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Alterations of the gut microbiota in coronavirus disease 2019 and its therapeutic potential

Gut Microbiota and COVID-19

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a serious threat to global health. SARS-CoV-2 infects host cells primarily by binding to angiotensin-converting enzyme 2, which is coexpressed in alveolar type 2 cells and gut epithelial cells. It is known that COVID-19 often presents with gastrointestinal symptoms and gut dysbiosis, mainly characterized by an increase in opportunistic pathogens and a decrease in beneficial commensal bacteria. In recent years, multiple studies have comprehensively explored gut microbiota alterations in COVID-19 and highlighted the clinical correlation between dysbiosis and COVID-19. SARS-CoV-2 causes gastrointestinal infections and dysbiosis mainly through fecal-oral transmission and the circulatory and immune pathways. Studies have shown that the gut microbiota and its metabolites can regulate the immune response and modulate antiviral effects. In addition, the gut microbiota is closely related to gastrointestinal symptoms, such as diarrhea, a common gastrointestinal symptom among COVID-19. Therefore, the contribution of the gut microbiota in COVID-19 should not be overlooked. Strategies targeting the gut microbiota via probiotics, prebiotics and fecal microbiota transplantation should be considered to treat this patient population in the future. However, the specific alterations and mechanisms as well as the contributions of gut microbiota in COVID-19 should be urgently further explored.

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Key Words: COVID-19; SARS-CoV-2; ACE2; Gut microbiota; Dysbiosis; Lung

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Core Tip: ¹Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global health threat. SARS-CoV-2 ¹infects host cells through binding to angiotensin-converting enzyme 2. COVID-19 patients exhibit gut dysbiosis. Here, the gut microbiota alterations in COVID-19 are summarized. ²The pathways and possible mechanisms of dysbiosis caused by SARS-CoV-2, as well as the impact of the gut microbiota and its metabolites on the inflammatory response and antiviral effects during the course of the disease, are also described. Therefore, targeting the gut microbiota should be considered ³a promising strategy for COVID-19 ²prevention, treatment, and prognostic assessment. ¹

¹⁵INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a new acute infectious disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the *Coronaviridae* family^[1]. SARS-CoV-2 ³²infects host cells through the binding of the S protein to angiotensin-converting enzyme 2 (ACE2) ⁶and interacts with transmembrane serine protease 2 (TMPRSS2), which cleaves the viral S-protein, allowing efficient viral fusion^[2,3]. Interestingly, ACE2 is coexpressed in alveolar type 2 cells and intestinal and colonic epithelial cells, especially in small intestinal enterocytes^[4,5]. Correspondingly, in addition to infecting the respiratory system and causing respiratory symptoms (e.g., fever, dry cough, dyspnea, myalgia, headache, etc.), ²⁹SARS-CoV-2 also infects the gastrointestinal tract, where it replicates abundantly^[6]. Overwhelming evidence substantiates the detection ²⁹of viral RNA in fecal samples or rectal swabs from COVID-19 patients^[6-8]. It is estimated that up to 48.1% of COVID-19 patients had fecal samples positive for viral RNA^[7], even when the virus was not detected in respiratory and/or sputum samples^[9]. Moreover, researchers have found

56 that the gut microbiota composition of COVID-19 patients exhibits significant alterations (dysbiosis), mainly characterized by 34 an increase in the abundance of opportunistic pathogens and a decrease in the abundance of beneficial commensal bacteria⁷⁰[10]. Gut dysbiosis is closely associated with gastrointestinal symptoms and disease severity⁶⁵[11,12]. Therefore, the crosstalk between the gut microbiota and COVID-19 is gaining attention.

2 The gut microbiota has become a hot research topic in recent years. The resident microbial composition of the human gut mainly includes bacteria, archaea, viruses and fungi¹³. The human gut microbiota consists of more than 10^{14} bacteria and comprises approximately 500 to 1000 species. Gut bacteria in healthy individuals are mainly *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes*¹³. The complex gut microbiota communities have 18 important genomic and enzymatic properties and perform a critical role in the immune system, which protects against pathogens and helps maintain gut microbiota homeostasis. Gut microbiota homeostasis is essential for maintaining human health. Conversely, dysbiosis can lead to metabolic disturbance, immune dysfunction and systemic inflammation and has been linked to various diseases¹⁴.

2 Therefore, this review mainly summarizes the gut microbiota alterations and the possible mechanisms of dysbiosis in COVID-19. Furthermore, we highlight the theoretical basis 2 that the gut microbiota can be considered a promising therapeutic target in COVID-19, potentially interfering with immune and inflammatory responses and antiviral effects. Finally, we also reviewed multiple interventions 12 targeting the gut microbiota, such as prebiotics, probiotics and fecal microbiota transplantation (FMT), which could optimize COVID-19 treatment.

3 GUT DYSBIOSIS EXISTS IN COVID-19 AND IS ASSOCIATED WITH DISEASE SEVERITY

Gut dysbiosis in COVID-19 patients has received widespread attention in recent years (Table 1). The 1 gut microbiota is significantly altered in COVID-19 patients receiving and not receiving medication compared to that in non-COVID-19

individuals^[12]. Gut dysbiosis persists throughout the course of the disease and even after viral clearance^[10,12]. Yeoh *et al*^[12] suggested that members of the *Bacteroidetes* phylum and *Actinobacteria* were predominant in COVID-19 and non-COVID-19 individuals, respectively. In an American cohort study, *Peptoniphilus*, *Corynebacterium* and *Campylobacter* were identified as the most enriched genera in COVID-19 patients^[23]. Immunomodulatory gut bacteria, such as *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Bifidobacterium adolescentis* and *Bifidobacterium longum*, were depleted in COVID-19, and their depletion was correlated with an elevation in the levels of inflammatory cytokines and markers (CXCL10, IL-10, TNF- α , CCL2, and CRP)^[12]. However, Tao *et al*^[16] found that COVID-19 patients had higher abundances of *Clostridium*, *Veillonella*, *Streptococcus*, *Fusobacterium*, *Lactobacillus*, *Escherichia* and *Bifidobacterium* and lower abundances of *Bacteroidetes*, *Sutterella*, *Faecalibacterium*, *Coprococcus* and *Parabacteroides* than controls. The increased richness of *Streptococcus* exacerbates the risk of opportunistic pathogenic infections^[24].

Moreover, it is widely thought that gut dysbiosis is closely associated with COVID-19 severity. The basal gut microbiota of healthy individuals and its alterations during SARS-CoV-2 infection influence host susceptibility to SARS-CoV-2 and disease recovery. The abundances of *Coprobacillus*, *Clostridium ramosum* and *Clostridium hathewayi* were significantly and positively associated with COVID-19 severity, while the abundances of *Bifidobacterium bifiduz*, *Alistipes onderdonkii* and *F. prausnitzii* were negatively associated^[10,12]. However, even different members of the same phylum, such as Firmicutes, have opposing correlations in influencing disease severity and regulating ACE2 expression^[10]. ACE2 presents a critical role in gut microbial ecology, gut inflammation and innate immunity^[25]. Moreover, the abundances of other bacteria, including *Bacteroides dorei* (*B. dorei*), *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*), *Bacteroides ovatus* (*B. ovatus*), *Ruminococcus*, *Clostridium citroniae*, *Bifidobacterium*, and *Haemophilus parainfluenzae*, were negatively associated with viral load in fecal samples from SARS-CoV-2-infected patients^[10]. Interestingly, *B. dorei*, *B. thetaiotaomicron*, and *B. ovatus* can

downregulate ACE2 expression in the murine gut^[10,26], suggesting that Bacteroides species may play a protective role against SARS-CoV-2 infection by interfering with ACE2 production. The abundances of Erysipelotrichaceae bacterium, Prevotella copri and Eubacterium dolichum have been reported to be positively correlated with fecal viral load^[10, 19]. Furthermore, Prevotella, Enterococcus, Enterobacteriaceae, and Campylobacter can contribute to higher infectivity and worse prognosis in COVID-19^[27]. For instance, Prevotella is associated with enhanced T helper 17 (Th17)-mediated mucosal inflammation, stimulating the production of cytokines and subsequently promoting neutrophil recruitment and inflammation^[28]. Although it has been established that an altered gut microbiota composition is prevalent in COVID-19, conflicting results have been reported due to the heterogeneity of the gut microbiota itself, the sample size, and so on. Geographic and demographic differences also appear to affect the conclusions and dysbiosis recovery after SARS-CoV-2 infection^[10,29]. Therefore, targeting specific gut microbiota alterations represents a potential strategy to alleviate disease severity in COVID-19.

POTENTIAL PATHWAYS AND MECHANISMS OF SARS-COV-2-INDUCED GUT INFECTION AND DYSBIOSIS

Generally, SARS-CoV-2 is principally transmitted via respiratory droplets and close contact, subsequently inducing diverse symptoms^[30]; however, this paradigm does not explain how SARS-CoV-2 causes gut infection and dysbiosis. An *in vitro* study reported that the virus could be transmitted by the fecal-oral route through contaminated water, food, etc.^[30,31]. Since then, a new SARS-CoV-2 transmission route has been revealed (Figure 1). Notably, many viral activities are greatly diminished or even lost after passing through the gastrointestinal tract since gastric and intestinal fluids (low pH, rich in bile and digestive enzymes) can destroy the viral lipid envelope, inhibiting infectivity. For example, SARS-CoV has long been thought to be inactivated under acidic conditions (pH < 3). However, SARS-CoV-2 seems to overcome this obstacle since virus have been detected in the feces of infected individuals^[30,31], and viruses isolated

from feces can survive for an additional 1-2 days^[32]. Therefore, SARS-CoV-2 may remain infectious in feces, particularly when patients have diarrhea^[1]. Furthermore, virus-containing sputum swallowed by COVID-19 patients may be another pathway of gut infection because viscous sputum can protect the virions, preserving virus infectivity^[33]. However, in the absence of evidence of fecal viral titers and viral viability in sewage and contaminated food, the capability of SARS-CoV-2 to be transmitted by the fecal-oral route requires further confirmation.

Moreover, circulatory and immune pathways are reportedly critical for SARS-CoV-2 to cause gut infection and dysbiosis (Figure 1). Studies conducted on SARS showed that coronaviruses damage lung tissue and then migrate to the systemic circulation, where they migrate to gut cells through the circulatory and lymphatic systems^[34]. The virus can be found in the blood samples and gut of COVID-19 patients; in addition to those in epithelial cells, viral components are mainly present in intestinal lymphocytes and macrophages^[31, 35]. Therefore, it is speculated that SARS-CoV-2 may be transported from the lungs to other tissues, including the gastrointestinal tract, through transport via immune cells, similar to influenza virus^[35, 36]. Subsequently, SARS-CoV-2 invades gut epithelial cells by binding to ACE2^[37], causing the release of cytokines and chemokines and triggering a gut inflammatory response characterized by neutrophil, macrophage, and T-cell infiltration, which further promotes dysbiosis^[38-40]. In addition, the amino acid transport function of ACE2 is related to the microecology in the gut. B⁰AT1, a molecular ACE2 chaperone, mediates the absorption of neutral amino acids in the intestinal epithelium^[41]. Studies have confirmed that SARS-CoV-2-induced downregulation of B⁰AT1 on gut epithelial cells contributes to gut barrier disruption and dysbiosis, promoting pathogen invasion and COVID-19 exacerbation^[42-44]. De Oliveira *et al*^[45] hypothesized that the internalization of SARS-CoV-2 causes ACE2 downregulation, leading to mTOR inhibition and intestinal autophagy activation. Interestingly, autophagy can regulate the gut microbiota, and increased autophagy has been associated with diarrhea^[45, 46]. There is currently no evidence, however, that the ACE2/mTOR/autophagy pathway is involved in the pathogenesis of COVID-19.

Paradoxically, *Coprobacillus*, which is mostly correlated with the COVID-19 severity, has been shown to increase colonic expression of ACE2^[26,47]. Therefore, the net expression of ACE2 in the gut remains largely unclear, warranting further investigation.

Misuse and overuse of antibiotics, common in initially treating COVID-19 patients^[48], significantly impact the gut microbiota (Figure 1). Yeoh *et al*^[12] found that COVID-19 patients treated with and without antibiotics had different gut microbiota composition. In addition, antibiotics attenuated the antiviral activity of commensal-enhanced type I interferons (IFN-I)^[49]. Therefore, antibiotics are unlikely to improve patient outcomes without comorbid bacterial infections but instead exacerbate and prolong gut dysbiosis in this patient population.

THE IMPACT OF THE GUT MICROBIOTA AND ITS METABOLITES ON THE INFLAMMATORY RESPONSE AND ANTIVIRAL ACTIVITIES

Immune and inflammatory responses are important pathophysiological mechanisms in the pathogenesis of COVID-19. SARS-CoV-2 invades cells through ACE2 and TMPRSS2, where it replicates rapidly, producing and releasing large numbers of viruses and inducing excessive inflammatory cytokine release ("cytokine storm"), such as IL-6, IL-1 β , TNF- α and IFN^[41,50] (Figure 2). Excessive production of proinflammatory cytokines is pathologically associated with acute respiratory distress syndrome, extensive tissue damage, and even death. Viral replication after SARS-CoV-2 invades cells induces immune cells to recognize and bind viral pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), followed by the activation of the NF- κ B, IRF3 and JAK/STAT signaling pathways to induce the expression of proinflammatory factors, IFNs and IFN-stimulated genes (ISGs)^[41,51,52]. In addition, killing or damage of cells by SARS-CoV-2 results in the release of danger-associated molecular patterns (DAMPs), activating RLRs and NLRs and subsequently facilitating proinflammatory factor expression^[41,51]. Dysbiosis after SARS-CoV-2 infection further damages the gut barrier and promotes the production of inflammatory factors such as CXCL10, IL-2, IL-4, IL-6, IL-10, IL-18 and TNF- α ^[16,53,54]. For example, *B.*

²¹ *Lactobacillus reuteri* and *Akkermansia muciniphila* were positively associated with IL-1 β , IL-6 and CXCL8^[12]. Subsequently, opportunistic pathogens and inflammatory factors infiltrate the circulation and cause systemic inflammation and infection^[55]. Therefore, SARS-CoV-2 infection promotes gut inflammation to aggravate dysbiosis, which in turn exacerbates inflammation and disease progression, forming a vicious cycle.

² The gut microbiota and its metabolites influence the host immune response, inflammation and the development and regression of pulmonary infectious diseases, such as influenza A virus and *Streptococcus pneumoniae*^[56]. A healthy microbiome protects against respiratory viral infections^[57,58]. During respiratory viral infection, the ²⁰ gut microbiota regulates the host immune response ³⁸ via the gut-lung axis, which refers to the crosstalk between the gut microbiota and lung. Therefore, endotoxins and ⁴² microbial metabolites can affect the lung through the blood circulation, and conversely, lung inflammation will affect the gut microbiota. It has been reported that gut dysbiosis significantly increases mortality from respiratory viral infections, which may be associated with dysregulated immune responses; increased secretion of ⁴¹ IFN- γ , IL-6, and CCL2; and decreased Treg cell counts in the lung and gastrointestinal tract^[59]. The gut ⁶⁴ microbiota facilitates inflammasome activation, pro-IL-1 β and pro-IL-18 expression, and DC migration, which are critical for protective immunity post-influenza virus infection^[60] (Figure 2). In addition, microbial-associated molecular patterns (MAMPs) are transmitted to parenteral tissues to activate PRRs in immune cells and influence innate immune responses^[61]. The gut microbiota reportedly regulates the expression of IFN-I receptors in respiratory epithelial cells that limit viral replication and increase resistance to viral infections through IFN- α and IFN- β ^[58,62]. The gut commensal microbiota promotes the IFN-I response and ISG expression by inducing IFN- β expression mediated by TLR4-TRIF signaling through colonic lamina propria DCs, thereby enhancing antiviral capacity^[62]. The gut microbiota can also affect respiratory mucosal immunity through multiple mechanisms. On the one hand, activated immune cells within the mucosa can reach and affect distant mucosal sites, highlighting the ²⁷ beneficial effects of the gut microbiota during respiratory viral infections^[63,64]. On the

other hand, the gut microbiota affects the secretion of cytokines and growth factors by the gastrointestinal mucosa, which reach the systemic circulation and act on other mucosal tissues^[63,65]. Furthermore, severe COVID-19 clinical symptoms or complications and higher mortality are more likely to arise in elderly individuals or in patients with concomitant underlying disease, such as cardiovascular disease, diabetes, and cancer^[66]. Critically ill patients with COVID-19 often require invasive ventilator-assisted ventilation. However, gut microbiota intervention can reduce the demand for invasive mechanical ventilation in critically ill patients and reduce the incidence of ventilator-associated pneumonia^[67], although this finding needs further validation in COVID-19 patients. Therefore, restoring gut microbiota homeostasis is essential for inhibiting the inflammatory response and enhancing antiviral effects.

Gut microbiota metabolites are signaling molecules and substrates for metabolic reactions^[68]. Microbial metabolites are absorbed by the gut mucosa and participate in mucosal immune regulation, a process known as "metabolic reprogramming"^[69,70]. For example, short-chain fatty acids (SCFAs, mainly including acetate, propionate, and butyrate) can reach distant organs through the bloodstream to exert immunomodulatory and immunoglobulin expression-inducing effects, as well as anti-inflammatory and antiviral effects^[70,71] (Figure 2). Butyrate facilitates the polarization of M2 macrophage and exerts anti-inflammatory effects by increasing arginase 1 (ARG1) and decreasing TNF, Nos2, IL-6, and IL-12b expression^[41,72]. Butyrate also inhibits histone deacetylase activity or increases the Foxp3 promoter transcription in naive T cells, thereby promoting the naive T cells differentiation into Treg cells^[73,74]. Treg cells can suppress autoinflammatory responses and protect tissues from damage caused by pathological immune responses^[41]. Furthermore, butyrate restored CD8+ T-cell function in mice and inhibited proinflammatory cytokine production as well as eosinophilic lung infiltration^[49]. Unfortunately, a reduced richness of butyrate-producing bacteria such as *F. prausnitzii* and *Clostridium* species has been found in COVID-19 patients^[15,17,20,75]. Propionate promotes Treg cell proliferation by activating G-protein-coupled receptor 43 (GPR43)^[76]. Moreover, microbe-produced SCFAs (acetate) enhance B-cell metabolism

and gene expression, promoting the production of anti-SARS-CoV-2 antibodies and thereby inhibiting disease progression^[77]. Deaminotyrosine (DAT) produced by *Clostridium orbiscindens* augments IFN-I signaling to protect the host from viral infection^[57]. In addition, riboflavin is a product of gut microbiota constituents, which can activate mucosal-associated T cells (MAIT) *via* restrictive major histocompatibility complex (MHC)-related protein-1 (MR1)^[78,79]. MAIT cells, as innate sensors and mediators of the antiviral response, exert antiviral response to SARS-CoV-2 through the gut-lung axis^[80]. Antimicrobial peptides secreted by bacteria can promote virolysis, block cell–virus fusion, and induce adaptive immune responses, and thus they have been proposed as viable alternative therapeutic treatments for infections by viruses, such as MERS-CoV^[81]. Therefore, COVID-19 severity is tightly correlated with the levels of gut microbiota metabolites. However, studies on the direct action of gut microbiota metabolites in COVID-19 remain scarce.

REPROGRAMMING THE GUT MICROBIOTA AS A STRATEGY TO AMELIORATE GASTROINTESTINAL SYMPTOMS AND REDUCE DISEASE SEVERITY

The gut microbiota is closely related to various human gastrointestinal diseases and symptoms, such as gastrointestinal cancer, stomachache, diarrhea, and flatulence^[82,83]. For instance, *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, *Clostridium difficile*, and *Aeromonas* are the main pathogens that cause diarrhea^[84,85]. Reprogramming the gut microbiota *via* probiotics, prebiotics and FMT is well documented to effectively treat gastrointestinal diseases. Moreover, probiotic/prebiotic administration has been shown to protect against viral infections in multiple studies (e.g., those caused by influenza virus, rhinovirus, respiratory syncytial virus and coronaviruses). For example, Johnson *et al*^[86] found that *Bacillus subtilis* peptidoglycans reduced the infectivity of coronavirus. Peptidoglycan-associated surfactin can disrupt virion integrity, such as in influenza, Ebola, Zika, and Mayaro^[86]. Therefore, probiotics, prebiotics and FMT can restore ecological homeostasis

by regulating the gut microbiota, representing an effective alternative approach to ameliorate or suppress COVID-19 severity.

Probiotics, such as *Lactobacillus*, yeast, *Bifidobacterium*, *Enterococcus*, and *Bacillus*, are live microorganisms that benefit the host by colonizing the human body. Probiotics maintain healthy gut homeostasis and exert antiviral effects through the gut-lung axis^[87,88] and have been widely used to treat gastrointestinal diseases, including diarrhea (acute, antibiotic-associated and *C. difficile*-associated) and adult inflammatory bowel disease (IBD)^[89-91]. On the one hand, probiotics can strengthen Treg cell and natural killer cell activity and suppress proinflammatory cytokines, such as TNF- α , CRP, IL-1 β , IL2, IL-6, IL7, MCP1, and LDH^[92,93]. *Lactobacillus* exerts antiviral activity through direct probiotic-virus interactions, production of antiviral metabolites, and stimulation of the immune system^[94]. On the other hand, probiotics are beneficial for enhancing epithelial barrier function and improving gut microbial diversity. Furthermore, probiotics combat and block harmful bacterial strains in the gut or enhance beneficial signaling pathways^[95,96]. Existing evidence shows that probiotic miRNA modulation and regulation of signaling pathways such as NF-kB and STAT1 ameliorate COVID-19 complications^[97,98]. Probiotics have the potential to interact with ACE2; for example, some lactobacilli release peptides with a high affinity for ACE2^[93,99]. *Lactobacillus spp.* and *Bifidobacterium spp.* exhibit the strongest anti-respiratory virus activity via an immunomodulatory mechanism^[56]. In January 2020, relevant Chinese government departments recommended the addition of probiotics in COVID-19 treatment to improve gut microbiota homeostasis and protect against subsequent bacterial infections. However, evidence detailing the efficacy of probiotics in treating COVID-19 is limited, although relevant clinical trials are being conducted. Moreover, the safety of probiotics in COVID-19 patients should be emphasized to ensure that their use does not induce new gastrointestinal symptoms or secondary infections.

Prebiotics are substrates that are selectively utilized by host microorganisms to provide health benefits^[100]. Unfortunately, little information is available on using prebiotics to treat respiratory infections. In a clinical trial of prebiotics, KB109 was

found to regulate gut microbiome composition and increase the production of SCFAs in the gut (NCT04414124). For instance, butyrate and propionate obtained from the fermentation of prebiotics affect the differentiation or function of T cells, macrophages, and DCs^[93]. In addition, prebiotics selectively stimulate beneficial bacterial growth or enhance the activity of indigenous probiotics^[93]. Accordingly, the role of prebiotics in COVID-19 patients should be further emphasized.

FMT refers to the transplantation of a functional microbiota from healthy individuals into the gastrointestinal tract of patients to treat intestinal and extraintestinal diseases^[84]. An expanding body of evidence suggests that FMT can effectively treat many diseases, such as cancer, liver diseases, *C. difficile* infection, irritable bowel syndrome (IBS), and IBD^[101-103]. Since COVID-19 patients exhibit a depletion of beneficial commensal bacteria, FMT represents a theoretically promising strategy to mitigate disease severity. Regrettably, convincing clinical evidence for the effect of FMT in COVID-19 is lacking, and its safety and efficacy warrant further investigation.

CONCLUSION

COVID-19 was previously identified as a respiratory infectious disease. However, accumulating clinical studies have subsequently found that a large proportion of patients have gastrointestinal symptoms, such as abdominal pain, diarrhea, vomiting, and acid reflux, as well as significant gut microbiota alterations^[7,30]. The gut microbiota not only significantly affects the COVID-19 development and disease severity but also reflects the susceptibility of COVID-19 patients to long-term complications^[104]. Studies have confirmed that dysbiosis increases the poor prognosis of COVID-19^[104]. However, controversial results exist regarding gut microbiota alterations in COVID-19 patients. A variety of factors, such as sex, age, basic health status, medication use, genetics, ethnicity, and geographic location, can affect the composition of the gut microbiota and lead to individual differences and varying responses to SARS-CoV-2 infection. Therefore, further exploration of the specific gut microbiota alterations in COVID-19

patients and the clinical correlation between the gut microbiota and COVID-19 will be a very challenging and valuable research direction in the future.

Unfortunately, direct evidence for the contribution of the gut microbiota in COVID-19 remains lacking. For example, the exact mechanism of dysbiosis remains unclear. Moreover, the regulation of host immune and inflammatory responses and antiviral effects by the gut microbiota during SARS-CoV-2 infection relies largely on inferences or conjectures from previous studies. Given that specific drugs for COVID-19 remain enigmatic, vaccines represent the most effective prevention and control strategy; however, the continuous mutation of the virus has exacerbated this conundrum. Therefore, gut microbiota intervention through probiotics, prebiotics and FMT is undoubtedly one of the promising cosupplementation strategies for the future treatment of COVID-19, but large-scale studies are lacking and there are no corresponding treatment guidelines. The therapeutic prospects of the gut microbiota in COVID-19 are promising, but there is still a long way to go to realize its potential.

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Figure Legends

Figure 1. Potential pathways and mechanisms of gut infection and dysbiosis induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Fecal-oral transmission of SARS-CoV-2 in contaminated water and food, or by swallowing virus-laced sputum, results in gut infection and dysbiosis. SARS-CoV-2 infects alveolar type 2 cells and damages lung tissue before invading the gut through circulating immune cells and the lymphatic system and infiltrating gut epithelial cells via angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), subsequently triggering gut inflammation and further promoting dysbiosis. Internalization of SARS-CoV-2 Leads to downregulation of ACE2, resulting in inhibition of mTOR and subsequent activation of gut autophagy, which modulates the gut microbiome.

However, gut microbiota top associations with disease severity in coronavirus disease 2019 (COVID-19) patients, such as *Coprobacillus*, have been shown to upregulate ACE2 expression in the gut. The improper use of antibiotics can also lead to dysbiosis.

Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes “cytokine storm” and the impact of gut microbiota and its metabolites on inflammatory response and antiviral effects. SARS-CoV-2 invades cells through angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) and replicates frantically, inducing innate immune cells (e.g. macrophages, DCs) to recognize and bind pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs). Subsequently, the expression of pro-inflammatory factors, IFNs and IFN-stimulated genes (ISGs) is induced through the NF- κ B, IRF3 and JAK/STAT signaling pathways, thereby promoting excessive inflammatory cytokine release. Killing or damaging cells by SARS-CoV-2 releases danger-associated molecular patterns (DAMPs) that facilitate the expression of pro-inflammatory factors via activation of RLRs and NLRs. In addition, dysbiosis damages the gut barrier (tight junctions) and promotes the production of inflammatory factors such as CXCL10, IL-2, IL-4, IL-6, IL-10, IL-18 and TNF- α .

Gut microbiota facilitates inflammasome activation, pro-IL-1 β and pro-IL-18 expression, and DCs migration, thereby promoting protective immunity post viral infection. microbial-associated molecular patterns (MAMPs) are transmitted to the parenteral tissues to activate PRRs and affect innate immune responses. Gut microbiota regulates IFN-I receptors expression in respiratory epithelial cells and exerts antiviral effects through IFN- α and IFN- β . Immune cells, cytokines, and growth factors in the gut mucosa reach the respiratory tract to regulate immunity and exert antiviral effects. Butyrate promotes M2 macrophage polarization, upregulating arginase 1 (ARG1), and downregulating TNF, Nos2, IL-6, and IL-12b exerts anti-inflammatory activity. Propionate promotes Treg cell proliferation by activating G-protein-coupled receptor 43 (GPR43), thereby inhibiting autoinflammatory responses and protecting tissues from

damage caused by pathological immune responses. Acetate promotes the production of SARS-CoV-2 antibodies by B cells, thereby inhibiting the development of COVID-19. The gut microbiota produces Deaminotyrosine (DAT) to enhance IFN-I signaling and protect the host from viral infection. Riboflavin, produced by the gut microbiome, activates ¹ mucosal-associated T cells (MAIT) *via* major histocompatibility complex (MHC)-related protein-1 (MR1) ³. MAIT cells participate in the immune response against SARS-CoV-2 through the gut-lung axis, thereby exerting antiviral efficacy.

Table 1. Alterations of the gut microbiota composition in coronavirus disease 2019 patients.

Study	Method	Increased abundance of gut microbiota	Decreased abundance of gut microbiota	Main conclusion	Ref.
Gu et al. (2020)	16S rRNA	Streptococcus, Rothia, Veillonella, Actinomyces, Erysipelatoclostridium	Ruminococcaceae family, Lachnospiraceae family, Agathobacter, Fuscatenibacter, Roseburia	<ul style="list-style-type: none"> ● Gut microbiota has potential value as a diagnostic biomarker and a therapeutic target for COVID-19. [45] ● Fecal microbiota alterations are associated with fecal virus levels and COVID-19 severity. [5] ● Symbionts were depletion and opportunistic pathogens were enrichment in COVID-19 patients. [15] ● Gut dysbiosis persists in COVID-19 patients after virus clearance. [8] ● IL-18 level was higher in the fecal samples from COVID-19 patients. [10] ● Dysbiosis may contribute to SARS-CoV-2-induced production of inflammatory cytokines and cytokine storm in the gut. [16] ● Specific gut microbiota can be considered diagnostic biomarkers for COVID-19. [17] ● The Ec/E ratio can be used to predict death in critically ill patients. ● The guts of COVID-19 patients are [18] 	
Zuo et al. (2020)	shotgun	22-stridium, hawthayii, Actinomyces viscosus, Bacteroides nordii	Eubacterium, Faecalibacterium prausnitzii, Roseburia, Lachnospiraceae		
Tao et al. (2020)	16S rRNA	Streptococcus, Clostridium, Lactobacillus, Bifidobacterium	Bacteroidetes, Roseburia, Faecalibacterium, Coprococcus, Parabacteroides		
Tang et al. (2020)	q-PCR	Enterococcus, Enterobacteriaceae	2 Faecalibacterium prausnitzii, Clostridium butyricum, Clostridium leptum, Eubacterium rectale		
Zuo et al. (2020)	shotgun	5 Candida albicans, Candida auris, Aspergillus flavus	-		

Yeoh et al. (2021)	shotgun	Actinobacteria, 1 Ruminococcus gnavus, Ruminococcus torques, Bacteroides dorei,	Bifidobacterium adolescentis, Faecalibacterium prausnitzii, Eubacterium rectale,	<ul style="list-style-type: none"> Immunomodulatory gut bacteria were depleted in COVID-19 patients. Gut dysbiosis persists in COVID-19 patients after virus clearance. Gut microbiota composition was associated with disease severity. Personalized microbiome affects disease outcomes in COVID-19 patients. 	[12]
Wu et al. (2021)	16S rRNA	Streptococcus, Weissella, Enterococcus, Rothia, Lactobacillus, Actinomycetes, Granulicatella	Blautia, Coprococcus, Collinsella	<ul style="list-style-type: none"> Targeting the gut microbiota has potential to prevent and treat COVID-19. 	[19]
1 Zuo et al. (2021)	shotgun	26 Collinsella aerofaciens, Collinsella tanakaei, Streptococcus infantis, Morganella morganii	Parabacteroides merdae, Bacteroides stercoris, Alistipes onderdonkii, Lachnospiraceae bacterium 1_1_57FAA	<ul style="list-style-type: none"> Elimination of gut SARS-CoV-2 activity and modulation of gut microbiome composition should be considered new treatments for Covid-19. 	[20]
Ly et al. (2021)	ITS sequencing	-	Ascomycota (Aspergillaceae, Candida parapsilosis, Talaromyces wortmannii); Basidiomycota (Malassezia yamatoensis, Rhodotorula mucilaginosa, Moesziomyces	<ul style="list-style-type: none"> The 3' end of SARS-CoV-2 genome was highly covered than the 5' end. Total gut fungal burden was significantly elevated in patients infected with SARS-CoV-2. Altered gut fungi and microbiota are closely related to patient clinical characteristics. 	[21]

<div>16</div> aphidis, <i>Trechispora</i> sp. and <i>Wallemia sebi</i>)				<div>14</div> Suskun et al. (2022)	<div>16</div> Bifidobacterium adolescentis, <i>Dorea</i> <i>formicigenerasus</i> , <i>Eubacterium dolichum</i> , <i>Eggerthella lenta</i>	16S rRNA	Faecalibacterium prausnitzii	<ul style="list-style-type: none"> First evaluate the microbiota composition in inflammatory syndrome in children cases. 	<div>22</div>
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COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

30%

SIMILARITY INDEX

PRIMARY SOURCES

- | | | |
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Internet | 226 words — 4% |
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