligranulum*, and *Anaerobutyricum*. They metabolize carbohydrates *via* the butyryl-CoA: acetate CoA-transferase pathway and butyrate kinase terminal enzymes to produce most of butyrate. Although, in minor fractions, amino acids can also be utilized to generate butyrate *via* glutamate and lysine pathways. Butyrogenic microbes play a vital role in various gut-associated metabolisms. Butyrate is used by colonocytes to generate energy, stabilizes hypoxia-inducible factor to maintain the anaerobic environment in the gut, maintains gut barrier integrity by regulating Claudin-1 and synaptopodin expression, limits pro-inflammatory cytokines (IL-6, IL-12), and inhibits oncogenic pathways (Akt/ERK, Wnt, and TGF-β signaling). Colonic butyrate producers shape the gut microbial community by secreting various anti-microbial substances, such as cathelicidins, reuterin, and β-defensin-1, and maintain gut homeostasis by releasing anti-inflammatory molecules, such as IgA, vitamin B, and microbial anti-inflammatory molecules. Additionally, butyrate producers, such as *Roseburia*, produce anti-carcinogenic metabolites, such as shikimic acid and a precursor of conjugated linoleic acid. In this review, we summarized the significance of butyrate, critically examined the role and relevance of butyrate producers, and contextualized their importance as microbial therapeutics.

KEYWORDS

butyrate producers, microbial homeostasis, gut epithelial barrier, immunomodulation, gut inflammation, colorectal cancer, gut-organ axis

Role of butyrate-producing gut-commensals

The human gut harbors an enormous number of microbes, approximately 38×10^{12} in total (Sender et al., 2016), comprising genetic material that is comparable to the human genome itself (Manson et al., 2008). This complex gut microbiome contains both aerobic and anaerobic commensal microbes, but anaerobic microbes constitute 99% of the gut microbiota (Nagpal et al., 2017). The gut environment is predominantly anaerobic, providing a suitable ecological niche for anaerobic commensals. The gut microbiome is host-specific, and even among healthy individuals, it varies with geographical location, race, ethnicity, and diet (Gupta et al., 2017). These host-specific gut communities interact with each other through a number of metabolites, which in turn promote gut health (Lin and Zhang, 2017; Krautkramer et al., 2021). Gut microbes also affect the overall health of the host by participating in various metabolic pathways, regulating gene expression, and synthesizing beneficial bioactive compounds, such as short-chain fatty acids (SCFAs), amines, secondary bile acids, and vitamins. In the gut, SCFAs are the major beneficial metabolites produced by gut microbes through metabolizing indigestible dietary fibers. SCFAs are fatty acids with fewer than six carbon atoms and comprise three major forms, i.e., acetate (60%), propionate (20%), and butyrate (20%) (Chambers et al., 2018). Among them, butyrate has been considered of significant importance, as it is involved in several functions of physiological importance, such as trans-epithelial transport, amelioration of mucosal inflammation, alleviation of oxidative stress, enforcement of the epithelial barrier, and protection against colorectal cancer (CRC) (Hamer et al., 2008). The microbial origin butyrate is mainly synthesized by certain anaerobic commensal microbes belonging to the Clostridium cluster (Clostridium_IV and Clostridium_XIVa) of the phylum Firmicutes (Manson et al., 2008). In addition, it is also known that certain commensals convert bacterial metabolites such as lactate and acetate into butyrate *via* the acetyl-CoA pathway (Bui et al., 2015; Belzer et al., 2017).

In the gut, colon is the primary site of fermentation of indigestible fibers by fibrolytic, butyrate-producing microbes, such as *Roseburia intestinalis*, *Faecalibacterium prasunitzi*, *and Eubacterium,* which are sensitive to the presence of oxygen (Manson et al., 2008). Colonic butyrate is actively transported to colonocytes by monocarboxylate transporters, where the majority (~70%) of transported butyrate is used to generate energy *via* the citric acid cycle. Non-metabolized butyrate, on the other hand, is transported to the hepatic portal system (Zheng et al., 2017) where butyrate acts as an energy source for hepatocytes, and from there, it is transported to peripheral tissue and systemic circulation. The concentration of butyrate in portal circulation is around 30 μM, and falls near 0.2–15 μM in the systemic circulation, which is almost 2% of the colonic butyrate concentration (Dalile et al., 2019).

The lower level of butyrate producers is continuously found to be associated with various ailments, such as *Roseburia* in colorectal cancer and inflammatory bowel disease (Sun et al., 2020; Wu et al., 2022), butyrate-producing *Coprococcus* in pregnant preeclampsia patients (Altemani et al., 2021), and *Faecalibacterium* in gut inflammation (Fujimoto et al., 2013). Therefore, the level of butyrate producers should be considered to be of therapeutic importance, which has even promoted its oral administration in various studies (Vieira et al., 2012; Chen et al., 2018; Liu et al., 2019). Additionally, butyrate producers are present in the human gut, and their proportion can be enhanced by selecting a suitable diet and healthy lifestyle, thus facilitating the maintenance of overall gut health.

Microbial butyrate and its fate in the gut

Studies suggest that initial butyrate-producing communities, i.e., initial butyrate producers in infant gut, such as Clostridiaceae, Lachnospiraceae, and Ruminococcaceae spp., might be introduced into the human gastrointestinal tract *via* resistant microbial endospores (Appert et al., 2020). A recent study on a Swiss-cohort confirmed that *Eubacterium hallii*, a member of the family Lachnospiraceae, is one of the earliest butyrate producers in the gut of infants (Schwab et al., 2017). This is also supported by a study on Swiss, Venezuela, Malawi, and USA populations, which confirmed the human milk oligosaccharide metabolizing ability of *Eubacterium Hallii* (Schwab et al., 2017). The majority of butyrate producers are gram-positive and come under Clostridium clusters IV and XIVa of the phylum Firmicutes (Manson et al., 2008; Table 1). These microbial communities comprise a significant population of butyrate-producers, including various butyrogenic species of *Eubacterium*, *Faecalibacterium*, and *Roseburia* (Manson et al., 2008; Louis and Flint, 2009). Among all butyrate producers, *Faecalibacterium prausnitzii* is most abundant in fecal samples (~ 5%) (Miquel et al., 2013), and its proportion can increase up to 13–17.6% (Manson et al., 2008). Other major butyrate producers in fecal gut microbiota are *Eubacterium rectale*, *Eubacterium Hallii*, and *Roseburia intestinalis*, which can constitute up to ~13% (Rivière et al., 2016), 2.4% (mean, 0.6%), and 0.9–5% (mean, 2.3%), respectively (Hold et al., 2003). In smaller fractions, various other butyrate producers are also present in the gut, which produce butyrate by utilizing different dietary oligosaccharides, polysaccharides, and metabolic intermediates (Table 1). Although the majority of butyrate-producing microbes belong to the phylum Firmicutes, studies have suggested that certain members of the phyla Actinobacteria, Bacteroidetes, Fusobacteria, and Proteobacteria can also produce butyrate (Vital et al., 2014). During fermentation, butyrate producers cause substrate-level phosphorylation of the dietary substrate to generate energy in the form of ATP, which results in the formation of multiple

TABLE 1 Major butyrate producers in the human gut and their relevance.

Butyrate producer					
Phylum	Sub-cluster	Genus	Species	Relevance	Reference
Firmicutes	Clostridium IV Or Clostridium leptum group	*Faecalibacterium*	*F. prasunitzi*	Most abundant butyrate producer	Louis and Flint (2009)
		Subdoligranulum	*S. variabile*	Metabolizes calprotectin	Kamp et al. (2022)
		Anaerotruncus	*A. colihominis*	Degrade mucin	Raimondi et al. (2021)
		Ruminococcus	*R. bromii*	Key fermenter of resistant starch	Ze et al. (2012)
			R. callidus	Degrades complex polysaccharides such as starch or xylan	Chassard et al. (2012)
			R. champanellensis	Most efficient cellulolytic bacterium in human colon	Chassard et al. (2012)
	Clostridium XIVa or Clostridium coccoides group	*Roseburia*	*R. intestinalis*	Major Xylan degrader in human gut	Leth et al. (2018), Mirande et al. (2010)
			R. faecis	Utilizes fructose, glucose, maltose, cellobiose, raffinose, xylose, sorbitol, melibiose and amylopectin starch; but not Arabinose, and sucrose	Duncan et al. (2006)
			R. hominis	Utilizes arabinose, fructose, glucose, maltose, cellobiose, xylose and glycerol; but not Sucrose, sorbitol, oat spelt xylan, amylopectin starch and inulin (dahlia)	Duncan et al. (2006)
			R. inulinivorans	Utilizes inulin (dahlia), fructose, glucose, and maltose cellobiose, and amylopectin; but not rabinose, raffinose, xylose, glycerol, sorbitol and oat spelt xylan	Duncan et al. (2006)
		Anaerostipes	*A. caccae*	Utilizes Lactate to produce butyrate	Duncan et al. (2004)
			A. hadrus	Utilizes D-Lactate (not L-Lactose) and acetate to produce butyrate	Allen-Vercoe et al. (2012)
			A. butyraticus	Utilizes fructooligosaccharide (FOS) to produce butyrate	Endo et al. (2022)
			A. rhamnosivorans	Utilizes lactate and acetate for butyrate generation	Bui et al. (2019)
		Butyrivibrio	*B. fibrisolvens*	Utilizes cellulose	Rodríguez Hernáez et al. (2018), Paillard et al. (2007)
		Eubacterium	*E. rectale*	Metabolizes sulfonated monosaccharide (sulfoquinovose) present in green vegetables; Dahlia inulin is specifically catabolized	Hanson et al. (2021)
			E. ramulus	Metabolizes variety of flavonoids	Schneider and Blaut (2000), Braune et al. (2001)
			E. hallii	Utilizes glucose and the intermediates acetate and lactate, for butyrate generation	Engels et al. (2016a)
			E. limosum	Transformation of 8-prenylanringenin (phyto-estrogen) from iso-xanthohumol	Possemiers et al. (2008)
		Coprococcus	*C. cactus*	Metabolizes fructose; cross-feed on fermentation products (acetate, lactate) to produce butyrate	Reichardt et al. (2014), Alessi et al. (2020)
			C. eutactus	Metabolizes β-glucan, cellobiose and lichenan	Alessi et al. (2020)
			C. comes	Metabolizes glucose	Alessi et al. (2020)
		Anaerobutyricum	*A. soehngenii*	Utilizes D-and L-lactate and acetate to produce butyrate	Gilijamse et al. (2020)

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end-products, including butyrate (Louis and Flint, 2009). In the human gut, the majority of microbial butyrate is synthesized from carbohydrate metabolism *via* butyryl-CoA: acetate CoA-transferase pathway (but) and butyrate kinase (buk) pathway, of which the but-pathway is predominant (Vital et al., 2013); (but) and (buk) are derived from the genes encoding enzymes involved in the terminal steps of microbial butyrate synthesis (Altemani et al., 2021). *Radioisotope analysis of human fecal microbiota has shown that the* majority of butyrate in the gut is produced from carbohydrates through the Embden-Meyerhof-Parnas pathway (glycolysis) *via* acetyl-CoA (Miller and Wolin, 1996; Louis and Flint, 2009; Figure 1). *During this process, two molecules of acetyl-CoA combine to form a butyrate molecule* (Miller and Wolin, 1996), and the transformation of crotonyl-CoA to butyryl-CoA is the main energy generation step (Tsukuda et al., 2021; Figure 1). *In addition to carbohydrates, in minor fraction, butyrate can also be synthesized from proteins via glutamate, lysine, glutarate, and 4-aminobutyrate pathways* (Louis and Flint, 2017; Vital et al., 2017; Mallott and Amato, 2022). Furthermore, butyrate is transported into colonocytes in the gut epithelium *via* monocarboxylate transporter 1 (MCT1) (Cuff et al., 2002), where it participates in various activities, including stabilization of hypoxia-inducible factor (HIF), inhibition of histone deacetylase (HDAC), and regulation of specific G-protein coupled receptors, which will be discussed later.

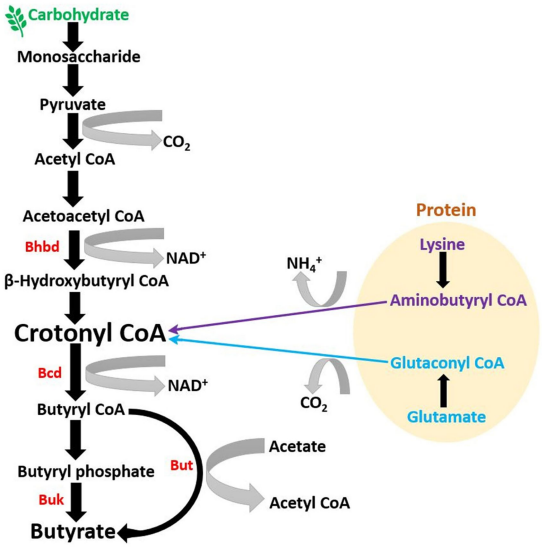

FIGURE 1
Microbial pathway to generate butyrate in gut: Majority of butyrate in the colon is generated by the metabolization of dietary fibers, primarily of carbohydrate origin (BHBD, β-hydroxybutyryl-CoA dehydrogenase; Bcd, butyryl-CoA dehydrogenase; But, butyryl-CoA: acetate CoA-transferase; Buk, butyrate kinase).

Impact of butyrate producers on neighboring gut microbial communities

In the gut, butyrate-producing microbial communities play a crucial role in maintaining a healthy gut environment as they restrict the entry and establishment of other microbes, especially pathogenic microbes. Butyrate is used by colonocytes to generate energy which increases epithelial oxygen consumption (Litvak et al., 2018). As a result, the presence of butyrate producing bacteria helps maintain an anaerobic environment in the gut, which further prevents the colonization of opportunistic aerobic pathogens, such as *Salmonella and E. coli* (Manson et al., 2008; Parada Venegas et al., 2019). Butyrate also regulates the production of cathelicidins, a polycationic peptide that participates in mammalian innate immunity and exhibits broad-spectrum antimicrobial activity against potential gut pathogens (van Vliet et al., 2010; Kościuczuk et al., 2012; van Harten et al., 2018). Moreover, butyrate-producing bacteria such as *E. hallii* produces reuterin, a broad-spectrum antimicrobial agent with yeast inhibition activity (Engels et al., 2016b) while metabolizing glycerol to 3-hydroxypropionaldehyde (Figure 2). These anti-microbial agents limit the incursion or abundance of potential pathogens and thus, help maintain a healthy gut microbiome.

Butyrate produced in the gut shapes the gut microbial community *via* regulating IgA secretion and by limiting the hyperresponsiveness of macrophages toward colonic commensals to maintain their abundance (Chang et al., 2014; Isobe et al., 2020). Butyrate regulates colonic macrophages present in the lamina propria by inhibiting HDAC, and limits the generation of proinflammatory IL-12 and IL-6, as well as antimicrobial nitric oxide from lipopolysaccharide-stimulated macrophages (Chang et al., 2014; Kibbie et al., 2021). Butyrate enhances the GPCR-independent antimicrobial activity of macrophages *via* metabolites, as evidenced by a study that showed that macrophages grown in the presence of microbial butyrate upregulated the expression of antimicrobial protein calprotectin but showed lowered expression of anti-inflammatory IL-10 (Schulthess et al., 2019; Jukic et al., 2021; Figure 2). Additionally, microbial butyrate significantly enhances the ability of macrophages to eliminate possible pathogens, such as *Salmonella enterica* and *Citrobacter rodentium* (Flemming, 2019). Thus, butyrate bolsters gut defense against invasive pathogens without causing tissue-damaging inflammation or hyper-responsiveness. Butyrate-induced macrophages also exhibit higher levels of AMP, an inducer of AMP-kinase (AMPK), which inhibits mammalian target of rapamycin (mTOR), the master regulator protein kinase of autophagy, which is associated with cancer, insulin resistance, and other diseases (Schulthess et al., 2019; Figure 2).

In vitro and *in vivo* studies have also shown that butyrate producers participate in vitamin biosynthesis, especially vitamin B complex biosynthesis. For example, *Eubacterium hallii* produces vitamin B12, which is symbiotically utilized by *Akkermansia* to produce propionate (Belzer et al., 2017; Pham et al., 2021;

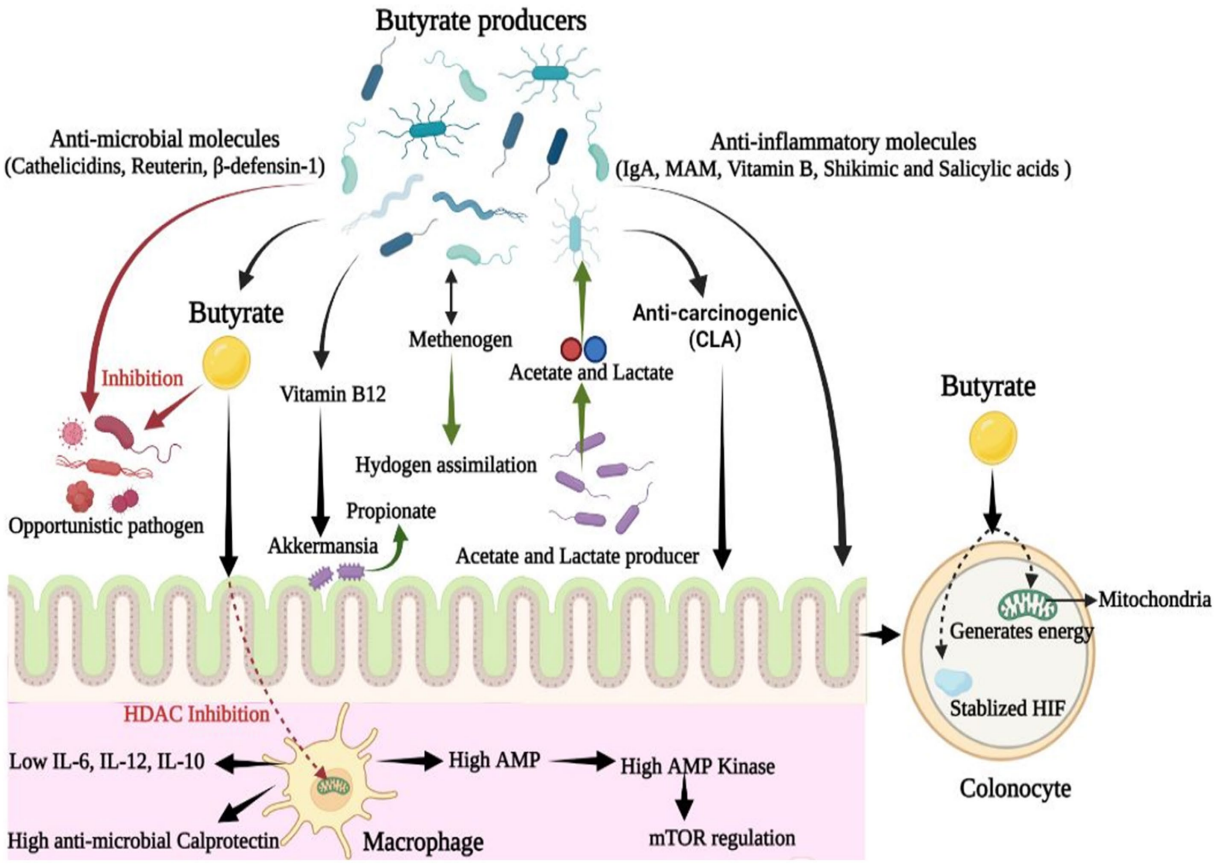

FIGURE 2
Dynamic role of butyrate producing microbial communities in gut: Along with butyrate, butyrate-producing communities also produce various bioactive molecules that are anti-microbial, anti-inflammatory, and anti-carcinogenic in nature. These molecules are of therapeutic importance in alleviating gut-associated disorders and maintaining gut-homeostasis (CLA, Conjugated Linoleic Acid; IL, Interleukin; MAM, Microbial Anti-inflammatory Molecule).

Figure 2). The vitamin B complex acts as an essential cofactor in various metabolic activities and is also associated with the regulation of immunological homeostasis in the host (Yoshii et al., 2019). A cross-feeding relationship is also reported between butyrogenic genera, such as *Faecalibacterium*, *Roseburia*, *Anaerostipes*, *Eubacterium*, and probiotic *Bifidobacterium* (Rivière et al., 2016). For example, *Bifidobacterium* produces lactate and acetate, which are further utilized by butyrogenic microbes, such as *E. Hallii*, to generate butyrate; this in turn supports the abundance of Bifidobacterium (Louis and Flint, 2009; Schwab et al., 2017). Similarly, *Anaerostipes hadrus* and *Anaerobutyricum hallii*, members of the family Lachnospiraceae, utilize lactate and acetate to produce butyrate in the gut (Duncan et al., 2004).

Importance of butyrate producers in maintaining the gut epithelial barrier

The intestinal epithelium is a single-layer structure covered by a mucous layer and functions as the first line of defense against gut pathogens. The cells of intestinal epithelium are interconnected with tight junctions. The intestinal epithelium contains mucous-secreting goblet cells that provide barrier protection by secreting mucus, which also functions as a reservoir of immunoglobulin IgA and antimicrobial peptides (Martens et al., 2018). The mucous layer is composed of mucin, and in colon *MUC2* is the primary mucin-producing gene (Martens et al., 2018). The mucous layer adhering to the gut epithelium is thick and limits the microbial growth near the epithelial layer, whereas the outer mucous layer is less dense and suitable for the growth of different commensals, such as *Akkermansia muciniphila*, *Faecalibacterium*, *and Eubacterium rectale* (Maier et al., 2015; Martens et al., 2018). Some harmful microbes can decrease mucus thickness by degrading it, thereby allowing pathogens to enter the gut; for example, *Vibrio cholerae* secretes hemagglutinin protease that possesses mucolytic activity. Cholera-causing bacteria also secrete zonula occludens toxin, which further hampers epithelial integrity by acting on tight junctions (Martens et al., 2018). Another microbe, *Clostridium perfringens*, disrupts tight junctions by secreting endotoxins (Saitoh et al., 2015). Additionally, decreased abundance of butyrate producers leads to compromised defense and dysfunctional gut

epithelium as observed in the case of *Clostridium difficile* infection (Antharam et al., 2013).

Faecalibacterium, a major butyrate producer in the human gut, enhances mucus formation by increasing goblet cell differentiation and expression of genes related to mucin glycosylation (Wrzosek et al., 2013). Furthermore, clinical studies have demonstrated rapid recovery in patients with cholera after oral administration of resistant starch, a butyrate precursor (Canani et al., 2011). In addition, butyrate produced by bacteria in the gut accelerates mitochondria-dependent oxygen consumption in gut epithelial cells, which stabilizes HIF. Butyrate itself also inhibits HIF-prolyl hydroxylase that degrades HIF (Wang et al., 2021). Stabilized HIF regulates the tight junction protein claudin-1, *MUC2* expression, and generation of antimicrobial peptide beta defensin-1 (DEFB1) (Zheng et al., 2017; Wang et al., 2021). Butyrate also regulates the immunological aspect of barrier function as it tightens the intestinal epithelial cell barrier *via* inducing anti-inflammatory cytokine IL-10RA-dependent suppression of claudin-2 protein, which forms paracellular channels in tight junctions and increases gut permeability (Zheng et al., 2017; Zhu et al., 2019). A recent study also demonstrated the role of butyrate in the regulation of actin-binding protein synaptopodin (SYNPO), which is expressed in gut epithelial tight junctions and is crucial for gut-barrier integrity (Wang et al., 2020).

Protective role of butyrate producers against bowel inflammation

Based on their severity, inflammatory diseases of the gut can be categorized into irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). IBS is characterized by cramps, bloating, diarrhea, and/or constipation (Camilleri et al., 2016). There are no biological markers to confirm it; moreover, this condition does not pose major discomfort to the patients. Normally, IBS patients are identified using a questionnaire prepared by medical staff (Werlang et al., 2019). In contrast, IBD is a generic term for more severe conditions, such as Crohn's disease and ulcerative colitis (Franzosa et al., 2019), which cause inflammation and ulcers in the intestine, rectal bleeding, anemia, and diarrhea. Incidentally, decreased butyrate levels have often been reported in both IBS and IBD. In the case of IBD, butyrate producers play important roles as they increase mucus production from goblet cells to strengthen the intestinal mucous barrier and regulate the expression of tight junction proteins *via* butyrate to restrict the harmful penetration through the gut (Pozuelo et al., 2015; Pascal et al., 2017; Dalile et al., 2019; Schirmer et al., 2019). Similarly, in the case of IBS lower number of butyrate producers result in a reduced availability of butyrate and thus decrease the gut permeability (Camilleri et al., 2016).

Butyrate maintains the anaerobic environment in the colon by enhancing colonocyte oxygen consumption and stabilizing HIF, while its absence facilitates the buildup of potentially harmful bacteria and molecules, such as *Salmonella*, *E. coli*, and nitric oxide (NO), respectively (Parada Venegas et al., 2019). The reduced proportion of butyrate producers is also associated with a decreased count of methanogens, which disposes of the excess hydrogen (H_2) produced in the form of CH_4 during dietary fermentation, one of the possible reasons for the bloating experienced by IBS and IBD patients (Pozuelo et al., 2015; Chong et al., 2019). Studies have reported that among SCFAs, butyrate alone is responsible for gut motility, possibly *via* regulating serotonin, and can be used to increase propulsive gut movement, making it a suitable microbial therapeutic for patients with IBS (Vincent et al., 2018). An induced-colitis study in a murine model confirmed the decrease in butyrate-producing Clostridium clusters and reduced butyrate levels in the gut, which facilitated gut epithelial oxygenation and growth of *Salmonella enterica serovar Typhimurium* (S. *Typhimurium*), a known cause of foodborne gut inflammation and diarrhea (Rivera-Chávez et al., 2016; Anderson and Kendall, 2017; Litvak et al., 2019). Similarly, a reduced proportion of butyrate producers in the gut increases the expansion of aerobic Enterobacteriaceae, which is a common marker of gut dysbiosis (Matamouros et al., 2018; Parada Venegas et al., 2019). Studies have demonstrated a decreased count of butyrate-producing *Faecalibacterium* and *Roseburia* in the gut of ulcerative colitis patients (Sartor, 2011; Franzosa et al., 2019). On the other hand, the culture supernatant of *Faecalibacterium* was reportedly effective against IBD (Crohn's disease) and colitis in murine models, and *Faecalibacterium* was found to secrete an anti-inflammatory peptide (MAM, m.wt. 15 KDa), which inhibits pro-inflammatory NF-κB signaling to arrest colitis (Quévrain et al., 2016). Additionally, *Faecalibacterium* inhibits colitis by producing anti-inflammatory shikimic and salicylic acids (Miquel et al., 2015). In another study, a combination of six different butyrate producers (*B. pullicaecorum* 25–3T, *F. prausnitzii*, *Roseburia hominis*, *Roseburia inulinivorans*, *Anaerostipes caccae*, and *E. hallii*) reportedly enhanced butyrate production in IBD fecal microbiota by 5–10% and enhanced higher gut-barrier integrity, as examined in the Caco-2 cell line (Geirnaert et al., 2017). Similarly, patients with *Clostridium difficile* infection, which has a high mortality rate and increases the chances of acquiring hospital-acquired diarrhea, also exhibited a significant depletion in butyrate producers such as *Roseburia*, *Anaerostipes*, *Blautia*, and *Faecalibacterium*, along with lowered butyrate levels (Antharam et al., 2013). By contrast, in the case of mucositis, microbial butyrate enhances mucosal healing to accelerate the recovery of inflamed gut epithelium by stimulating the migration of gut epithelial cells (van Vliet et al., 2010).

By acting as a ligand, microbial butyrate participates in anti-inflammatory reactions to cease the inflammation and maintain gut homeostasis through the aryl hydrocarbon receptor (AhR) and various G-protein coupled receptors (GPCRs) such as GPR109a, GPR43, and GPR41 (Marinelli et al., 2019; Yip et al., 2021). AhR and GPCRs are transcription factors that control the transcriptional machinery of various immunoregulators following

their activation. AhR exhibits the anti-inflammatory effect by enhancing anti-inflammatory IL-10 secreting B and Th2 cells, with a decline in pro-inflammatory Th1 and Th17 cells (Dong and Perdew, 2020; Abdulla et al., 2021). Among GPCRs, butyrate-activated GPR109a promotes differentiation of Treg cells and enhances anti-inflammatory IL-10 producing Th2 cells and plasma levels of IL-10, which in turn inhibits pro-inflammatory IL-17 (Akitsu and Iwakura, 2018; Martens et al., 2018). Upon butyrate activation, GPR43 reduces CD4 T-cell proliferation and limits the secretion of pro-inflammatory cytokines such as IL-17 and IL-22 (Kibbie et al., 2021). In addition, butyrogenic clostridia such as *Clostridium butyricum* limit IBD-associated inflammation by increasing Treg cell differentiation through microbial butyrate, which exerts its effects *via* transforming growth factor-β (TGF-β) (Ihara et al., 2017).

Relevance of butyrate producers in CRC and tumorigenesis

Colorectal cancer (CRC) begins with a growth of the inner lining of the colon and rectum, which can later transform into cancerous polyps (Das et al., 2017; Salmerón et al., 2022). Evidence has shown that alterations in the gut microbiota are closely associated with CRC progression (Xie et al., 2020). Microbiome profiles of CRC patients exhibit a decrease in major butyrate-producing genera, including *Roseburia, Clostridiales, Faecalibacterium,* and members of the Lachnospiraceae family, and administration of butyrate-producing *Clostridium butyricum* was effective in decreasing the proliferation of cancerous cells and enhancing cancer cell apoptosis (Zou et al., 2018; Stoeva et al., 2021). Similarly, a lower abundance of *Eubacterium ventriosum* is a potential biomarker for CRC patients (Mukherjee et al., 2020), and its administration in CRC patients has been patented,[1] indicating its significant therapeutic importance. Additionally, gut commensals such as *Butyricicoccus pullicaecorum*, *Butyrivibrio fibrisolvens*, *Ruminococcus bromii*, and members of the family Lachnospiraceae also produce sodium butyrate upon fermenting dietary fibers, which inhibits CRC cell proliferation by regulating immune cells such as natural killer cells and macrophages, and causes apoptosis (Xi et al., 2021).

Luminal butyrate inhibits CRC mainly through HDAC inhibition by inactivating oncogenic pathways, such as mitogen-activated protein kinase (MAPK), Akt/ERK signaling, Wnt signaling pathway, and TGF-β signaling (Li et al., 2017; Geng et al., 2021). Butyrate-mediated inhibition of HDAC3 blocks the activation of Akt and ERK1/2, which are required for CRC cell migration and invasion (Li et al., 2017). Similarly, Wnt is a hydrophobic glycoprotein ligand that participates in various cellular processes, and aberration in Wnt signaling can cause CRC (Patel et al., 2019). An aberrant Wnt pathway can be suppressed by the butyrate-dependent activation of GPR109, as exhibited by *Clostridium butyricum*, but further investigation is required to confirm its direct or indirect role (Chen D. et al., 2020). Similarly, TGF-β is an immunosuppressive cytokine that regulates cell proliferation, differentiation, growth, and apoptosis, and any decrease in the inhibitory activity of TGF-β can lead to cancer, including CRC (Ku et al., 2007). Recent *in vivo* findings have reported significant expression of TGF-β after ingestion of dietary sodium butyrate, which can help combat CRC (Liu et al., 2014). Usually, cancer cells have a higher glucose demand and metabolic rate to support accelerated cell growth, which makes glycolysis inhibitors a promising anticancer drug candidate (Figure 3). Besides being an HDAC inhibitor, microbial butyrate differentially inhibits glucose transport, glycolysis, and DNA synthesis in cancerous colonocytes *via* inhibiting GLUT1 and glucose-6-phosphate dehydrogenase (G6PD) through the GPR109a-AKT pathway (Geng et al., 2021). GLUT1 is a glucose transporter, while G6PD is a key enzyme that produces ribose-5-phosphate for nucleotide synthesis (Geng et al., 2021). Microbial butyrate also inhibits CRC by increasing the 2-oxoglutarate level, which in turn downregulates proinflammatory cytokines such as IL-6, IL-22, IL1-β, and TNF-α (Wang et al., 2021). Furthermore, colonic butyrogenic microbes such as *Roseburia* and *Butyrivibrio* metabolize linoleic acid to produce the precursor of conjugated linoleic acid (CLA) (Devillard et al., 2007; Louis and Flint, 2009), which induces apoptosis and has been reported as an effective anti-carcinogenic molecule in various studies, including CRC (den Hartigh, 2019). *Roseburia* species, which are among the most active linoleic acid metabolizers, also produce vaccenic acid, which is known to be beneficial for the host (Devillard et al., 2007).

In contrast, some studies have reported an association between microbiota-derived butyrate and CRC upregulation (Okumura et al., 2021). This is a butyrate-paradox, wherein butyrate can act differently in normal and cancerous colonocytes. This is due to a metabolic shift of cancerous cells toward glycolysis, also called Warburg effect. In colonocyte mitochondria, butyrate is not metabolized to the same extent as in normal cells, and therefore, accumulates in the nucleus where it inhibits HDAC (Bultman and Jobin, 2014; Bultman, 2016; Hajjar et al., 2021; Figure 3). A similar paradox was observed in the microbial regulation of the PI3/Akt pathway, which is a major signaling cascade involved in the regulation of normal cellular activities, such as cell proliferation, growth, motility, and survival; however, its aberrant activation is associated with cancer (Luo et al., 2003; Prossomariti et al., 2020). Studies have reported that the PI3-Akt pathway is activated in 60–70% of CRC patients, and inhibitors of this pathway are considered therapeutic (Malinowsky et al., 2014). In the dysbiotic gut of CRC patients, the abundance of rare *Porphyromonas* species, such as *P. gingivalis* and *P. asaccharolytica*, may promote CRC *via* butyrate-mediated activation of the PI3/Akt pathway (Okumura et al., 2021).

Relevance in gut-organ axis

Butyrate producers are associated with various gut-organ axes, such as the gut-brain, gut-lung, gut-liver, gut, kidney, and gut-heart

1 https://patents.google.com/patent/WO2016019506A1/en

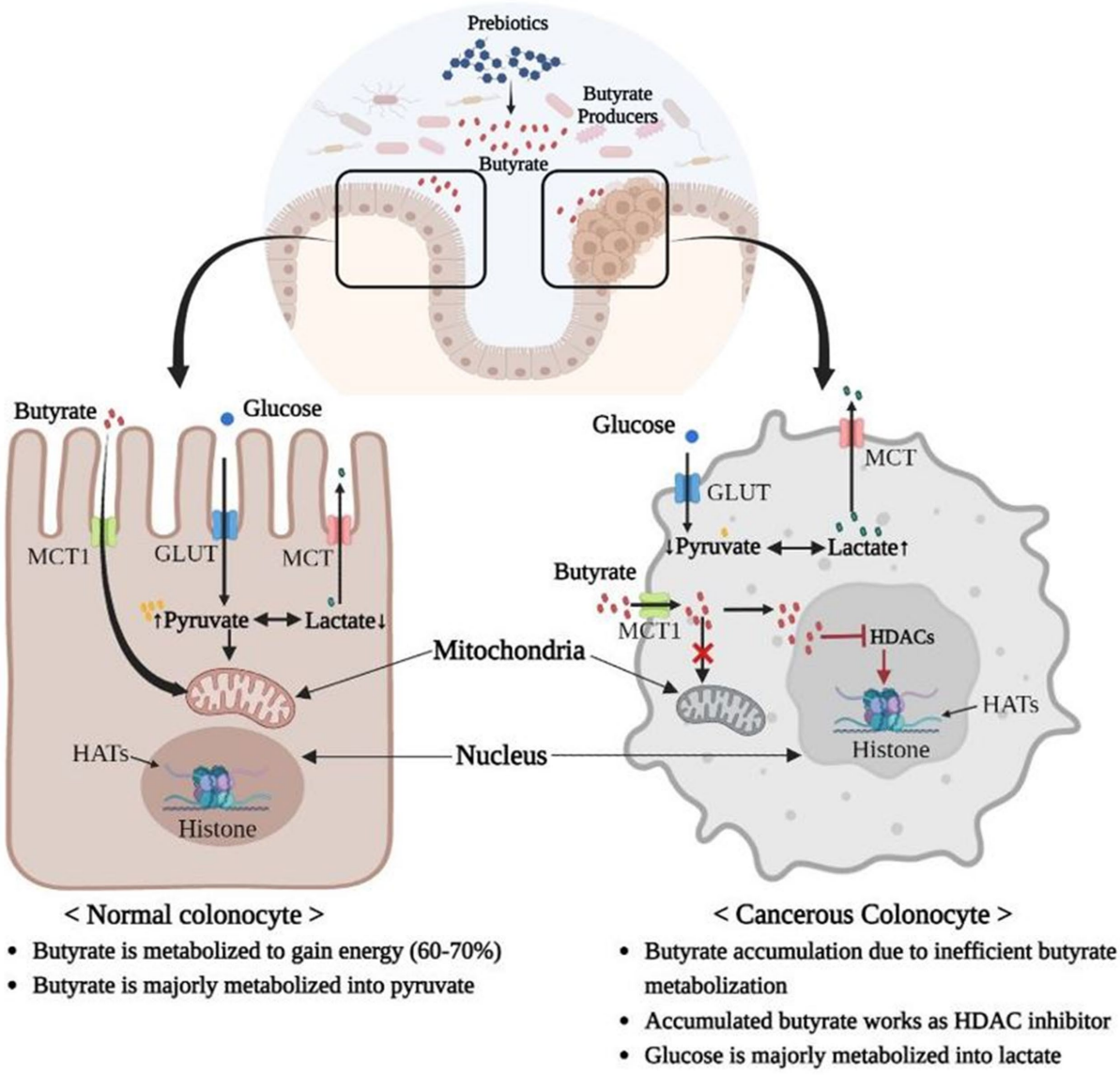

FIGURE 3
Warburg Effect: Inefficient butyrate metabolization by mitochondria of cancerous colonocytes leads to accumulation of butyrate, which in turn acts as an HDAC inhibitor and induces cancer. Additionally, majority of glucose is converted into lactate in cancerous colonocytes owing to their higher glycolysis rates, which is less energy efficient compared to phosphorylation of pyruvate in mitochondria *via* the TCA cycle. Therefore, cancerous colonocytes need higher glucose inflow and a higher rate of glycolysis to survive (MCT, Monocarboxylate Transporter; GLUT, Glucose Transporter; HDAC, Histone deacetylase, HAT; Histone acetyltransferase).

axes (Ahlawat and Asha, 2021). In such complex relationships, butyrate producers act as microbial regulators and exert their effects through their metabolites. As in the gut-brain axis, microbiota-induced expression of AhR in gut neurons allows them to respond to the environment of the gut lumen while simultaneously connecting their functional output to the gut (Obata et al., 2020). As stated earlier, butyrate acts as a ligand for AhR, making butyrate producers a relevant community in the gut-brain axis. Studies have identified the antidepressant effects of the butyrate-producing genera *Butyricimonas* and *Coprococcus* and their depletion in depressed individuals (Yang et al., 2017; Valles-Colomer et al., 2019). Similarly, *Faecalibacterium* and *Coprococcus* are robustly associated with better mental health (Valles-Colomer et al., 2019). Metagenomic analysis of fecal samples from a Belgian cohort identified butyrate-producing *Alistipes* and *Roseburia* as potential producers of serotonin (Valles-Colomer et al., 2019), which is a neurotransmitter expressed abundantly in the gut where it regulates bowel movement, secretion (McLean et al., 2007), and glucose homeostasis (Singh et al., 2022). Studies also confirmed the gut-lung axis, as it's been found that gut dysbiosis is closely related to the occurrence of asthma and pulmonary diseases. In infants reduced gut microbial diversity is

reported to increases the risk of asthma and infectious respiratory diseases (Bisgaard et al., 2011; Abrahamsson et al., 2014). Specially, reduced abundance of butyrogenic *Faecalibacterium* in the gut is closely related with the increased risk of atopy and asthma (Dang and Marsland, 2019). In addition, during a viral infection such as influenza, through GPCR41 receptors, microbial butyrate enhances the Ly6C-monocytes in the lungs, which differentiate into alternatively activated macrophages (AAMs) that alleviate the immunopathological response in the lungs by limiting the neutrophil influx into the airways (Dang and Marsland, 2019).

The gut microbiome is also involved in the gut-liver axis because the liver receives approximately 70% blood supply from the gut, and even shows the presence of higher microbial liposaccharide (LPS) levels in the portal and hepatic circulation during chronic liver ailments (Compare et al., 2012). Microbial butyrate maintains the integrity of the gut barrier and inhibits the inflow of antigens (LPS). In murine studies, butyrate supplementation in the form of tributyrin was found to be effective in alleviating alcohol-induced liver injury (Cresci et al., 2017; Singhal et al., 2021). Alcohol-induced dysbiosis significantly reduces the members of Firmicutes and Lachnospiraceae with a lower abundance of butyrate-producing genera such as *Anaerostipes*, *Coprococcus*, and *Roseburia* (Singhal et al., 2021). A study based on a large human population ($n = 1{,}148$) also identified a significantly lower abundance of the genus *Faecalibacterium* in patients with non-alcoholic fatty liver disease (NAFLD) (Iino et al., 2019). Additionally, the butyrate-producing strain (MIYAIRI 588) of *Clostridium butyricum* reportedly suppresses oxidative stress and hepatic inflammatory indices in NAFLD (Endo et al., 2013).

Metabolites of protein fermentation, such as choline, phosphatidylcholine, and carnitine, are metabolized by the gut microbiota into trimethylamine, which is further converted into trimethylamine-N-oxide (TMAO) in the liver by hepatic flavin-containing monooxygenase (FMO) (Tong et al., 2022). TMAO is known to cause chronic kidney disease (CKD) and induces cardiovascular diseases such as atherosclerosis and coronary heart disease (Evenepoel et al., 2017). Although, a study also suggested that a low dose of TMAO might reduce cardiac dysfunction (Huc et al., 2018). Other than that, butyrate can lower the circulating cholesterol through reverse cholesterol transport by stimulating secretion of apoA-IV-containing lipoprotein (Chen W. et al., 2020). In addition, butyrate also enhances the secretion of glucagon-like peptide-1 (GLP-1) from the gut, which decreases blood pressure (Yadav et al., 2013). While, in CKD, the levels of uremic toxins such as indoxyl sulfate and p-cresyl sulfate are abnormally high, which can also lead to hypertension (Chen et al., 2019). Studies have reported decreased abundance of major butyrate producers such as *Roseburia*, *Faecalibacterium*, and *Coprococcus* in CKD patients (Jiang et al., 2017; Yang et al., 2018). In a murine study, CKD treatment with traditional medicine was found to be mediated by the butyrate-producing microbe Lachnospiraceae-NK4A136 *via* the gut-kidney axis (Tong et al., 2022). In addition to maintaining gut integrity to limit the level of uremic toxins, butyrate improves renal inflammation and dysfunction in patients with CKD.

Impact of selective dietary interventions to enhance butyrate producers

Prebiotic administration positively affects butyrate producers, as they metabolize prebiotics into butyrate. Prebiotics are also beneficial in treating diarrhea and cholera, as prebiotic (e.g., resistant starch) administration accelerates recovery *via* microbial butyrate (Canani et al., 2011). Indigestible dietary fibers are commonly used as prebiotics, but other bioactive molecules, such as polyphenols, can also function as prebiotics to generate butyrate. Polyphenol intervention significantly increases the abundance of butyrate producers such as *Faecalibacterium* and members of the Ruminococcaceae family (Del Bo et al., 2021). Among other polyphenols, the impact of catechins, anthocyanins, and proanthocyanidins as prebiotics is more evident because they increase the abundance of *Roseburia* and *Faecalibacterium* spp. (Alves-Santos et al., 2020). Other phenolic compounds such as caffeic acid, chlorogenic acid, and rutin are also reported to increase microbial butyrate (Catalkaya et al., 2020). Additionally, the microbial accessibility of different prebiotics also varies among butyrate producers; therefore, the administration of different prebiotics can selectively enrich specific butyrate producers (Table 2). Other than prebiotics, synbiotic treatments can also be administered to promote butyrate production in the gut (Gurry, 2017). Synbiotics contain a combination of prebiotics and probiotics, and their synergistic effects are more prominent than those of prebiotics and probiotics used individually (Singh et al., 2021). Synbiotic treatment with *Bacillus subtilis* DSM 32315 and L-Alanyl-L-glutamine improved butyrate levels and enhanced the major butyrate producers such as *Faecalibacterium prausnitzii*, both *in vitro* and in humans (tom Dieck et al., 2022). Similarly, another study reported the prevalence of butyrate-producing *Eubacterium* and *Pseudobutyrivibrio* upon synbiotic administration of fiber-enriched yogurt (Jaagura et al., 2022).

Strain and strategies for tomorrow

Butyrate-producing gut microbes are of significant therapeutic importance and are believed to be niche-specific next-generation probiotics. Multiple butyrate-producing probiotic strains of *Clostridium butyricum* (Stoeva et al., 2021) and *Butyricicoccus pullicaecorum* (Geirnaert et al., 2014; Boesmans et al., 2018) have been used as they exhibit good bile tolerance, viability, and metabolic activity (Table 3). Microbes of interest or butyrate producers can also be genetically manipulated to increase their butyrate-producing capacity. For example, heterologous genes required for butyrate production from acetyl-CoA can be introduced by inactivating the gene encoding the conversion of acetyl-CoA to acetate and the gene encoding the aldehyde/alcohol dehydrogenase for ethanol production or simply disrupting a CoA transferase gene, which may be an alternative route for acetate production (Ueki et al., 2014; Suo

TABLE 2 Impact of different fiber and bioactive metabolites on various gut butyrate producers.

Dietary substance	Monomer unit	Affected microbe	Model	Reference
Human milk oligosaccharides (HMOs)	β-d-galactose (Gal), β-d-glucose (Glc), β-d-N-acetyglucosamine (GlcNAc), α-l-fucose (Fuc), and the sialic acid α-d-N-acetylneuraminic acid (Sia)	*Roseburia*↑ *Eubacterium*↑	Human	Pichler et al. (2020)
Inulin	D-Fructose	*Faecalibacterium* ↑;	Human; Humanized mice	Healey et al. (2018), Van den Abbeele et al. (2011)
		Roseburia intestinalis ↑		
		Eubacterium rectale ↑		
		Anaerostipes caccae ↑		
Xylan	D-xylose	*Roseburia intestinalis* ↑	*In vitro*	Leth et al. (2018)
Fructooligosaccharide	D-fructose	*Faecalibacterium* ↑	Human	Tandon et al. (2019)
		Ruminococcus ↑		
		Oscillospira ↑		
Galacto-oligosaccharides	Galactose	*Anaerostipes caccae* ↑	Murine	Sato et al. (2008)
Polyphenols	Phenol	*Anaerobutyricum hallii*↑	Human	Del Bo et al. (2021)
		Butyricicoccus spp.↑		
		Faecalibacterium prausnitzii↑		
Pectin	Galacturonic acid	*Faecalibacterium*↑	*In vitro*	Bang et al. (2018), Chung et al. (2016)
		Eubacterium eligens		
Guar gum (Galactomannan polysaccharide)	Galactose and Mannose	Clostridium coccoides group↑	Human	Ohashi et al. (2015)
		Roseburia/Eubacterium rectale group↑		
		Anaerobutyricum halli↑		
		Butyrate-producing bacterium strain SS2/1↑		
Alginate	D-mannuronic acid and L-guluronic acid	*Bacteroides ovatus* ↑	*In vitro*	Li et al. (2016)
		Bacteroides xylanisolvens ↑		
Arabinoxylan	D-xylosyl	*Roseburia/Eubacterium rectale* group↑	Murine	Damen et al. (2011)
Stachyose	Galactose, Glucose, and Fructose	*Faecalibacterium*	*In vitro*	Zhao et al. (2021)
Lactulose	Galactose and Fructose	*Anaerostipes*	*In vitro*	Bothe et al. (2017)

et al., 2018). Additionally, a co-culture strategy, that is an interactive microbial population of more than two microbes, can also be implemented to achieve higher levels of butyrate and increased abundance of butyrate producers in the gut. Co-culture of *F. prausnitzii* and *Bifidobacterium catenulatum* with fructooligosaccharides as an energy source resulted in a higher viable cell count and butyrate production (Kim et al., 2020). Moreover, butyrate producers of animal origin (ruminants), such as cellulose-degrading *Ruminococcus albus and R. flavefaciens* (Flint et al., 2008; Chassard et al., 2012), can also be considered to study their impact on human hosts.

Conclusion

The present review critically examined all aspects of butyrate-producing gut microbial communities and their possible impact on host health to better understand their therapeutic significance. We considered the significance of butyrate producers and butyrate in the gut to understand their importance as microbial therapeutics. Although butyrate is an important metabolite, butyrate producers are much more important as they actively control the gut microbiome *via* various anti-microbial and anti-inflammatory molecules, and

TABLE 3 Butyrate producers that can be used as microbial therapeutic to maintain microbial homeostasis and gut health.

Microbes	Model	Reference
Butyricicoccus pullicaecorum 25-3^T	Human	Boesmans et al. (2018)
Faecalibacterium prausnitzii A2-165	Murine	Martín et al. (2015)
Eubacterium Hallii DSM 3353	Human	Engels et al. (2016a)
Eubacterium Hallii DSM 17630	Human	Engels et al. (2016a)
Eubacterium limosum KIST612	Bio-fermenter	Litty and Müller (2021)
Co-culture of *Clostridium hylemonae DSM 15053*; or *Coprococcus comes ATCC 27758*; or *Roseburia hominis A2-183*; or *Eubacterium rectale ATCC 33656*; or *Eubacterium biforme DSM 3989* and *Clostridium ljungdahlii*	Dynamic metabolic modelling	Li and Henson (2021)
Butyricicoccus pullicaecorum 1.20; *Roseburia hominis* DSM 16839; *Roseburia inulinivorans* DSM 16841; *Anaerostipes caccae* DSM 14662; *Eubacterium hallii* DSM 3353	Fed batch fermenter and Caco-2 cell line	Geirnaert et al. (2017)
Clostridium butyricum (CGMCC0313.1)	Murine	Pan et al. (2019)
Clostridium butyricum (MIYAIRI 588)	Murine	Endo et al. (2013), Pan et al. (2019)
Clostridium butyricum Prazmowski	Murine	Wu et al. (2022)
Ruminococcus albus	Caco-2 cell line	Park et al. (2017)

by synthesizing vitamin B. Butyrate-producing microbial communities inhibit cancer growth by secreting anti-carcinogenic substances and regulate tumorigenesis *via* butyrate. Butyrate producers are promising next-generation probiotics, and their counts in the gut can be regulated by dietary interventions to benefit the host. Moreover, butyrate producers can also be genetically manipulated to enhance butyrate synthesis, making them suitable microbial therapeutic agents. We also see the possibility of introducing new butyrate communities to the gut, which are alien to the human gut, to study their impact and to analyze any possible health effects. However, detailed studies are required to cease all safety concerns regarding the introduction of animal or soil origin butyrate producers in the human gut.

Author contributions

VS conceptualized, analyzed, and wrote the draft. GL and HS participated in writing and project management. HK and EK supervised the manuscript. TU and J-HS supervised, reviewed, and approved the manuscript. 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.

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EDITED BY
Junling Shi,
Northwestern Polytechnical University, China

REVIEWED BY
Yi Xu,
Hefei University of Technology, China
Nadia Andrea Andreani,
Max Planck Institute for Evolutionary Biology, Germany

*CORRESPONDENCE
Riadh Hammami
✉ riadh.hammami@uottawa.ca

†These authors have contributed equally to this work

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Neuromicrobiology, an emerging neurometabolic facet of the gut microbiome?

Saba Miri[1†], JuDong Yeo[1†], Sarah Abubaker[1] and Riadh Hammami[1,2*]

[1]School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada, [2]Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

The concept of the gut microbiome is emerging as a metabolic interactome influenced by diet, xenobiotics, genetics, and other environmental factors that affect the host's absorption of nutrients, metabolism, and immune system. Beyond nutrient digestion and production, the gut microbiome also functions as personalized polypharmacy, where bioactive metabolites that our microbes excrete or conjugate may reach systemic circulation and impact all organs, including the brain. Appreciable evidence shows that gut microbiota produce diverse neuroactive metabolites, particularly neurotransmitters (and their precursors), stimulating the local nervous system (i.e., enteric and vagus nerves) and affecting brain function and cognition. Several studies have demonstrated correlations between the gut microbiome and the central nervous system sparking an exciting new research field, neuromicrobiology. Microbiome-targeted interventions are seen as promising adjunctive treatments (pre-, pro-, post-, and synbiotics), but the mechanisms underlying host-microbiome interactions have yet to be established, thus preventing informed evidence-based therapeutic applications. In this paper, we review the current state of knowledge for each of the major classes of microbial neuroactive metabolites, emphasizing their biological effects on the microbiome, gut environment, and brain. Also, we discuss the biosynthesis, absorption, and transport of gut microbiota-derived neuroactive metabolites to the brain and their implication in mental disorders.

KEYWORDS

gut-brain axis, gut microbiome, microbial neurometabolites, neurotransmitter, GABA, SCFAs, dopamine, serotonin

1. Introduction

Over the past few decades, increasing attention has been paid to the gastrointestinal microbiome as one of the key elements contributing to the regulation of host physiology (de Vos et al., 2022). The microbiome has recently been redefined to pertain not only to the community of microorganisms but also their theatre of activity, including microbial structures, metabolites, and mobile genetic elements, whereas the microbiota is an assemblage of microbial communities associated with a habitat (Berg et al., 2020). The metabolic activities of gut symbionts go beyond simply assisting in digestion and nutrient production, or modulating and protecting the intestinal barrier, and have important implications for one health (Berg et al., 2020). Over the past decade, gut neuromicrobiology has emerged as an exciting area of research that encompasses understanding the link between the gut microbiome, its neurometabolic interactome, and its association with brain health and diseases (de la Fuente-Nunez et al., 2018). Indeed, appreciable evidence highlight that alterations in the diversity and the metabolic activity of the gut

microbiome, also known as "dysbiosis," are linked to multiple psychiatric and neurological disorders (de la Fuente-Nunez et al., 2018).

The gut-brain axis is a bi-directional communication system linking the gut microbiome to the brain and plays a crucial role in neuronal development, cognitive regulation, mental state, emotional regulation, behavior, and brain function (Cryan et al., 2020; Agirman and Hsiao, 2021). Gut-brain axis activity can be modulated by broadly two approaches: "top-down" and "bottom-up" (Figure 1). A combination of endocrine (cortisol), immune (cytokines), and neural (vagus and enteric nervous systems) pathways are involved in these two approaches. In the top-down approach, the brain recruits these mechanisms in order to influence the composition of the microbiota in the gut. It is known that the hypothalamus-pituitary–adrenal axis regulates cortisol secretion under stress conditions, and cortisol directly affects immune cells (including the secretion of cytokines) both locally in the gut and systemically. Also, cortisol affects gut permeability and barrier function, as well as the composition of the gut microbiota (Cryan and Dinan, 2012). In the bottom-up approach, the gut microbiota signals the brain through immune regulation (production of cytokines) and the production of microbial neuroactive metabolites and neurotransmitters. Through this approach, for instance, the level of systemic tryptophan and the stimulation of the vagus and enteric nerves play a significant role in the communication between the gut microbiome and the brain. Appreciable evidence suggests that the gut microbiota produce a broad spectrum of neuroactive metabolites (Valles-Colomer et al., 2019; Lai et al., 2021), particularly neurotransmitters and their precursors, highlighting a potential involvement in neuroendocrinology-based mechanisms, illustrated by the bottom-up pathway in Figure 1. For example, spore-forming bacteria secrete their metabolites, stimulating serotonin biosynthesis in enterochromaffin cells (Yano et al., 2015). Moreover, some neurotransmitters and their precursors produced by the gut microbiota and enteroendocrine cells are transferred to the bloodstream and could reach the brain. Figure 1 shows the importance of the microbiome and produced neuroactive metabolites in the gut-brain axis, especially in the "bottom-up" pathway.

In recent years, an increasing number of studies have reported on the biosynthesis of gut microbiome-derived neurotransmitters [i.e., γ-aminobutyric acid (GABA), serotonin, dopamine, norepinephrine, etc.] and other neuroactive metabolites that could impact brain functions and condition (Cox and Weiner, 2018; Cryan et al., 2020). For instance, some research groups found that gut dysbiosis and the following interference in releasing monoamine cause severe major depressive disorder (MDD) in an animal model, proving a deep relationship between the gut microbiome and mental disorders (Heijtz et al., 2011; Neufeld et al., 2011; Clarke et al., 2013). Therefore, microbially-produced neuroactive metabolites could be an integral part of the gut microbiome–host crosstalk mechanisms, thus, eliciting various health-promoting effects. Despite recent research progress, multiple questions surrounding gut neuromicrobiology remain unsolved. Why and how do some specific gut microbes harbor the genes responsible for producing neuroactive molecules but not others? Is it an intra-kingdom or inter-kingdom quorum sensing signaling mechanism or both? What are the possible routes of delivery of these neuroactive metabolites to the gut environment and brain? In this

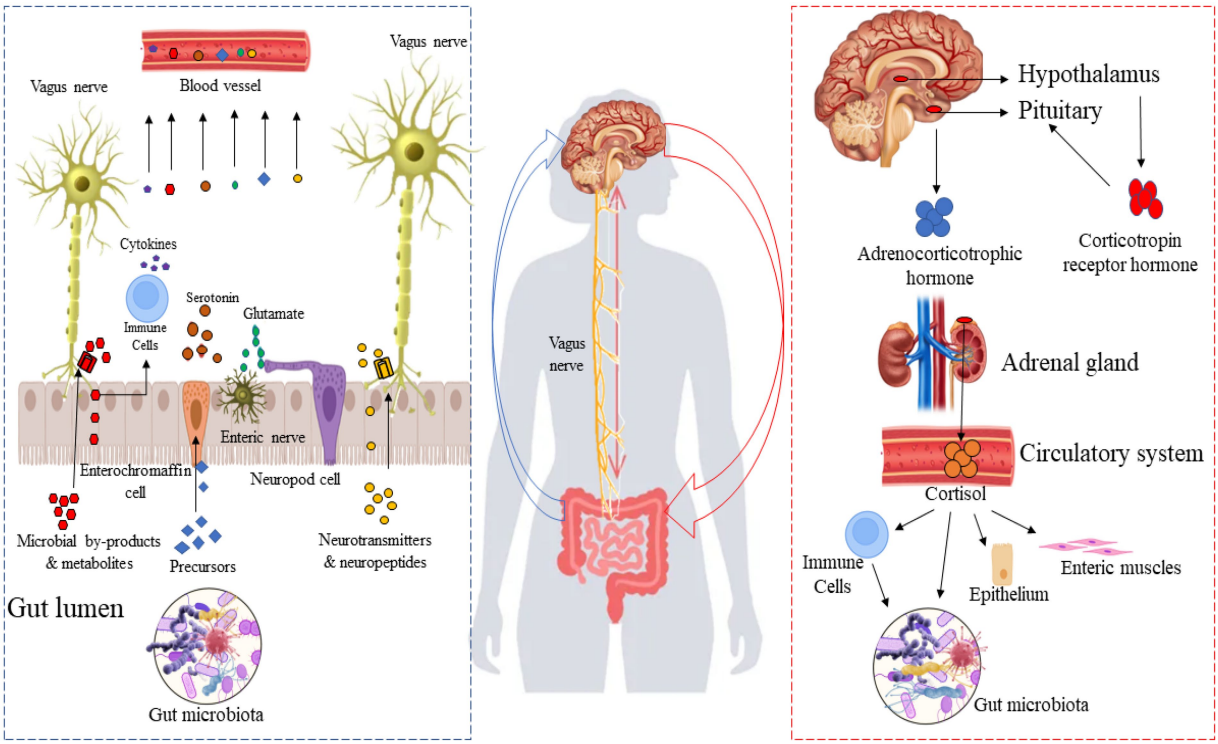

FIGURE 1
Top-down and bottom-up pathways between the gut microbiota and the brain. Right side: Gut microbiota-derived neurotransmitters and their precursor in the gut microbiome-brain axis; left side: the hypothalamus-pituitary–adrenal axis.

review, we discuss the diversity, biosynthesis, transport, and interplay of microbiome-produced neuroactive metabolites with the gut-brain axis.

2. Microbiota-produced neurotransmitters and related metabolites

2.1. Diversity within gut neurotransmitter-producing bacteria

A consideration of some of the more well-studied neuroactive gut microorganisms demonstrates their considerable phylogenetic and neuroactive diversity (Figure 2). As detailed below, multiple neurotransmitters secreted by the gut microbiome have been reported; as such, gut neuromicrobiology has been proposed as a separate field of study in recent years. As shown in Figure 2, some bacterial strains can produce more than one main neurotransmitter. It is often difficult to correlate neurotransmitter production with phylogeny (Figure 2) due to the possible adaptation of bacteria through horizontal gene transfer. Indeed, the gut environment is one of the most favorable ecological niches for lateral gene transfer, which is characterized by stable temperatures, continuous food supply, stable physicochemical conditions, a high concentration of bacterial cells and phages, and ample opportunities for conjugation of these cells and phages on food particles and host tissues (Lerner et al., 2017). In response to selective pressures in the gut, bacteria may undergo genetic restructuring, but the transfer of neuroactive genes has not yet been documented so far.

2.2. Synthesis of neurotransmitters by gut microbiota

2.2.1. γ-Aminobutyric acid

GABA, a nonprotein amino acid generated by the decarboxylation of glutamic acid, is a naturally occurring amino acid, and it functions as a neurotransmitter at the inhibitory synapses of the vertebrate and invertebrate nervous system. GABA plays a crucial role in controlling neuronal excitability in the nervous system and has shown many other physiological functions. It is important to mention that a wide range of GABA-binding proteins are present in gut-associated bacteria and are thought to be critical in bacterial and inter-domain communication (Valles-Colomer et al., 2019). The low level of GABA in the brain causes severe psychiatric and neurological disorders, including depression, anxiety, insomnia, and epilepsy (Luscher et al., 2011; Gabbay et al., 2017; Erjavec et al., 2021). Some evidence revealed that the gut microbiome affects the level of GABA and subsequently influences mental health. For instance, Bravo et al. (2011) reported that *L. rhamnosus* elevated the abundance of $GABA_{B1b}$ mRNA ($GABA_B$ produces slow and prolonged inhibitory signals) while decreasing the level of $GABAA_{\alpha 2}$ mRNA

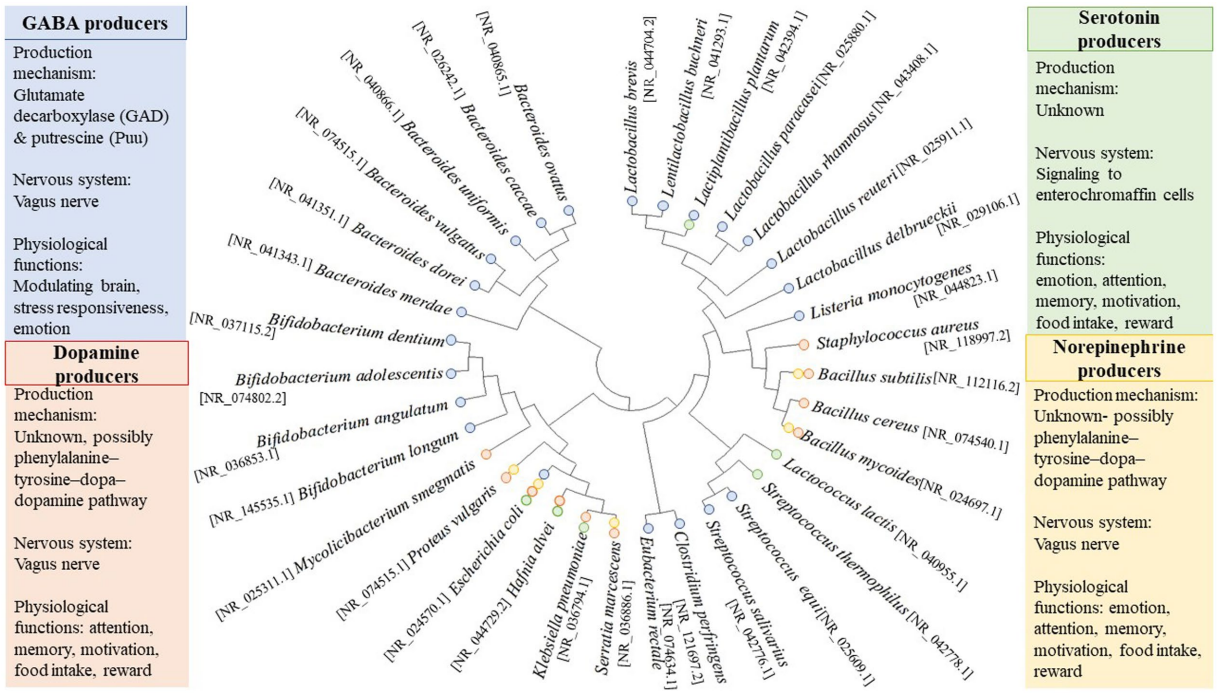

FIGURE 2
Phylogenetic diversity of neurotransmitter-producing bacteria. Sequences are based on published whole-genome or partial sequences from the NCBI Reference Sequence (NCBI RefSeq Targeted Loci Project, Direct Submission, National Center for Biotechnology, Information, NIH, Bethesda, MD 20894, USA). GeneBank accession numbers for the 16S rRNA sequence are shown in the bracket. The phylogenetic tree was constructed using MEGA 11 software (version 11.0.10). Briefly, the evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1,000 replicates represents the evolutionary history of the taxa analyzed (Felsenstein, 1992). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura, 1992) and are in the units of the number of base substitutions per site. This analysis involved 34 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1,606 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

($GABA_A$ mediates fast inhibitory signals) in the cortex of mice, leading to the inhibition of anxiety and depression-like behaviors (Bravo et al., 2011; Terunuma, 2018). In mammalians, approximately 25–50% of neurons contain GABA as a primary inhibitory neurotransmitter in their central nervous system (CNS; Peters et al., 2019). In this section, we focused on the biosynthesis of GABA in potential gut microbes. The biosynthesis of GABA has been reported in various microorganisms (Figure 2). Microbial species can produce GABA either using the glutamate decarboxylase (GAD) or putrescine (Puu) pathways (Diez-Gutiérrez et al., 2020). Most bacteria use the GAD pathway, while the Puu pathway is considered a minor route for synthesizing GABA (Diez-Gutiérrez et al., 2020). Mainly, *Lactobacillus* spp., *Bifidobacterium* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Aspergillus oryzae* produce GABA through the GAD pathway (Huang et al., 2014; Das and Goyal, 2015; Sano et al., 2016), while the Puu pathway is described only for *Escherichia coli* (Cha et al., 2014) and *Aspergillus oryzae* (Akasaka et al., 2018). The GAD pathway is initiated by Glu/GABA antiporters encoded by a *gadC* gene (Gao et al., 2019). As a result of the action of this antiporter, glutamate or monosodium glutamate is pumped into the microorganism (Choi et al., 2013). *gadB* gene encodes the GAD enzyme, which catalyzes the transformation of Glu to GABA. This enzyme consists of six repetitive subunits containing a conserved lysine residue that binds to pyridoxal-5-phosphate (Yu et al., 2019). However, Lyu et al. (2019) reported that the *gadA* gene plays the same role as *gadB* in GAD expression, while the deletion of *gadB* has more effect on reducing GABA production (Lyu et al., 2019).

The putrescine pathway begins with the transport of Puu into the cell *via* an antiporter encoded by the *puuP* gene (or *ycjJ*). Then, Puu undergoes two paths; (1) direct conversion to γ-aminobutyraldehyde catalyzed by a Puu-amino-transferase encoded by *patA* gene (*ygjG*) and subsequent oxidation to GABA by a γ-aminobutyraldehyde-dehydrogenase encoded by *patD* gene (*ydcW* gene). (2) Transformation to γ-glutamyl-Puu catalyzed by γ-glutamate-putrescine-synthetase encoded by a *PuuA* gene and then two oxidation reactions for the production of γ-Glu-GABA by γ-Glutamyl-oxidase and a γ-glutamyl-γ-butyraldehyde dehydrogenase encoded by *puuB* (*ycjA*) and a *puuC* genes, respectively. Then, γ-Glu-GABA hydrolase (encoded by *puuD* gene) degrades γ-Glu-GABA into GABA (Wu et al., 2017). It is noteworthy that GABA can degrade by following the Puu pathway and entering the tricarboxylic acid cycle (TCA). In this path, GABA converts to succinic semialdehyde catalyzed by GABA-aminotransferase (encoded by *gabT* gene) and subsequently converted into succinate yield by a succinic semialdehyde dehydrogenase encoded by a *gabB* gene (Yu et al., 2019). Then the succinate is introduced into the TCA cycle (Kurihara et al., 2010).

GABA shunts and polyamine pathways are metabolic pathways that enable microorganisms to produce and maintain optimal levels of GABA (Cui et al., 2020). Some gut commensal microbes produce GABA, such as *Bacteroides*, *Bifidobacterium,* and *Lactobacillus* genera, as listed in Figure 2. Strandwitz et al. (2019) reported several GABA-producing bacteria, including *Bacteroides caccae*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides dorei*, *Bacteroides uniformis*, *Parabacteroides merdae*, *Bifidobacterium adolescentis*, and *Eubacterium rectale* in which they showed a discrepancy in GABA-producing capacity depending on pH of the liquid medium used for growing those bacteria, with *B. caccae*, *B. vulgatus*, and *B. ovatus* being the most GABA producers (Strandwitz et al., 2019). Recently, Sultan et al. (2022) reported a high GABA production (3–6 mM) for *B. finegoldii, B. caccae, and B. faecis*, three human gut isolates having a distinctive signature operon compared to low GABA-producing isolates. Previously, Barrett et al. (2012) reported on the GABA-producing capacity of *Lactobacillus* and *Bifidobacterium* from the human gut. Out of 91 tested bacteria, the authors found one *Lactobacillus* strain and four strains of *Bifidobacterium* capable of producing GABA, with *Levilactobacillus brevis* DPC6108 being the most potential producer strain (Barrett et al., 2012). Likewise, Pokusaeva et al. (2017) reported that commensal *Bifidobacterium dentium* generates GABA through the enzymatic decarboxylation of glutamate by glutamate decarboxylase beta (gadB) in the rat fecal retention model (Pokusaeva et al., 2017). Besides, chronic treatment of mice with *Lacticaseibacillus rhamnosus* attenuates depression and anxiety-like behavior by producing GABA and regulating GABA receptors such as $GABA_{A\alpha 2}$ and $GABA_{B1b}$ in the brain (Bravo et al., 2011). Aside from the above microorganisms, several lactobacilli, *Monascus purpureus*, and *Streptococcus salivarius* subsp. *thermophilus* have also been reported as efficient GABA-producing microbes in the gut environment (Cui et al., 2020). A recent study showed that *Lentilactobacillus curieae* produces GABA through two distinct pathways: (1) Transamination of succinic semialdehyde by GABA transaminase; and (2) decarboxylation of L-glutamate by 5-Oxopent-3-ene-1,2,5-tricarboxylate decarboxylase (HpaG; Xie et al., 2022).

2.2.2. Dopamine

Dopamine, 3,4-dihydroxyphenethylamine, is a primary catecholaminergic neurotransmitter that plays a significant role in brain physiological functions (i.e., emotion, attention, memory, motivation, food intake, and reward; Kleinridders and Pothos, 2019). Dopamine dysregulation was strongly associated with psychiatric and neurological disorders, such as anxiety, depression, autism, Parkinson, and Alzheimer's (Moraga-Amaro et al., 2014; Bäuerl et al., 2018; Eltokhi et al., 2020). Although the brain is the main site of dopamine synthesis, enteric neurons and intestinal epithelial cells produce approximately 50% of total dopamine in the gastrointestinal tract (Eisenhofer et al., 1997). The mechanism of dopamine synthesis is well-known through the phenylalanine–tyrosine–dopa–dopamine pathway. In this pathway, L-phenylalanine is converted to L-tyrosine by phenylalanine hydroxylase, which mainly occurs in the liver and kidney (Møller et al., 2000). L-tyrosine (from the diet or the liver and kidney) can cross the blood–brain barrier (BBB) and enter the brain. In the brain, it converts to (s)-3,4-dihydroxyphenylalanine (L-dopa) by tyrosine hydroxylase, then the transformation of L-dopa is completed to dopamine by dopa decarboxylase (Seeman, 2010). Tyrosine hydroxylase is considered one of the most important enzymes due to its role as the rate-limiting enzyme in the biosynthesis of catecholamines. It is a monooxygenase that contains iron and requires tetrahydrobiopterin (BH_4) as a cofactor (Nagatsu et al., 2019). There is growing evidence pointing out that the intestinal microbiome contains bacteria that produce BH_4 and that phenylalanine–tyrosine–dopa–dopamine metabolic pathways also exist in microorganisms. Therefore, bacteria may contain homologs of the enzyme genes that mammals use to produce dopamine (Iyer and Ananthanarayan, 2008; Belik et al., 2017). As shown in Figure 2, several bacteria have been reported to produce dopamine in the gut, including bacilli, *E. coli*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus*, *Hafnia alvei*, *Klebsiella pneumoniae* (Tsavkelova et al., 2000; Cryan and Dinan, 2012). However, the detailed mechanism of dopamine biosynthesis by the gut microbiome has not yet been fully elucidated.

2.2.3. Serotonin

Serotonin, a monoamine neurotransmitter, is involved in various brain functions such as modulating mood, reward, cognition, memory,

learning, and many physiological processes, including vasoconstriction and vomiting (Berger et al., 2009). The altered expression, production, and function of serotonin in the brain result in the pathogenesis of mental illnesses, such as anxiety and depressive disorders (Helton and Lohoff, 2015). Several local effects are also conferred by gut-produced serotonin (5-hydroxytryptamine), including stimulating gut motility. The primary serotonin synthesis pathway occurs *via* enteric enterochromaffin cells, in which tryptophan hydroxylase 1 (Tph1) takes part in the reaction as the rate-limiting enzyme for serotonin synthesizing (Kwon et al., 2019). Indeed, most serotonin is present around enterochromaffin cells in the gastrointestinal tract and enteric nerves after their biosynthesis from tryptophan (Spiller, 2008; Gershon, 2013; Mawe and Hoffman, 2013). The production capacity of serotonin by the enterochromaffin cells is beholden to the available level of tryptophan needed for the synthesis; thus, maintaining the abundant amount of tryptophan in the gastrointestinal tract is crucial to synthesize an adequate level of serotonin. So far, many research groups have explored serotonin-producing bacteria in the gut, including *E. coli* K-12, *Lactiplantibacillus plantarum* FI8595, *Lactococcus lactis* subsp. *cremoris* MG 1363, *Streptococcus thermophilus* NCFB2392, *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. (Shishov et al., 2009; Cryan and Dinan, 2012). As opposed to eukaryotes, little is known about the serotonin synthesis pathway in bacteria. Several bacteria have been identified to encode for eukaryote-like aromatic amino acid hydroxylase and aromatic amino acid decarboxylase, although the serotonin production pathway has not yet been investigated in most of these bacteria (Gonçalves et al., 2022).

Gut microbiota also indirectly take part in the production of serotonin: for instance, enterochromaffin cells produce serotonin once they receive signals through gut microbiome-produced metabolites that upregulate expression of the *tph1* gene (Legan et al., 2022). Indeed, germ-free mice (GF) have substantially reduced colonic Tph1 mRNA expression, serum serotonin levels, and increased serotonin-selective reuptake transporter mRNA expression compared to control mice (Sjögren et al., 2012). In another study, gut microbiome was shown to play a role in the production of serotonin by comparing three mice groups: GF mice, GF mice colonized with human gut bacteria, and normally raised mice with mouse microbiomes. The colonized mice with human gut bacteria and normally raised mice expressed higher levels of colonic Tph1 mRNA and protein along with an increase in colonic serotonin level compared to GF mice. There was no difference in enterochromaffin cell density between the three groups, so the gut microbiome could directly regulate serotonin levels in the gastrointestinal tract (Reigstad et al., 2015). Likewise, the gut microbiome release short-chain fatty acids and bile acids, inducing serotonin production in the enterochromaffin cells (Reigstad et al., 2015; Legan et al., 2022). Although Legan et al. (2022) provided some evidence of the direct and indirect effects of the gut microbiome on host serotonin systems, they also mentioned that no serotonin-producing human commensal has not yet been reported (Legan et al., 2022).

2.2.4. Norepinephrine

Norepinephrine is a catecholamine that plays roles in learning, attention, cognition, and memory, in addition to its function in alertness, arousal, and sensory detection (Borodovitsyna et al., 2017). Disturbances in norepinephrine neurotransmission in the CNS are increasingly associated with developing psychiatric and neurological diseases (Vazey and Aston-Jones, 2012; Bäuerl et al., 2018), although pathophysiological implication remains limited (Moret and Briley, 2011). The biosynthesis of this neurotransmitter takes place mainly at the adrenal medulla and postganglionic neurons by the multiple enzymatic reactions in which the structural changes of tyrosine, a precursor molecule, to dopamine occurs primarily in the cytoplasm, while the alteration of dopamine to norepinephrine by dopamine β-monooxygenase takes place in the neurotransmitter vesicles (Zahoor et al., 2018). Bacteria such as *Bacillus mycoides*, *Bacillus subtilis*, *Proteus vulgaris*, and *Serratia marcescens* have been reported as norepinephrine-producing microorganisms (Tsavkelova et al., 2000), while *E. coli* K-12, *Bacillus* spp., and *Saccharomyces* spp. have also displayed noradrenalin-producing ability (Shishov et al., 2009; Cryan and Dinan, 2012). Sperandio et al. (2003) reported that norepinephrine is responsible for the quorum-sensing ability of the bacterial population (Sperandio et al., 2003). Wu and Luo (2021) also considered norepinephrine as one of the five main signaling molecules in the classical quorum-sensing system involved in interkingdom communication (Wu and Luo, 2021). The bacterial adrenergic receptors QseC (encoded by the *qseC* gene) and QseE (encoded by *qseE*) are membrane-bound histidine kinases that sense epinephrine and norepinephrine (Kendall and Sperandio, 2016). QseC quorum-sensing sensors have been associated with changes in bacterial motility and activation of virulence genes in several bacteria, including enterohemorrhagic *E. coli* and *Salmonella enterica* serovar Typhimurium (Karavolos et al., 2008; Kendall and Sperandio, 2016). It is documented that bacterial quorum-sensing sensors also sense the host hormones norepinephrine/epinephrine so that they may be interchangeable in the crosstalk between the microbiota and human gut (Li et al., 2019; Wu and Luo, 2021).

Although the related biosynthesis pathway of these neurotransmitters involving the gut microbiome remains unclear, it is assumed that the above bacteria may possess the relevant enzyme, such as dopamine β-monooxygenase needed for converting dopamine into norepinephrine. Shishov et al. (2009) reported that bacterial cells could produce and degrade monoamine neuromodulators *via* enzyme systems that are presumably similar to those found in animals (Shishov et al., 2009).

2.3. Neurotransmitter precursors and their biosynthesis pathways

The gut microbiome is primarily known to perform a fundamental function in metabolizing indigestible material consumed by the host, thus contributing to optimum energy production. Accordingly, human colonic bacteria have access to 5–12 grams of proteinaceous material daily. Therefore, amino acids, an essential part of the human diet, serve not only as the basic building blocks of proteins and peptides but also as the precursors to a wide variety of bioactive molecules essential for signaling pathways and metabolic processes. Given the diversity of amino acids and the complex mechanisms involved in metabolic pathways, we will focus here on amino acids that serve as precursors for neurotransmitters.

2.3.1. Tryptophan and its metabolites

As an essential aromatic amino acid, tryptophan is found in several common foods, such as milk, fish, cheese, chocolate, bananas, bread, and wine. It is composed of an indole group and a β carbon. For more than a century, it has been known that certain bacteria can produce amino acids, a trait that has been significantly exploited in the food and feed industry. Since the 1980s, the development of the amino acid

industry has been vibrant and has centered primarily on amino acids for feed supplements, which constitute 56% of the total market. The remaining 44% were primarily used in the agriculture, pharmaceutical, food, and cosmetic industries (Lim et al., 2019). A number of studies have indicated that lactic acid bacteria (LAB) possess genes for amino acid synthesis in addition to their well-established proteolytic system. An increasing understanding of the functions and properties of amino acid-producing bacteria has led to increasing commercial interest and diverse commercial applications. LABs are also considered an excellent candidate for amino-acid production for feed supplements (Lim et al., 2019). It is known that some gut bacteria, including *E. coli*, can produce tryptophan, but there is no evidence that bacteria-derived tryptophan contributes significantly to host health (Krautkramer et al., 2021). Since tryptophan is not produced by animal cells, humans must obtain it from an exogenous source through their diet. It has been reported that members of *Clostridium* spp. and *Tannerella* spp. co-occurred with tryptophan biosynthesis and contained genes for tryptophan biosynthetic pathways (Kaur et al., 2019; Valles-Colomer et al., 2019; Aleti et al., 2022). Generally, five enzymes encoded by seven genes (*trpA-F*), typically arranged in a single cluster, are involved in tryptophan biosynthesis in microbes (Crawford, 1989). Gut microorganisms can convert tryptophan to several signaling molecules, including serotonin, melatonin, tryptamine, and other indole derivatives. As mentioned above, tryptophan metabolism is a major pathway leading to the production of serotonin in the gut environment. It is noteworthy that gut-produced serotonin may indirectly impact central serotoninergic pathways, even if they do not cross the BBB, by modulating tryptophan and tryptamine availability (Agus et al., 2018). Some members of the human gut microbiota, such as *Clostridium sporogenes,* have been identified to decarboxylate tryptophan to produce tryptamine, a chemical that modulates host neurological activity (Williams et al., 2014). In addition, tryptophan is also the precursor to melatonin, which acts as an antioxidant and free radical scavenger in microorganisms while having positive effects on human health and could regulate the circadian sleep–wake rhythm if it crosses the BBB. It is noticeable that melatonin is mainly produced in the pineal gland (Danilovich et al., 2021).

In addition, 90% of the circulating tryptophan is metabolized through the kynurenine pathway in the human body (Jenkins et al., 2016). Kynurenine has importance in generating cellular energy in the form of nicotinamide adenine dinucleotide (NAD^+; Savitz, 2020). In the first step of the kynurenine pathway, tryptophan is converted to N-formylkynurenine by indoleamine 2,3-Dioxgenase 1 and 2 (IDO-1 and IDO-2) and tryptophan-2,3-dioxygenase (TDO), then converted to kynurenine by formamides (Kennedy et al., 2017). Lastly, kynurenine is metabolized into NAD^+ by different enzymes such as kynurenine aminotransferases (KATs), kynurenine monooxygenase (KMO), and 3-hydroxyanthralinic acid dioxygenase (HAAO; Schwarcz and Pellicciari, 2002). Więdłocha et al. (2021) recently reviewed the current knowledge on the effect of gut microbiota on the kynurenine pathway and their relation with specific psychiatric disorders such as schizophrenia, Alzheimer's disease, bipolar disorder, depression, autism spectrum disorders, and alcoholism (Więdłocha et al., 2021). Authors mentioned that gut bacteria are capable of synthesizing kynurenine pathway enzymes analogous to TDO, formamidase, KATs, and KMO, which affect this pathway further (Kurnasov et al., 2003; Więdłocha et al., 2021). Synthesis of B6 and B12 vitamins are also dependent on gut microbiome activity. These compounds are cofactors to kynurenine pathway enzymes (Oxenkrug et al., 2013).

Indoles is also one of the derivatives of tryptophan metabolisms. It is documented that the bacterial metabolism of tryptophan generates more than 600 indoles in the gut (Regunathan-Shenk et al., 2022). Indoles are structurally related to neuroactive substances such as serotonin and Lysergic acid diethylamide (LSD). The structural similarity of these compounds has led to increased interest in their potential as neurotoxins. However, studies showed that administration of uremic indoles showed no altering CNS function (Himmelfarb and Sayegh, 2010). Walters and Sperandio (2006) mentioned that indole is considered a bacterial quorum-sensing system in the gut and acts as a signaling molecule. The same authors highlighted that indole could contribute to adapting bacterial cells to nutrient-poor environments where amino acid catabolism is an important energy source (Walters and Sperandio, 2006). A recent study showed that *Lactobacillus reuteri* isolated from murine gut microbiomes metabolize host dietary tryptophan into indole derivatives, kynurenines, and cresol and imidazoles, which may be involved in the regulation of CNS autoimmunity (Montgomery et al., 2022).

2.3.2. Glutamate and its metabolites

Prokaryote and eukaryote organisms produce glutamate as a part of their intra- and inter-kingdom signaling. A portion of the free glutamate in the lumen comes from bacterial synthesis. For instance, several bacteria, such as *Corynebacterium glutamycum*, *L. plantarum*, *L. paracasei*, and *L. lactis,* were reported to produce glutamate (Mazzoli and Pessione, 2016). Although, glutamate plays a fundamental role as an excitatory neurotransmitter in the central nervous system (CNS) and in the enteric nervous system (ENS), where it is synthesized by neurons and glia (Miladinovic et al., 2015). It has been demonstrated that Gram-positive and Gram-negative bacteria use glutamate as a substrate for synthesizing GABA *via* decarboxylation by glutamate decarboxylase (GAD; Tsai and Miller, 2013). Therefore, we mainly considered microbiota-produced glutamate as a precursor for GABA, as mentioned above, and a signaling molecule in this section. A comprehensive evaluation of the microbiome-gut-brain axis and glutamate as a neurotransmitter/neuromodulator has been elegantly reviewed elsewhere (Baj et al., 2019). Authors mentioned that glutamatergic pathways may contribute to interkingdom communication in the gut microbiota (Baj et al., 2019).

Ionotropic (iGlu) and metabotropic (mGlu) glutamate receptors are the two major types of glutamate receptors. Studies have identified at least 100 prokaryotic potassium channels containing putative glutamate binding domains, of which 22 have homology with vertebrate iGlu receptors (Ger et al., 2010). This point allows hypothesizing that glutamate can play a role as inter-bacterial and inter-kingdom signaling molecules and glutamate-producing bacteria can modulate signaling pathways both locally and systemically. There are some evidence that the modulation of glutamatergic receptors along the microbiome-gut-brain axis affects several physiological responses in the brain and the gut, potentially having significant consequences for diseases involving dysfunctions of this communication pathway (Filpa et al., 2016; Mazzoli and Pessione, 2016). It is noteworthy that more investigations are needed to identify gut bacteria able to produce, sense, and respond to glutamate.

Previously, probiotics administered to mice resulted in a long-lasting increase in levels of glutamine/glutamate in the brain, suggesting that the gut microbiome may control enzymatic biosynthesis pathways involved in the production of glutamate in the brain since the BBB impedes the passage of amino acids into the CNS under physiological conditions (Janik et al., 2016). As mentioned

above, GABA is synthesized in the gut environment from glutamate through the enzymatic activity of GAD. In addition, the gut microbiome may indirectly affect glutamatergic pathways along the microbiome-gut-brain axis by controlling the metabolic process for L-tryptophan (Agus et al., 2018). It is relevant to mention that decarboxylation of glutamate to GABA is an important survival mechanism for bacteria in the stomach's extreme acidity (Feehily and Karatzas, 2013).

3. Other microbiota-produced neuroactive metabolites

Several metabolites produced by the gut microbiome contribute to the host physiology and homeostasis through, for instance, serving as substrates for reactions or signaling molecules. Although elucidating host-microbiome interactions remains challenging due to the high diversity of produced metabolites and the extent of crosstalk among gut microbes, several actionable microbial targets relevant to host health have been identified through metabolite-focused research (Krautkramer et al., 2021). Here, we mainly discuss only metabolites reported to have mental effects.

3.1. Short-chain fatty acids

Extensive research studied the production and metabolism of short-chain fatty acids (SCFAs) by gut microbes. SCFAs are a subclass of fatty acids, ranging from one to six carbon atoms, and they are generated by the gut microbiota fermentation of nondigestible polysaccharides/fibers (Krautkramer et al., 2021). The main route of SCFA production in the colon occurs *via* saccharolytic fermentation of carbohydrates not absorbed in the small intestine, mainly nondigestible polysaccharides/fibers. Butyrate is also formed from amino acid metabolism, and produced SCFAs contribute to the decrease in the pH of the colon (Louis and Flint, 2017). The most common SCFAs found in the human body are acetate, propionate, and butyrate, along with less amount of fumarate, valerate, and caproate, and their levels reach nearly 500–600 mmol per day in the gut depending on the composition and amount of fiber in the diet (Macfarlane and Macfarlane, 2003). In some studies, SCFAs modulated neurotransmitter and neurotrophic factors levels (Silva et al., 2020). Acetate has been shown to alter glutamine, glutamate, and GABA levels and stimulate the production of anorexigenic neuropeptides in the hypothalamus (Frost et al., 2014). Butyrate was also reported with antidepressant properties and effects on social dominance (Hao et al., 2019; Wang, T. et al., 2022). Likewise, propionate, a precursor in lipid biosynthesis, has neuroprotective effects (Hu et al., 2018). In this research, propionate was found to protect against haloperidol-induced neurite lesions and prevent the reduction of neuropeptide Y (Hu et al., 2018). Moreover, SCFAs influence the expression of tryptophan 5-hydroxylase 1 that is responsible for the synthesis of serotonin as well as tyrosine hydroxylase, which takes part in the biosynthesis of dopamine, adrenaline, and noradrenaline; thus, SCFAs play a crucial role in brain neurochemistry by affecting the production of neurotransmitters (Reigstad et al., 2015; Yano et al., 2015; Dalile et al., 2019). Even though the detailed mechanism of their action in the CNS remains unclear, some animal studies have shown that SCFAs have a widespread influence on significant neurological and behavioral processes and may be engaged in important steps of neurodevelopmental and neurodegenerative disorders (Dalile et al., 2019; Fung et al., 2019).

Metagenomic approaches have been widely used to determine individual bacterium responsible for generating SCFAs in the colon. The production routes for propionate, butyrate, and lactate are more conserved and substrate-specific than the acetate production pathways; for instance, limited bacterial genera are involved in propionate production (Reichardt et al., 2014). Many studies have been carried out to identify SCFAs-producing microorganisms and their substrates, and are presented in Table 1. A report listed SCFAs-producing gut microbiomes along with dietary sources used for fermentation (Cheng et al., 2021). The authors found 11 gut commensals that possess a potential capacity to produce SCFAs in the colon, including *Bifidobacterium* spp., *Eubacterium* spp., *Ruminococcus* spp., *Prevotella* spp., *Faecalibacterium* spp., *Collinsella* spp., *Atopobium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Clostridium cluster* XIVa, and *Roseburia* spp. (Cheng et al., 2021). Basson et al. (2016) also provided a list of acetate-, propionate-, butyrate- and lactate-producing gut microbiomes (Basson et al., 2016). It is reported that *Akkermansia muciniphila* is a representative propionate-producing organism (Naito et al., 2018). Moreover, Ze et al. (2012) showed that *Ruminococcus bromii* significantly contributes to butyrate production in the presence of resistant starch in the colon (Ze et al., 2012). Besides, Chang et al. (2021) combined bioinformatics to scan gut-inhabiting *Clostridia* genomes pathways and *in vitro* assay to detect fatty acid amides, revealing that these metabolites might mimic human signaling molecules to modulate their host (Chang et al., 2021). Wang, T. et al. (2022) recently demonstrated that most dominant hosts are characterized by butyrate-producing core microbes, and that colonization of *Clostridium butyricum* alone is adequate to restore the host's dominance (Wang, T. et al., 2022). In addition, SCFAs commonly have chemical structures similar to the diffusible signal factors (DSF) families. Some Gram-negative bacteria use DSFs as quorum-sensing signals for biofilm formation and virulence. SCFAs, as DSFs mimic, can inhibit bacterial biofilm or other dependent gene expressions in the quorum-sensing system, influencing autoinducer signals (Kumar et al., 2020). Furthermore, SCFAs can be used by other bacteria or pathogens as sources of nutrients or aid colonization, virulence, and invasion. For instance, SCFAs promote adhesion, flagellum growth, and virulence of *Salmonella* Typhimurium by upregulating the expression of T3SS gene (Lawhon et al., 2002).

3.2. Neuroactive peptides

Peptide YY, glucagon-like peptide 1, gastric inhibitory peptide, cholecystokinin, oxytocin, corticotropin-releasing factor, and ghrelin are only found in gut produced by the stimulation of the enteric bacterial microbiome. In the systemic circulation, gut peptides can bind cognate receptors on vagus nerve terminals and immune cells, enabling indirect communication between the gut and the brain. Intestinal microbiome composition influences gut peptide concentrations and enteric signals (Lach et al., 2018). The neuropeptide Y family is the brain's most abundant family of peptides and is expressed across the gut-brain axis, such as enteric neurons, primary afferent neurons, sympathetic neurons, and several neuronal pathways throughout the brain (Holzer and Farzi, 2014). In the brain, neuropeptide Y, for instance, is expressed by a multitude of neuronal systems in regions spanning from the medullary brainstem to the cerebral cortex. Gut peptides YY and pancreatic polypeptides are mainly released by enteroendocrine cells, where peptide

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TABLE 1 SCFAs-producing microorganisms and substrates associated with bacterial fermentation.

SCFAs type	Bacterial strains	Substrate	Potential neuroactivity	Deficiency effect	Ref.
Acetate	*Bacteroides* (*B. thetaiotaomicron*)	Cellulose, hemicellulose, pectin, fructans, mucins, mucopolysaccharides	Cognitive functions	Depletion of acetate-producing bacteria resulted in the reduction of synaptophysin in the hippocampus as well as learning and memory impairments in diabetic mice	Basson et al. (2016), Zheng et al. (2021)
	Ruminococci	Celluloses			
	Bifidobacteria	Milk oligosaccharides, fructose, lactose			
	Clostridia				
	Proteobacteria (*Desulfovibrio pigler*)				
	Eubacteria				
	Fusobacteria				
	Peptoccocci				
	Peptostreptococci				
	Propionibacteria				
	Veillonella				
Propionate	*Bacteroides*	Cellulose, hemicellulose, pectin, fructans, mucins, mucopolysaccharides	Effect on anxiety and stress behaviors	Minimal variation in the abundance of butyrate and propionate was observed in the gut of depressed individuals compared to healthy controls; however, antidepressant-like effects of sodium propionate were reported	Liu et al. (2015), Basson et al. (2016), Hoyles et al. (2018), Li et al. (2018)
	Clostridium cluster IX				
	Propionibacteria				
	Veilonella				
	Akkermansia municiphilla	Mucin and mucopolysaccharides			
Acetate, propionate, and butyrate	*Faecalibacterium* spp. *Prevotella* spp. *Bifidobacterium* spp. *Eubacterium* spp. *Ruminococcus* spp. *Collinsella* spp. *Atopobium* spp. *Enterococcus* spp. *Lactobacillus* spp. Clostridium cluster XIVa	Pectin, fructans			
	Roseburia spp.	Hemi-cellulose, bacterial polysaccharides			
		Milk oligosaccharides, fructose, lactose			
Butyrate	*Roseburia* spp.	Hemi-cellulose, fructose, fructans	Neuroprotective effects	The long-term supplementation of acetate, propionate, and butyrate in drinking water for chronic cerebral hypoperfusion mice models revealed a positive neuroprotective effect by reducing inflammation and hippocampal neuronal apoptosis following bilateral occlusion of the common carotid artery.	Basson et al. (2016), Xiao et al. (2022)
	F. prusnitztii				
	E. rectale				
	E. hallii				
	R. bromine				
	Anaerostipes				
	Ruminococcus bromii				Ze et al. (2012)
	Lachnospiraceae	Plant polysaccharides			Sun et al. (2021)
Lactate	*Bifidobacterium* spp.	Milk oligosaccharides, fructose, lactose	Antidepressant effect	To the best of our knowledge, no study has examined the relationship between lactate production in the gut microbiome and its deficiency effect. However, there is a well-established interchange of lactate between the periphery and the CNS.	Basson et al. (2016), Caspani et al. (2019)
	Collinsella aerofaciens				

YY is released by the L cells of the ileum and colon in response to food intake. Gut peptides can be activated by their cognate receptors in vagal afferents to signal the brain stem (Latorre et al., 2016). A recent study has identified dipeptides (Phe-Val and Tyr-Val) and their biosynthetic gene clusters in the human microbiome (Cao et al., 2019). These molecules play a critical role in quorum sensing (cell-to-cell communication) to promote the growth of beneficial *Bifidobacterium* and maintain cell density (Hatanaka et al., 2020). A previous study showed that the Phe-Phe produced by *Clostridium* sp. can inhibit host cellular proteins, particularly cathepsins, by chemical modifications causing inflammation (Guo et al., 2017). Another study showed that three quorum sensing peptides (BIP-2, PhrANTH2, PhrCACET1) could selectively penetrate BBB, and two of them influx into the mouse brain (Wynendaele et al., 2015). Since gram-positive bacteria mostly use peptides as signal molecules, this may highlight the potential benefits of probiotics and the human microbiome in depression, anxiety, and stress (Luna and Foster, 2015). This topic is undoubtedly an area of research that requires further exploration.

Other studies showed that some bacterial strains could modulate the expression of gut peptides. For example, Ko et al. (2022) reported that the administration of *L. plantarum* SBT2227 promotes sleep in *Drosophila melanogaster* through the induction of neuropeptide F (a homolog of mammalian neuropeptide Y; Ko et al., 2022). On the other hand, different types of proteases are produced by the gut microbiome, which results in the generation of a large number of peptides during the digestion of food proteins. In the case of simulated gastrointestinal digestion *in vitro*, some studies have shown the production of bioactive peptides (Wu et al., 2021). For instance, Capriotti et al. (2015) showed that hundreds of peptides with various biological activities were produced from soybean proteins in the simulated gastrointestinal digestion. It has been found in other studies that these peptides were stable and remained intact, allowing them to reach their target sites and exert their potential health benefits (Miri et al., 2019; Virgilio, 2019). However, little is known about the interaction mechanism of peptides produced by the gut microbiome and enteroendocrine cells and their interactions with brain physiology.

3.3. Bile acids

The liver synthesizes primary bile acids primarily from cholesterol metabolism, a process that is in part mediated and controlled by the gut microbiome. It is thought that microbial enzymes are responsible for deconjugating and dehydroxylation of conjugated primary bile acids to produce secondary bile acids that function as signaling molecules (Wahlström et al., 2016). Due to the possibility that gut bacteria may control the composition of the brain's bile acid pool, bile acids may serve as a communication link between the gut microbiome and the brain (Monteiro-Cardoso and Corlianò, 2021). It is well-documented that the vagal nerve modulates brain function indirectly through neurotransmitters, which are unlikely to cross the BBB. However, studies demonstrated that bile acids could cross the BBB and are therefore capable of directly signaling through the brain's bile acids receptors. Still, little is known about the molecular mechanisms involved and the physiological functions of microbiome-derived bile acids in the central nervous system.

3.4. Vitamins

Most gut microorganisms have the ability to synthesize *de novo* and metabolize vitamins, including vitamin K2 (menaquinone), vitamin A (retinol), as well as water-soluble B-vitamins, such as B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (Folate), and B12 (cobalamin; Das et al., 2019; Rudzki et al., 2021). Several biochemical processes, such as the metabolism of neurotransmitters, require the B vitamins as coenzymes. Microbial-produced B vitamins and their role in CNS and their effect on gut bacteria are summarized in Figure 3. B vitamins play an important role in neuroprotection, myelin formation, energy production, mitochondrial function, and cellular respiration, as well as exert antioxidant and anti-inflammatory properties (Rudzki et al., 2021). Das et al. (2019) studied the abundance of vitamin biosynthetic gene(s) and consumption of vitamins through uptake transporter(s) using human fecal metagenomic data collected from four different countries (i.e., China, USA, Spain, and Denmark; Das et al., 2019). The authors showed that the range of total gene abundances remained constant across healthy populations in all studied countries. Based on their estimation, 49% of vitamin-related pathways are found in the *Firmicutes* phylum, 19% in the *Proteobacteria* phylum, 14% in the *Bacteroidetes* phylum, and 13% in the *Actinobacteria* phylum (Das et al., 2019; Rudzki et al., 2021). Moreover, a comprehensive analysis of 256 common human gut bacteria genomes revealed that 40–65% of these bacteria could produce some or all of the B vitamins. This prediction was validated by published data in 88% of cases (Magnúsdóttir et al., 2015). It is also important to note that gut microbial metabolism of B vitamins is age dependent. There has been evidence that infant gut microbiomes are enriched for genes involved in *de novo* folate biosynthesis, whereas adult gut microbiomes are enriched for genes involved in folate metabolism and its reduced form tetrahydrofolate (Yatsunenko et al., 2012).

3.5. Other potential neurochemical compounds

Recently, Sultan et al. (2022) reported the presence of several neurotransmitter-related compounds or their precursors, such as arachidonyl-dopamine (NADA), gabapentin, and N-acylethanolamines inside gut microbiome-secreted extracellular vesicles (MEVs; Sultan et al., 2022). Dopamine, a representative human neurotransmitter, was also found in these MEVs as a conjugated form with arachidonic acid. N-acylethanolamines (NAEs), such as palmitoyl-ethanolamide (PEA) and linoleoyl-ethanolamide (LEA), have been reported as effective neuroprotective agents (Sun et al., 2007; Schomacher et al., 2008). Also, NADA is an endocannabinoid with widespread physiological and pharmacological activities, including modulation of neuropathic pain, inflammatory hyperalgesia, and immune and vascular systems (Grabiec and Dehghani, 2017). Two potential biosynthetic pathways for NADA have been proposed, though no conclusive evidence exists. First, NADA biosynthesis pathways could involve the conjugation of *N*-arachidonoyl tyrosine to *N*-arachidonoyl-L-DOPA by tyrosine hydroxylase (TH), which would then be converted to NADA by L-amino acid decarboxylase (AADC). Hu et al. (2009) reported the possibility that fatty acid amide hydrolase (FAAH) has the potential to be involved in the direct conjugation of dopamine with arachidonic acid liberated from arachidonoyl-ethanolamide (AEA), the blockade of which significantly decreases *in vivo* the production of NADA (Hu et al., 2009). According to the same authors, FAAH functions either as a rate-limiting enzyme that liberates arachidonic acid from AEA, a conjugation enzyme, or both (Hu et al., 2009). Previous comparative analyses of FAAH enzymes from bacteria, yeast, and mammals showed a strong evolutionary relationship.

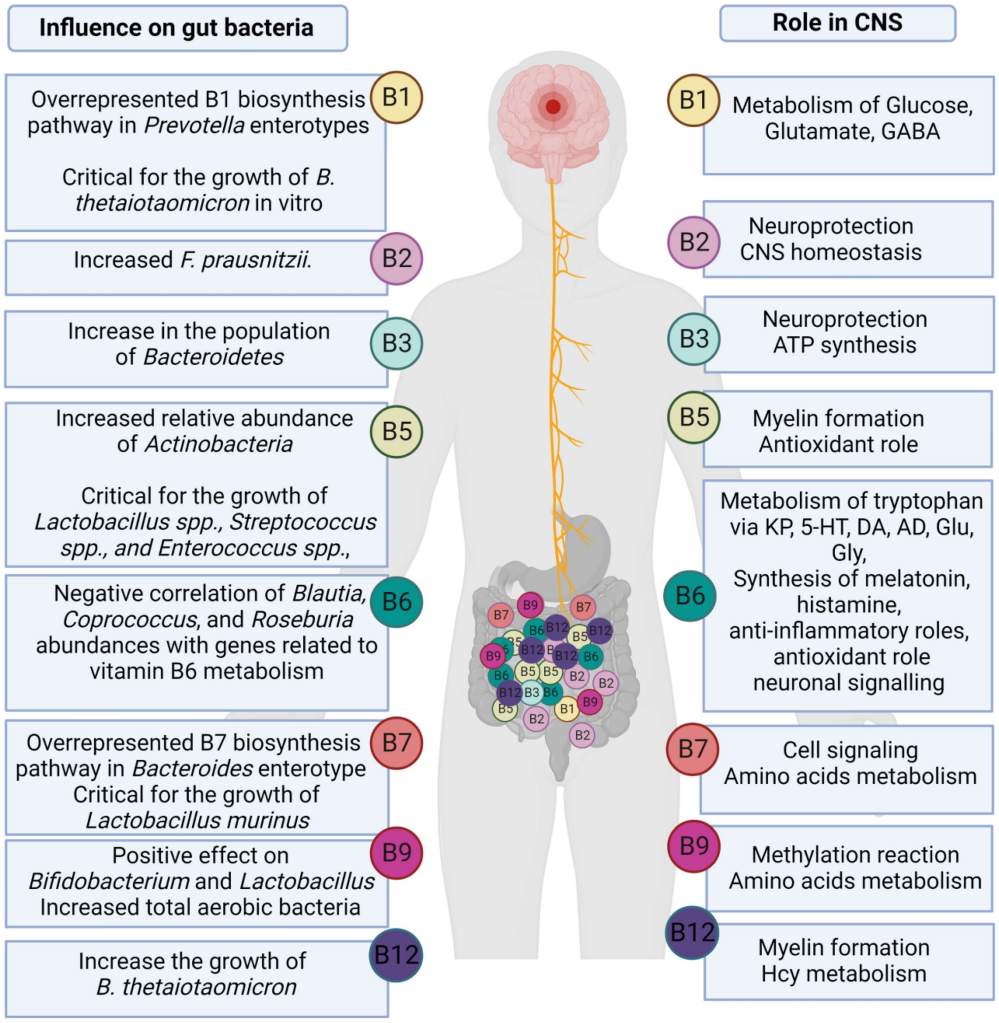

FIGURE 3
The role of microbially-produced B vitamins in CNS and gut microbiome.

The alignment of bacterial amidases and mammalian FAAH cDNA confirmed the existence of a highly conserved region known as the signature sequence (Mayaux et al., 1990; Cravatt et al., 1996). This evidence implies the potential presence of genes coding for FAAH enzymes in the gut microbiome, but this has not yet been reported.

4. Impact of neuroactive compounds on the gut environment

Neurochemicals, such as GABA, serotonin, dopamine, or their precursors and derivatives, are microbially metabolized by gut commensals and being considered major modulators of the gut environment, including the enteric nervous system (Sarkar et al., 2016). Neuroactive molecules, such as GABA, once secreted into the intestinal environment by bacteria, possibly induce epithelial cells to release molecules that, in turn, modulate neural signaling within the enteric nervous system and consequently signal the brain function and behavior of the host. For instance, *Bifidobacterium dentium* ATCC 27678, a GABA-producing bacterium, was shown to modulate sensory neuron activity in a rat fecal retention model of visceral hypersensitivity, suggesting that GABA-producing bacteria may represent future therapeutics for recurrent abdominal pain and functional bowel disorders (Pokusaeva et al., 2017). The GABA neurochemical was detected in the cytoplasm and brush border of epithelial cells in the rat jejunum and colon (Wang, 2004). The exposure of GABA to epithelial cells selectively stimulated *MUC1* expression in isolated pig jejunum (Braun et al., 2015) and increased the expression of tight junctions and transforming growth factor beta (TGF-β; Sokovic Bajic et al., 2019) while decreasing IL-1β-mediated inflammation *in vitro* (Sokovic Bajic et al., 2019), providing a protective effect against the disruption of the intestinal barrier. GABA-producing bacteria are believed to modulate the gut microbiome and interact with the brain *via* GABAergic signaling *via* vagal afferent neurons (Pokusaeva et al., 2017). The GABAergic system involves GABA receptors, neurons, and enzymes that regulate the immune system to release inflammatory cytokines and attenuate pain. The contribution of the GABAergic system in the pathogenesis of mood disorders is now well-recognized (Northoff and Sibille, 2014; Romeo et al., 2018). Additionally, probiotic bacteria can alter GABA receptor mRNA expression in the brain, which is associated with reduced anxiety and depression (Holzer and Farzi, 2014). Importantly, GABA has also been identified as an essential growth factor that solely can induce the growth of unculturable gut microorganisms (Strandwitz et al., 2019). Indeed, bacteria are known

to both produce and consume GABA (Strandwitz et al., 2019). GABA consumption has been studied less than GABA production, however, Feehily and Karatzas (2013) found that GABA is converted to succinate for use in the TCA cycle (Feehily and Karatzas, 2013). Dover & Halpern also described GABA as a source of nitrogen and carbon in *E. coli* (Dover and Halpern, 1972). GABA-producing bacteria also could modulate the gut microbiome structure and metabolism. In our recent study, we have shown the potential of *Bifidobacterium animalis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*, three GABA-producing bacteria, to promote gut health (Mousavi et al., 2022). While these GABA-producing probiotic candidates had no change in gut microbiome diversity, *ex-vivo* supplementation induced an increase of the *Bacteroidetes*, a key gut population having anti-inflammatory properties (Mousavi et al., 2022). The relative abundance of *Bacteroides*, a major GABA-producing genus in the gut, was also negatively correlated with depression-associated brain signatures (Strandwitz et al., 2019), indicating a significant role of microbiota-derived GABA in brain functionality. Also, *Bacteroides* spp. were linked with higher levels of serotonin, and myoinositol, which is pivotal in maintaining signaling between the enteric and central nervous systems (Mudd et al., 2017). Likewise, Mason et al. (2020) have reported depletion of *Bacteroides* in depression and anxiety (Mason et al., 2020). The oral administration of *B. fragilis* reduced gut permeability, microbiome dysbiosis, and several behavioral abnormalities in a mice model of autism spectrum disorder (ASD), thus highlighting the potential of microbial interventions for the treatment of gut microbiota-mediated neurological disorders (Hsiao et al., 2013).

Interestingly, Wang, J. et al. (2022) demonstrated that pollutants-treated zebrafish could be rescued from the disorder of intestinal peristalsis by using an exogenous treatment containing 100 μg/L of serotonin (5-hydroxytryptophan). They also suggested that *Lactobacillus rhamnosus* GG could normalize gut motility *via* increasing serotonin secretion (Wang, J. et al., 2022). It is estimated that 90–95% of the body's serotonin is located within the gastrointestinal tract. The gut microbiome produces a significant amount of serotonin (Kelly et al., 2015). At the same time, these levels of serotonin affect the gut microbiome. Researchers found that increased levels of serotonin promote the colonization of gut bacteria. In other studies, dopamine and norepinephrine have also been shown to affect the gut microbiome. For instance, *E. coli* grows more rapidly when dopamine and norepinephrine are present. It also exhibits an increase in biofilm formation, motility, and virulence in the presence of norepinephrine (Yano et al., 2015; Strandwitz, 2016). In addition to the ability to produce histamine, gut bacteria could degrade it. It is important to note that if more histamine is produced than is degraded, this could create symptoms of histamine intolerance. Eventually, this results in gut inflammation (Shulpekova et al., 2021). Moreover, microorganisms that produce SCFAs in the gut have been demonstrated to suppress gut motility. These findings support the theory that the microbiome participate in gut motility regulation through gut-to-brain signaling (Kelly et al., 2015; Muller et al., 2020). The understanding of the effect of neuroactive compounds on gut microbiome composition and activity is still limited despite significant efforts. Some recent studies mentioned that serotonin has a quorum-sensing effect on probiotic *Enterococcus faecium* NCIMB10415 and *Campylobacter jejuni*, a pathway that can modulate their behavior and subsequent interaction with the gut epithelium (Lyte et al., 2021; Scardaci et al., 2022). Due to the critical role of gut microorganisms in the production of neuroactive compounds and mental health, further research in this area is necessary.

5. Transport mechanisms of gut microbiota-produced neuroactive metabolites to the brain

It has long been assumed that gut-produced neurotransmitters, such as GABA, are unlikely to cross the BBB, but the investigations that have built this paradigm are often conflicting and vary widely in their used methods (Boonstra et al., 2015). However, recent research points out that gut microbiota-derived neurometabolites may cross intestinal barriers and reach distal organs, such as the brain. A fecal transplant from lean to obese individuals illustrated such gut microbiome-host interplay, which resulted in increased plasma levels of GABA (Kootte et al., 2017). For instance, gut microbiota-derived GABA is potentially transported through different pathways to the brain. The intestinal GABA absorption may occur *via* the transcellular pathway with the support of the relevant carrier proteins, and Nacher et al. (1994) reported that GABA could share a transporter with β-alanine in rat intestine models. GABA in the plasma can enter the BBB through GABA transporters such as GABA transporter types 1, 2, 3, and 4 (GAT1, GAT2, GAT3, and GAT4, respectively), which are also widely distributed to other organs, including the liver and kidneys (Nacher et al., 1994). The plasma membrane GABA transporters in the brain play a crucial role in maintaining the extracellular GABA level around the synapse (Liu et al., 2015). The GABA transporter is an active voltage-dependent system in which the inward electrochemical gradient of Na^+ ions significantly affects the activity of the GABA transporter instead of ATP (Scimemi, 2014). Furthermore, the GABA transporter shows a weak micromolecular affinity to GABA molecules and requires Cl^- ions in the extracellular matrix (Scimemi, 2014). Still, the exact transportation mechanism of GABA from the intestinal tract to the brain is not well understood. Likewise, most neurotransmitters, such as dopamine, norepinephrine, and acetylcholine, present in blood circulation cannot penetrate the BBB due to the absence of relevant transporters (Chen et al., 2021). However, the precursors of the above neurotransmitters, such as tyrosine and tryptophan, can penetrate BBB; thus, they can be transferred to the corresponding cells and used to synthesize corresponding neurotransmitters in the brain.

The SCFAs produced by the gut microbiota-mediated fermentation of fiber are absorbed through the colonocytes *via* monocarboxylate transporters (MCTs) and sodium-coupled MCTs (SMCTs), which are known as active transport (Vijay and Morris, 2014). SCFAs are transported *via* MCT1 transporters in an H^+-dependent (electroneutral manner), while they are also transported through the electrogenic and sodium-dependent SMCTs, known as SCFA anion transport (Stumpff, 2018). Most SCFAs introduced into the colonocytes are metabolized by entering the citric acid cycle in the mitochondria to produce ATP and energy (Schönfeld and Wojtczak, 2016). However, some portions of SCFAs in the colonocytes are not metabolized, which leads to their introduction into the portal circulation, used as an energy source for hepatocytes, except for acetate, which is not metabolized in the liver (Schönfeld and Wojtczak, 2016). This indicates that only a limited amount of colon-derived SCFAs is allowed to enter the systemic circulation and other organs and tissues; namely, only 36, 9, and 2% of gut-derived acetate, propionate, and butyrate, respectively, reach the blood plasma and peripheral tissues (Boets et al., 2015).

Bloemen et al. (2009) reported that the respective average levels of acetate, propionate, and butyrate in the portal blood of humans were 260, 30, and 30 μM (Bloemen et al., 2009). However, the penetration capacity of SCFAs in the BBB has not been well investigated to date, indicating that more research is needed to better understand the effects of gut microbiota-derived neuroactive metabolites on brain functions.

Recently, secreted microbiota extracellular vesicles (MEVs) have been proposed as a potential new carrier for the transportation of gut microbiota-derived neuroactive compounds to the brain (Sultan et al., 2021, 2022; Figure 4). Accumulating evidence suggests that MEVs are significant mediators in the intercellular signaling mechanism that could be an integral part of microbiome-host communications (Sultan et al., 2021). MEVs are small membrane-bound phospholipid vesicles that encase a spectrum of biologically active molecules (i.e., proteins, mRNA, miRNA, DNA, carbohydrates, and lipids) that protect them from lytic enzymes and RNases in the extracellular environment (Al-Nedawi et al., 2015) and facilitate their horizontal transfer across both short and distant locations, such as the brain (Choi et al., 2015; Sultan et al., 2021). For instance, *Akkermansia muciniphila*-produced extracellular vesicles were reported to induce serotonin secretion in both the colon and hippocampus of mice, suggesting MEVs' potential as signaling molecules in the gut–brain axis (Yaghoubfar et al., 2020). Besides, MEVs may cross intestinal barriers and reach distal organs, such as the liver and adipose tissues, inducing insulin resistance and glucose intolerance (Choi et al., 2015). A reported increased level of systemic LPS-positive bacterial MEVs in humans with intestinal barrier dysfunction provides evidence of their capacity to reach the systemic circulation (Tulkens et al., 2020) and deliver and elicit various immunological and metabolic responses in different organs, including the brain. From another point of view, the phospholipid nature of MEVs itself may directly influence neuronal

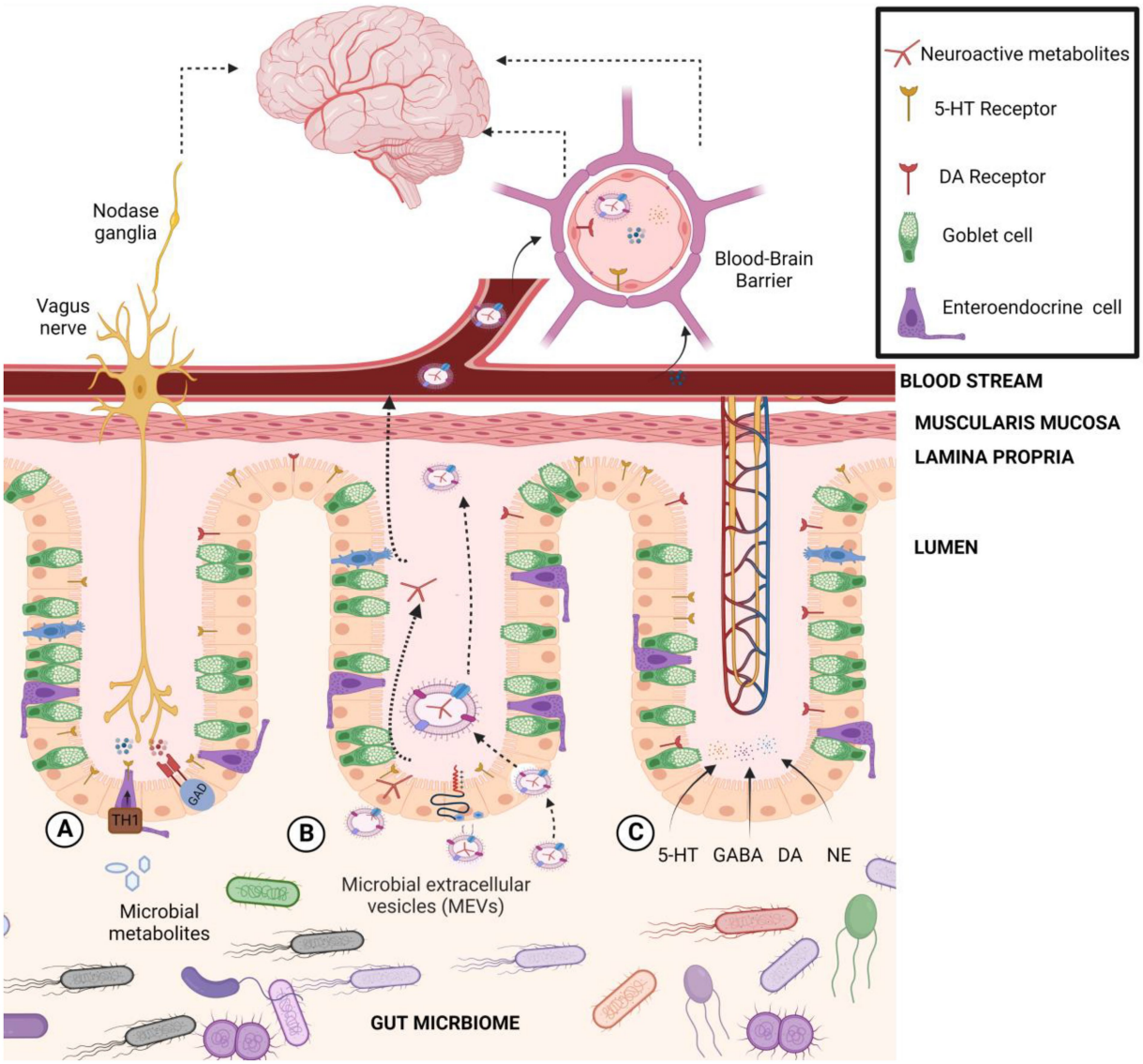

FIGURE 4
The transportation pathways of gut microbiota-derived neuroactive compounds to the brain. **(A)** Indirect transportation: gut microbiome regulates or induces host biosynthesis of neurotransmitters in cells like serotonin (5-HT) through tryptophan hydroxylase 1 (Tph1) or GABA through glutamate decarboxylase (GAD). **(B)** Microbial extracellular vesicle transportation: MEVs may bind to the cell receptor and deliver their contents to the host cell, activate a cell response, or be fully incorporated into the host cell's cytoplasm. **(C)** Direct transport: Microbially modulated neurotransmitters could interact with receptors or circulate systemically to reach the blood–brain barrier.

function under stress-related conditions (Donoso et al., 2020). For instance, *Lactiplantibacillus plantarum*-secreted extra vesicles exhibited an antidepressant-like effect in chronic restraint stress-treated mice (Choi et al., 2019). MEVs released by *Bacteroides fragilis* contain GABA and its intermediates α-ketoglutarate and glutamate as part of their content (Zakharzhevskaya et al., 2017). MEVs containing neuroactive compounds from *B. fragilis* may explain the observation of a previous study that showed the oral administration of this bacteria reduced gut permeability, microbiome dysbiosis, and several behavioral abnormalities in a mice model of autism spectrum disorder (ASD; Hsiao et al., 2013). Also, *Bacteroides*, a significant GABA-producing genus in the gut, was linked with higher levels of serotonin, and myoinositol, which is pivotal in maintaining signaling between the enteric and central nervous systems (Mudd et al., 2017). The relative abundance of *Bacteroides* was negatively correlated with depression-associated brain signatures (Strandwitz et al., 2019), indicating a significant role of microbiome-secreted GABA in brain functionality. Likewise, Mason et al. (2020) have reported depletion of *Bacteroides* in depression and anxiety (Mason et al., 2020). Recently, metabolomics profiling of MEVs content isolated from human gut microbiome revealed presence of a wide array of embedded metabolites, including neurotransmitter-related compounds such as arachidonyl-dopamine (NADA), gabapentin, glutamate and N-acylethanolamines (Sultan et al., 2022). The same authors reported that gut *Bacteroides* isolates (*B. finegoldii*, *B. faecis,* and *B. caccae*) produce high GABA levels (4.5–7 mM range) in supernatants, and importantly, GABA was detected inside secreted microvesicles at 2.2–4 μM. Such vesicles can transfer their cargo to the host cells such as Caco-2, RIN14B, and hCMEC/D3 cells, which showed capacity to internalize labeled MEVs through an endocytic mechanism (Sultan et al., 2022). These results provided novel insights on the shuttle role of MEVs for neuroactive molecules to the brain as a new signaling mechanism in microbiota-gut-brain axis communications. MEVs should be considered of utmost importance as delivery vehicles for host neuroactive compounds to the intestinal mucosa and other organs in the body such as the brain, thus, affecting the host's mental health.

6. Conclusion and future perspectives

One of the most intriguing and controversial topics in microbiome research is the relationship between gut microbial metabolism and mental health. Accumulating evidence showed that the gut microbiome produces a broad spectrum of neuroactive compounds, including neurotransmitters and their precursors, highlighting a potential involvement in neuroendocrinology-based mechanisms. One of the key challenges facing this field is the identification of neuroactive compounds originating from the host rather than the gut microbiome, which can be challenging due to complex biological communications between the gut microbiome and the brain. It is also difficult to determine the extent to which gut microbial metabolism directly influences central nervous system activity. This limitation may be attributed partly to the lack of a clear understanding of the general rate at which microbial molecules are transported into the brain. Indeed, the direct effects of microbial metabolites on the central nervous system function are difficult to distinguish from other communication pathways (such as immunological or neuronal pathways) that could confound *in vivo* studies. Some of these neuroactive compounds can travel through portal circulation to interact with the host's enteric nervous system, influence metabolism, or affect local neuronal cells of the ENS and afferent pathways of the vagus nerve that signal directly to the brain. When neurotransmitters cannot pass the BBB, their bacterial precursors do (such as tyrosine and tryptophan); thus, they can be located in the corresponding cells and synthesized into neurotransmitters in the brain. However, recent studies highlighted that secreted microbiome extracellular vesicles are potential new carriers for the transportation of gut microbiota-derived neuroactive compounds to the brain. In addition, most of the studies focusing on these relationships have relied heavily on simplified animal models, which cannot adequately simulate the complexity of the mechanism of microbial-produced neuroactive. Therefore, more studies on the mechanism, biosynthesis, absorption, and transportation of gut microbiota-derived neurotransmitters to the brain are needed. More analytical and statistical frameworks are needed to acquire and integrate multi-omics data types for a systematic approach to this extensively complex system. As described above, gut microbial neuroactive metabolites have various health-promoting effects. Despite recent research progress, multiple questions surrounding this field of gut neuromicrobiology remain unsolved. Indeed, there is a limited understanding of how gut microbes orchestrate the microbiome-gut-brain axis, a prerequisite for developing evidence-based microbiota-targeted interventions. Future research needs to progress from phenomenological studies to a mechanistic understanding of the microbiome-host dialogue and how these microbes impact host neurobiological functions. Future studies integrating metabolomic and metagenomic profiles with functional and behavioral outcomes will help us bridge this gulf of understanding toward translation into specific microbiota-targeted interventions. While further investigations remain necessary before the possibilities for evidence-based therapeutic applications, this review provided an overview of the biosynthesis and transport of gut microbiome-derived neurotransmitters and their precursors and interplays with the microbiome-gut-brain axis.

Author contributions

SM, JY, and RH designed and wrote the first draft of this article. SM, SA, and RH reviewed and edited the manuscript. 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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EDITED BY
Junling Shi,
Northwestern Polytechnical University, China

REVIEWED BY
Mara Mihai,
Carol Davila University of Medicine and Pharmacy, Romania

*CORRESPONDENCE
Xiang Wen
✉ xiangwen_wcums@163.com

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Role of the skin microbiota and intestinal microbiome in rosacea

Weitao Zhu[1], Michael R. Hamblin[2] and Xiang Wen[3]*

[1]Clinical Medicine (Eight-Year Program), West China School of Medicine, Sichuan University, Chengdu, China, [2]Laser Research Centre, Faculty of Health Science, University of Johannesburg, Doornfontein, South Africa, [3]Department of Dermatology, West China Hospital, Sichuan University, Chengdu, China

Rosacea is a chronic inflammatory cutaneous disorder of uncertain etiology that mainly affects the centrofacial region, including cheeks, nose, chin, forehead, and eyes. The pathogenesis of rosacea remains unclear because it involves several complex factors. Additionally, the potential treatment methods need to be explored. We reviewed the common bacterial species in the skin microbiota and gut microbiota of rosacea patients such as *Demodex folliculorum*, *Staphylococcus epidermidis*, *Bacillus oleronius*, *Cutibacterium acnes*, and *Helicobacter pylori* and identified their role in the pathogenesis. Besides, we summarized the influence factors such as temperature and age on rosacea patients. We also systematically reviewed the commonly used clinical treatment methods, including antibiotics, probiotics. as well as their treatment mechanism and application precautions.

KEYWORDS

rosacea, skin microbiota, gastrointestinal microbiome, influence factors, treatment

Introduction

Rosacea is a chronic inflammatory cutaneous disorder of uncertain etiology that mainly affects the centrofacial region, including cheeks, nose, chin, forehead, and eyes. There are four subtypes of rosacea, which are erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea, and ocular rosacea (Wilkin et al., 2002). However, these subtypes can progress from one type to another, so the current clinical recommendation is to classify rosacea according to clinical presentation, as patients with rosacea can have different clinical signs and symptoms. The newest research has classified rosacea symptoms into recurrent flushes or transient erythema, persistent erythema, morphological changes, papules, pustules, and telangiectasia (van Zuuren et al., 2021). The pathogenesis of rosacea involves several complex factors. Not only genetic factors but also environmental factors have been linked to rosacea. There are several flare triggers in patients with rosacea, including temperature changes, heat, cold, exercise, ultraviolet radiation, spicy food, and alcohol (Buddenkotte and Steinhoff, 2018). These factors can make patients more susceptible to skin disorders because they alter the skin's epidermal barrier function or disrupt immune function (Park et al., 2021). Rosacea is associated with many systemic complications such as gastrointestinal disease, cardiovascular disease, neurological disease, psychiatric disease, and autoimmune disease, but the exact pathogenesis of rosacea remains unclear (Holmes et al., 2018; Figure 1). The classification of rosacea is shown in Figure 1.

In the pathogenesis of rosacea, there has been extensive discussion on the skin microbiota and its related inflammatory effects. Many different communities of microorganisms have been studied in the skin, formed by hundreds of microbial species occupying different environmental niches in the skin (Xu and Li, 2019). The skin microbiota is essential for regulating inflammation and immune responses. The epidermis, dermis, and deeper subcutaneous tissue together form a physical and chemical barrier against external pathogens (Chen et al., 2021). Temporary non-specific immune cells and highly specific long-acting immune components constitute the skin immune barrier (Chaplin, 2010). The bacteria, fungi, viruses, and arthropods that live on the human skin together

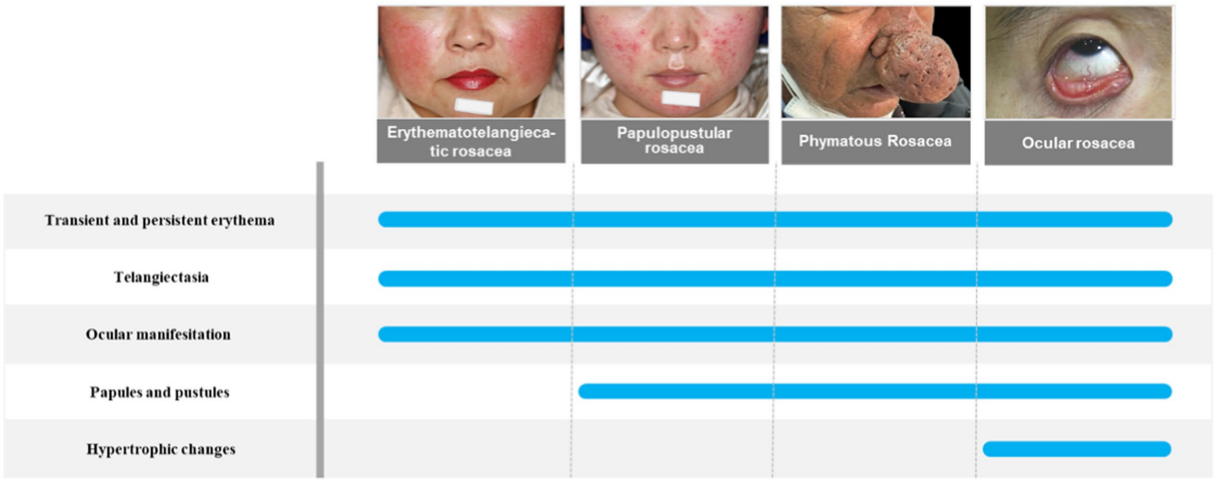

FIGURE 1
The classification of rosacea.

make up the human skin microbiome, all of which have been found to play a role in regulating immune responses. Some of these can cross the skin barrier and interact with deeper cells. If the skin microbiome is disturbed by internal or external factors, it can interfere with the function of the immune barrier to maintain homeostasis. Microorganisms in the skin not only trigger the release of certain antimicrobial peptides, but also regulate components of the complement system, and aggravate skin inflammation by accumulating neutrophils and producing interleukins (Park and Lee, 2018). However, the skin is not only affected by its own microorganisms, because recent studies have suggested that the skin can be affected by the gastrointestinal microbiome. The most frequently mentioned comorbidity is gastrointestinal disease among all kinds of rosacea. It has been gradually recognized that commensal microbes may play a significant part in the development of certain cutaneous disorders, and it is also believed that a weakened external barrier to pathogens leads to dysregulation of the skin microecology (Lam et al., 2022). Therefore, in this review, we summarize reports about the association between rosacea and the skin microbiota and gastrointestinal microbiota and provide an overall picture of the impact of rosacea treatment on the skin and gut microbiota.

Studies of the skin microbiome of patients with rosacea

Like most organ systems, the microbiota within the skin is indispensable for promoting efficient immune function. Researchers have identified several microbes as potential contributors to the development of rosacea; these are *Demodex folliculorum*, *Staphylococcus epidermidis*, *Bacillus oleronius*, and *Cutibacterium acnes* (Holmes, 2013).

Demodex folliculorum are microscopic mites which are usually found at the base of the eyelashes. The adult mites are cigar-shaped with four legs to grasp cylindrical structures like eyelashes. Demodex infection can cause activation of the immune system, inflammation, and follicular changes that may lead to disease (Fromstein et al., 2018).

Staphylococcus epidermidis is a Gram-positive biofilm-producing symbiotic bacteria and is the most important member of coagulase-negative staphylococci, widely present on human skin and mucosa, *S. epidermidis* is one of the most abundant colonizers on human skin. It could attach to foreign objects and form biofilms, which contributes to its ability to cause infectious disease (Yuan et al., 2020).

The *Bacillus* genus is a group of Gram-positive rod-shaped bacteria that can produce endospores under adverse conditions, making them widespread in nature. Bacillus species include some pathogens of clinical interest, bacterial contaminants in food, and some are used as industrial organisms to produce various enzymes (Owusu-Darko et al., 2017).

Cutibacterium acnes is a lipophilic anaerobic Gram-positive bacterium belonging to the *Cutibacterium* spp. family. It is a part of the skin commensal flora and is generally found in hair follicles and sebaceous glands, and can also exist in the oral mucosa, nose, urogenital tract, and large intestine (Achermann et al., 2014).

Demodex mites are associated with the presence of other microbiota in the skin. *Firmicutes*, *Actinobacteria*, and *Proteobacteria* were the most represented phyla in these Demodex related microbiota. Studies comparing rosacea patients with healthy standardized skin surface biopsies to study Demodex-associated microbiota, reported that *Proteobacteria* and *Firmicutes* were more abundant at the phylum level, whereas actinobacteria were less abundant (Murillo et al., 2014). By analyzing the microbial β-diversity, the researchers found that the patient-to-sample cluster was less pronounced, while the treatment-to-sample cluster was least pronounced. *Staphylococcus*, *Cutibacterium*, *Pseudomonas*, *Corynebacterium*, *Acinetobacter*, and *Snodgrasella* were the main bacterial groups at the genus level in untreated rosacea patients (Tutka et al., 2020). *Keratomyces acnes* (Rainer et al., 2020) and *S. epidermidis* (Woo et al., 2020b) are the most diverse bacteria on the skin of patients with rosacea.

When focused on the species level, *S. epidermidis* was the most common bacterial species, followed by *Stenotrophomonas rootophilus*, *C. acnes*, and *Corynebacterium tuberculostearicum* (Woo et al., 2020b). Previous studies had revealed diversity in the microbiota among different subtypes of rosacea. The phylum profile in papulopustular rosacea microbial communities was significantly different from erythematotelangiectatic rosacea. *Actinomycetes* accounted for only about one tenth of all clones in the papulopustular rosacea community, while most clones were found in erythematotelangiectatic rosacea. On the other hand, the proportions of *Proteobacteria* and *Firmicutes* in

papulopustular rosacea communities were increased compared with erythematotelangiectatic rosacea (Murillo et al., 2014).

Many studies have shown that the innate immune system is aberrantly activated by some skin microorganisms through Toll-like receptor 2 (TLR 2). After TLR 2 expression, antimicrobial peptides can be abnormally produced, and the expression and activity of serine kallikrein were also increased (Picardo and Ottaviani, 2014). Furthermore, TLR 2 can elicit erythema, telangiectasia, and infammation *via* expression of cytokines, chemokines, proteases, and pro-angiogenic factors (van Zuuren et al., 2021). Moreover, rosacea skin evidently showed increased cathelicidin expression, which was expressed by leukocytes as well as epithelial cells, compared to normal skin. This can lead to several unwanted downstream effects such as leukocyte chemotaxis, vasodilatation, angiogenesis, and extracellular matrix deposition (Weiss and Katta, 2017). At the same time, these effects may eventually lead to the development of a long-lasting non-infectious skin condition. *C. acnes* may play a role in protecting healthy skin (Barnard et al., 2020). It could prevent other microorganism from colonizing the skin because it breaks down sebum into free fatty acids (Marples et al., 1971).

The skin microbiome is a variable phenomenon, that alters with age, sex, environmental factors, and the use of cosmetics and antibiotics. There are differences in the pathogenesis of papules and pustules between acne and rosacea, which have been shown to be caused by age affecting the skin microbiome. Some studies have suggested that the severity of rosacea increases with age (Woo et al., 2020b). Under different temperature conditions, members of the normal skin microbiota that do not normally cause disease, such as *S. epidermidis,* can replicate at different rates and can also secrete more virulence factors (Dahl et al., 2004). *Staphylococcus epidermidis* strains isolated from the skin of rosacea patients were found to produce more protein at 37°C than at 30°C. Research has suggested that sudden changes in temperature can lead to worsening rosacea symptoms. The increased mobility and survival of *Demodex mites* at higher temperatures may explain that heat contributes to the worsening of rosacea (He et al., 2018). Bacteria behave differently at varying temperatures and produce different bacterial products. Skin temperature is likely to influence the activity of other skin microbiota, such as aerobic bacteria, anaerobic bacteria, and *Demodex mites*.

Study on the gastrointestinal microbiota of patients with rosacea

The human gut, like the skin, is home to countless microbes. Intestinal bacterial species such as *Lactobacillus*, *Escherichia coli*, *Bifidobacterium,* and *Streptococcus thermophilus* help to maintain human health, while others are more likely to cause disease, such as *Clostridium difficile*, *Campylobacter*, *Enterococcus faecalis*, and *Helicobacter pylori*.

Probiotics are living beneficial microbial species, but one way for a host to provide useful substrates for probiotic bacteria is the consumption of prebiotics, for example, foodstuffs or supplements containing certain saccharides (fructose, glucose, galactose, inulin, lactulose, sorbitol, or xylitol), These compounds can affect the intestinal microbiota and improve the environment of the skin, by increasing the number of beneficial gut microbes (Szántó et al., 2019).

Helicobacter pylori colonizes the human stomach and duodenum and is a microaerophilic Gram-negative bacterial species (Zeng et al., 2015). It can lead to a lifelong infection that is difficult to eradicate and may infect more than half of the human population worldwide. *Helicobacter pylori* can produce cytotoxins and cause gastric mucosal inflammation by proliferating and producing nitric oxide. It can alter physiological processes such as vasodilation, inflammation, and immune regulation (Mahmud et al., 2022). Rosacea is also associated with *H. pylori* seropositivity (Holmes, 2013). One mechanism for this theoretical association has been suggested to be that *H. pylori* can cause skin inflammation and flushing by the activity of cytotoxins and gastrin (Holmes, 2013), while other mechanisms have also been proposed. An autoimmune mechanism involving cross-reactive antibodies has also been hypothesized. This is based on systemic effects due to increased mucosal permeability to digestive tract antigens, or impaired vascular integrity (Wedi and Kapp, 2002). *Helicobacter pylori* infection has been found to be a risk factor for rosacea, but the association between them is weak. However, researchers reported there was a strong association between a positive C13-urea breath test and rosacea, and the C13-urea breath test is accepted as high diagnostic value for *H. pylori* infection (Jørgensen et al., 2017). This may be due to differences in the way *H. pylori* was diagnosed in the past. Besides, various strains of *H. pylori* have different virulence factors, which might lead to the divergence in the reported results (Woo et al., 2020a). Studies have also linked rosacea to overgrowth of various bacteria in the small intestine (Woo et al., 2020b).

A recent concept called the gut-skin axis has been proposed to explain the pathogenesis of many chronic inflammatory disorders, which proposes that skin homeostasis and allostasis are influenced by gastrointestinal health, through a complicated interplay between the immune system, metabolic system, and nervous systems (O'Neill et al., 2016). The gut microbiome has a bidirectional regulatory effect on host immunity, which is considered the primary regulator of the gut-skin axis (Forbes et al., 2016). Disturbances in the gut microbiome could affect the equilibrium of the immune system.

Some studies have analyzed the composition of the gut microbiota and found that there are significant differences between rosacea patients and control groups (Nam et al., 2018). There is ongoing debate about the effect of digestive diseases on rosacea. In rosacea patients' intestinal bacterial overgrowth, irritable bowel syndrome and chronic inflammatory bowel disease may be more common (Daou et al., 2021). One study found that altered levels of the mammalian synthetic AMP pheromone, plantaricin A could also play a part in rosacea (Nakatsuji and Gallo, 2012).

The link between skin microbiota and gastrointestinal microbiome

A complicated link between the alimentary tract, brain and skin has been recognized because patients have been found to improve their skin conditions after oral consumption of probiotics or prebiotics, but researchers have yet to thoroughly investigate the link (Tan-Lim et al., 2021). Changes in gastrointestinal microecology are often accompanied by the diagnosis of psychological disorders such as depression and anxiety. It is known that various neurotransmitters or neuropeptides can be induced by psychological stressors (Salem et al., 2018). This may increase intestinal permeability and therefore lead to enteric and systemic inflammation.

The activation of the plasma kallikrein–kinin system could also be influenced by intestinal bacteria (Kendall, 2004). Researchers have

reported the increased stimulation of the plasma kallikrein–kinin system in patients with intestinal inflammation and rosacea (Parodi et al., 1980).

Impact of treatments on the cutaneous and gut microbiome

Treatment for rosacea usually involves education, including avoiding ultraviolet light exposure, extreme temperatures, diet and alcohol. In addition, skin-irritating cosmetics should be avoided and daily use of sunscreen is recommended because ultraviolet exposure can cause severe effects on the skin. Studies have suggested that the signs and symptoms of rosacea should be treated based on the patient phenotype. For individual major symptoms such as transient and persistent erythema, inflammatory papules or pustules, telangiectasia, or lumps, a first-line treatment followed by a general skin-care regimen should be recommended. Several first-line treatments are listed as follow. Transient erythema: α-adrenergics (topical) and beta blockers (oral). Persistent erythema: brimonidine (topical), IPL and PDL. Inflammatory papules/pustules: azelaic acid (topical), ivermectin (topical), doxycycline (oral) and metronidazole (topical). Telangiectasia: electrodessication, IPL, and lasers. Phyma: doxycycline (oral) and Isotretinoin (oral). If there are multiple symptoms in a single patient, a variety of drugs could be used simultaneously to treat them. If treatment is unsatisfactory within a certain period, another treatment, or the addition of another first-line drug is recommended. The type of treatment and the patient's preference determine whether to continue treatment (Schaller et al., 2017).

Facial erythema can be treated with topical β-blockers or 2-epinephrine agonists, while oral β-blockers have also been shown to be effective (Logger et al., 2020). In severe infections which oral antibiotics have failed to improve, or which relapse after discontinuation of antibiotics, oral low-dose isotretinoin therapy could be effective. Research has suggested that bacteria sensitive to antibiotics may directly or indirectly cause papules and pustules (Dahl et al., 2004). Antibiotic treatment makes the disease less severe and increases the amount of *Weissella confusa*, a potentially beneficial microbe (Ferček et al., 2021). Studies have found that when rosacea is treated with topical or systemic antibiotics, papules and pustules tend to disappear rapidly. Papules and pustules also disappear rapidly when patients are treated with a range of chemically different antibiotics. Treatment can include erythromycin, clindamycin, ampicillin, metronidazole, clarithromycin, and any of the sulfonamides. The apparent disappearance of papules and pustules in patients treated with chemically different antibiotics suggests that bacteria do play a role in the pathogenesis (Dahl et al., 2004). In patients with rosacea, abnormalities in the hair follicles or the microenvironment of the skin surface can lead to worsening disease (Dahl et al., 2004). Coagulase-negative *staphylococci* produce and secrete proteins in the skin or follicles of patients with rosacea, which may lead to increased inflammation and to papules, pustules and dermatitis.

Many dermatologists treat rosacea patients with papules and pustules with topical or systemic antibiotics. Systemic antibiotics must be used continuously in patients with numerous papules and pustules. The anti-inflammatory activity of systemic antibiotics can lead to the disappearance of papules and pustules in rosacea patients.

Tetracycline has several mechanisms of action, such as antibacterial activity, regulation of innate immunity, inhibition of proinflammatory mediators and protease enzymes, etc. However, it is unclear which is the most relevant mechanism for the eliminatiopapules or pustules. Current studies suggest that an imbalance in the intestinal microbiota can lead to inflammatory skin diseases. Because intestinal bacteria may lead to disturbed immune responses, the use of oral metronidazole treatment can improve both inflammatory enteritis and rosacea symptoms (Vera et al., 2018).

Both minocycline and doxycycline were found to treat rosacea with similar results. Minocycline is a broad-spectrum antibiotic used to treat skin infections caused by many bacteria. The most common non-cutaneous adverse event in the treatment of rosacea with minocycline was viral upper respiratory tract infection, while the most common cutaneous adverse event was pruritus (Martins et al., 2021). Studies found that the skin microbiome α-diversity of rosacea patients treated with oral doxycycline was basically the same before and after systemic antibiotic treatment (Woo et al., 2020b). After treatment of rosacea with doxycycline for six weeks, there was a significant increase in the abundance of a bacterium called *Weissella confusa*. Between rosacea subjects and healthy controls, the researchers found that gut microbiome α-diversity was basically the same (Nam et al., 2018). When it came to the diversity of gut microbiota samples, their results were also the same. In one recent study, treatment with doxycycline significantly reduced the severity of rosacea and the number of inflammatory papules or pustules. Doxycycline (40 mg orally) was as effective as minocycline (100 mg orally) and there was no difference in the rate of adverse events (van Zuuren et al., 2019). Delayed release doxycycline 40 mg MR was as effective as 100 mg, with fewer side effects (Del Rosso et al., 2008). Several reports have used sub-antimicrobial doses of doxycycline hyclate 20 mg (SDD). One study used 20 mg of SDD twice daily for eight weeks to treat 50 patients with various stages of rosacea. On average, the inflammatory lesions were reduced by 80% to 100% and the erythema was reduced by 50% (Bikowski, 2003).

Some studies have shown that 0.75% metronidazole gel can be used as a first-line topical treatment for the treatment of rosacea. Researchers used 0.75% metronidazole gel twice a day for 12 weeks in the treatment of rosacea and found that inflammatory lesions and erythema were significantly improved, by 79% for papules and 94% for pustules (Miyachi et al., 2022). Reactive oxygen species and oxidative stress are closely associated with a range of skin conditions. Topical metronidazole can both reduce the production of reactive oxygen species and exert its efficacy in rosacea related diseases through anti-inflammatory and immunomodulatory pathways.

Topical 1% ivermectin can effectively reduce *Demodex mite* density and had a significant effect on rosacea (Ebbelaar et al., 2018). It could also be observed under reflectance confocal microscopy that *Demodex follicularis* would undergo morphological changes through the action of ivermectin, such as "phantom mites." Mite density decreased significantly after treatment and clinical improvement. Topical permethrin, benzyl benzoate and crotamine have also been shown to affect *Demodex* populations (Forton and De Maertelaer, 2020). Studies have been conducted to treat rosacea with 1% ivermectin cream once daily. Of 910 participants who received ivermectin, 615 showed improvement, with a post-treatment improvement rate of 68% (van Zuuren et al., 2019). Benzyl benzoate and crotamiton have also been shown to be effective.

The long-term use of broad-spectrum antibiotics can lead to the emergence of resistant strains, more adverse events and compliance problems. Sarecycline is a novel tetracycline derivative with narrow spectrum activity targeting Gram-positive bacteria, especially *Bacillus acnes* (Bunick et al., 2021). In a 12-week study of 72 subjects who received oral administration of sarecycline once daily according to body weight,

TABLE 1 Some microorganisms closely related to rosacea in intestinal microbiome and skin microbiota.

Authors	Microbes in skin microbiota	Microbes in intestinal microbiota
Holmes (2013)	*Demodex folliculorum Staphylococcus epidermidis Bacillus oleronius Cutibacterium acnes*	*Helicobacter pylori*
Fromstein et al. (2018)	*Demodex folliculorum*	
Yuan et al. (2020)	*Staphylococcus epidermidis*	
Owusu-Darko et al. (2017)	*Bacillus oleronius*	
Achermann et al. (2014)	*Cutibacterium acnes*	
Murillo et al. (2014)	*Firmicutes Actinobacteria Proteobacteria*	
Tutka et al. (2020)	*Staphylococcus Cutibacterium Pseudomonas Corynebacterium Acinetobacter Snodgrasella*	
Rainer et al. (2020)	*Keratomyces acnes*	
Woo et al. (2020a)	*Staphylococcus epidermidis Stenotrophomonas rootophilus C. acnes Corynebacterium tuberculostearicum*	
Barnard et al. (2020)	*Cutibacterium acnes*	
Dahl et al. (2004)	*Staphylococcus epidermidis*	
He et al. (2018)	*Staphylococcus epidermidis Demodex mites*	
Zeng et al. (2015)		*Helicobacter pylori*
Mahmud et al. (2022)		*Helicobacter pylori*
Jørgensen et al. (2017)		*Helicobacter pylori*
Woo et al. (2020b)		*Helicobacter pylori*
Bunick et al. (2021)	*Bacillus acnes*	
Hacini-Rachinel et al. (2009)		*Bifidobacteria Lactobacillus*
Pinchuk et al. (2001)		*Bacillus subtilis Helicobacter pylori*

the results showed that sarecycline was effective in treating papules and pustules in adults with rosacea, with an efficacy of 80% (Rosso et al., 2021).

Although rosacea can be treated with effective oral or topical antibiotics, sulfur compounds can change the facial microbiota (van Zuuren et al., 2015) and there is no conclusive evidence that these changes in the skin microbiota are effective in treating the disease. The effects of antibiotic treatment on the gut microbiota are both short-term and long-term. Although antibiotic treatment may be effective in the short term, most skin diseases are associated with long-term disturbances in the microbiota, so this treatment strategy may not be optimal (de Gunzburg et al., 2018).

Some studies have found that topical application of probiotics could directly affect the skin microbiota and immune response (Yu et al., 2020). The effect of topical probiotics on various skin conditions has not been fully explored. Topical and oral probiotics have both been shown to be effective in treating some local diseases. Besides, a combination of topical and oral probiotic treatment may be the most effective (Knackstedt et al., 2020). In general, treatment with probiotics may improve the skin barrier function, reduce inflammation, and reduce the dysregulation of the skin microbiome by restoring a healthy balance of cytokines. For example, TLR2 may be upregulated in rosacea and could be a possible target for probiotics (Tripathi et al., 2019). Besides, oral probiotics can regulate the intestinal microfora and indirectly affect cutaneous conditions (Yu et al., 2020). The consumption of *Bifidobacteria* and *Lactobacillus* to affect the gut can also be used to treat certain cutaneous conditions (Hacini-Rachinel et al., 2009). *Bacillus subtilis* produces spores to colonize the gastrointestinal tract and alter the mucosal barrier microbiome, thereby eradicating *H. pylori* to reduce rosacea symptoms and associated gastrointestinal problems (Pinchuk et al., 2001). The microorganisms in the intestinal microbiome and skin microbiota described in this review are shown in Table 1.

Conclusion

Human skin provides a suitable environment for the growth of both beneficial and pathogenic bacteria. It has been shown that rosacea is associated with disturbances in the microbiome of the skin and gut. Therefore, treating rosacea with antibiotics or microbiome modulation has been an attractive approach to disease management. Most dermatologists treat rosacea patients with papules and pustules with topical or systemic antibiotics. Thus, research on changes in the skin and gut microbiota in rosacea patients could contribute to a better understanding of the development and prognosis of the disease.

The role of the gut microbiota in the pathogenesis of rosacea should be further explored. In future studies, the relative abundance of microbial distribution at the strain level will need to be analyzed and different DNA sequencing techniques will need to be used to confirm the various findings. In addition, the clinical complications of rosacea often occur and the pathogenesis and treatment of complications still needs to be further explored, to better manage this disease.

Author contributions

WZ contributed to data acquisition, analysis, data interpretation, and manuscript drafting. XW contributed to data acquisition, analysis, supervised the review, and revised the manuscript for intellectual content. MH critically edited the article for content and presentation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MH declares the following potential conflicts of interest. Scientific Advisory Boards: Transdermal Cap Inc., Cleveland, OH; Hologenix Inc. Santa Monica, CA; Vielight, Toronto, Canada; JOOVV Inc., Minneapolis-St. Paul MN; Sunlighten, Kansas City, MO; Consulting; USHIO Corp, Japan; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany; Klox Asia, Guangzhou, China. Stockholding: Niraxx Light Therapeutics, Inc., Irvine CA; JelikaLite Corp, New York NY.

The remaining 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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EDITED BY
Junling Shi,
Northwestern Polytechnical University,
China

REVIEWED BY
Nar Singh Chauhan,
Maharshi Dayanand University,
India
Anne-Sophie Bergot,
Diamantina Institute,
The University of Queensland,
Australia

*CORRESPONDENCE
Fernanda Sales Luiz Vianna
✉ fslvianna@gmail.com

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Role of gut microbiota in infectious and inflammatory diseases

Mirian Ferrão Maciel-Fiuza[1,2,3,4], Guilherme Cerutti Muller[1], Daniel Marques Stuart Campos[3,4], Perpétua do Socorro Silva Costa[1,2,5], Juliano Peruzzo[6,7], Renan Rangel Bonamigo[6,7,8], Tiago Veit[4,9] and Fernanda Sales Luiz Vianna[1,2,3,4,7]*

[1]Postgraduate Program in Genetics and Molecular Biology, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brazil, [2]Instituto Nacional de Genética Médica Populacional, Porto Alegre, Brazil, [3]Genomics Medicine Laboratory, Center of Experimental Research, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [4]Laboratory of Immunobiology and Immunogenetics, Department of Genetics, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brazil, [5]Department of Nursing, Universidade Federal do Maranhão, Imperatriz, Brazil, [6]Dermatology Service of Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [7]Postgraduate Program in Medicine, Medical Sciences, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brazil, [8]Postgraduate Program in Pathology, Universidade Federal De Ciências Da Saúde de Porto Alegre, Porto Alegre, Brazil, [9]Department of Microbiology, Immunology and Parasitology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Thousands of microorganisms compose the human gut microbiota, fighting pathogens in infectious diseases and inhibiting or inducing inflammation in different immunological contexts. The gut microbiome is a dynamic and complex ecosystem that helps in the proliferation, growth, and differentiation of epithelial and immune cells to maintain intestinal homeostasis. Disorders that cause alteration of this microbiota lead to an imbalance in the host's immune regulation. Growing evidence supports that the gut microbial community is associated with the development and progression of different infectious and inflammatory diseases. Therefore, understanding the interaction between intestinal microbiota and the modulation of the host's immune system is fundamental to understanding the mechanisms involved in different pathologies, as well as for the search of new treatments. Here we review the main gut bacteria capable of impacting the immune response in different pathologies and we discuss the mechanisms by which this interaction between the immune system and the microbiota can alter disease outcomes.

KEYWORDS

gut microbiota, microbiome, infectious diseases, inflammation, cytokines, immune modulation

1. Introduction

The human gut microbiota is a community of microorganisms that includes viruses, bacteria, archeas, fungi and protozoa, and the microbiome is the collective genomes of microorganisms, their metabolites, and proteins in a specific environment (Budden et al., 2019). In humans, the intestine harbors the greatest number of microorganisms and the greatest number of species in relation to other places in the body (Quigley, 2013). They consist of over

1,500 species, which colonize the digestive tract within minutes of birth, establishing a symbiotic or mutualistic relationship with epithelial and lymphoid tissue (Robles-alonso et al., 2013; Horta-Baas et al., 2017; Lourido et al., 2017; Budden et al., 2019; Mitev and Taleski, 2019). The intestinal microbiota is predominantly composed of bacteria, containing especially the phyla Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Figure 1), being the most microorganisms found in the colon (Acharya et al., 2017; El-Mowafy et al., 2021). These microorganisms produce a variety of metabolites from the anaerobic fermentation of exogenous dietary components and endogenous compounds generated by microorganisms and host. The generated metabolites, such as short-chain fatty acids (SCFAs), interact with host cells and influence immune responses (Hooper et al., 2002; Rooks and Garrett, 2016). Therefore, this is a dynamic and complex ecosystem that helps the proliferation, growth, and differentiation of epithelial cells to fight against infections and stimulate the immune system (Hara and Shanahan, 2007; Sultan et al., 2021a).

There is mounting evidence indicating that the gut microbiome plays an important role in modulating host physiology, with studies linking gut microbiome composition and functions to differential inflammatory, neurological, and even behavioral activities (Dinan and Cryan, 2017; Wipperman et al., 2021). The intestinal microbiota has several functions, including protection against pathogens by colonization of the mucosal surface and production of antimicrobial substances (Mills et al., 2019), aiding in digestion and metabolism (Rothschild et al., 2018), controlling the proliferation and differentiation of epithelial cells (Wiley et al., 2018), changing insulin resistance and affecting its secretion (Kelly C. J. et al., 2015; Kelly J. R. et al., 2015), influencing brain-intestinal communication and thus influencing host neurological functions (Zheng et al., 2019; Gomaa, 2020). Thus, disturbances in the intestinal microbial population can result in an imbalance of the homeostasis, promoting the development of pathologies (Mori et al., 2021). Several intrinsic factors can influence the composition and function of the gut microbiota, such as birth form, age, host genetics and innate and adaptive immunity. Extrinsic factors such as diet, lifestyle, geographic region, presence of allergens or pathogens and antibiotic therapy can also determine the type of microorganism found (Rodrı et al., 2015; Martinez and Taddei, 2018; Hasan and Yang, 2019). Dysbiosis is a term used to describe a quantitative and/or qualitative change in the composition of the microbiota (Passos and Moraes-Filho, 2017). Dysbiosis can be caused by many of daily activities, such as dietary patterns, hygiene habits, physical activity, and medication use (Mitev and Taleski, 2019). When there is a dysbiotic state, the functioning of the microbiota is affected and can induce a disease state (Schwiertz, 2016; Lee and Kim, 2017). In this review, we provide an overview of the current understanding of the role of the gut microbiota in the regulation of the immune system and the modulation of serum cytokines in the most common and/or most studied autoimmune and inflammatory diseases, and in viral and mycobacterial infections.

2. Microbial metabolites and immune system

The human gastrointestinal tract is the main site of interactions between microorganisms and the host's immune system. In this interaction, the microbiota contributes to the physiological functions of the host while the host provides nutrition and habitat (Caricilli, 2014). The gut microbiota is essential not only for the degradation and fermentation of feed, but also for defense against pathogens, either by competing for nutrients and adhesion sites, or by secreting antimicrobial peptides (Moens and Veldhoen, 2012; Kamada et al., 2013; Takiishi et al., 2017). Experiments conducted in germ-free animals (GF) have demonstrated that colonization of the microbiota early in life is necessary for the proper development of the immunity. In the lack of gut microbiota, the immune system of the intestinal mucosa is underdeveloped, with, for example, reduced number of functional regulatory CD4+ CD25+ T cells, resulting in a reduced

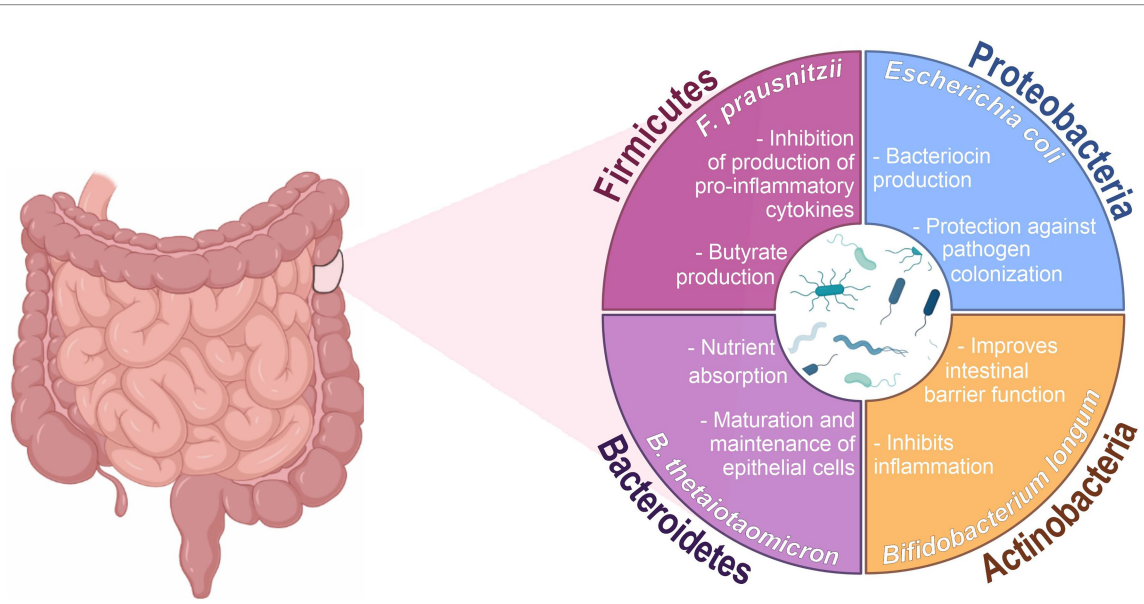

FIGURE 1
Main phyla and functions associated with the intestinal microbiota. Created with BioRender.com and coreldraw.com.

capacity to fight pathogenic bacteria (Hara and Shanahan, 2007; Sommer and Bäckhed, 2013; Takiishi et al., 2017). In addition, the balance between proinflammatory interleukin (IL)-17-producing effector T helper (Th17) cells and Forkhead box P3 (Foxp3$^+$) regulatory T (Tregs) cells in the gut requires signals from gut bacteria, and those signals are dependent on gut microbiota composition (Ivanov and Littman, 2010). For instance, GF animals colonized with *Bacteroides fragilis* had the balance between Th1 and Th2 cells restored, thanks to the production of polysaccharide A (Mazmanian et al., 2005). Polysaccharide A is a bacterial product that influences T cell activation through interaction with Toll-like Receptor 2 (TLR2). It inhibits Th17 differentiation and favors Treg activity, thus favoring immune tolerance (Round et al., 2011). Resident bacteria, especially Clostridia-related species, have been associated with development of Th cells and induction of Treg cells (Gaboriau-Routhiau et al., 2009; Atarashi et al., 2011; D'Amelio and Sassi, 2018).

The intestinal microbiota produces a diverse repertoire of metabolites from food by modifying host products and by *de novo* synthesis. Among them, short-chain fatty acids (SCFAs) are the most described in the regulation of the immune system (D'Amelio and Sassi, 2018). SCFAs result from fiber fermentation in the colon and include acetic acid, butyric acid, and propionic acid, which cross the intestinal epithelium and interact with host cells, influencing immune responses (Takiishi et al., 2017). In addition to their metabolic functions, these substrates have several regulatory functions. SCFAs are inhibitors of histone deacetylases (HDACs) and ligands for G protein-coupled receptors (GCPRs), also called free fatty acid receptors (FFAR). SCFA-guided inhibition of HDACs tends to promote a tolerogenic and anti-inflammatory cell phenotype that is essential for maintaining immune homeostasis (Rooks and Garrett, 2016).

Studies with exposure of peripheral blood mononuclear cells (PBMCs) and neutrophils to SCFAs showed inhibition in the production of the pro-inflammatory cytokine tumor necrosis factor (TNF) and in the activation of nuclear factor-κB (NF-κB; Usami et al., 2008; Rooks and Garrett, 2016). SCFAs also influence peripheral T cells, especially regulatory T cells, through HDAC inhibition. Tao et al. (2007) reported that inhibition of HDAC9 increased the expression of Foxp3$^+$ and number of Treg cells, improving suppressor function of Foxp3$^+$ Treg cells under homeostatic conditions and amplified attenuation of Treg cell-mediated colitis in mice. Some SCFAs such as butyrate and propionate also modulate antigen presentation by inhibiting dendritic cell development through inhibiting HDACs (Bernard et al., 2002; Wang et al., 2008; Singh et al., 2010; Liu L. et al., 2012) and interacting with FFAR (Singh et al., 2010; Arpaia et al., 2013; D'Amelio and Sassi, 2018).

Furthermore, by regulating the activity of hypoxia-inducible factor (HIF), butyrate and propionate are associated with the maintenance of intestinal homeostasis (Kelly C. J. et al., 2015). HIF is the main regulator of oxygen homeostasis in response to hypoxia (Brahimi-Horn and Pouysségur, 2007; Rocha, 2007). It is a transcription factor formed by a heterodimeric protein, composed of α and β subunits. The β subunit, also called the aryl hydrocarbon receptor nuclear translocator (ARNT), is not influenced by oxygen and is stably expressed. The α subunit, composed of three subunits (HIF-1α, HIF-2α, HIF-3α), is directly regulated by the presence of oxygen (EMA et al., 1997; Muz et al., 2009; Dengler et al., 2014). In a situation of tissue normoxia, HIF-1α is continuously synthesized and degraded through the 26S proteasome system. In contrast, under hypoxic conditions, HIF-1α stabilizes and bound to HIF-1β, initiates transcription of its target genes. In the intestine, these target genes are basally regulated to maintain the epithelial barrier and include genes crucial for cellular energetics (Glover et al., 2016), barrier function (Furuta et al., 2001), mucin production (Louis et al., 2006), microbial defense (Kelly et al., 2013), and xenobiotic clearance (Wartenberg et al., 2003). Therefore, HIF-1α stabilization maintains the structure of the epithelial barrier (van Itallie and Anderson, 2014), stimulates CD4+ T cells and IL-22 production (Yang et al., 2020) and, increases the expression of MUC2, MUC3 and intestinal trefoil factor (ITF), which is essential for the epithelial restoration of the colon (Louis et al., 2006; Dilly et al., 2016; Glover et al., 2016; Ma S. et al., 2022). Thus, SCFAs play an important role in regulating the host–microbe interaction, modulating the HIF, which directly influences this crosstalk.

In addition to SCFAs, other metabolites produced by the gut microbiota have important immunomodulatory functions, such as indole derivatives, which are derived from tryptophan, and polyamines, originated from dietary arginine. Indole derivatives promote the integrity of the enteric epithelium and the defense against microorganisms, inducing the multiplication of intestinal goblet cells, and the secretion of antimicrobial peptides, and mucins (D'Amelio and Sassi, 2018). Tryptophan derivatives also promote the differentiation and function of anti-inflammatory macrophages, Treg cells and IL-22 producing innate lymphoid cells 3 (ILC3). IL-22 acts in the maintenance of intestinal epithelial cells (IECs), regulates the equilibrium of the commensal microbiota and protects against infection by *Citrobacter rodentium* (Liang et al., 2006; Su et al., 2022). In mice, ILC3s induce fucosylation, which is an important glycosylation mechanism in IECs. This induction may be dependent on commensal bacteria, using IL-22, and independent of these bacteria, requiring lymphotoxin. The absence of intestinal fucosylation leads to increased susceptibility to *Salmonella typhimurium* infection. Therefore, ILC3s play an important role in modulating the intestinal microenvironment through the regulation of epithelial glycosylation, protecting against infection by pathogenic bacteria (Goto et al., 2014). Polyamines, such as putrescine, are found in many cells and play a role in gene transcription, translation, proliferation, and cell death. Polyamines are essential for host cell functions; for example, intestinal epithelial cells depend on these molecules to maintain high proliferation rates. They assist the development and maintenance of the intestinal epithelium and the inhabiting immune cells (Rooks and Garrett, 2016; D'Amelio and Sassi, 2018).

Most evidence suggests that intestinal microbiota metabolites and antigens can influence the immune system. Therefore, dysbiosis, characterized by alterations in the microbiome resulting in an imbalance in the microbiota, can contribute to the development of some immunological and inflammatory pathologies, both at the intestinal level, such as the well-documented Inflammatory Bowel Disease (IBD; Lane et al., 2017), and in other regions of the body, such as in rheumatoid arthritis (RA; Maeda et al., 2016). Indeed, many organs distant from the intestine, such as the skin, brain, and lungs, which are not in direct contact with the intestinal microbiota, can be affected by dysbiosis and its repercussions in the immune point of view. This suggests that the gut microbiota actually has the capacity to interact with the immune system in a systemic manner. For this, the gut microbiota needs to send microbial signals that are transmitted

through the intestinal epithelium. These signals can be structural components of the bacteria or the metabolites themselves produced by the gut microbiota that can diffuse through the circulation and directly affect distant organs or by signaling nerves or hormones in the gut (Schroeder and Bäckhed, 2016; D'Amelio and Sassi, 2018).

3. Gut microbiota and cytokine modulation

The gut microbiota is mainly composed of the phyla Bacteroidetes and Firmicutes, which comprise approximately 90% of the microbial population in humans (Eckburg et al., 2005; Qin et al., 2010). Bacteroidetes vary in relative abundance among individuals, but they normally make up half of the gut microbiome. The members of this phylum reside especially in the distal intestine, where they function in the fermentation of indigestible carbohydrates. The predominant genera of Bacteroidetes in the human gastrointestinal tract are *Bacteroides*, *Prevotella* and *Porphyromonas* (Qin et al., 2010; Huttenhower et al., 2012).

Pro- and anti-inflammatory cytokines play an important role in regulating the host's immune response to the intestinal bacteria's own compositional variations and, therefore, in maintaining intestinal balance. For example, **interleukin-10** (IL-10) production by Tregs is essential in maintaining intestinal homeostasis, as it prevents excessive inflammation. *Lactobacillus rhamnosus* and *Lactobacillus reuteri* have been shown to induce IL-10 production by Tregs (Liu Y. et al., 2012; Jang et al., 2019). **Interleukin-17A** (IL-17A), produced by Th17 cells, is an important mediator of innate and adaptive immune response, but can also contribute to inflammation and tissue damage. Some members of the *Bacteroides*, such as *B. fragilis*, have been shown to induce Treg differentiation from CD4+ T cells, and thus decrease IL-17A production (Round et al., 2011). On the other hand, *Prevotella* spp. have been associated with an increase in IL-17A production in CD4+ T cells (Figure 2; Maeda et al., 2016). **Interleukin-22** (IL-22), also produced by Th17 cells, and innate lymphoid type 3 (ILC3) cells and is involved in defense against extracellular pathogens. IL-22 production is also associated with epithelial regeneration and repair. *Akkermansia muciniphila*, a common human gut bacterium, has been shown to induce IL-22 production by ILC3 cells (Bachmann et al., 2022; Zheng et al., 2023). **Interferon gamma** (IFN-γ) production is a typical response of Th1 cells and is associated with protective immunity against intracellular pathogens. Supplementation with different species of *Lactobacillus* increased IFN-γ production by T lymphocytes, macrophages, and dendritic cells (Won et al., 2011; Dimitrijevic et al., 2014; Kim et al., 2015; Saliganti et al., 2015; Yang et al., 2015). **Interleukin-12** (IL-12), which is a key factor in the polarization of CD4+ T cells to the Th1 phenotype, can also be modulated through the gut microbiota. Several strains of *Lactobacillus* also have been associated with an increase in IL-12 production (Christensen et al., 2002; Lee et al., 2013, 2015; Kim et al., 2015). Therefore, the intestinal microbiota can modulate and be modulated by cytokines produced in the intestine. Directly and indirectly influencing host immune responses in states of health and disease. However, the impact of the different compositions of the intestinal microbiota on the modulation of cytokine production and consequent inflammatory response needs to be better elucidated.

4. The gut microbiota and autoimmune/inflammatory diseases

The immune system is a collection of cells, tissues and organs that work together in complex ways to protect the body from invaders. It is composed of several blood cells such as dendritic cells (DCs), T cells and B cells, lymphoid organs such as bone marrow, and lymph nodes, and molecules as antibodies, complement and cytokines. The function of the immune system is to eliminate infectious microorganisms and cancer cells, and to aid repair tissue after injury, thus contributing to the maintenance and reestablishment of homeostasis. In autoimmune diseases, the balance between pathogen recognition and self-attack prevention is compromised. As a result, control of inflammation is lost and continued activation of the immune system occurs even in the absence of infection (Wahren-Herlenius and Dörner, 2013; Kuwabara et al., 2017).

Autoimmune and inflammatory diseases are characterized by dysregulated immune response, with production in abnormal amounts of autoantibody-producing B cells, autoreactive T cells, and augmented production of pro-inflammatory cytokines (Raphael et al., 2015; Kamali et al., 2019). Genetic and environmental factors including geographic location, immunological disorders and viral infections favor the development of autoimmune diseases. Furthermore, dysbiosis of the intestinal microbiota has been associated with these pathologies through several mechanisms, which can impact the regulation of the human immune system (Table 1). For example, molecular mimicry (when self-antigens and foreign antigens share similar sequences or structures) impacts on the permeability of the intestinal mucosa and may be associated with initiation and amplification of disease progression. While certain microbiota compositions could prevent autoimmunity in genetically susceptible individuals, disturbances or alterations in this composition may trigger the autoimmune process (Kawajiri and Fujii-Kuriyama, 2017; Xu et al., 2019).

4.1. The gut microbiota and inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of complex multifactorial inflammatory diseases that affect the gastrointestinal tract (Xavier and Podolsky, 2007; Jergens et al., 2021). It comprises two main classes: ulcerative colitis (UC) and Crohn's disease (CD), which have different clinical, endoscopic, immunological, and histopathological characteristics (Jergens et al., 2021; Sultan et al., 2021a). UC is the most common form of IBD, which affects more than 5 million individuals worldwide (Alatab et al., 2020; Yang et al., 2021). Its inflammation is limited to the mucous layer, causing superficial damage restricted to the wall of the rectum and colon (Kobayashi et al., 2020; Arukha et al., 2021). CD is characterized by irregular transmural inflammation that extends through the intestinal wall into the serous layer, and it affects mainly the terminal ileum, but it can affect any part of the gastrointestinal tract (Arukha et al., 2021; Jergens et al., 2021).

Both diseases are characterized by an imbalance between anti and proinflammatory signals and the displacement of leukocytes to the intestinal epithelium. However, the T cells populations involved in the immune responses seem to be different depending on the disease, which may explain the different phenotypes observed in clinical practice (Ramos and Papadakis, 2019). UC is thought to occur due to

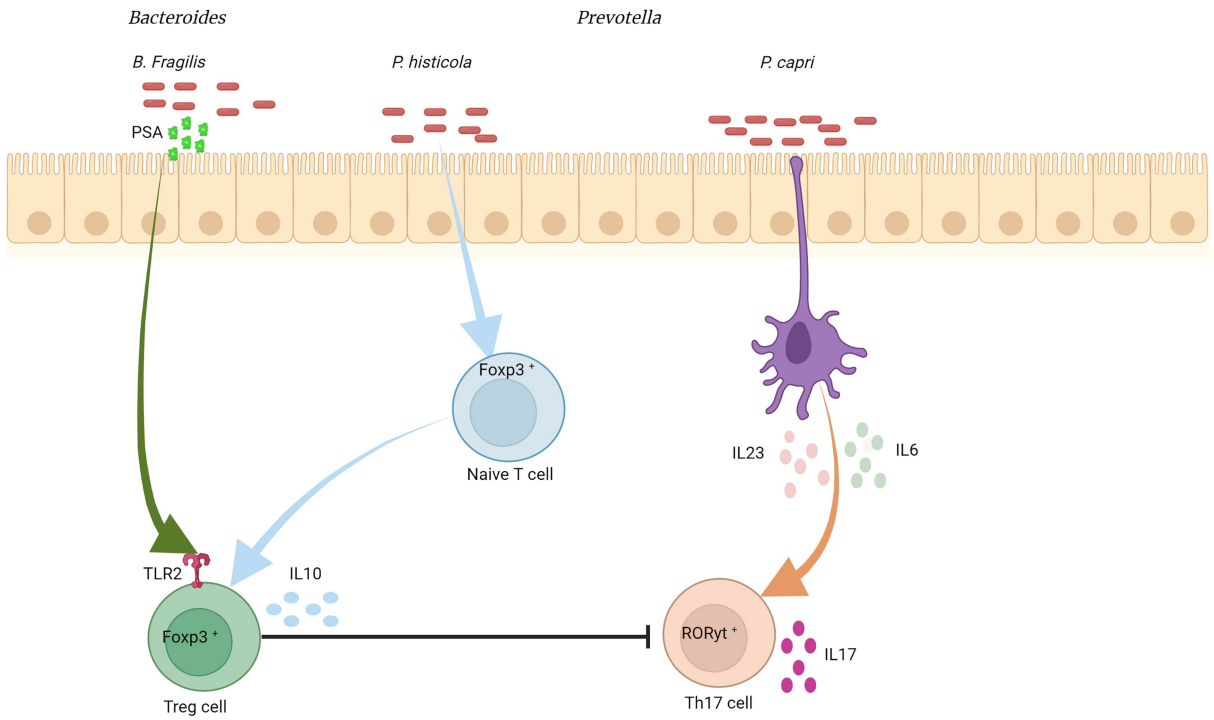

FIGURE 2
Bacteroides/Prevotella colonization and cytokine modulation. *Bacteroides fragilis* was associated with the induction of an anti-inflammatory response, inducing the differentiation of CD4+ T cells into Treg cells, which produce IL-10 and suppress Th17. This differentiation into Treg is mediated by TLR2 (from CD4+ cells), which recognizes polysaccharide A (from the bacterial outer membrane), activating a signaling cascade. *Prevotella copri* stimulates dendritic cells to express high levels of IL-6 and IL-23, which may increase the number of intestinal Th17 cells. *P. histicola* suppresses serum levels of pro-inflammatory cytokines such as IL-2, IL-17, and TNF-α, by increasing Treg cells in the gut and reducing Th17 cell responses. Created with Biorender.com.

TABLE 1 Autoimmune diseases and alteration of the gut microbiota composition.

Disease	Species	Increase microbiota species	Depletion microbiota species
SLE	Human[1], Mouse[2], Human and Mouse[3]	Genus: *Bacteroides* (Wei et al., 2019)[1], *Rhodococcus*, *Eggerthella*, *Klebsiella*, *Prevotella*, *Eubacterium*, and *Flavonifractor* (He et al., 2016)[1]	Phylum: *Firmicutes* and *Bacteroidetes* (He et al., 2016; HEVIA et al., 2014; Rodríguez-Carrio et al., 2017;[1] van der Meulen et al., 2019)[1]
RA	Human[1], Mouse[2], Human and Mouse[3]	*Prevotella copri* (Scher et al., 2013; Maeda et al., 2016)[3,1]	Genus:*Bacteroides* (Scher et al., 2013; MAEDA et al., 2016)[3,1]
		Lactobacillus salivarius (Zhang et al., 2015)[1]	*Haemophilus* spp. (Zhang et al., 2015)[1] and Genus *Faecalibacterium* (Chen et al., 2016)[3]
		Collinsella aerofaciens and *Eggerthella lenta* (Chen et al., 2016)[3]	
IBD	Human[1], Mouse[2], Human and Mouse[3]	Phylum *Proteobacteria*, Family *Enterobacteriaceae*, *Bilophila* and certain members of phylum *Bacteroidetes* (Zhou et al., 2018)[1]	*Akkermansia muciniphila* (PNG et al., 2010)[1]
			Bifidobacterium spp. (Joossens et al., 2011; Andoh et al., 2012)[1], *Lactobacillus* spp. (OTT et al., 2004)[1], and *F. prausnitzii* (Sokol et al., 2009; Joossens et al., 2011; Andoh et al., 2012)[1]

[1]Human model.
[2]Mouse model.
[3]Human e Mouse model.
SLE, systemic lupus erythematous; RA, rheumatoid arthritis; IDB, inflammatory bowel disease.

an imbalance of intestinal immunity related to Th2 cytokines, while CD is associate to a Th1 and Th17 cytokine profile (Heller et al., 2005). In CD, differentiation into Th1 and Th17 occurs by induction of cytokines IL-12, IL-18, IL-23 and transforming growth factor beta (TGFβ) produced by macrophages and other antigen-presenting cells (APCs; Ramos and Papadakis, 2019). In UC, increased secretion of IL-5, which is Th2 specific, is related to more effective activation of B cells and stimulation of immune responses when compared to the Th1 response observed in CD (Ramos and Papadakis, 2019). Although the exact mechanism of causing IBD remains unknown, it is broadly accepted that the pathogenesis of the disease involves the interaction of genetic susceptibility and environmental factors in the microbiome, which, through an impaired intestinal epithelium, will lead to excessive immune activation, responsible for the clinical observed in

patients (Ma et al., 2019; Ramos and Papadakis, 2019; Sultan et al., 2021a). Thus, genetically susceptible subjects are thought to produce a disordered immune response to their gut microbiota, leading to chronic inflammation and repetitive damage to the intestinal mucosa (Sartor, 2008; Jergens et al., 2021).

IBD is one of the diseases most associated with dysbiosis of the gut microbiota (Xu et al., 2019). Patients with IBD show loss of microbial diversity and stability and an increase in Proteobacteria and certain members of Bacteroidetes (Bautzova et al., 2018; Xu et al., 2019; Barbara et al., 2021). Among the components of Proteobacteria, adherent/adhesive *Escherichia coli* strains have been associated with IBD (Darfeuille-Michaud et al., 2004; Sokol et al., 2006). Adherent invasive *E. coli* was associated with CD, while diffusely adherent *E. coli* was associated with UC (Chloé Mirsepasi-Lauridsen et al., 2019). Adherent invasive *E. coli* is able to adhere to the intestinal mucosa, invade and replicate within IECs, translocate through the intestinal barrier and move to deeper tissues (Darfeuille-Michaud, 2002; Barnich et al., 2007). Furthermore, adherent invasive *E. coli* survives within macrophages, induces TNF-α production, and promotes granulomatous inflammatory response (Barnich et al., 2007; Meconi et al., 2007). Diffusely adherent *E. coli*, on the other hand, is able to adhere to the colonic mucosa and induce inflammatory responses characterized by induction of cytokine secretion, including IL-8, TNF-α and IL-1β and by promoting increased intestinal permeability (Servin, 2005; le Bouguénec and Servin, 2006). These data suggest that *E. coli* strains may play a key role in the pathogenesis of IBDs (Chloé Mirsepasi-Lauridsen et al., 2019).

Unlike *E. coli*, *Akkermansia muciniphila* has been shown usually reduced in the intestine of patients with IBD, resulting in an increase in the overall population of mucosal bacteria (Png et al., 2010; Barbara et al., 2021). IBD patients also have a lower abundance of *Lactobacillus* spp. (Ott et al., 2004), *Bifidobacterium* spp. (Joossens et al., 2011; Andoh et al., 2012), and *F. prausnitzii* (Sokol et al., 2009; Joossens et al., 2011; Andoh et al., 2012) resulting in reduced SCFAs concentrations when compared to healthy individuals (Huda-Faujan et al., 1967; Sultan et al., 2021a). Through its ability to produce butyrate *F. prausnitzii* performs anti-inflammatory activity. Butyrate improves intestinal barrier function and regulates the balance between Treg and Th17 cells (Zhou et al., 2018). Furthermore, Regner et al. reported that intestinal intraepithelial lymphocytes (IELs) and cytokines produced by these cells correlated with the relative abundance of various bacterial taxa. IELs from individuals with UC and CD produce different cytokines when compared to controls. In UC, IELs secrete increased amounts of IL-1β, while in CD there is increased secretion of IL-17A, IFN-γ and TNF-α (Figure 3; Regner et al., 2018). IELs are T cells that are in close contact with gut bacteria and can be influenced by differences in gut microbiota components (Regner et al., 2018; Xu et al., 2019). Together, these data suggest that dysbiosis in IBD patients could lead to the loss or impairment of microbial functions necessary to maintain intestinal epithelial barrier integrity, possibly causing increased inflammatory responses and spread of pathogens to intestinal tissues. However, is still unknown if these changes are a cause or consequence of IBD (Barbara et al., 2021).

4.2. The gut microbiota and rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by a constant immune response that results to chronic inflammation and destruction of cartilage and bones. It is a serious chronic disease that affects about 1% of the world's population, being more common in women than in men (Horta-Baas et al., 2017; Li Y. et al., 2019; Bergot et al., 2020). The mechanisms involved in the etiopathogenesis of the disease are complex and encompass both innate and adaptive immune responses, involving APCs, generation of autoreactive T cells and production of autoantibodies, such as rheumatoid factor (Horta-Baas et al., 2017; Pan et al., 2020). In addition, an association of the intestinal microbiome with the development and progression of RA has been demonstrated (Mangalam et al., 2021). An altered gut microbiota has been associated with loss of tolerance to autoantigens and in the increase of inflammatory episodes that cause damage to the joints (Xu et al., 2019). Furthermore, patients with RA have a reduction in the diversity of the gut microbiota when compared to controls and this is correlated with duration of illness and levels of autoantibodies produced (Chen et al., 2016; Xu et al., 2019).

Some individuals with early rheumatoid arthritis (who have not treated with antirheumatic drugs) have a greater relative abundance of *Prevotella copri* and a decrease in the number of *Bacteroides* in the gut (Scher et al., 2013; Maeda et al., 2016; Schwiertz, 2016; Maeda and Takeda, 2017). A study in China identified that RA patients had an increase in the abundance of *Lactobacillus salivarius* in the gut, teeth, and saliva. In contrast, *Haemophilus* spp. were decreased in these patients at all sites evaluated (Zhang et al., 2015). In another study, patients with RA also had decreased intestinal microbial diversity, which correlated with antibody production and illness duration. RA patients showed an increase in the relative abundance of *Collinsella aerofaciens* and *Eggerthella lenta* and a decrease in *Faecalibacterium* (Chen et al., 2016; Maeda and Takeda, 2019). In *in vitro* experiments, the genus *Collinsella* increased intestinal permeability and induced IL-17A expression, suggesting that the expansion of the microorganisms of this genus increases proinflammatory conditions, thus being an arthritogenic candidate in the human intestine (Figure 3; Nielen et al., 2004; Xu et al., 2012; Chen et al., 2016; Jiao et al., 2020). The reduction in the abundance of *Faecalibacterium* may be associated with a reduction in the production of butyrate, a final metabolite of fiber breakdown that presents an anti-inflammatory property, maintaining the integrity of the intestinal epithelium (Kim et al., 2016; Zhong et al., 2018; Gioia et al., 2020).

Intestinal microbiota involvement appears to vary in different subsets of RA patients (Chiang et al., 2019). However, despite the discrepancies found in different studies, *P. copri*, *L. salivarius*, and *Collinsella* are predominant in recent early RA and may be associated with its pathogenesis. Differences in patient characteristics, such as genetic background, environmental exposures and different treatment regimens may explain the variety of candidate arthritogenic bacteria (Chiang et al., 2019; Maeda and Takeda, 2019).

4.3. The gut microbiota and systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple structures in the body (Luo et al., 2018; Muhammad Yusoff et al., 2020). It is characterized by persistent inflammation in organs and presents several clinical manifestations, including skin rash, neurological disorders, glomerulonephritis, and severe vasculitis (Xu et al., 2019; Gu et al., 2020). SLE is more frequent

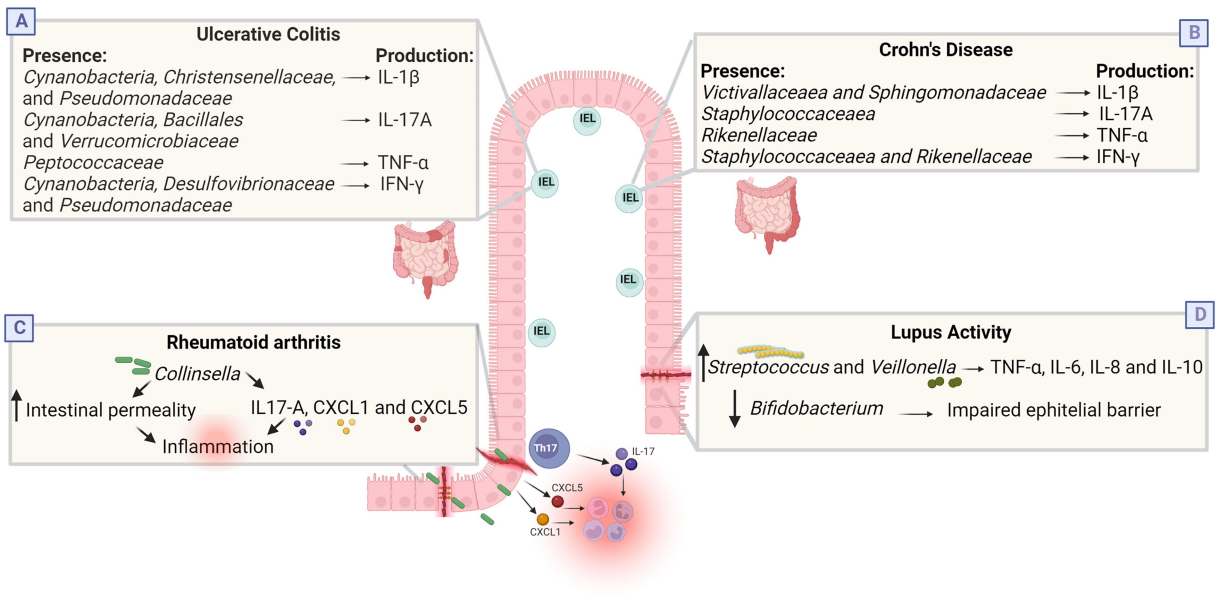

FIGURE 3
Dysbiosis in inflammatory and infectious diseases. **(A,B)** Cytokines produced by intestinal intraepithelial lymphocytes (IEL) correlate with the relative abundance of some bacterial taxa in IBD. In the UC group, there was positive correlation between the abundances of *Cynanobacteria*, *Christensenellaceae*, and *Pseudomonadaceae* and IL-1β; between *Cynanobacteria*, *Bacillales*, and *Verrucomicrobiaceae* and IL-17A; between *Peptococcaceae* and TNF-α; and between *Cynanobacteria*, *Desulfovibrionaceae*, and *Pseudomonadaceae* and IFN-γ. In the CD group, there was positive correlation between the abundances of *Victivallaceaea* and *Sphingomonadaceae* and IL-1β; between *Staphylococcaceaea* and IL-17A; between *Rikenellaceae* and TNF-α; and between *Staphylococcaceaea* and *Rikenellaceae* and IFN-γ. **(C)** Culture with *Collinsella* reduces the expression of junction proteins, increasing intestinal permeability, and influences the secretion of IL-17A, CXCL1 and CXL5, which can trigger neutrophil recruitment and NFkb activation, possibly increasing pro-inflammatory conditions in RA. **(D)** Lupus activity was positively associated with the genera *Streptococcus*, *Campylobacter* and *Veillonella*, and negatively correlated with *Bifidobacterium*. *Streptococcus* combined with *Veillonella* enhance the TNF-α, IL-8, IL-6, and IL-10 response while *Bifidobacterium* is associated with improved gastrointestinal barrier function and suppression of pro-inflammatory cytokines. Together, these changes possibly induce an inflammatory state. Created with Biorender.com.

in women, being triggered by the interaction between different factors, such as genetic predisposition, hormonal changes, environmental factors, and epigenetics. Despite this, the exact etiology and pathogenesis of the disease remain unknown (Luo et al., 2018; Guo et al., 2020).

Several immunological alterations have been reported in human and animal models of SLE, including autoreactive B and T cells, abnormal levels of pro-inflammatory cytokines and impaired immune complex clearance. This loss of self-tolerance plays a fundamental role in the occurrence and development of the disease (Luo et al., 2018; Muhammad Yusoff et al., 2020). Ineffective elimination and/or excessive formation of neutrophil extracellular traps (NETs), characterized by fibrous networks made up of nuclear and granular components that protrude from the membrane of activated neutrophils, is involved in the pathogenesis of SLE (Berthelot et al., 2017; Kaufman et al., 2017; Pan et al., 2020). In addition to this mechanism, Th1, Th2, and Th17 cell dysfunction have been related to the occurrence and development of the disease. Under normal circumstances, Th1 and Th2 cells maintain an immune balance. However, the imbalance between these cells contributes to the pathogenesis of the disease. As for Th17 cells, IL-17 produced by these cells, associated with B-cell growth factor, positively regulates the differentiation and survival of B cells, stimulating humoral immunity to produce antibodies. Thus, SLE is characterized by intense production of autoantibodies, deposition of antigen–antibody complex and activation of the complement system in tissues, leading to the accumulation of self-reactive monocytes, neutrophils, and lymphocytes (Tsokos et al., 2016; Muhammad Yusoff et al., 2020; Pan et al., 2020).

The failure in immunological tolerance characteristic of SLE can be also promoted by dysbiosis or aberrant intestinal immunity (Jiao et al., 2020). Despite differences in dysbiosis patterns in the disease, studies have reported a reduction in the Firmicutes/Bacteroidetes ratio compared to healthy controls (Hevia et al., 2014; Rodríguez-Carrio et al., 2017; van der Meulen et al., 2019). As an example, in a Chinese population, in fecal samples from patients with SLE, a decrease in bacterial richness, a reduction in the Firmicutes/Bacteroidetes ratio and an increase in the relative abundance of *Bacteroides* was identified (Wei et al., 2019). In addition, an abundance of other genera has been demonstrated in individuals with SLE: *Rhodococcus*, *Eggerthella*, *Klebsiella*, *Prevotella*, *Eubacterium*, and *Flavonifractor* (He et al., 2016; Xu et al., 2019).

Dysbiosis may also be associated with the activity or remission phase of SLE, since affected individuals seem to exhibit characteristic patterns of dysbiosis in the intestinal microbiota in parallel with disease activity. Lupus activity was positively associated with the genera *Streptococcus*, *Campylobacter* and *Veillonella* and the species *S. anginosus* and *V. dispar*, while the genus *Bifidobacterium* was negatively correlated with disease activity (Li Y. et al., 2019). *Streptococcus* and *Veillonella* genera appear to have pro-inflammatory effects. *Streptococcus* combined with *Veillonella* obtained from the human intestine inhibited the production of IL12p70 and increased

the response of TNF-α, IL-8, IL-6, and IL-10 (Figure 3; Van Den Bogert et al., 2016). Furthermore, through molecular mimicry, some *Streptococcus* species induce the activation of B cells and specific CD4+ T cells through antigen presentation (Blank et al., 2007). Therefore, these genera can interfere with the mucosal immune system and induce cross-reaction with host tissue, potentially being involved in enhancing the host's immune response in SLE (Wang et al., 2022).

4.4. The gut microbiota and inflammatory skin diseases

There is increasing evidence that gut health exerts profound effects upon non-gastrointestinal diseases, including those of the skin (Searle et al., 2020). Intestine and skin are immunological barriers and constitute the environment for physiological microbiota (Polkowska-Pruszyńska et al., 2020). The concept of **gut–skin axis** has been implicated in the pathogenesis of many chronic inflammatory diseases. It suggests that the gastrointestinal system directly affects the skin homeostasis and allostasis through interactions between the immune, metabolic, and nervous systems (Wang and Chi, 2021). Gut dysbiosis has been implicated in many dermatologic conditions.

Intestinal microbiota dysbiosis has been shown in **psoriatic patients** and it correlates to the severity and status of the disease (Huang et al., 2019; Buhaș et al., 2022). Moreover, psoriatic patients showed less diversity in gut microbiota when compared to controls (Schade et al., 2022). It was hypothesized that the differential plenty of bacteria may be the reason for the gut dysbiosis in psoriasis instead of the number of bacterial species (Thye et al., 2022). A link between gut dysbiosis and butanoate metabolism and butyrate production has also been proposed, since it has been implicated in the regulation of various inflammatory factors, including TNF-α, IL-10, and Il-1β (Buhaș et al., 2022). It has been hypothesized that the presence of *Escherichia coli* could be related to psoriasis, since it was increased in intestinal flora of psoriatic patients. *E. coli* is known to be responsible for the production of TNF-α and other proinflammatory cytokines and also have been related to the etiology of IBD (discussed above), which is known to be related to psoriasis (Wen et al., 2023). Although the immunological and inflammatory responses in psoriatic patients seem to be affected by intestinal dysbiosis, the composition of the microbiota profile still needs more investigation since the results are heterogeneous (Buhaș et al., 2022).

The relationship between **atopic dermatitis** and gut microbiota was also studied. Various observational studies showed different results regarding the diversity and the composition of the gut microbiota in atopic dermatitis patients (Widhiati et al., 2021). Lower intestinal bacterial diversity has been associated with an increased risk of atopic disease (Polkowska-Pruszyńska et al., 2020). This dysbiosis results in a reduction of short-chain fatty acids production, like acetate, propionate, and butyrate. They are known to be potent anti-inflammatory in many diseases, including atopic dermatitis, through inhibition of Th2 and activation of regulatory T cells (Alam et al., 2022). These changes can cause a disruption in the integrity of the gut epithelial barrier, leading to an increased intestinal permeability and favoring toxins and gut microorganisms to penetrate the body circulation and contribute to skin inflammation. When these reach the skin, a strong Th2 reaction may be induced, causing further tissue damage (Moniaga et al., 2022). The use of probiotics was also studied, and some results point to an improvement on the severity of the atopic dermatitis (Petersen et al., 2019). Its role is based on their ability to balance the intestinal microbiota, protecting the gut barrier function, and decreasing the production of the pro-inflammatory cytokines IL-4, IL-5, IL-13, and TNF-α, which are closely related to atopic dermatites (Fang et al., 2021).

Microbial diversity is significantly decreased in **acne** patients when compared to controls(Deng et al., 2018). A decrease in *Lactobacillus*, *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, and *Allobaculum* was found in patients with acne (Yan et al., 2018). *Lactobacillus* and *Bifidobacterium* are probiotic genera that balance the intestinal microbiota and also strengthen the intestinal barrier (Lee et al., 2019). Furthermore, the influence of dietary habits in acne supports the existence of gut-skin axis (Polkowska-Pruszyńska et al., 2020). Similar results were seen in **rosacea** patients, which present with similar quantity of bacteria, but a reduced richness on the composition (Chen et al., 2021). *Acidaminococcus*, *Megasphaera* e *Lactobacillales* were genus more prevalent in rosacea patients, while *Peptococcaceae*, *Methanobrevibacter*, *Slackia*, *Coprobacillus*, *Citrobacter* e *Desulfovibrio* were reduced when compared to controls (Nam et al., 2018). Another study found increased abundance of *Rhabdochlamydia*, *Bifidobacterium*, *Sarcina*, *CF231*, *Ruminococcus* in rosacea patients and reduced quantity of *Lactobacillus*, *Roseburia*, *Megasphaerae*, *Acidaminococcus*, *Hemophilus*, *Citrobacter* and *Clostridium* (Chen et al., 2021).

In patients with **hidradenitis suppurativa** (HS), a reduction diversity was observed when compared to controls (McCarthy et al., 2022). One of the greatest differences were high degrees of *Ruminococcus gnavus* and *Clostridium ramosum*, which have already been related to Crohn's disease (McCarthy et al., 2022). Different compositions in intestinal microbiota between HS patients and controls have also been demonstrated, with lower abundance of Firmicutes phyla (Kam et al., 2021). However, that was a pilot study and further investigation is still needed to corroborate these results. On the other hand, no differences in diversity were observed in another recent study, although there were some bacterial features differences (Lam et al., 2021). One interesting finding was the presence of *Robinsoniella* in 59% of HS patients and in none of the healthy controls (Lam et al., 2021). The gut dysbiosis described in these studies leads to an increased production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 by the intestinal epithelia which is followed by an increase in circulating inflammatory cytokines namely IFN-γ, and TNF-α. These cytokines end in an inflammatory process in the skin that involves MMP expression and are directly related to HS lesion formation (Molnar et al., 2020). It is also known that as HS, other diseases like psoriasis and IBD run with increased IL-17, and also present with gut microbiota alterations (Matusiak et al., 2017).

In **alopecia areata** (AA), two studies failed to demonstrate differences in diversity between patients and controls (Moreno-Arrones et al., 2020; Lu et al., 2021). However, at the genus level, abundance of *Blautia*, *Pseudomonas*, *Collinsella*, *Megasphaera*, and *Dorea* was found in AA patients (Lu et al., 2021). Other study found an elevated presence of *Holdemania filiformis*, Lachnospiraceae, Erysipelotrichaceae, *Parabacteroides johnsonii*, *Bacteroides eggerthii*, *Clostridiales vadin* BB60 group, *Eggerthellaceae* and *Parabacteroides distasonis*, while in controls, *Phascolarctobacterium succinatutens*, Clostridiales family XIII, *Dorea longicatena*, *Phocea massiliensis*, *Turicibacter sanguinis, Streptococcus thermophilus* and *Flavonifractor*

plautii in patients with AA were notably more abundant (Moreno-Arrones et al., 2020). Moreover, improvement of AA symptoms was reported after fecal microbiota transplantation, reinforcing the association between intestinal microbiome composition and AA pathogenesis (Rebello et al., 2017; Xie et al., 2019). It is hypothesized that the gut microbiota could also interfere with **wound healing** by interfering with healing factors like tissue oxygenation levels, blood pressure, inflammation, and the immune system (Patel et al., 2022). Nevertheless, little is known about the intestinal microbiota composition in patients with chronic ulcers.

The impact of the gut microbiota is being studied in several other skin conditions, like **vitiligo**, **lichen sclerosus**, **seborrheic dermatitis,** and **skin cancer**, including the response to immunotherapy, like in **cutaneous melanoma** (Bzioueche et al., 2021; Chattopadhyay et al., 2021; Spencer et al., 2021). Although the relationship between the gut microbiota and these diseases is already established in most of them, there is still a gap to be filled in order to better understand its impact and mechanisms. The knowledge of such influence could shed some light on potential therapeutic allies, like probiotics, diet, and even fecal microbiota transplantation.

5. The gut microbiota and viral infections

As with each infection, each pathogen can induce a different immune response, as the process of activating these responses takes place. In a viral infection, the main cells involved in the process of fighting the virus are cytotoxic cells, which may be linked either to innate immunity (NK) or to adaptive immunity (CD8+), always with the fundamental antiviral action of the cytokine IFN-γ (Mazzoni et al., 2020).

It is well known that a healthy commensal microbiota is critical to protecting the host against a several of infections, either by direct elimination or by indirect suppression, inside or outside the intestine (Rothschild et al., 2018). The mucosal epithelium is the main entry route for many pathogens, which can cause an important dysbiosis by affecting the intestinal mucosal barrier (Rigo-Adrover et al., 2018). During viral infection in mucosal tissue, viruses may encounter the host's commensal microbiota. Depending on the profile of this microbiota, it is possible that it is beneficial to the host, defending it from infections, as well as it is possible that it creates an environment conducive to viral infection (Table 2; Pfeiffer and Virgin, 2016; Schuijt et al., 2016).

The commensal microbiota may help to promote viral infection by, for instance, facilitating viral gene recombination, thus allowing an increase in viral infectious capacity (Combe et al., 2015). The microbiota also may influence viral infection through other indirect mechanisms, such as stimulating the creation of immunoregulated environments through the production of IL-10 by Treg cells and the inhibition of cytokines such as IFN-γ and TNF-α, which disrupts the immune system's ability to act properly to fight the viral infection (Basic et al., 2014; Robinson et al., 2014; Zhao and Elson, 2018).

On the other hand, intestinal microbiota is fundamental for the maturation of the immunological system and can also cooperate with it to prevent and fight infections. For instance, commensal populations can induce the immune system to produce antiviral products, such as IFN (Yitbarek et al., 2018). Among the different viral infections

TABLE 2 Viral diseases and alteration of the gut microbiota composition.

Disease	Species	Increase microbiota species	Depletion microbiota species
HBV	Human[1], Mouse[2]	Genus: *Enterococcus*, Family: *Enterobacteriaceae* (Lu et al., 2011)[1], genus *Faecalibacterium* and *Gemella* (Wang et al., 2017) [1]	Genus: *Bacteroides* (Sender et al., 2016; Wang et al., 2017)[1]
			Species: *Bifidobacteria*, and *Lactobacilli* (Cosseau et al., 2008; Lu et al., 2011; Xu et al., 2012; Kakiyama et al., 2013; Aly et al., 2016; Wang et al., 2017; Inoue et al., 2018; Sultan et al., 2021b)[1]
HCV	Human[1], Mouse[2]	Genus: *Prevotella*, *Succinivibrio*, *Catenibacterium*, *Megasphaera*; and family *Ruminococcacea* (Sultan et al., 2021b)[1]	Genus: *Bacterioides*, *Dialister*, *Bilophila*, *Streptococcus*, *Parabacterioides*; and families of *Enterobacteriaceae*, *Erysipelotrichaceae* and *Rikenellaceae* (Sultan et al., 2021b)[1]
		Family *Enterobacteriaceae*, Genus *Bacterioides* (Cosseau et al., 2008; Ponziani et al., 2018)[1]	Phylum *Firmicutes*, Family *Ruminococcaceae* and *Lachnospiraceae* (Cosseau et al., 2008; Lu et al., 2011; Xu et al., 2012; Kakiyama et al., 2013; Aly et al., 2016; Wang et al., 2017; Inoue et al., 2018; Sultan et al., 2021b)[1]
		Phylum *Proteobactérias*; Genus *Veillonella*, *Prevotella*, *Faecalibacterium*, *Acinetobacter*; *Streptococcus viridans*, *Streptococcus salivarius*; Families *Staphylococcaceae*, *Enterococcaceae*, *Veillonellaceae*, *Phascolarctobacterium* (Inoue et al., 2018; Ponziani et al., 2018)[1]	
		Family *Streptococcaceae* and *Lactobacillaceae* (Kakiyama et al., 2013; Tuomisto et al., 2014; Chen et al., 2016; Inoue et al., 2018)[1]	
COVID-19	Human[1], Mouse[2]	Genus: *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces* (Gu et al., 2020)[1]	Genus: *Agathobacter*, *Fusicatenibacter*, *Roseburia*, family *Ruminococcaceae* (Gu et al., 2020)[1]
			Species: *Faecalibacterium prausnitzii* and *Eubacterium* rectale (Yeoh et al., 2021)[1]

[1]Human model.
[2]Mouse model.
HBV, hepatitis type B virus; HCV, hepatitis C virus.

positively and negatively affected by the intestinal microbiota, and which are capable of also altering it, viral hepatitis (mainly HBV and HCV) and SARS-CoV-2 have been highlighted in the literature and will be discussed in further detail.

5.1. The gut microbiota and viral hepatitis

Viral hepatitis occurs due to infections of hepatitis A B, C, D, and E viruses, which are considered a public health issue, mainly in low and middle-income countries. Hepatitis type B virus (HBV) and Hepatitis C virus (HCV) are considered the most important etiological agents of hepatitis, whose infection can result in serious liver problems, including liver cirrhosis (LC), hepatocellular carcinoma (HCC) and liver failure. These clinical conditions can usually progress slowly and silently through various clinical stages as long these liver viruses have ways of preventing their detection by the host's immune system, a characteristic called viral escape (Visvanathan et al., 2007; Lemon et al., 2018; Yang et al., 2018). Hepatitis A and E viruses, on the other hand, cause acute infection that can resolve independently of any intervention, unless the infected individuals are in an immunocompromised condition (Lemon et al., 2018).

Dysbiosis of the intestinal microbiota can be exploited by viral hepatitis as an escape mechanism of the immune system (Inoue et al., 2018; Li et al., 2018). Sender et al. (2016) and Wang et al. (2017) showed that the level of *Bacteroides* was lower in patients with hepatitis B compared to healthy people. Lu et al. (2011) suggested that cirrhosis could impact the dysbiosis process, leading to a worsening of the patient's clinical condition. The intestinal microbiota also can be greatly affected during the different stages of HCV infection. During the asymptomatic phase, an increase in bacteria of the genera *Prevotella*, *Succinivibrio*, *Catenibacterium*, *Megasphaera* and from the *Ruminococcacea* family has been observed, as well as a reduction in bacteria from the genera *Bacteroides, Dialister, Bilophila, Streptococcus, Parabacterioides,* in addition to the following bacterial families: Enterobacteriaceae, Erysipelotrichaceae and Rikenellaceae (Sultan et al., 2021b). Studies have indicated evidence of dysbiosis since the onset of HCV infection, such as increased concentration of bacteria from the Enterobacteriaceae family and bacteria from the genus *Bacteroides* (Cosseau et al., 2008; Ponziani et al., 2018). Different studies have suggested HCV infection-related dysbiosis, can be intensified by the increased presence of bacteria from the phylum Proteobacteria Firmicutes and Bacteroidetes (Inoue et al., 2018; Ponziani et al., 2018). However, in dysbiosis related to chronic HBV infection, changes occur in the concentration of bacteria of the *Enterococcus* genus and the Enterobacteriaceae family, which may be increased (Lu et al., 2011; Wang et al., 2020).

In chronic HCV infection, bacteria from the Firmicutes phylum and Ruminococcaceae and Lachnospiracea families may occur, while in chronic HBV infection, Bifidobacteria, from the genus *Bifidobacterium,* and the intestinal *Lactobacilli*, from the genus *Lactobacillus*, seem to be less present due to dysbiosis process in chronic infection. On the other hand, in the development of cirrhosis (due to HCV infection), there may be an increase in the proliferation of bacteria from the genera *Enterobacteriaceae*, *Staphylococcaceae*, *Veillonellaceae* and *Bacteroides*, in addition to the phylum Proteobacteria (Cosseau et al., 2008; Xu et al., 2012; Kakiyama et al., 2013; Aly et al., 2016; Wang et al., 2017; Inoue et al., 2018; Sultan et al., 2021b).

According to Wang et al. (2017), the dysbiosis observed in chronic HBV infection is similar to that found in cirrhosis, with an increase in bacteria from the *Enterococcus*, *Faecalibacterium* and *Gemella* genera, and from the Enterobacteriaceae family, in addition to a decrease in *Bifidobacteria* and *Lactobacilli* in the intestinal microbiota. However, in the evolution to Hepatocellular Carcinoma, the dysbiosis caused by HCV seems to be distinct from the dysbiosis found in cirrhotic patients and chronic patients, as only the species *Streptococcus salivarius* and the families Streptococcaceae, Lactobacillaceae and Enterobacteriaceae seem to be elevated, while only Ruminococcaceae and Lachnospiraceae would have a reduction (Kakiyama et al., 2013; Tuomisto et al., 2014; Chen et al., 2016; Sanduzzi Zamparelli et al., 2017; Inoue et al., 2018).

The dysbiosis process may be accompanied by liver inflammation, allowing the evolution to cirrhosis and hepatocellular carcinoma due to action of pro-inflammatory cytokines with a Th1/Th17 profile (Rigo-Adrover et al., 2018). Dysbiosis in patients with cirrhosis and hepatocellular carcinoma can strongly affect the permeability of the mucosal tissue, allowing the induction of the innate immune system of the liver. Thus, it is possible that the liver damage found in these patients is not only due to the antigen-specific cellular immune response in response to viral action, but also due to pathogen-associated molecular patterns (PAMPs), which also can trigger an innate immune response and, eventually, tissue damage. For example, patients chronically infected with HBV may have a reduction in the presence of *Lactobacilli* and *Bifidobacteria* in their intestinal microbiota. Both bacterial families are rich in unmethylated CpG DNA, which directly triggers the CpG DNA-TLR9 pathway and the immune response to the liver virus. Unmethylated CpG DNA are recognized as PAMPs by TLR9, which is expressed in several mononuclear cells, stimulating the innate and adaptive immune response (Wu et al., 2011; Xu et al., 2012; Ries et al., 2013).

5.2. The gut microbiota and SARS-CoV-2 infection

Infection by SARS-COV-2, which causes the pathology called Covid-19, is still under intensive investigation due to its unique characteristics. In addition to COVID-19 being a respiratory viral infection, different clinical pictures, and a major feature of the infection's aggravation is the cytokine storm and the development of an intense inflammatory response (Vabret et al., 2020).

The pathophysiology of this infection is directly related to this intense inflammatory response. Thus, the severity of the disease is often not only related to the viral infection, but also to the exacerbated immune response of the host. Patients with severe COVID-19 exhibit elevated levels of inflammatory markers such as IL-6, IL-8, C-Reactive Protein (CRP) and lactate dehydrogenase (LDH). SARS-CoV-2 utilize the angiotensin-converting enzyme receptor 2 (ACE2) to penetrate the host's target cell. ACE2 is highly expressed not only in the respiratory tract but also in various other tissues, including the gastrointestinal tract. This important aspect of infection is further evidenced by the fact that ACE2 is important in controlling inflammation and the intestinal microbiota (Lamers et al., 2020; Vabret et al., 2020; Zuo et al., 2020). After virus entry, various

inflammatory signaling pathways are activated within cells and inflammatory products are released. Among these products, type I Interferons (IFN-I) are essential in the first line of defense, creating an antiviral environment that makes it difficult for the perpetuation of the virus. However, SARS-COV-2 has the ability to evade the immune system by inhibiting the production of IFN-I (Lamers et al., 2020; Tay et al., 2020; Vabret et al., 2020; Zuo et al., 2020; Yeoh et al., 2021).

SARS-Cov-2 has already been detected in fecal samples and there is evidence that this virus replicates in enterocytes, which could promote alterations in the intestinal microbiota in patients who developed COVID-19 (Lamers et al., 2020). Zuo et al. (2020) identified persistent changes in the fecal microbiome of patients with COVID-19 during their hospital stay, compared to controls. These changes in the fecal microbiota were associated with fecal levels of virus and gravity of COVID-19. Furthermore, bacterial species of Bacteroidetes appeared to be negatively correlated with the severity of COVID-19. Species of the genus *Bacteroides,* such as *B. dorei*, were inversely correlated to the fecal viral load of SARS-COV-2, and it is possible that *B. dorei* induces suppression of ACE2 expression (Zuo et al., 2020). On the other hand, Agathobacter, Fusicatenibacter, Roseburia, and Ruminococcaceae were less present in COVID-19 patients, being negatively correlated with CRP, procalcitonin and D-dimer levels. A reduction in the presence of bacterial with immunomodulatory activity, such as *Eubacterium rectale*, *Faecalibacterium prausnitzii*, and *Bifidobacterium* was observed. Conversely, CRP and D-dimer levels were positively correlated with the increased expression of *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces* bacteria (Gu et al., 2020). In summary, the composition of the intestinal microbiota in patients with COVID-19 has been correlated to the severity of the disease. Dysbiosis may remain present in the patient's intestinal microbiota even after recovery from SARS-COV-2. The alteration of these bacterial groups was also associated with the elevation of the cytokines TNF-α, CXCL10 (C-X-C Motif Chemokine Ligand 10), CCL2 (C-C motif chemokine ligand 2) and IL-10. So, it is possible that this dysbiosis is related to the more severe version of the pathology of COVID-19, where there is an intense production of proinflammatory cytokines (Yeoh et al., 2021).

6. The gut microbiota and mycobacteria infections

6.1. Tuberculosis

Tuberculosis (TB) is an infectious disease caused by the alcohol-acid-resistant bacillus *Mycobacterium tuberculosis* and is considered one of the main neglected diseases in the world (Eribo et al., 2020). It is a highly transmissible disease spread by aerosol droplets containing bacilli, usually during sneezing or coughing (Eribo et al., 2020; Global Tuberculosis Report, 2020). It is believed that in most individuals the infection results in clinically asymptomatic latent tuberculosis infection (Eribo et al., 2020; Mori et al., 2021). The bacillus predominantly infects the lungs, causing pulmonary tuberculosis. However, it can also invade extrapulmonary organs such as lymph nodes, bones, and meninges (Ko et al., 2000; Hu et al., 2019). Although 90%–95% of individuals infected with *M. tuberculosis* remain protected throughout their lifetime, 5%–10% of people develop active tuberculosis (Nadeem et al., 2020). Immune, host genetic and environmental predisposing factors, such as HIV infection and diabetes, have been associated with the disease (Eribo et al., 2020; Mori et al., 2021). During active tuberculosis, symptoms include cough, fever, weight loss and hemoptysis (Lyon and Rossman, 2016).

The gut microbiota has been reported as a host factor that may be associated with tuberculosis (Hudrisier et al., 2018; Khan et al., 2019). Studies have shown remarkable differences between the gut microbiota of TB patients and healthy controls (Luo et al., 2017; Hu et al., 2018). Hu et al. (2018) reported a decrease in microbiome diversity, mainly associated with changes in the relative abundance of Bacteroides in the gut microbiota of Chinese patients with TB. In another study, an important decrease in the number and diversity of the microbiota was observed, with a remarkable reduction in SFCA-producing bacteria such as *Roseburia inulinivorans*, *Bifidobacterium adolescentis*, and *Akkermansia muciniphila* (Hu et al., 2019).

Luo et al. (2017) divided patients analyzed by them according to time of diagnosis and treatment time into new tuberculosis patients and recurrent tuberculosis patients. New tuberculosis patients showed an increase in Actinobacteria and Proteobacteria, while recurrent patients showed a reduction in Bacteroidetes, containing several beneficial commensal bacteria in fecal samples. The phylum Proteobacteria contains several gram-negative bacteria and opportunistic pathogenic species (Luo et al., 2017). The lipopolysaccharide (LPS) component of the cell wall of these bacteria can trigger the activation of pro-inflammatory macrophages (M1) and other innate immune cells (Sommer and Bäckhed, 2013; Mori et al., 2021). M1 macrophages are characterized by high antigen presentation and expression of IL-12, IL-23 and TNF-α (X). Therefore, this group of bacteria can induce an inflammatory response locally and at distant sites if the epithelial barrier is disturbed (Sommer and Bäckhed, 2013; Mori et al., 2021). Since any damage to the intestinal barrier can cause microbial translocation into the blood and produce a sustained inflammatory response, it might also impact lung disease (Ma P. J. et al., 2022). In the same research, *Prevotella* and *Lachnospira* were considerably reduced in new and recurrent tuberculosis patients compared to healthy subjects (Table 3; Luo et al., 2017; Li W. et al., 2019; Liu et al., 2021). Furthermore, *Prevotella* was positively correlated with the number of peripheral CD4+ cells in NTB and negatively correlated with RTB (Luo et al., 2017). Taken together, these data suggest that specific intestinal microorganisms may modulate the host immune system and be related to patient prognosis and outcome, especially in cases of impaired intestinal barrier (Luo et al., 2017; Li W. et al., 2019).

6.2. Leprosy

Leprosy is a chronic granulomatous mycobacteriosis with high infectivity and low pathogenicity, and, like tuberculosis, is considered one of the main neglected diseases. The disease is caused by *Mycobacterium leprae* and occurs in a variety of clinical forms that depend on the immune status of the host (Costa et al., 2018; Pinheiro et al., 2018). The disease especially affects the skin and peripheral nerves, but it can also affect the eyes, upper respiratory tract mucosa, bones, and testicles (Desikan and Iyer, 1972; Pinheiro et al., 2018). Classically, it is characterized with a Th1/Th2 paradigm, presenting a cytokine profile

TABLE 3 Tuberculosis and alteration of the gut microbiota composition.

Disease	Species	Increase microbiota species	Depletion microbiota species
TB	Human[1], Mouse[2]		Genus *Bacteroides* (Hu et al., 2019)[1]
			Species: *Roseburia inulinivorans*, *Bifidobacterium adolescentis* and *Akkermansia muciniphila* (Hu et al., 2019)[1]
NTB	Human[1], Mouse[2]	Phylum: *Actinobacteria* and *Proteobacteria* (Luo et al., 2017; Li W. et al., 2019)[1]	Genus *Prevotella* and family *Lachnospira* (Luo et al., 2017; Li W. et al., 2019)[1]
RTB	Human[1], Mouse[2]		Phylum *Bacteroidetes*, genus *Prevotella* and family *Lachnospira* (Luo et al., 2017; Li W. et al., 2019)[1]

TB, tuberculosis; NTB, new tuberculosis patients; RTB, recurrent tuberculosis patients.

that varies according to the type of Th response. However, studies have also shown differences across the disease spectrum for Th9, Th17, Th25 and Treg lymphocytes (de Sousa et al., 2017; Froes et al., 2022).

Leprosy has been associated with dysbiosis of the skin microbiota. Atypical human skin taxa were identified in leprosy lesions, with the genera *Burkholderia*, *Pseudomonas* and *Bacillus* being overrepresented (Silva et al., 2018), while the *Staphylococcus* genus, which is inhabitant and abundant in healthy people skin, was underrepresented in these lesions when compared to healthy controls (Silva et al., 2015; Bayal et al., 2019). A study evaluated the constitution of the skin microbiome in lepromatous skin lesions (and matched adjacent uninjured areas) sampled from a cohort of Brazilian patients. The researchers found in both samples from infected leprosy patients (injured and uninjured tissue) less diversity compared to the skin of healthy individuals (Silva et al., 2018). This lower diversity could be imputed to the impact of the microorganism itself or to a systemic change resulting from the ongoing treatment regimen (Bayal et al., 2019).

Two main types of reactions can occur in leprosy patients, the reverse reaction and erythema nodosum leprosum (ENL). The reverse reaction is an acute inflammatory episode in the skin and nerves characterized by an accentuated of the cellular immune response against *M. leprae*. ENL is a systemic inflammatory process characterized by an increase in the levels of pro-inflammatory cytokines, such as TNF-alpha, IL-6, and IL-1B, derived from Th17 lymphocytes (Costa et al., 2018; Froes et al., 2022). Taking into account that the gut microbiota influences the homeostasis of various populations of T cells in the gut, including Tregs, Th1 and Th17 (Gaboriau-Routhiau et al., 2009), directing the pattern of local and systemic immune response (Schirmer et al., 2016; Thursby and Juge, 2017), it is plausible to assume that the host's gut microbiota may be associated to the variety of clinical responses present in leprosy and to the inflammatory state in leprosy reactions. However, so far there are no studies evaluating the role of the intestinal microbiota in leprosy and in the development of leprosy reactions.

7. Discussion and conclusion

The microorganisms present in the human intestine play a key role in the process of development, maturation, and maintenance of the action of the body's defense cells. They play a key role in host homeostasis under basal conditions. In addition to the competition with different pathogens for different niches, they induce protective responses through the modulation of inflammatory responses with the production of cytokines. Allied to the stimulation of protection against pathogens, the tolerance mechanisms promoted by a healthy intestinal microbiota, which involve the reduction of physiological impairment originated by interplay with microorganisms, constitute a strategy of equal significance for the conservation of the host's health that enable the co-evolution of microorganism-host interactions. Despite this, small disparity in this interplay can have negative health implications and dysbiosis can lead to increased susceptibility to infections, as well as an imbalance in the host defense system, resulting in multifactorial inflammatory diseases.

Evidence of the ways in which microbiota influences host physiology suggests that interplay between microbiota and cytokine pathways may be critical to sustaining host inflammation both in the gut and at extraintestinal sites. As an example, IL-17 can modulate, and be modulated by the composition of the microbiota (Valeri and Raffatellu, 2016; Douzandeh-Mobarrez and Kariminik, 2019). Mice with microbiota depleted by antibiotic have reduced levels of IL-17 in the lamina propria of the small intestine (Hill et al., 2010) and mice with RA treated with antibiotics show reduced Th17 cells and IL-17 levels in the gut, reducing the consequences of arthritis (Rogier et al., 2017). These data exhibited the relevance of the intestinal microbiota in modulating the production of IL-17 and associated the overexpression of this cytokine to the development of RA (Eyerich et al., 2017).

In addition, in IBD, RA, COVID-19 and tuberculosis there is a reduction in the abundance of butyrate-producing bacteria (Sokol et al., 2009; Chen et al., 2016; Hu et al., 2019; Yeoh et al., 2021). Especially *Faecalibacterium* species, which are diminished in three of these pathologies (Sokol et al., 2009; Chen et al., 2016; Yeoh et al., 2021). As mentioned earlier, butyrate is an SCFA that plays a fundamental role in conserving the integrity of the intestinal mucosa and in the balance between Treg and Th17 cells (Kim et al., 2016; Zhou et al., 2018). Thus, the change in the abundance of this bacterium could be related to a greater exposure of the host's immune cells to intestinal bacteria, generating excessive activation of the immune system and an imbalance between T cells. Therefore, resulting in the intense inflammatory response involved in these diseases, whether autoimmune or infectious (IBD, AR and COVID; Sokol et al., 2009; Chen et al., 2016; Yeoh et al., 2021). Taken together, these findings exemplify the importance and influence that the gut microbiota can exert on the host's immune response.

The microbiota-host cytokine relationship is a dynamic and complex process, where several factors can have a major effect on inflammation. The cytokine interaction patterns of the microbiota

are stimulus-specific, cytokine-specific and cytokine and stimulus-specific, and are therefore disease-specific. In addition to the influence of the gut microbiome, environmental and host factors (genetic and non-genetic) also have an impact on cytokine modulation. Based on the data reviewed here, we can suggest that the gut microbiota has an important relevance in the outcome of infectious and inflammatory diseases by directing Th cell responses and by producing proinflammatory cytokines. Inflammatory diseases in which the intestinal microbiota has not been investigated, such as leprosy and its reactions, which are characterized by an intense increase in pro-inflammatory cytokines, may be at least in part related to alterations in the gut microbiota. Mainly because patients undergoing treatment for leprosy, for example, receive antibiotic treatment for up to 12 months (Costa et al., 2018). Therefore, it could lead to significant changes in the intestinal microbial community, with possible consequences for the modulation of immune responses and the development of inflammatory reactions. However, further studies are needed to clarify whether dysbiosis is the cause or consequence of the pathologies studied here and other diseases with an inflammatory background. Seeking to understand which changes in the intestinal microbiota or metabolites influence the variability of human cytokine responses in immunological diseases.

8. Future perspectives

As reviewed in this article, the gut microbiota has been associated with many diseases by inducing immune responses in different inflammatory conditions. Here, we provide a comprehensive overview of some of the possible cytokine modulation mechanisms by the microbiota already reported. However, it is fundamental taking into account that most studies use sequencing and analysis of 16S rRNA to infer to role of the microbiota in the health-disease interface. In order to obtain an in-depth view of the role of intestinal bacteria in the host's immune system, it is essential that a greater number of studies assess, in addition to the composition, the metabolic patterns of the intestinal microbiota through metabolomic analyses. As highlighted here, microbial metabolites can directly or indirectly influence the pathophysiological states of the host, developing both pro- and anti-inflammatory effects. The future challenge will be to understand microbiota-metabolite-host interactions at the molecular level and in its entirety. A better understanding of these interactions can open perspectives for understanding and of biological pathways, as well as for adjuvant treatments based on probiotics containing immunoregulatory bacteria, prebiotics, which influence the growth of beneficial bacteria populations, or even a simple intervention in the diet.

Author contributions

MM-F and GM contributed to the conception of the study, searching the literature, creating graphical illustrations, and writing the manuscript. DC contributed searching the literature and designed the tables. JP and RB contributed writing the manuscript. PS and TV contributed correcting the manuscript. FV contributed devising the concept, writing, and correcting the manuscript. 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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EDITED BY
Junling Shi,
Northwestern Polytechnical University, China

REVIEWED BY
Ying Shi,
Zhejiang University, China
Karolina Skonieczna-Żydecka, Pomeranian Medical University, Poland

*CORRESPONDENCE
Xiaohui Wu
✉ wuxiaohui1971@sina.com
Wei Wei
✉ wei.wei@whu.edu.cn
Xiang Li
✉ li.xiang@whu.edu.cn

†These authors have contributed equally to this work and share first authorship

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The role of the gut microbiome and its metabolites in cerebrovascular diseases

Hongyu Xu[1,2†], Ziyue Xu[1,2†], Shengrong Long[1,2†], Zhengwei Li[1], Jiazhi Jiang[1,2], Qiangqiang Zhou[1,2], Xiaopeng Huang[1,2], Xiaohui Wu[1*], Wei Wei[1,2*] and Xiang Li[1,2*]

[1]Department of Neurosurgery, Zhongnan Hospital, Wuhan University, Wuhan, Hubei, China, [2]Brain Research Center, Zhongnan Hospital, Wuhan University, Wuhan, Hubei, China

The gut microbiome is critically involved in maintaining normal physiological function in the host. Recent studies have revealed that alterations in the gut microbiome contribute to the development and progression of cerebrovascular disease *via* the microbiota-gut-brain axis (MGBA). As a broad communication network in the human body, MGBA has been demonstrated to have significant interactions with various factors, such as brain structure and function, nervous system diseases, etc. It is also believed that the species and composition of gut microbiota and its metabolites are intrinsically linked to vascular inflammation and immune responses. In fact, in fecal microbiota transplantation (FMT) research, specific gut microbiota and downstream-related metabolites have been proven to not only participate in various physiological processes of human body, but also affect the occurrence and development of cerebrovascular diseases directly or indirectly through systemic inflammatory immune response. Due to the high mortality and disability rate of cerebrovascular diseases, new treatments to improve intestinal dysbacteriosis have gradually attracted widespread attention to better ameliorate the poor prognosis of cerebrovascular diseases in a non-invasive way. This review summarizes the latest advances in the gut microbiome and cerebrovascular disease research and reveals the profound impact of gut microbiota dysbiosis and its metabolites on cerebrovascular diseases. At the same time, we elucidated molecular mechanisms whereby gut microbial metabolites regulate the expression of specific interleukins in inflammatory immune responses. Moreover, we further discuss the feasibility of novel therapeutic strategies targeting the gut microbiota to improve the outcome of patients with cerebrovascular diseases. Finally, we provide new insights for standardized diagnosis and treatment of cerebrovascular diseases.

KEYWORDS

gut microbiota, gut microbial metabolites, intestinal dysbacteriosis, cerebrovascular diseases, microbiota-gut-brain axis

1. Introduction

Cerebrovascular diseases refer to conditions that cause brain tissue damage due to intracranial blood circulation disorders caused by various reasons (Thomas, 1996). The predominant clinical manifestations are transient ischemic attack (TIA), stroke, cerebral arteritis, and cognitive impairment (Mehanna and Jankovic, 2013; Dichgans and Leys, 2017). Stroke is the most common clinical manifestation of cerebrovascular diseases. In particular,

ischemic stroke resulting from cerebrovascular diseases is the most prevalent cause. According to the current statistics, stroke caused by cerebrovascular diseases has become the second leading cause of death in industrialized countries and the most common reason for permanent acquired disability (O'Donnell et al., 2016). Therefore, increasing studies have concentrated on risk factors for cerebrovascular diseases (Boehme et al., 2017; Cipolla et al., 2018; Claeys et al., 2020). Early intervention in the associated risk factors can reduce the incidence of cerebrovascular disease. At present, hypertension, diabetes, smoking and gender have been identified as the main risk factors for cerebrovascular diseases. (Muhammad et al., 2021; Tsai et al., 2021). Simultaneously, with the application of multi-omics approaches (McCombie et al., 2019; Chen et al., 2021), numerous studies, notably the human microbiome project (HMP) and metagenomics of the human intestinal tract (MetaHIT) have emerged and provided a comprehensive reference for the composition of the human gut microbiota (Peterson et al., 2009; Arumugam et al., 2011). Since then, research has uncovered the function of microbiomes in varieties of diseases, mainly including cancer immunotherapy (Li et al., 2019), systemic inflammatory diseases (Clemente et al., 2018), and cardiovascular system diseases (Jie et al., 2017). Recent studies have revealed that the gut microbiota has evolved into an inseparable and symbiotic relationship with the host during the evolutionary process (Zou et al., 2022).

The composition of the human gut microbiome is dynamically balanced, and it also plays essentials roles in the human body: the circulating metabolism of various nutrients, the formation of the intestinal immune protection system, the promotion of the development of the nervous system (Yadav et al., 2018; Adak and Khan, 2019; Schoeler and Caesar, 2019). Once intestinal dysbacteriosis is under certain circumstances, it is a severe blow to the homeostasis of the gut microbiota and the health of the body. GBA refers to the two-way communication exchange network between the brain and gut microbiome, composed of the brain, intestines, and gut microbiota (Cryan et al., 2019). Recent studies have shown that ecological imbalances of the gut microbiota can disrupt the integrity of the intestinal barrier, allowing pathogens and toxic metabolites to invade the systemic circulation, resulting in the dysregulation of GBA. The ensuing immune system dysregulation and neuroinflammation can induce neurotoxic misfolded proteins to accumulate around neurons, eventually triggering neuronal death. At the same time, central nervous system involvement can aggravate intestinal dysbacteriosis through defective autophagy-mediated, thus forming a vicious circle mediated by defective autophagy and immune system disorders (Chidambaram et al., 2022). Many studies have indicated that intestinal dysbacteriosis has become an extremely significant risk factor for the onset and development of cerebrovascular diseases (Benakis et al., 2020a; Zhu S. et al., 2020).

The mammalian gut microbiota includes bacteria, viruses, fungi, yeasts, and bacteriophages (Rutsch et al., 2020), in which bacteria are the main components of the gut microbiome. Current research divides the gut microbiota into four main categories: Bacteroidota, Actinomycetes, Pseudomonadota, and Bacillota (Wolter et al., 2021). Communication between the gut microbiome and the brain has recently received widespread attention. The concept of GBA also has emerged (Pellegrini et al., 2020). Interactions between the brain, intestines, and gut microbiota regulate the physiological processes of the human body. It has been confirmed that nervous system diseases from early brain development to old age are closely related to GBA (Socała et al., 2021). Multiple anatomical structures, systems, and metabolic pathways are involved in establishing a bidirectional connection between the gut microbiota and the brain, such as neuroendocrine (*via* the HPA axis), neuroimmune system, and the sympathetic and parasympathetic arms of the autonomic nervous system including the enteric vagus nerve system and the immune system (Carabotti et al., 2015; Rao and Gershon, 2016), proposed the concept of GBA, which demonstrates bidirectional communication and mutual influence between the gut and brain through the gut microbiome in immune (Li et al., 2019), endocrine (Régnier et al., 2021) and neuromodulation (Quigley, 2017). With the further deepening of the study of gut microbiota, intestinal dysbacteriosis and gut microbiota metabolites are not merely risk factors. They also strongly correlate with the prognosis and treatment of cerebrovascular diseases (Osadchiy et al., 2019; Sorboni et al., 2022). This review discusses the research progress of several most common cerebrovascular disorders. The gut microbiota introduces the close relationship between cerebrovascular diseases and the gut microbiota and its metabolites. In addition, we look forward to the possible research directions in the future and provide new ideas for further research on the diagnosis and treatment of cerebrovascular diseases.

2. Gut microbiota and metabolites

2.1. Gut microbiota

The gut microbiome comprises more than 1,500 species distributed in more than 50 phyla (Gomaa, 2020). Bacteroides and firmicutes, followed by Proteus, Fusobacterium, Ciliate, Actinomycetes, and Verrucous bacteria, have been reported to be the most dominant species of the gut microbiome, accounting for 90% of the total human microbiome (Passos and Moraes-Filho, 2017). Therefore, its abundance ratio is an essential indicator of the degree of intestinal dysbacteriosis (Kuziel and Rakoff-Nahoum, 2022). The role of the gut microbiota in the human body goes far beyond its function of promoting the digestion and absorption of food. Current research has confirmed that the gut microbiota can participate in various life activities, such as behavioral cognition (Mohajeri et al., 2018), endocrine regulation (Farzi et al., 2018), and immune response (Sadler et al., 2020). The gut microbiota produces biologically active metabolites that affect many aspects of host life activity and are widely considered the largest endocrine organ in the human body (Witkowski et al., 2020). Several factors can alter the composition and function of the gut microbiome, including host genetics, diet, age (Odamaki et al., 2016), birth pattern (Nagpal et al., 2017), and antibiotics (Hasan and Yang, 2019). Among these numerous environmental factors, diet is considered the most crucial factor determining the diversity and composition of the human gut microbiota (Wu et al., 2011; David et al., 2014). Changes in the composition and function of the gut microbiota can affect intestinal permeability, digestion and metabolism, and immune responses, resulting in metabolic disorders, vascular inflammation, immune responses associated with the nervous system, and more (Al Bander et al., 2020; González Olmo et al., 2021). Therefore, current studies have shown that the gut microbiota is closely related to obesity, diabetes, hypertension, Parkinson's disease, Alzheimer's disease and other diseases (Durack

and Lynch, 2019). Based on a series of case–control and CeVD (Cerebral small vessel disease) animal model studies, a significant correlation between cerebrovascular disease and gut microbiota has been demonstrated. It can be seen that the gut microbiota can play a role similar to that of metabolic organs, producing a series of bioactive factors through metabolic pathways that act on the host and thus affect the occurrence and development of cerebrovascular diseases (Haghikia et al., 2018).

2.2. Gut microbial metabolites

Gut microbial metabolites mainly come from the food the host cannot or does not have time to digest and the endogenous mucus secreted by the intestinal epithelial cells. After the action of the gut microbiota, many metabolites that are harmful or beneficial to the human body are produced, such as short-chain fatty acids (SCFA), bile acids (BA), choline metabolites, vitamins, etc. Among them, SCFA, Trimethylamine N-oxide (TMAO), lipopolysaccharide (LPS) and BA have been widely confirmed to participate in various inflammatory responses, immune responses, signaling and other processes (Martin-Gallausiaux et al., 2021; Matsushita et al., 2021), thereby affecting the occurrence and development of cerebrovascular diseases.

2.3. Short-chain fatty acids

SCFA is the main product of dietary fiber fermentation in the colon, and the flora that produces SCFA mainly includes anaerobes, bifidobacteria, eubacteria, streptococci, and lactobacilli (Sadler et al., 2020). Adults produce approximately 500–600 mmol of SCFA in their gut daily. Acetate, propionic acid, and butyric acid are the most abundant SCFA in the human body and the most abundant anions in the colon (Kim et al., 2022). Because acetate, butyrate and propionate in SCFA easily cross the blood–brain barrier (BBB), and SCFA has neuroactive properties and its impact on other intestinal-brain signaling pathways, including immune and endocrine systems, SCFA may be directly or indirectly involved in the occurrence and development of cerebrovascular diseases and exert its biological role (Clarke et al., 2014; Stilling et al., 2016; Wenzel et al., 2020). SCFAs possess favorable anti-inflammatory and chemopreventive properties. SCFAs are also considered as tumor inhibitors to exert anti-cancer and anti-inflammatory effects in cerebrovascular disease. Among them, the anti-cancer and anti-inflammatory effects of propionate and butyrate have been confirmed (Säemann et al., 2000; Verhaar et al., 2020). Current research confirms that SCFA is not only involved in cerebral angiogenesis but is also active in the management of complications, sequelae, and post-stroke recovery(Chen et al., 2019; Lee et al., 2020; Sadler et al., 2020; Huang Q. et al., 2022).

2.3.1. Trimethylamine N-oxide

TMAO, one of the gut microbial metabolites most associated with cerebrovascular diseases, is an amine oxide produced by choline, betaine, and carnitine, which is mainly obtained through the intake of foods rich in choline, L-carnitine, and phosphatidylcholine (Ascher and Reinhardt, 2018). It is processed by the human gut microbiota, converted into trimethylamine (TMA), and then enters the liver through the portal system. It is oxidized to TMAO by Flavin Monooxygenase 3 (FMO3), and then released into the bloodstream for action. The association between TMAO levels and diseases is still controversial. TMAO has been proven to directly lead to platelet hyperreactivity and enhance thrombosis, thus increasing the risk of cardiovascular and cerebrovascular accidents (Zhu et al., 2016). The clinical research on hypertensive people in China has shown that higher TMAO level was associated with an increased risk of the first stroke. Patients in the upper tertiles had a 34% higher risk of the first stroke than those in the lowest tertiles. They also found that patients with low folate and high TMAO had the highest stroke rate (Nie et al., 2018). TMAO has been confirmed to have elevated levels in the blood of patients with atherosclerosis, hypertension, type 2 diabetes, stroke, cognitive impairment and other cardiovascular and cerebrovascular diseases (Heianza et al., 2017; Zhu Y. et al., 2020). However, a case–control study by Yin et al. (2015) has come to the opposite conclusion. They found that patients with atherosclerotic ischemic stroke and TIA episodes showed significant dysregulation in their gut microbiota and reduced levels of TMAO in their blood. There is also a lot of convincing evidence of an association between TMAO and inflammation. Chen et al. (2017) have shown that TMAO can significantly trigger oxidative stress and activate NLRP3 inflammasomes by inhibiting the SIRT3-SOD2-mitochondrial ROS signaling pathway, thereby promoting vascular inflammation leading to endothelial cell dysfunction. At the same time, it has been found that TMAO can enhance leukocyte recruitment and the expression of pro-inflammatory cytokines IL-1β, IL-18, and TNF-α, and reduce the expression of the anti-inflammatory cytokine IL-10 (Chen et al., 2017). In addition, due to individual differences in the distribution of gut microbiota, the secretion level of TMAO is also different (Kim and Jazwinski, 2018) and related to major unconscionable cerebrovascular events. Therefore, TMAO has potential research value in predicting the risk of cardiovascular and cerebrovascular diseases (Ke et al., 2018).

2.3.2. Bile acids

BA is a kind of substance produced by gut microbiota mediating and regulating cholesterol metabolism, and is synthesized in the liver mainly through the action of cytochrome P450 family enzymes, such as CYP7A1, CYP27A1, CYP8B1, and CYP7B1 (Winston and Theriot, 2020). Total bile acids (TBA) in the human body can be divided into primary and secondary bile acids. Circulating BA produced in the liver and intestines can reach the brain by diffusing or crossing the BBB through BA transporters. At least 20 bile acids have been found in the brain, including conjugated and unconjugated BA (Pan et al., 2017). Therefore, the content of BA in the body is also related to the occurrence of cerebrovascular diseases. Recent studies have implicated BA in cerebrovascular disease in both positive and negative functions and are directly involved in the physiological activities and pathological processes of the brain (Weng et al., 2022). For instance, taurine deoxycholic acid (TUDCA) has been proven to be a protective BA in brain diseases with anti-apoptotic, anti-inflammatory and antioxidant characteristics (Palmela et al., 2015). In stark contrast, some BAs, such as CDCA and DCA, act as risk metabolites to alter BBB permeability by disrupting the tight junctions of rat brain microvascular endothelial cells (RBMECs; Lirong et al., 2022). Overall, BA metabolism and the BA pool are engaged in a straightforward interface between the gut microbiota and cerebrovascular disease, integral to internal environmental homeostasis.

2.3.3. Lipopolysaccharide

LPS is a major component of the outer membrane of gram-negative bacteria, also known as endotoxin. The essential source of endotoxin is the death and disintegration of gut microbiota, which can form a protective barrier around bacteria to evade the action of antibiotics, acts on host cells, produces inflammatory cytokines, and causes endotoxemia and sepsis (Maldonado et al., 2016). The lipid A component of LPS is the primary pathogen-related molecular model (PAMP), which can interact with Toll-like receptor 4 (TLR4) (Ciesielska et al., 2021). When LPS is transferred from the intestinal tract to circulation, LPS forms a complex with LBP binding protein, and LBP can bind to CD14 on monocytes. This may lead to the production of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6 (Sun et al., 2016). Recent studies have confirmed that inflammation is essential in developing cerebrovascular diseases, especially stroke. It is also related to the pathophysiological process of ischemia and the overall outcome after stroke (Anrather and Iadecola, 2016). Therefore, LPS is involved in the occurrence and development of stroke.

3. Regulation of gut microbial metabolites on interleukin

The gastrointestinal (GI) tract is considered the largest immunological organ in the body, having a central role in regulating immune homeostasis. The Human GI tract contains approximately 100 trillion bacteria, making it an important site of interaction between microorganisms and the host immune system (Rooks and Garrett, 2016). The host's immune system dynamically balances anti-inflammatory and pro-inflammatory cytokines by interacting with the microbiota to regulate the action of effector cells and immune cells (Takiishi et al., 2017). Interleukin is a critical cytokine family which participates in many processes, such as the maturation, activation, proliferation, and regulation of immune cells, and also participates in many physiological and pathological reactions of the body. Some gut microbial metabolites such as SCFA, LPS, and BA have been elucidated in the related mechanism of an interleukin-mediated inflammatory immune response. Following dysregulation of gut microbial homeostasis, there is a massive release of intestinal inflammatory factors such as helper T-type (Th)1, Th17 and interleukin IL-6. The release of inflammatory factors results in altered intestinal permeability, barrier dysfunction and transit from the peripheral blood to the BBB. Ultimately, they act on the cerebrovascular system and take a pivotal role in the development, progression and prognosis of cerebrovascular disease.

3.1. Regulation of the SCFA on interleukin

SCFA serves as an essential fuel for intestinal epithelial cells (IEC), regulating IEC proliferation, differentiation and the function of subpopulations. For instance, SCFA exerts influence on intestinal motility by affecting the secretion of hormones from enteroendocrine cells, enhancing intestinal barrier function as well as host metabolism (Martin-Gallausiaux et al., 2021). Recent studies have partially clarified that SCFA regulates the expression of interleukin, thus affecting gut immunity and promoting the occurrence and development of diseases. Recent studies have found that SCFA induced the activation of microbial antigen-specific TH1 cells through G-protein coupled receptors 43 (GPR 43) and activates STAT3 and mTOR, thus up-regulating transcription factor B lymphocyte-induced maturation protein 1 (Blimp-1) and finally promoting the production of IL-10 (Sun et al., 2018). Yang et al. (2020) have shown that SCFA can promote the production of IL-22 by CD4T cells and innate lymphoid cells (ILCs) through G-protein receptor 41 (GPR41) and inhibiting histone deacetylase (HDAC). At the same time, they also found that butyric acid up-regulates the production of IL-22 by promoting the expression of aryl hydrocarbon receptor (AhR) and hypoxia-inducible factor 1α (HIF1α). In addition, propionate has shown that it acts directly on γδ T cells to inhibit their production of IL-17 in a histone-deacetylase-dependent manner. Moreover, the production of IL-17 by human IL-17-producing γδ T cells from patients with inflammatory bowel disease (IBD) is regulated by propionate (Dupraz et al., 2021).

3.2. Regulation of the LPS on interleukin

As a common endotoxin, LPS can activate monocytes, macrophages, endothelial cells, and epithelial cells through a cell signal transduction system and synthesize and release various cytokines and inflammatory mediators (Mohammad and Thiemermann, 2020). Then it causes a series of immune responses and participates in the occurrence and development of multiple diseases (Qin et al., 2007). The primary mechanism of LPS in immune response has been thoroughly studied: When LPS is released into the blood through intestinal epithelium in a pathological state, lipopolysaccharide-binding protein (LBP) can be combined with LPS and transported to the surface of myeloid cells. When LPS is released into the blood through intestinal epithelium in a pathological state, lipopolysaccharide-binding protein (LBP) can be combined with LPS and transported to the surface of myeloid cells. MCD14 on the surface of myeloid cells binds to it, forming the LPS-LBP-CD14 triple complex. Then it was transported to the protein complex of TLR4-MD2, and the triple complex combined with TLR4 with the help of MD-2 to activate TLR4. The activated TLR4 activates the intracellular signal transduction pathway through conformational changes. Intracellularly, IL-1R-related protein kinase (IRAK) aggregates into receptor complexes through MyD88 and MyD88 adaptor protein analogs, which activates IRAK phosphorylation. Afterward, IRAK dissociates from the complex and transmits the signal to TRAF6. The activated TRAF6 can signal transduction by activating nuclear factor-κ B-induced kinase (NIK) and transforming growth factor β-activated kinase 1 (TAK1), and activating the corresponding NF-κB and mitogen-activated protein kinase (MAPK). Eventually, it causes the release of IL-1, IL-6, and TNF-α and participates in inflammatory reactions (Cohen, 2002; Kumar et al., 2022).

3.3. Regulation of the BA on interleukin

As a cholic acid derivative synthesized by the liver, BA is involved in many physiological and pathological processes, such as metabolism, immunity, and inflammation, playing a significant role in regulating intestinal physiological function and the disease process (Holtmann

et al., 2021). Among many inflammatory cytokines, BA against NLRP3 inflammasome is not only a key mediator of host defense but also a key regulator of intestinal homeostasis (Holtmann et al., 2021). Recent studies have confirmed that BA can activate NLRP3 inflammasome to trigger the release of inflammatory factors IL-1β and IL-18 (Zhen and Zhang, 2019), promote the inflammatory process, and restore the imbalance of body homeostasis induced by PAMP (Haneklaus and O'Neill, 2015). In addition, NLRP3 inflammasome can mediate the production and release of the inflammatory factor IL-1β, and mediate cell apoptosis by triggering Caspase-1 to produce gasdermin D (Shi et al., 2017). Furthermore, the targeted preparation for NLRP3 inflammasome can effectively reduce the intestinal inflammatory response caused by BAs and is expected to reduce the occurrence of chronic autoimmune diseases such as inflammatory bowel disease. In addition, Glycodeoxycholic acid (GDCA) and TUDCA have also been proven to induce group 3 internal lymphoid cells (ILC3s) to promote the secretion of IL-22 by up-regulating GATA3 expression (Figure 1).

4. Gut microbiota and metabolites in cerebrovascular diseases

4.1. Stroke

Stroke has become a global health problem, the second leading cause of death and the third leading cause of disability (Hossmann, 2006; Kalaria, 2018). According to a systematic analysis of the worldwide burden of disease published in The Lancet, stroke became the leading cause of death from the disease in China in 2017 (Zhou et al., 2019). In humans, stroke is classified as ischemic or hemorrhagic based on the underlying neuropathology. Numerous studies have found that the gut microbiota acts on the homeostasis of the human environment through metabolic pathways and immune responses, affecting the occurrence and development of stroke (Huang and Xia, 2021; Xu et al., 2021; Peh et al., 2022).

Atherosclerotic cerebral infarction is one of the most common causes of stroke worldwide (Weinberger, 2005). Atherosclerotic cerebral infarction has been shown to be strongly correlated with TMAO. Its main mechanisms are (a) TMAO can partially increase the expression of atherosclerotic scavenger receptors CD36 and A (SRA) in macrophages, hinder cholesterol transport, and promote macrophage and foam cell formation. On this basis, mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways promote endothelial inflammatory response (Seldin et al., 2016; Zhang X. et al., 2020). (b) TMAO can reduce the production of cholesterol 7α-hydroxylase, thereby reducing the production of bile acids, causing cholesterol to accumulate in cells. At the same time, up-regulating the expression of the vascular cellular adhesion molecule-1 (VCAM-1) can promote monocyte adhesion, activate protein kinase C (PKC) and p-NF-κB, and further lead to the formation of atherosclerotic plaque (Ma et al., 2017), thus increasing the risk of cerebrovascular events. Furthermore, a prospective cohort study also confirmed that an elevation in inflammation-associated monocytes caused by elevated TMAO levels can raise the risk of stroke and compromise the severity of stroke (Zhu et al., 2018, 2021). At the same time, TMAO can also reflect the human gut microbiota, which suggests that we can reduce the risk of cerebrovascular diseases and the prognosis of adverse

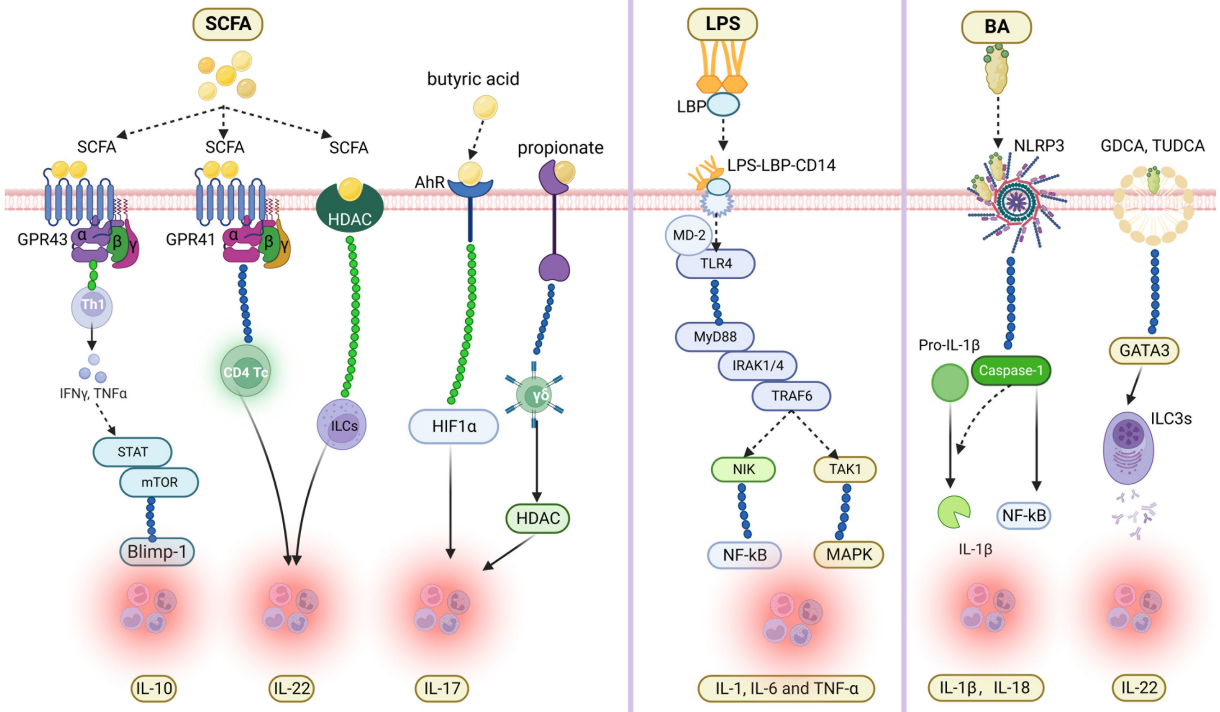

FIGURE 1
Molecular mechanism of gut microbial metabolites regulating the expression of specific interleukin in inflammatory immune response.

cerebrovascular disorders by regulating the gut microbiota. For instance, we can prevent and treat cerebrovascular diseases by regulating TMAO levels by controlling the composition of TMAO-related bacteria in the gut microbiota. Probiotic preparations specifically tailored for this purpose are expected to form the foundation of treatment strategies for cerebrovascular diseases.

Furthermore, the crucial involvement of SCFA in stroke has also gained a high profile recently. It was observed that hemorrhagic transformation (HT), a life-threatening stroke complication in MCAO rats, correlated with inflammatory response and serum levels of SCFA (Huang Q. et al., 2022). They found that the total SCFA, specifically butyrate and valeric acid, was significantly lower in HT rats than in non-HT rats. At the same time, SCFA has also been linked to stroke treatment. Studies have shown that SCFA levels in ischemic stroke rats are reduced, and it has been shown that ischemic stroke can be effectively treated by transplanting SCFA-rich feces and supplementing it with butyric acid (Chen et al., 2019). Interestingly, Lee et al. (2020) also found that transplanting feces containing higher SCFA levels or related bacteria could effectively alleviate nerve defects and inflammation after stroke in elderly male mice, and promote post-stroke recovery in elderly mice. At the same time, studies have shown that SCFA can promote post-stroke recovery by altering the recruitment of brain-resident immune cells in the brain (Sadler et al., 2020). By increasing the level of systemic SCFA, it is expected to be applied to clinical diagnosis and treatment to improve the poor prognosis of stroke patients.

Some studies have shown the relationship between TBA levels and the severity and prognosis of acute ischemic stroke (AIS). Huang L. et al. (2022) found that TBA levels in patients admitted to the hospital with AIS were inversely associated with mortality within three months. Moreover, another research showed that higher TBA was associated with smaller hematoma volume and lower clinical severity (Wang K. et al., 2018). Therefore, serum TBA levels are likely to play a protective role in the severity and poor prognosis of ischemic stroke. Lowering serum TBA levels through diet and medications may predict lower mortality and fewer stroke sequelae in stroke patients. In addition, some current studies have also confirmed that conjugated and unconjugated bile acids are related to the occurrence and development of stroke. In conjugated bile acids, a clinical trial has shown that higher concentrations of deoxycholic acid (DCA), lithocholic acid (LCA), and cholic acid (CA) in feces in stroke patients are associated with higher survival after stroke. They also found that decreased bile acid excretion (BAE) may be an independent risk factor for stroke (Charach et al., 2020). For stroke severity or morbidity, Bian et al. (2019) found that DCA could improve acute cerebral infarction (ACI) induced nerve damage by inverting the Nrf2 signaling pathway. In unconjugated bile acids, a study of metabolite analysis in young stroke patients found that Glycochenodeoxycholic acid (GCDCA) concentrations were significantly higher in the stroke group than healthy controls (Liu et al., 2021). Besides, Wu et al. (2020) have demonstrated that TUDCA could attenuate neuronal apoptosis and improve neurological functions through TGR5/ SIRT3 signaling pathway after spontaneous subarachnoid hemorrhage (SAH). Interestingly, another research also has shown that TUDCA enhanced cerebral blood flow, reduced BBB permeability, inhibited the ER stress through the PERK/eIF2α/ATF4/CHOP signaling pathway, blocked the Caspase-12-dependent ER-stress mediated apoptosis, resulting in significantly improved neurological function of mice subjected to SAH (Chen X. et al., 2020). Accordingly, TUDCA is expected to be the first-line anti-apoptosis drug for SAH patients and reduce the related neurological sequelae. These results all suggest that BA is likely to have the potential to predict stroke outcomes in stroke patients.

Intestinal dysbacteriosis is also closely related to stroke treatment and prognosis. A recent study has identified a new way of regulating the GBA. Benakis et al. (2016) have shown that intestinal dysbacteriosis affects the outcome of ischemic stroke by altering dendritic cell activity and immune homeostasis in the small intestine, leading to an increase in regulatory T cells and a decrease in IL-17γδ T cells. Their findings shed new light on the immune mechanisms of stroke. Studies by Ling et al. (2020) suggest that Enterobacteriaceae, in particular, may be able to predict post-stroke cognitive impairment (PSCI), a common neuropsychiatric complication of stroke, while being used as a clinical biomarker for PSCI. For the treatment of stroke, several studies have pointed out that increasing the intake of SCFA can play a therapeutic role in stroke mice (Chen et al., 2019; Lee et al., 2020). In addition, Benakis et al. (2020b) have shown that mice treated with antibiotic cocktails significantly reduce infarct volume in the acute phase of stroke after changing the gut microbiota while improving neuromotor function in mice. Consequently, this evidence demonstrated the importance of the gut microbiota in the short-time and long-term outcomes of ischemic stroke. At the same time, microbiome-targeted therapies related to specific microbial enzymatic pathways may provide a better prognosis for patients at high risk of stroke. It has also been proposed to regulate the composition of the gut microbiota by oral administration of specific probiotics or by fecal microbiota transplantation (FMT) and to treat ischemic stroke by increasing beneficial metabolites such as SCFAs (Ling et al., 2020). Klimiec et al. (2016) observed that plasma endotoxin activity rises during ischemic stroke and is associated with worse short-term outcomes. Another research has also shown that metabolic endotoxemia can promote neuroinflammation after focal cerebral ischemia (Kurita et al., 2020). Therefore, the application of antibiotics against endotoxemia may be a new treatment strategy to improve the outcome of stroke. However, it should be noted that long-term use of antibiotics may lead to drug resistance. Studies by Tang et al. (2013) showed that plasma TMAO levels decreased significantly after taking broad-spectrum antibiotics to inhibit gut microbiota, and then increased again after stopping treatments. The advent of FMT has brought new hope (Wang et al., 2019; Zhang W. et al., 2020) for treating various diseases, which can effectively avoid intestinal dysbacteriosis caused by antibiotic treatment. In the future, whether antibiotics and FMT can be considered for treating stroke will be an exceedingly exciting research direction. At the same time, immunotherapy for the intestinal mucosal barrier also provides new ideas for treating stroke patients in the future.

4.2. Cerebrovascular malformation

Cavernous angiomas (CAs) are characterized by dysmorphic dilated vascular capillaries, or caverns, lined by endothelium (Yin et al., 2015; Zhu Y. et al., 2020). Cavernous hemangiomas (CCMs) are relatively common cerebrovascular malformations and a common clinical cause of hemorrhagic strokes and seizures (Spiegler et al., 2018). CCMs arise due to loss of function mutations in three genes, KRIT1 (aka CCM1), CCM2, and PDCD10 (aka CCM3), that encode

components of a single, heterotrimeric, adaptor protein complex (Tang et al., 2019). The current standard treatments for CCMs are still symptomatic and surgically resected. Unfortunately, there is no specific drug for CCMs (Akers et al., 2017). Recent studies have shown that lipopolysaccharides (LPS) from Gram-negative bacteria (GNB) in the gut microbiome can drive the development of CCM disease by activating TLR4 and MEKK3 signaling in brain endothelial cells (Tang et al., 2017). Their study confirmed the central role of the gut microbiome and endothelial response to GNB in the pathogenesis of CCMs while demonstrating that the gut microbiome is the primary source of TLR4 ligands needed to stimulate CCMs formation in mice.

The minor differences in gut microbiota may significantly impact the progression of CCMs disease in this animal model. Previous studies have hypothesized the existence of the CCMs' gut-brain axis (Tang et al., 2017). Interestingly, the study by Tang et al. (2019) further demonstrated the presence of the CCMs gut-brain axis while identifying a central molecular component of the gut-brain axis in CCMs disease: the colonic mucus barrier. They concluded that the down-regulation of PDCD10 signaling in the brain endothelium and intestinal epithelium led to CCMs in mouse models. Surprisingly, their study also found that dexamethasone effectively inhibited the formation of CCMs in mice due to the combined action of brain endothelial cells and intestinal epithelial cells. Therefore, the activity of dexamethasone is probably based on its multiple critical molecular and cellular mechanisms in targeting CCMs' gut-brain axis. The recent research based on 16S rRNA gene sequencing technology, confirmed that CCMs patients have a unique gut microbiome, and LPS synthesis-related genes are more abundant in CA patients, consistent with intestinal LPS in driving CCMs disease (Polster et al., 2020). The study further demonstrated that CCMs patients with different disease characteristics have different gut microbiota, and the combination of plasma biomarkers and gut microbiome validated this idea. Future research can target gut microbiota and CCMs brain-gut axis-related targets to provide new strategies for treating CCMs. At the same time, combining the microbiome and circulating factors may also serve as biomarkers of potential disease severity and prognosis, providing new ideas for diagnosing CCMs. However, it should be noted that drugs targeting CCMs must fully consider the potential impact on the intestinal mucosal barrier function. Future research on targeted drugs should take more into account the existence of the intestinal mucosal barrier to effectively reduce the toxic side effects of targeted drugs.

4.3. Intracranial aneurysm

Intracranial aneurysm (IA) refers to the limitation and pathological expansion of the intracranial artery wall, which has emerged as the leading cause of SAH due to the risk of rupture (Macdonald and Schweizer, 2017). SAH caused by intracranial aneurysm rupture has the characteristics of a large number of occurrences, a wide range, and poor prognostic outcome. It has become a cerebrovascular disease that seriously endangers human health (Connolly et al., 2012). Despite extensive research in recent years, the exact mechanisms that lead to the pathogenesis of IAs are poorly understood. Therefore, there is an urgent need to find ways to diagnose and treat intracranial aneurysmal SAH to improve its poor prognosis.

Currently, the pathogenesis of IA is not completely clear, but the current evidence has confirmed that inflammation plays a significant role in it (Berge et al., 2016; Fennell et al., 2016). Recent research suggests that IA is partly caused by hemodynamically triggered endothelial cell dysfunction. This is followed by an inflammatory response of the vessels, accompanied by an increase in the activity of the inflammatory transcription factor NF-κB (Wei et al., 2011). The inflammatory response stimulates the phenotypic modulation of vascular smooth muscle cells (VSMCs) from a contractile to a pro-inflammatory/pro-matrix remodeling phenotype, followed by their degeneration, which may be crucial to IA formation and progression (Owens, 2007). At the same time, the gut microbiota also plays a crucial role in the development of many diseases through inflammation (Zhao et al., 2021; Cai et al., 2022). Therefore, the gut microbiota is also closely related to the occurrence and development of IAs. The findings of Shikata et al. (2019) are the first direct confirmation that the gut microbiota can influence the pathophysiology of IAs by modulating local inflammation. They found that antibiotics can reduce the effect of inflammation of cerebral arteries during IA formation and thus effectively reduce the formation of IAs. Metagenome-wide association studies (MWAS) performed serum metabolomics analysis of patients with IAs for the first time to identify microbial species associated with the unruptured intracranial artery (UIA), and further explored their effects on host amino acid and fatty acid metabolism (Shikata et al., 2019; Li et al., 2020). They reconfirmed the possible causal relationship between changes in the gut microbiota of UIA patients and more vital systemic inflammation. They also found that taurine can protect mice from the formation and rupture of IAs, while taurine supplementation can also reverse the progression of IAs. Not only does this study provide a new idea for the diagnosis and treatment of intracranial aneurysms, but it also shows that gut microbial metabolites may also impact the rupture of aneurysms. Another study provided new perspectives on intracranial ruptured aneurysm (Kawabata et al., 2022). Using 16S rRNA sequencing technology, they conducted a multicenter, prospective case–control study. For the first time, the relationship between gut microbiota dysregulation and intracranial rupture aneurysm has been elucidated: the gut microbiota characteristics of patients with the UIA and ruptured intracranial artery (RA) are significantly different. In addition, Campylobacter and Corynebacterium may be associated with intracranial brain aneurysm rupture. Surprisingly, they also elucidated for the first time the mechanism by which Campylobacter infection leads to the rupture of intracranial aneurysms, demonstrating its close association with inflammation and the MMP family. Finally, they concluded that Campylobacter could promote vascular remodeling and cell death of the cerebral artery wall by increasing inflammation-related cytokines, neutrophil-derived proteolysis, and oxidative stress. At the same time, it can finally lead to the rupture of IAs through the effects of hemodynamics and genetics. However, the current research on IAs and gut microbiota is still highly challenging. The diversity of gut microbiota is closely related to the environment, and the composition of gut microbiota in different regions is diverse. For example, one study reported that the gut microbiota of the Japanese population is exceedingly different from other populations (Park et al., 2021). Therefore, the current research may have specific limitations. In the future, we need to expand the scope of study further to understand other gut microbiota and the occurrence and development of IAs (Table 1).

TABLE 1 Gut microbiome metabolites in cerebrovascular diseases.

Metabolite		Associated bacteria	Research progress in cerebrovascular diseases
Short-chain fatty acids	Acetic acid	Anaerobic	Butyrate suppresses the production of pro-inflammatory cytokines, such as TNF-α, IL-12, and IF-γ, and upregulates the production of anti-inflammatory IL-10 by monocytes *in vitro* (Säemann et al., 2000).
	Propionic acid	Bacillus	SCFA can prevent vascular inflammation by activating GPR41/43 and inhibiting HDAC (Verhaar et al., 2020).
	Isobutyric acid	Bifidobacterium	Total SCFAs, especially butyrate and valeric acid, were significantly lower in the cecal contents of HT rats than in those of non-HT rats (Huang Q. et al., 2022).
	Butyric acid	Eubacteria	SCFA content in the blood of ischemic stroke rats decreased, and ischemic stroke can be effectively treated by transplanting feces rich in SCFA and supplementing butyric acid (Chen et al., 2019).
	Isovaleric acid	Streptococcus	Transplanting feces containing higher SCFA level or related bacteria can effectively alleviate the nerve defects and inflammation of old male mice after stroke, and promote the recovery of old mice after stroke (Lee et al., 2020).
	Valeric acid	lactobacillus	SCFAs modulate poststroke recovery *via* effects on systemic and brain resident immune cells (Sadler et al., 2020).
Choline metabolites	Methylamine	*F. prausnitzii*	TMAO induces vascular inflammation by activating the NLRP3 inflammasome through the SIRT3-SOD2-mtROS signaling pathway (Chen et al., 2017).
	Dimethylamine	Bifidobacterium	TMAO can up-regulate leukocyte recruitment and the expression of pro-inflammatory cytokines IL-1β, IL-18 and TNF-α, and reduce the expression of anti-inflammatory cytokine IL-10 (Chen et al., 2017).
	Trimethylamine		TMAO promotes early pathological process of atherosclerosis by accelerating endothelial dysfunction, including decreasing endothelial self-repair and increasing monocyte adhesion (Ma et al., 2017).
	Betaine		Elevated circulating TMAO during the aging process may deteriorate EC senescence and vascular aging, which is probably associated with repression of SIRT1 expression and increased oxidative stress (Ke et al., 2018).
	Trimethylamine N-Oxide		High levels of TMAO in the blood of stroke patients affect stroke severity (109).
			The increase of monocytes related to inflammation caused by the increase of TMAO level will lead to the increase of the risk of stroke (Zhang X. et al., 2020).
			TMAO has been confirmed to directly cause platelet hyperreactivity and enhance thrombosis, thereby increasing the risk of cardiovascular accidents (Zhu et al., 2016).
			Higher TMAO level is associated with increased risk of first stroke. Patients with low folate and high TMAO had the highest rate of stroke (Nie et al., 2018).
Bile acids	Conjugated bile acids:	Lactobacillus	Deoxycholic acid, cholic acid and lithocholic acid was higher in stroke-free patients compared to those who developed stroke (Quigley, 2017).
	CA, CDCA	Bifidobacterium	Decreased bile acid excretion is an independent risk factor for stroke (Charach et al., 2020).
	DCA, LCA	Enterobacter	Compared with the healthy control group, GCDCA concentration in stroke group was significantly higher (Liu et al., 2021).
	Conjugated bile acids:	Bacteroides	DCA can improve the nerve injury induced by acute cerebral infarction by reversely regulating Nrf2 signal pathway (Bian et al., 2019).
	GCA, TCA	Clostridia	The TBA level of AIS patients was negatively correlated with the mortality within 3 months (Huang L. et al., 2022).
	GCDCA, TCDCA		TUDCA could attenuated neuronal apoptosis and improve neurological functions through TGR5/SIRT3 signaling pathway after SAH (Wu et al., 2020).
			TUDCA improved cerebral blood flow, reduced BBB permeability, inhibited the ER stress through the PERK/eIF2α/ATF4/CHOP signaling pathway, blocked the Caspase-12-dependent ER-stress mediated apoptosis, resulting in significantly improved neurological function of mice subjected to SAH (Chen X. et al., 2020).
Lipopolysaccharide		Bifidobacterium	The lipid A component of LPS is the main PAMP, which can interact with TLR4 (Ciesielska et al., 2021).
		Klebsiella	LPS forms a complex with LBP binding protein, and bind to CD14 on monocytes. This may lead to the production of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6 (Sun et al., 2016).
		Enterobacter	An increased plasma LPS level constitutes a substantial risk factor for incident carotid atherosclerosis (Macrez et al., 2011).

(Continued)

TABLE 1 (Continued)

Metabolite		Associated bacteria	Research progress in cerebrovascular diseases
		Citrobacter	Plasma endotoxin activity rises during ischemic stroke and is associated with worse short-term outcome (Klimiec et al., 2016).
		Clostridium	Metabolic endotoxemia can promote neuroinflammation after focal cerebral ischemia (Kurita et al., 2020).
			Ex secreted from the LPS-stimulated macrophage RAW264.7 cell line (LPS-Ex) is effective in generating anti-inflammatory and neuroprotective effects by enhancing the microglial M2 polarization (Zheng et al., 2019).

4.4. Gut microbiota and metabolites in other vascular diseases

4.4.1. Pulmonary hypertension

Pulmonary Hypertension (PH) is a progressive and devastating disease characterized by pulmonary artery pressure greater than 25 mmHg. The leading cause of death was a right ventricular failure (Oliveira et al., 2020). Several studies have shown a close connection between PH and gut microbiota. The concept of the lung-gut axis (Wypych et al., 2019) has also promoted the research progress of PH. Hong et al. (2021) used a multinomial approach to study the correlation between the gut microbiota and host metabolome in PH and NPS 2143-treated rats, confirming changes in the gut microbiome in rats with PH. At the same time, there are differences between gut microbial metabolites in PH patients and ordinary people.

Interestingly, a recent study has discovered the association between TMAO and PH, finding that circulating TMAO was elevated in high-risk PH patients compared with healthy controls or low-risk PH patients. The use of 3,3-Dimethyl-1-butanol (DMB) significantly reduced right ventricular systolic blood pressure and the degree of pulmonary arterial muscularization in PH rats by reducing the content of TMAO (Huang Y. et al., 2022). At the same time, it was clarified that the reduction of TMAO can decrease the formation of pulmonary arterial muscularization by inhibiting the production of chemokines and cytokines and ultimately delaying the occurrence of PH. These findings deepen our understanding of the gut microbiota and PH, as well as confirm the existence of the gut-lung axis.

4.4.2. Portal hypertension

Portal hypertension is a pathological condition associated with liver injury, most commonly precipitated by cirrhosis. As the pressure in the portal vein rises, many fatal complications occur. Typically, the gut microbiome coordinates with the liver to maintain homeostasis in the body, and the concept of the gut–liver axis was born (Huang et al., 2021). Current research has confirmed that changes in the gut microbiota, as well as the intestinal mucosal barrier, may influence the degree of hepatic steatosis, inflammation, and fibrosis through multiple interactions with the host immune system and other cell types, leading to changes in portal venous pressure and ultimately influencing the progression of cirrhosis (Henao-Mejia et al., 2012). PAMP is the bacterial endotoxin known as lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria. The current study found that intraperitoneal injection of LPS has increased portal venous pressure (Steib et al., 2010), while increasing intestinal permeability. In addition, bacterial translocation, endotoxemia, and pro-inflammatory cytokines have been found to impair the contractility of mesenteric vessels in patients with cirrhosis and thereby increase portal venous pressure (Arab et al., 2018). For the treatment of portal hypertension, there are also some surprising results for the gut microbial metabolites BA. Regulation of BA nuclear receptors with the potent, selective FXR agonist Ocaliva (OCA) has improved portal hypertension through two different pathways. In both models, OCA therapy has been shown to reactivate signaling pathways downstream of FXR and reduce portal pressure by reducing intrahepatic total vascular resistance without developing systemic hypotension (Verbeke et al., 2014). Additionally, OCA has been shown to reduce bacterial translocation and reduce intestinal inflammation in rats with ascites cirrhosis (Úbeda et al., 2016). Therefore, the regulation of BAs signaling may be a new target for portal hypertension regulation in the future, closely related to the gut microbiota.

4.4.3. Vasculitis

Vasculitis refers to the infiltration of inflammatory cells in and around the blood vessel wall, accompanied by vascular endothelial cell injury, including cellulose deposition, collagen fiber degeneration, and endothelial cell and muscle cell necrosis. Many studies have shown the relationship between gut microbiota and vasculitis. Wang X. et al. (2018) first found that gut microbiota dysbiosis is associated with Henoch-Schönlein purpura (HSP) in children. The populations of Parabacteroidota and Enterococcus increased significantly in the gut microbiota of HSP patients, emphasizing the significance of gut microbiota dysbiosis in HSP. At the same time, Li et al. (2021) also found that the abundance of gut microbiota in children with IgA vasculitis was lower than that of normal children. Metabolomics has found that Bacteroidota, Bacillota, Proteus, and Actinomycetes are the four most abundant bacteria in children's gut microbiota. Pseudomonadota and actinomycetes have also been shown to be associated with organ involvement in IgA vasculitis. Similarly, with the deepening of research, other vasculitides, such as Kawasaki disease and Behcet's disease, have been confirmed to be related to the gut microbiota (Chen J. et al., 2020; Ma et al., 2021). Shortly, it is hoped that the field of the gut microbiome can be applied to the treatment of vasculitis, and the gut microbiota can be used as a biomarker to facilitate the early diagnosis and prognosis assessment of vasculitis diseases.

4.4.4. Summary

In addition, some recent studies have also found specific correlations between some other vascular diseases and gut microbiota. For example, it has been found that the composition of the gut microbiota in patients with diabetic angiopathy is significantly different from that of ordinary people (Iatcu et al., 2021). Disappointingly, although our understanding of the previous interaction between the gut microbiota and the host has deepened in recent years, we still need a comprehensive understanding of the

molecular mechanism of the GBA. At the same time, there are significant individual differences in gut microbiota itself, and there are differences in age, race, and sex that also limit the progress of related research (Bibbò et al., 2016; Takagi et al., 2019). In addition, current research is still blank for some vascular diseases such as moyamoya disease, arteriovenous fistula, and functional vascular diseases. However, it is undeniable that the recent research on gut microbiota and vascular diseases shows that a deeper understanding of gut microbiota can help us understand cerebrovascular diseases and also help us diagnose and treat cerebrovascular diseases, an exceedingly gratifying discovery (Figure 2).

5. Discussion

Cerebrovascular disease has high morbidity, disability rate, and mortality. Therefore, heart disease and malignant tumors constitute the three major causes of human death (Caprio and Sorond, 2019). Research on the gut microbiota and cerebrovascular disease has provided new insights into the effective prevention and treatment of cerebrovascular disease, thus reversing the traditional recognition of cerebrovascular disease and neuroinflammation. Although our research on the interaction between the gut microbiome and cerebrovascular diseases is still in its infancy, the results of various research results that continue to emerge are still surprising, especially the role of specific intestinal flora and its metabolites can delay the occurrence and progression of cerebrovascular diseases.

Currently, some new treatment strategies, such as FMT and phage therapy (Wang et al., 2019; Federici et al., 2022), can improve intestinal dysbacteriosis through probiotics, dietary intervention and other ways to treat cerebrovascular diseases, which have potential research value. Several studies using CeVD animal models have confirmed the role of FMT in the occurrence and treatment of cerebrovascular diseases. Intestinal T cells develop protective activity following transplantation of feces from a young population into mice with IS. Treg cells and IL-17T cells contribute to decreased inflammation, neurological deficits, and impairment of intestinal barrier function following stroke (Lee et al., 2020; Haak et al., 2021; Zou et al., 2022). In mice with ICH, transplantation of bacterial flora can affect T cells in the brain, reduce neuroinflammation following bleeding, and restore the average fluorescence intensity of the tight junction proteins occludin and claudin-1, thereby restoring intestinal barrier function (Wang et al., 2021). At the same time, as FMT increases the possibility of antibiotic-resistant bacterial infections, the advent of phage therapy could better address antibiotic resistance. The combination of FMT and Phage therapy in patients with cerebrovascular disease complicated with multiple drug-resistant infections caused by prolonged bed rest may better improve their poor prognosis and reduce the incidence of complications. In the future, targeted agents against the gut microbiota can be applied in a simple and non-invasive manner to the clinical

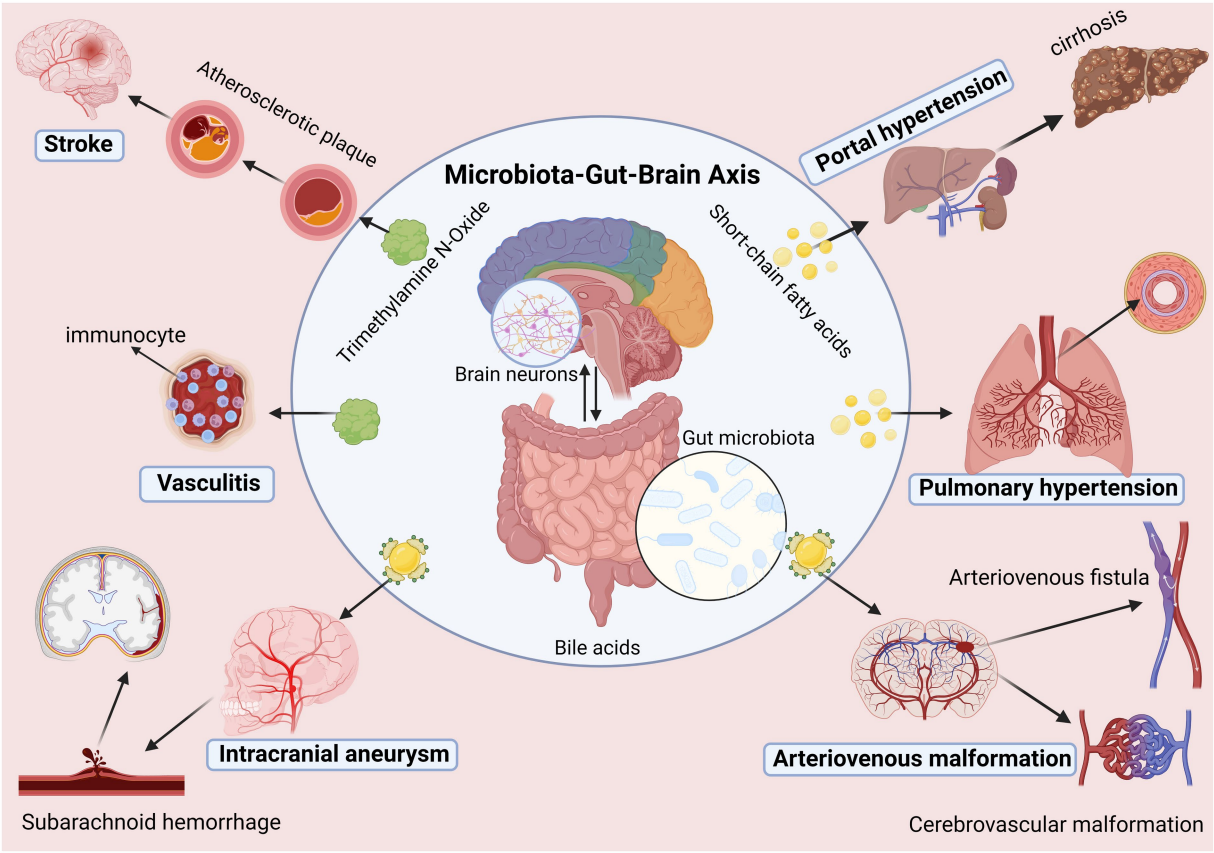

FIGURE 2
Microbiota-gut-brain axis is involved in the occurrence and development of cerebrovascular diseases.

diagnosis and treatment of cerebrovascular diseases. However, the current research on gut microbiota also has some limitations. The pathological state of stroke will inhibit the body's immune ability, thereby enhancing intestinal permeability and promoting microbiota translocation. The potential for transmission of antibiotic-resistant pathogens *via* FMT is dramatically increased, ultimately leading to fatal sepsis. A recent review of FMT safety found that serious adverse events occur in 2–6% of patients, depending on the route of administration (Wang et al., 2016). A uniform standard for screening and selection of FMT-related donors, fluid preparation and transplantation procedures are needed to effectively reduce the risk of potential infection, which is especially relevant for immunodeficient patients. In addition, most studies have been conducted on rodents and lack sufficient evidence of efficacy and long-term safety and evidence-based medicine (Walter et al., 2020; Gheorghe et al., 2021). FMT has inconsistent treatment outcomes due to differences in the route of administration (Ng et al., 2020). Therefore, the selection of the proper and efficacious method of administration is also a problem currently encountered. Consequently, we must be very cautious in analyzing and studying the impacts of gut microbiota on human beings. How to make a specific gut microbiota successfully target and colonize the human intestine will also become a problem that needs to be solved in the future.

This review summarizes the relevant research on the gut microbiome and cerebrovascular diseases in recent years, showing the close relationship between gut microbiota and cerebrovascular diseases. At the same time, we also elaborated on the relevant molecular mechanisms of the existing gut microbiota and its metabolites causing the occurrence and development of cerebrovascular diseases. However, the specific molecules, locations, and mechanisms acting on cerebrovascular diseases after intestinal dysbacteriosis still need to be further explored, which will become a research hotspot in the future. Due to some of the above limitations, the biomarkers of gut microbiota and its metabolites for early diagnosis, prognosis, and therapeutic targets of cerebrovascular diseases still need further more accurate and comprehensive research.

Author contributions

XL, WW, and XW proposed the ideas and drafted the outlines. HX, ZX, and SL performed the literature search and completed the manuscript. ZL, XH, JJ, and QZ helped revise the manuscript and provided support in need. All authors contributed to the design and writing of the 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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