Computational and Structural Biotechnology Journal 21 (2023) 3904–3911

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Mini-review

The microbiome in post-acute infection syndrome (PAIS)

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ARTICLE INFO

Keywords:
Post-acute infection syndromes
Microbiome
PASC
ME/CFS

ABSTRACT

Post-Acute Infection Syndrome (PAIS) is a relatively new medical terminology that represents prolonged sequelae symptoms after acute infection by numerous pathogenic agents. Imposing a substantial public health burden worldwide, PASC (post-acute sequelae of COVID-19 infection) and ME/CFS (myalgic encephalomyelitis/chronic fatigue syndrome) are two of the most recognized and prevalent PAIS conditions. The presences of prior infections and similar symptom profiles in PAIS reflect a plausible common etiopathogenesis. The human microbiome is known to play an essential role in health and disease. In this review, we reviewed and summarized available research on oral and gut microbiota alterations in patients with different infections or PAIS conditions. We discussed key theories about the associations between microbiome dysbiosis and PAIS disease development, aiming to explore the mechanistic roles and potential functions the microbiome may have in the process. Additionally, we discuss the areas of knowledge gaps and propose the potential clinical applications of the microbiome for prevention and treatment of PAIS conditions.

1. Introduction

As the COVID-19 pandemic wears on, increasing attention has been given to post-acute sequelae of COVID-19 infection (PASC), which is commonly referred to as long-COVID. PASC has imposed a significant burden on global public health, affecting a conservative estimate of 65 million people worldwide [1]. Patients with PASC show persistent symptoms that last for three months following COVID-19 infection that cannot be explained by alternative medical conditions [2]. However, such post-acute sequelae are not exclusive to SARS-CoV-2 infection, as prolonged sequelae symptoms have been documented to be associated with numerous infections. As tail-ends of infections, chronic sequelae caused by the inability to recover from acute infectious diseases are collectively called post-acute infection syndromes (PAIS). Still, PAIS is primarily unexplained in the medical community, featuring non-specific medical manifestations, lack of objective diagnostics, and mysterious mechanisms [3]. A long list of infectious agents, covering various bacterial, viral, and parasite species, is known to trigger the development of PAIS in a proportion of exposed individuals [4]. The majority of other PAIS show symptoms within 2–6 months of the initial infection, with one exception being that post-polio syndrome patients may have signs and symptoms that appear decades after initial polio infection [5]. Besides PASC, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is another major syndrome that overlaps with the concept of PAIS [6]. ME/CFS is a complex disease characterized by unexplained severe fatigue, with approximately 75% of cases reporting symptoms following an earlier infection. Throughout history, ME/CFS has also been named as post-polio syndrome and post-viral syndrome since patients develop chronic symptoms typically following infections with Polio virus, Epstein Barr virus, Influenza virus, or an array of other pathogens [7].

Currently, our understanding of PAIS is still in a nascent stage. Medical care providers are facing heavy challenges in the recognition, diagnosis, treatment, and prevention of PAIS, largely due to the lack of systematic research that examines the association between acute infections and chronic impairments. While condition-specific symptoms are often aligned with the pathogen tropism and the underlying pathogenesis of related diseases, PAIS patients generally present with a broad spectrum of symptoms in varying degrees of clinical manifestation [8–10]. Their conditions, however, are shared or characterized by a core set of symptoms, including disproportionate levels of fatigue, exertion intolerance, debilitating flu-like symptoms, chronic or recurrent joint

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https://doi.org/10.1016/j.csbj.2023.08.002
Received 12 May 2023; Received in revised form 3 August 2023; Accepted 3 August 2023
Available online 5 August 2023

pain, neurocognitive and sensory impairment, and myalgia/arthralgia [3]. Given the similarity of clinical presentations, a unified pathophysiology is needed to facilitate proper understanding and management of PAIS patients. Several mechanistic theories have been speculated to explain PAIS: 1) chronic immune system stimulation by persistent infection or unviable pathogen residues; 2) inflammation and coagulopathy caused by pathogen-induced autoimmunity; 3) irreversible organ injury causing characteristic symptoms in some PAIS conditions; and 4) dysregulation of the microbiota-gut-brain axis may cause chronic disease. It is worth noting that these theories are not mutually exclusive and may coexist or present with varying degrees of impact in different PAIS conditions [11].

The human microbiome generally refers to all microorganisms inhabiting the human body, encompassing their metabolic products and immediate environmental contexts as well. Recent advancements in high-throughput technology have enabled expeditious profiling and quantification of the human microbiota and their interactions with the environment, utilizing metagenomic, transcriptomic, and metabolomic approaches. It is known that human initial exposure to the microbial world occurs during vaginal birth, whereby neonates receive microbial species from the maternal birth canal, skin contact, and breastfeeding, playing a crucial and enduring role in developing the initial microbiome in neonates [12]. Furthermore, the human microbiome undergoes dynamic transformations throughout life, encapsulating diverse factors such as diet, environment, and lifestyle. The holistic microbiome functions as an additional organ system that plays essential roles in human health and development. The intestinal tract solely harbors a complex ecosystem comprising bacteria, fungi, parasites, and viruses, collectively forming the intestinal microbiome, which amounts to an estimated 100 trillion cells (10 times greater than the number of human cells) [13]. To preserve intestinal homeostasis, gut microbes are sequestered from the mucosal immune system by a protective mucous barrier that covers the intestinal epithelium [14]. It necessitates the intestinal immune system to distinguish the vast numbers of harmless or beneficial intestinal microbes from potential pathogens. The mucosal layer serves as the frontline of defense against infections while also providing a foundation for bacterial colonization by supplying a carbon source for the bacterial community [15]. The characteristics and mechanisms of how microbiota affects host susceptibility to infectious agents have already been well described [16,17]. Either gut dysbiosis or alterations in the gut microbiome may lead to the overgrowth of opportunistic bacterial pathogens. For instance, enteric viruses can alter the diversity and composition of the gut microbiome, resulting in gut dysbiosis and promoting opportunistic bacterial pathogens [18–20]. Similarly, bacterial infections have been observed to alter microbiome composition that *Porphyromonas gingivalis* causes the ecological dysbiosis in the oral cavity, consequently leading to periodontitis [21]. Numerous microorganisms from commensal human flora are also capable of transforming into opportunistic pathogens under conditions of microbiota dysregulation and immunosuppression. Staphylococcus aureus is a prevalent member of the normal nasal microbiome in 30% of human population, but it can alter its gene expression to promote a range of ailments, including skin infections and even life-threatening meningitis and endocarditis in severe cases [22]. In a mouse study, gut microbiota of antibiotic treated mice had a significant increase in opportunistic pathogens, which demonstrated the effects of antibiotic therapy on induced dysbiosis and then increased level of opportunistic pathogens [23]. Conversely, gut microbes are essential for restoring the immune system during the recovery from infection in both animal and human research [24]. It has been suggested that a persistent dysbiosis of the gut microbiota lasting for more than a month after disease subsidence maybe linked to the emergence of PASC [25]. Furthermore, common PAIS symptoms (such as fatigue, joint pain, sleep disturbance, diarrhea, anxiety, and headache) are long associated with microbiome dysbiosis, also suggesting that post-acute sequelae may arise from such dysbiosis after acute infection [26].

In summary, the state-of-art techniques used in microbiome research have increased our comprehension of the crucial, albeit underappreciated, roles played by gut microorganisms in both the pathogenesis and prognosis of PAIS. In this perspective, we summarize the latest findings on microbiota alterations in PAIS patients with different microbial sources. We also examine the potential mechanisms of immune interactions linking microbiome dysbiosis to disease development. Lastly, we highlight areas of knowledge gaps and explore the potential clinical applications of the microbiome for diagnosis, prevention, and treatment of PAIS conditions (Fig. 1).

2. Methods

To review and present the full picture of the interaction between microbiome and PAISs, we carried out an exhaustive search in PubMed (U.S. National Library of Medicine) publication database for relevant studies published prior to April 2023. The following keywords were used alone or in combination with a similar search strategy described elsewhere: “microbiome”, “microflora”, “microbiota”, “microorganism”, “dysbiosis”, and all individual PAIS conditions listed in the review by Choutka et. al. [3], Recent publications were preferred, but no limiting period was imposed in our screening. Furthermore, books, general newspapers, and Institutional Websites were reviewed for possible integration.

3. Microbiome dysbiosis

3.1. Microbiome dysbiosis in patients with acute infections

The host immune system works in partnership with microbiota to achieve homeostasis and resilience. When infection occurs, the exogenous pathogens disrupt host’s microbiome, and the impact can be pathogen-specific (Fig. 2). Recent studies of COVID-19 infection in varied populations consistently showed decreased oral and gut microbial diversity, reduced beneficial commensal bacteria, and elevated levels of opportunistic bacterial pathogens [27–29]. Specifically, the abundance of butyrate producing bacteria such as *Faecalibacterium prausnitzii*, *Clostridium butyricum*, *Clostridium leptum*, and *Eubacterium rectale* decreased, while the abundance of *Enterobacteriaceae* and *Enterococcus* increased [29]. Furthermore, studies of bronchoalveolar lavage fluid in COVID-19 patients also showed a decreased diversity in its microbiome, along with an increase in pathogenic bacteria from the oral and upper respiratory tracts [30]. Besides COVID-19 infection, elevated levels of *Enterococcus faecium*, *Clostridium ramosum*, *Coprobacillus*, *Lachnospiraceae*, and *Eubacterium ventriosum* were found in patients with community-acquired pneumonia (CAP) [29]. A cross-sectional study of COVID-19 patients, H1N1 patients, and matched healthy controls found that *Blautia*, *Bifidobacterium*, *Faecalibacterium*, and *Eubacterium* were decreased in influenza patients compared to healthy controls. Additionally, the microbiome in COVID-19 patients showed a higher relative abundance of the phyla *Actinobacteria* and *Firmicutes* compared to those with H1N1 infection [31]. Intriguingly, different subtypes of influenza viruses may have differing impacts on the gut microbiota [32]. The biological interpretation of the differences is still unknown, but it can be crucial knowledge towards deciphering the association between infection and the microbiome. Additionally, consistent changes in the gut microbiota have been reported in patients with chronic infectious diseases like HIV [33], HBV [34], and TB [35], which also lead to a reduction in microbial diversity and depletion of certain key bacterial species. Notably, specific changes observed in the microbiome may just reflect the complexity of conducting research, as variations in sample size, recruitment criteria, subject population, medical treatment, and methods for microbial classification can lead to varied conclusions. Similar observations were found in animal research as well [36]. A longitudinal study in mice found that infection with influenza H1N1 virus altered the composition and diversity of the lower

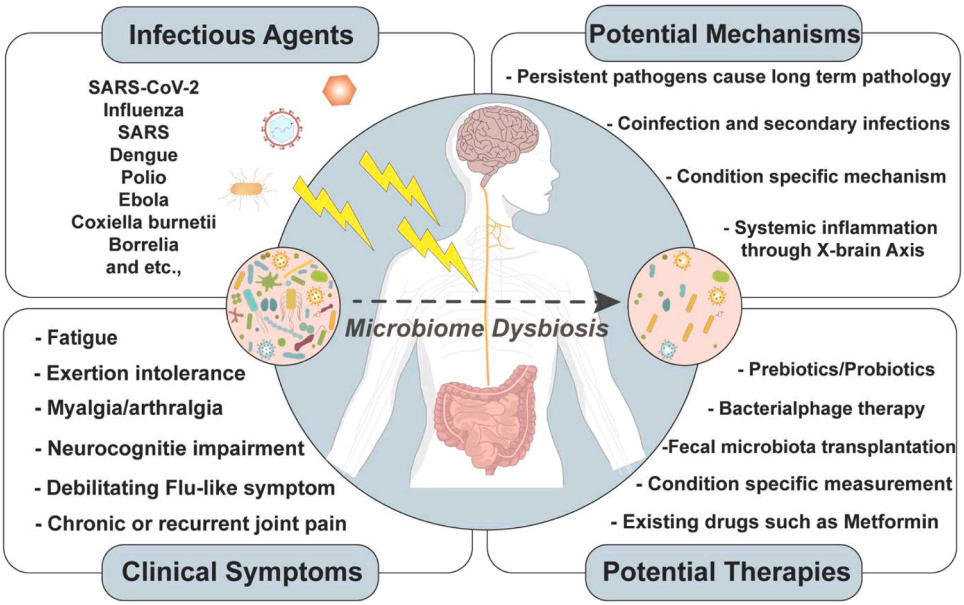

Fig. 1. Overview of the microbiome in PAIS.

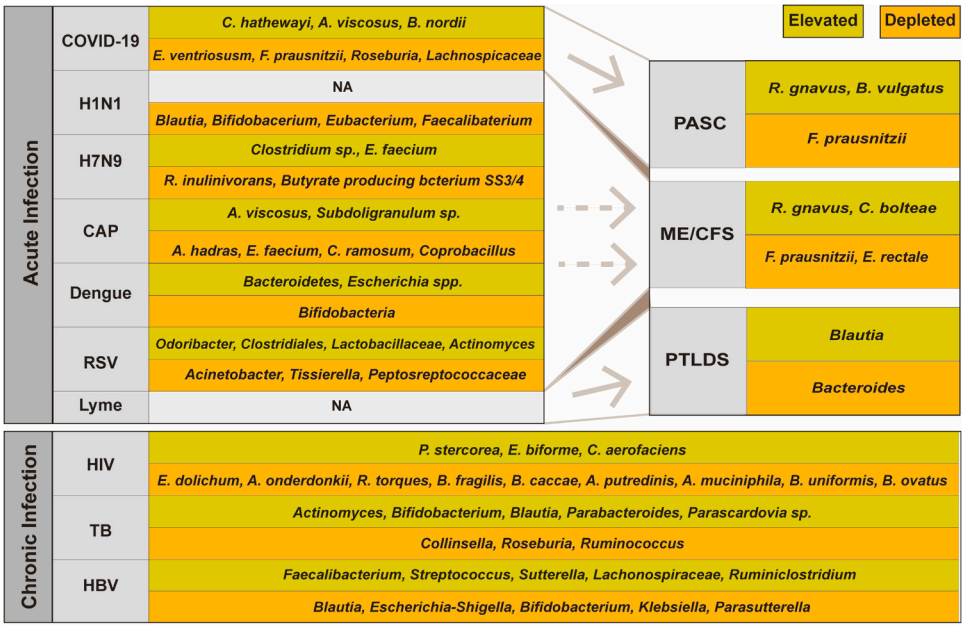

Fig. 2. The major taxa alterations in the gut bacteriome of acute infections, chronic infections and PAIS patients. Comparing to healthy controls, the major altered bacterial taxa identified in COVID-19 [27-29], H1N1 [31], H7N9 [32], CAP (Community-Acquired Pneumonia) [29], Dengue [39], RSV (Respiratory Syncytial Virus) [38], HIV (Human Immunodeficiency Virus) [33], HBV (Hepatitis-B Virus) [34], TB (Tuberculosis) [35], PASC (Post-acute Sequelae of COVID-19) [25,40-42], ME/CFS (Myalgic Encephalomyelitis/Chronic Fatigue Syndrome) [43-45] and PTLDS (Post--Treatment Lyme Disease Syndrome) [46] patients are listed in the figure. All the studies mentioned primarily focused on the gut bacteriome, except for the Dengue infection study, which was centered on the blood bacteriome

respiratory microbiome, resulting in an increase in the relative abundance of respiratory pathogenic streptococci and staphylococci. This dysbiosis did not return to normal during the recovery phase [37]. In another mice study, the overall gut microbiota composition after infection with either Respiratory Syncytial Virus (RSV) or Influenza A Virus appeared to be similar, which contradicts the findings of the aforementioned human studies [38], suggesting that the underlying mechanism is common to both infections, not a pathogen-specific immune effect.

3.2. Microbiome dysbiosis in patients with PAIS conditions

Under most circumstances, the infection-triggered microbial disturbance is anticipated to restore over time in convalescent individuals [39,47]. A longitudinal study investigating the oral and gut microbiomes of COVID-19 patients over a year after infection revealed a gradual recovery [40]. This recovery was accompanied by an increase in microbiome diversity, enhanced prevalence of butyrate producing microbes and Bifidobacterium, and a reduction in the presence of lipopolysaccharide (LPS) producing microbes. Despite this, persistent microbial dysbiosis was found in a proportion of patients with sequelae after acute infections. Given the urgency prompted by COVID-19 pandemic, the microbiome disturbance in PASC is an emerging medical condition that deserves greater attention. Together with PASC, the knowledge previously acquired from ME/CFS research is invaluable for advancing research efforts in PAIS [6].

Several studies have investigated the differences in gut microbiota composition between individuals who fully recovered from COVID-19 and those who developed post-acute sequelae of SARS-CoV-2 infection (PASC). The findings revealed discernible microbial differences at the time of diagnosis [25], three months later [41], six months later [42], and the discrepancy became more pronounced after one year [40]. The

microbial composition of gut samples collected at the admission showed potential for predicting PASC outcomes, indicating a connection between the microbiome and the recovery process. Unlike convalescents, patients who developed PASC failed to recover their microbiota richness to normal levels. In contrast to healthy individuals, patients with PASC had reduced bacterial diversity and abundance, characterized by elevated *Ruminococcus gnavus*, *Bacteroides vulgatus*, and depleted *Faecalibacterium prausnitzii*. The incidence of PASC was negatively correlated with the abundance of various commensal bacteria, which positively impact host immunity, including *Collinsella aerofaciens*, *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Blautia obeum*. It is still too early to conclude, but different symptoms in PASC patients seemly represent unique gut microbial features [25]. In addition, oral and nasal microorganisms exhibited immunomodulatory effects among PASC patients, which appeared to be correlated with both the duration of COVID-19 symptoms and PASC development [48].

Studies on gut dysbiosis in ME/CFS patients have identified a clear distinction among different cohorts [43–45]. Considering various confounding factors, multi-omics analysis on a cohort consisting of 106 patients with ME/CFS and 91 healthy controls has revealed notable differences in the diversity and abundance of the gut microbiome [43]. The abundances of *Faecalibacterium prausnitzii* and *Eubacterium rectale* were found to be reduced in individuals with ME/CFS, which were also confirmed with qPCR experiments. The qPCR results also revealed an intriguing finding that a higher fecal total bacterial load in ME/CFS patients comparing to controls. A negative association was observed between the abundance of *Faecalibacterium prausnitzii* and the severity of fatigue experienced by these individuals. Similar to PASC, dysbiosis of the oral microbiome was observed in ME/CFS individuals, which was characterized by an increased abundance of *Leptotrichia*, *Prevotella*, and Fusobacterium species [49]. A study on Post-Treatment Lyme Disease Syndrome (PTLDS) patients also suggested a unique microbiome signature, characterized by *Blautia*, *Staphylococcus aureus*, and *Roseburia*, which enabled accurate classification of over 80% of cases examined with machine learning methods [46]. Specifically, the signature is characterized by an increase in *Blautia*, a decrease in *Bacteroides*, along with several other alterations (Fig. 2).

Various fungi, viruses, and archaea coexist with bacteria, and their presences and potential functions cannot be neglected. Patients with long COVID-19 exhibited higher levels of fungal translocation from the gut or lung epithelium, which stimulated cytokine production when compared to patients without PASC or healthy controls [50]. This fungal-related coinfection has been linked to elevated mortality rates in COVID-19 patients, with approximately 54.6% case fatality rate associated with secondary fungal infections [51]. Evidence of active viral and fungal infections in individuals with ME/CFS who experience prolonged and unexplained symptoms was also indicated by previous studies [52], although no significant ME/CFS-specific differences were identified in our recent research utilizing unbiased high-throughput sequencing, capture sequencing, and both multiplex and targeted PCR assays [53].

4. Possible mechanisms

Our current understanding of how infection leads to microbiome dysbiosis is inadequate. To our best knowledge, it is only investigated once that the gut microbiome alteration was linked to immune-mediated inappetence by a mouse study [38]. It remains unexplained for why some individuals would fail to recover from acute infections with a continuing microbiome dysbiosis, as aforementioned in PAIS, and the underlying mechanism appears tightly related to the microbiome dysbiosis in acute infection and PAIS. In this regard, we hypothesize potential mechanisms of PAIS pathogenesis related to the dysregulation of the microbiome generated from the initial infection and ensuing immune responses.

Pathogens in either persistent or latent state can directly cause long-term pathology. Many viruses have been found to either regulate or closely correlate with genes involved in pro-inflammatory and anti-apoptotic pathways, thereby resulting in chronic neuroinflammation [54]. Even though their function is not yet apparent, Ebola viral particles were detected in semen samples several years after the initial infection [55], and Zika viruses were observed to persist for months in both cerebrospinal fluid and lymph nodes of rhesus monkeys following recovery [56]. In another study, influenza viruses were found to regulate the long-term expression of genes related to inflammation, neurons, and glia in mice up to a month after the initial infection [57]. Moreover, ample evidence of residual pathogens was noticed in known PAIS [3]. The prolonged presence of SARS-CoV-2 in the gastrointestinal tract of COVID-19 patients directly contributes to the degradation of the intestinal mucosal barrier through chain protein activity, implying a possible mechanism for long-term ecological dysregulation of the gut [58,59]. Similarly, Enterovirus protein and RNA were found in patients years after ME/CFS diagnoses in nearly thirty independent studies, suggesting at least a subgroup of ME/CFS is caused by enterovirus infection [60]. It is notable that numerous studies have indicated the virus could exist in bacterial organisms and then can be introduced into human cells [61, 62]. Such interactions could lead to a distinct and potent form of viral persistence, which may partially account for the observed gut dysbiosis in patients with PAIS.

Apart from the original infectious agents, mounting evidence shows that coinfection or secondary infection can worsen or prolong symptoms. For instance, SARS-CoV-2 infection often coincides with the reactivation of latent viruses or opportunistic bacterial pathogens, including Epstein Barr Virus (EBV) [63], Cytomegalovirus [64], various types of Herpes virus [65], and LPS-producing bacteria [66]. The resurrection of latent pathogens can cause profound damage to health [67]. HSV-2 reactivation can cause memory CD4 + T cells to temporarily open the blood-brain barrier, which may impair neural tissues' ability to deal with inflammatory cytokines and leukocytes, ultimately exacerbating neurological symptoms, and leading to PASC [68]. On the other hand, acyclovir is known as a medicine to treat HSV or EBV infections by blocking viral replication. A randomized controlled trial found that additional acyclovir treatment reduced the risk of death in patients with severe COVID-19 [69].

The gut-brain axis (GBA) is a bi-directional communication network between the enteric and central neural systems, influencing immune pathways with inflammatory signaling [70]. Given that over 70% of both microorganisms and immune cells reside in the intestinal tract, it is tempting to speculate on the critical role microbiome plays in modulating GBA pathway in PAIS [71]. A range of pathogens (Coronaviruses, HIV, Zika virus, Enteroviruses, Rotaviruses, Influenza virus, and others) are known to cause GBA disturbance by affecting gut bacteriome, virome and mycobiome, which further regulate the brain activity. Driven by the alteration of microbiome, GBS-associated key modulators, including short-chain fatty acids, 5-hydroxytryptamine, cytokine, cholecystokinin, and LPS have been found altered in different PASC and ME/CFS studies [72]. Furthermore, as one of the potential outcomes, fatigue appears to be linked to neuroinflammation, which is a main clinical manifestation in the PAIS [73]. In a mouse study, the torpor nucleus within the hypothalamus was found to be associated with fatigue, suggesting the activation of GBA in the brain may stimulate relevant fatigue nucleus [74]. The pathogen can either directly cause inflammation in the brain or initially trigger systemic inflammation through gut microbiome, then leading to inflammation and ultimately resulting in elevated inflammatory responses, immune dysregulation, and neurological symptoms in both PASC and ME/CFS [75]. Many gut bacterial species altered in the PAIS, such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bacteroides thetaiotaomicron*, were known to have immunomodulatory properties. For instance, *Faecalibacterium prausnitzii* was inversely correlated with severity of symptoms in ME/CFS [43]. In addition, elevated levels of *Prevotella*, *Veillonella* and other gram-negative bacteria were observed in the oral cavity of

COVID-19 patients, of whom many developed into PASC later. The altered pattern in oral microbiome is also observed in ME/CFS patients, suggesting the mediated systematic inflammation in PAIS by releasing LPS through the oral-brain axis [49].

Specific mechanistic pathway may underlie individual PAIS conditions. It has been hypothesized that antibiotics trigger ME/CFS through D-lactate toxicity, though no improvement in fatigue has been seen in recent studies by targeting the D-lactic acid producing bacteria with antibiotics or probiotics [52]. It is also notable that antibiotics may exert profound influence on the disease progression in both ME/CFS and PASC [1,54]. In COVID-19, ACE2 acts as a primary receptor for the virus to enter human cells, but it is also vital in regulating the renin-angiotensin-aldosterone system and controlling the composition of the intestinal microbiome to regulate immune responses. Alterations in the ACE2 balance have been linked to unfavorable outcomes for COVID-19 patients, ultimately leading to PASC [76,77]. It is also reported that *Bacteroides thetaiotaomicron* can affect PASC by regulating both ACE2 expression and anti-inflammatory activity [78]. A recent study has shown that SARS-CoV-2 can replicate in human gut bacteria before attaching to human cells [79], which demonstrated the possibility that SARS-CoV-2 can act as a bacteriophage infecting human microbiota. Additionally, differences in the prevalence and clinical severity of PASC and ME/CFS were observed between sexes. Among patients diagnosed with ME/CFS, the prevalence can be 4 times higher in female than in male, and male patients were also observed to have milder symptoms and a better quality of life compared to female patients [80]. Also, female gender was recognized as a key factor associated with PASC diagnosis [81]. It may indicate a potential role for sex hormones and chromosomes, particularly estrogens and testosterones, in PAIS development [82,83]. Since females consistently exhibit increased inflammatory responses compared to males after puberty, the interplay between sex hormones, microbiome, and PASC pathogenesis cannot be ignored. Future studies focusing on PASC among genders may assist in improving understanding of the mechanism [81]. Lastly, it is worth noting that the proposed hypotheses above may exhibit overlapping and interconnected mechanisms, and pathways in the etiopathogenesis of PAIS unrelated to the microbiome are not discussed in this review.

5. Potential therapeutic interventions targeting microbiome

When seemingly disparate findings from different PAIS are interpreted through the lens of these microbiome-based paradigms, a cohesive picture of the disease progression emerges. The pathogenesis of PAIS was triggered by varied pathogenic infections originating from pathogen-induced malfunction, which led to microbiome dysbiosis that varies depending on the patient's individual disease history and surroundings. Interventions to promote the human immune response may aid in the reversal of the inflammatory disease process in PAIS patients. Potential therapeutic approaches including prebiotics, probiotics, fecal transplants, antibiotic, bacteriophages therapy, and microbiome-derived metabolites may similarly modify host flora and decrease virus-induced intestinal inflammation, therefore, to prevent or alleviate PAIS development.

When consumed, probiotics are live microorganisms and prebiotics are typically high-fiber foods for human microflora to maintain or improve healthy microflora. The effects of probiotics/prebiotics therapy have been evaluated in several clinical and experimental human studies. A recent study demonstrated that treating patients with *Lactobacillus* probiotics and inulin can relieve acute and long-term symptoms among individuals with COVID-19 [84]. Another randomized controlled trial indicated probiotic intake had a positive effect on PASC by improving a variety of symptoms [85]. Prebiotics/probiotics can modulate the gut microbiome to help manage gastrointestinal symptoms. Increasing dietary fiber intake can cause structural alterations in gut microbiota, mitigating both gastrointestinal and non-gastrointestinal symptoms such as anxiety and palpitations in patients with PASC [86]. A case report showed a two-month treatment of high-fiber formula helped alleviate gastrointestinal symptoms by modulating the gut microbiota in one PASC patient [87]. There have been numerous studies involving treatment for ME/CFS as well [88]. However, it is still inconclusive to consider probiotics as a treatment for gastrointestinal symptoms in ME/CFS patients due to the overall poor study quality [89]. Nowadays, the effects of prebiotics/probiotics therapy have been assessed in ongoing clinical trials for both ME/CFS and PASC (NCT04741841, NCT04950803, and NCT05080244).

Fecal microbiota transplantation (FMT) is another therapeutic method using gut microorganisms from donated feces to treat the gut microbiome dysbiosis associated with pathological health conditions. It has achieved impressive therapeutic effects for recurrent *Clostridioides difficile* infection in 90% of cases [90]. In terms of treating ME/CFS, fecal transplantation has also been discussed. There are several preliminary indications that the microbiome might be an important player in the disease, especially its neurological symptoms [91]. Two controlled studies in double-blind randomized trials are presently underway (NCT04158427 and NCT03691987) to assess the effectiveness of FMT in treating ME/CFS. Meanwhile, there is no completed trial to evaluate the clinical efficacy of FMT treatment in post-COVID-19 neuropsychiatric conditions yet (NCT05556733).

Due to the possibility of COVID-19 leading to bacterial coinfection, antibiotics were frequently prescribed to patients. Antibiotic usage can eliminate bacterial pathogens while also affecting the normal symbiotic microbiota, and excessive use can lead to increased bacterial resistance and dysbiosis of the microbiota. Although numerous studies have suggested antibiotic usage is not associated with disease development in both PACS and ME/CFS [52,81], more studies are warranted to investigate the role of antibiotics in the PAISs, and antibiotic usage should be taken as a potential covariable in the analysis. Meanwhile, the issues of antibiotics encourage the search for alternative methods of limiting bacterial growth and restoring the normal balance of the microbiota in the human body. Bacteriophages are promising candidates as potential regulators of the microbiota, facing the increasing problem of bacterial resistance to antibiotics [92]. Bacteriophage cocktail for oral administration targeting multiple species of bacteria was used for the rehabilitation of PASC patients and showed significant improvements in saturation and respiratory rate as well as a decrease in inflammatory markers [93].

Furthermore, metformin is usually prescribed for diabetes to control blood sugar levels, partly by altering the gut microbiome. A recent study revealed that the administration of metformin induced a 42% reduction in COVID-19 sequelae relative to controls [94]. Increased *Escherichia coli* abundance due to metformin supplementation, as well as decreased *Intestinibacter bartlettii* abundance and increased SCFA production in humans, may be responsible for metformin-mediated health effects, specifically with regard to gut microbiota modifications [95]. It has been suggested that by activating AMP-activated protein kinase, metformin causes ACE2 to be phosphorylated, leading to a conformational change that might prevent SARS-CoV-2 from binding to the receptor. In vitro, metformin increases the mRNA expression of ACE2 and TMPRSS2 in human hepatocytes [96]. Lastly, the level of ACE2 activity is modulated by the local microbiome. The success of metformin also sheds light on other potential therapies for COVID-19 bowel physiology and PASC.

6. Future directions of research on the microbiome of PAIS

Consisting of PASC, ME/CFS, and other under-recognized post-acute infection sequelae, PAIS conditions represent vital global public health concerns. The overlap in symptoms, signs, and clinical features suggests a unified pathological mechanism. The associations among the gut microbiome, acute infection, and PAIS appear obvious, while current research has not yet established causality. With the various constellations of symptoms and severity observed in PAIS patients, multiple mechanisms may be involved. To solve the mystery and advance the

field, well-designed longitudinal animal and human studies that consider physical activity levels, antibiotic intake, vaccination status, and other potential confounding factors are crucial to systematically investigate the association between microbiome and the development of PAIS. Despite the complexity, the field is expanding with substantial opportunities, and current knowledge presents a unique opportunity to apply microbiome-relevant applications in prevention, diagnosis, and treatment of PAIS.

Author contributions

JL and CG contributed to conception of the review. CG, BY and JW wrote the manuscript. CG and BY contributed equally and share the first authorship. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key Research and Development Program of China (2018YFE0208000).

Declaration of Competing 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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