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Perspective

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Gut microbiota in COVID-19: key microbial changes, potential mechanisms and clinical applications

Fen Zhang[1,2,3,4], Raphaela I. Lau[1,2,3,4], Qin Liu[1,2,3], Qi Su[1,2,3], Francis K. L. Chan[1,2,3] & Siew C. Ng[1,2,3]

Abstract

The gastrointestinal tract is involved in coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The gut microbiota has important roles in viral entry receptor angiotensin-converting enzyme 2 (ACE2) expression, immune homeostasis, and crosstalk between the gut and lungs, the 'gut–lung axis'. Emerging preclinical and clinical studies indicate that the gut microbiota might contribute to COVID-19 pathogenesis and disease outcomes; SARS-CoV-2 infection was associated with altered intestinal microbiota and correlated with inflammatory and immune responses. Here, we discuss the cutting-edge evidence on the interactions between SARS-CoV-2 infection and the gut microbiota, key microbial changes in relation to COVID-19 severity and host immune dysregulations with the possible underlying mechanisms, and the conceivable consequences of the pandemic on the human microbiome and post-pandemic health. Finally, potential modulatory strategies of the gut microbiota are discussed. These insights could shed light on the development of microbiota-based interventions for COVID-19.

Sections

[1]Microbiota I-Center (MagIC), Shatin, Hong Kong S.A.R., China. [2]Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong S.A.R., China. [3]State Key Laboratory for Digestive Disease, Institute of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong S.A.R., China. [4]These authors contributed equally: Fen Zhang, Raphaela I. Lau.
e-mail: siewchienng@cuhk.edu.hk

Introduction

With the coronavirus disease 2019 (COVID-19) pandemic now in its third year, it seems possible that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will be here for the long term. However, there remain many unknowns, including the emergence of viral mutants, duration of natural and post-vaccination immunity, and reasons for persistent antigen-specific memory responses in COVID-19 (refs.[1,2]). Although SARS-CoV-2 primarily infects the respiratory tract, several lines of evidence point towards substantial involvement of the gastrointestinal tract, including the ability of SARS-CoV-2 to infect and replicate in intestinal enterocytes[3], increased expression of the viral entry receptor (angiotensin-converting enzyme 2 (ACE2) receptor) and of several membrane-bound serine proteases (such as transmembrane protease serine 2 (TMPRSS2) and TMPRSS4) in intestinal epithelial cells[4], as well as the presence of SARS-CoV-2 genomic and sub-genomic RNA in mucosal and faecal samples of individuals with SARS-CoV-2 infection[5–8]. In a longitudinal study of 113 patients with COVID-19, SARS-CoV-2 RNA was found in the faecal samples of nearly 50% of individuals during acute infection and faecal RNA shedding persisted for 7 months in 3.8% of patients[7]. Moreover, a wide range of gastrointestinal symptoms, including diarrhoea, nausea, vomiting, loss of appetite and abdominal pain, have been reported in patients with COVID-19, with a pooled prevalence of 17.6% according to a meta-analysis of 60 studies involving a total of 4,243 patients[9]. Another meta-analysis of 35 studies involving 6,686 patients suggested that COVID-19 with gastrointestinal involvement had a worse disease course[10] and, surprisingly, that gastrointestinal symptoms could even precede respiratory symptoms[11,12].

Emerging studies have begun to bridge the gap between the gut microbiome and the pathophysiology of COVID-19. In fact, the gastrointestinal tract is regarded as the largest immune organ in the body and its resident microbiota can regulate host immunity, defend against pathogens and support nutrient metabolism[13]. The composition of gut microbiota is determined and could be dynamically altered by numerous factors, including genes, diet, lifestyle, disease and ageing[14,15]. Gut dysbiosis with a reduction in microbial diversity is commonly linked to immune-mediated inflammatory and autoimmune diseases[16,17]. The gut microorganisms could also regulate local and systemic inflammatory activity[17], and studies have demonstrated that respiratory infections are associated with both compositional and functional alterations of the gut microbiota[18] through vital crosstalk between gut microorganisms and the pulmonary system, otherwise known as the 'gut–lung axis'[19]. The bi-directional interactions between the gut microbiota and the lungs have been linked with host immune responses to SARS-CoV-2 infection[20]. In particular, COVID-19 has been shown to be associated with microbiome alterations and gut barrier dysfunction in human studies, which could increase the translocation of bacterial products and toxins into the circulatory system and exacerbate the systemic inflammatory response[20,21]. Disruption of the gut microbiota could negatively influence the recruitment of immune cells to the lung, which could then increase the susceptibility of developing respiratory tract infections[22–24]. Furthermore, gut microorganisms could lead to a decreased expression of ACE2, a key regulator of innate immunity and microbial ecology, thereby influencing viral invasion and replication[25,26]. Overall, state-of-the-art microbiome analyses have advanced our understanding towards the critical but clinically underappreciated roles that gut microorganisms play in SARS-CoV-2 pathogenesis and COVID-19 severity and prognosis.

In this Perspective, we summarize the latest data on gut microbiota alterations in patients with COVID-19 globally and discuss evidence of the mechanical roles of these microorganisms in SARS-CoV-2 infectivity and disease outcomes. When available, we review clinical studies, in vitro and in vivo studies, systematic reviews, and high-quality narrative reviews on COVID-19 and the gut microbiome published between 31 December 2019 and 28 August 2022 (Supplementary Box 1). We also highlight knowledge gaps and share our viewpoints on the potential clinical applications of modulating the gut microbiota in the prevention and treatment of acute COVID-19 and post-acute COVID-19 conditions.

COVID-19 and gut microbiota

Acute COVID-19

Human studies. As of August 2022, a total of 46 human observational studies have reported alterations in gut microbiota composition in patients with COVID-19 at the time of diagnosis and during the disease course compared with individuals without COVID-19 (Supplementary Table 1). Amongst these studies, which originated from different populations in China, USA, Japan, Bangladesh, United Arab Emirates, Portugal, Germany and Hungary, the faecal microbiome of patients with COVID-19 showed decreased bacterial diversity[27–30] and reduced abundance of short-chain fatty acid (SCFA)-producing bacteria from the Lachnospiraceae, Ruminococcaceae and Eubacteriaceae families as well as increased opportunistic pathogens from Enterobacteriaceae families[27–29,31–37] compared with the faecal microbiome of healthy individuals. Specifically, the abundance of *Faecalibacterium*[27,28,31,33–35,37], *Eubacterium*[28,34,37,38], *Coprococcus*[27,35,36,38], *Ruminococcus*[27,28,35], *Lachnospira*[27,35,38] and *Roseburia*[27,35] was decreased, whereas that of *Enterococcus*[32,34–36], *Rothia*[28,32,35,36] and *Lactobacillus*[27,35,36] was increased. Intriguingly, a number of studies with a modest sample size of typically ~30 patients have reported that patients with COVID-19 had a distinct gut microbiome composition compared with patients with community-acquired viral pneumonia[39] and influenza virus infection[28,35]. In a study consisting of 62 patients with COVID-19, 33 patients with seasonal flu and 40 healthy individuals as controls, patients with COVID-19 had significantly decreased ($P = 0.0124$) bacterial diversity with enrichment of opportunistic pathogens, such as *Streptococcus*, *Veillonella*, *Fusobacterium* and *Escherichia*, as well as a higher faecal concentration of the pro-inflammatory cytokine IL-18 compared with patients with seasonal flu[35]. Another study of 30 patients with COVID-19 found a significantly higher relative abundance of phyla Actinobacteria (now known as Actinomycetota) and Firmicutes (now known as Bacillota) compared with 24 patients with H1N1 influenza infection[28]. Hospitalized patients with COVID-19 ($n = 15$) also showed an increased abundance of *Enterococcus faecium* and *Clostridium ramosum* compared with those hospitalized with other types of viral pneumonia ($n = 7$)[39]. Although the sample size of these studies remains small, these findings highlight that SARS-CoV-2 might have specific effects on the gut microbiota.

Although consistent gut microbial changes have been reported in patients with COVID-19, these studies had some limitations. First, most studies were cross-sectional and interindividual demographic differences could have greatly influenced the gut microbiome. Second, clinical information and symptoms at the time of sample collection were not always well presented and, in most studies, faecal samples before infection were not collected. Third, it remains unclear whether diet and medications, such as antibiotics and proton pump inhibitors, could influence these microbial changes specifically in the setting of COVID-19. Most studies have focused on adult populations and the effect of SARS-CoV-2 on the gut microbiome in children is comparatively less evident[33,37]. Lastly, the effect of variants of concern, such

as Omicron, on the gut microbiome is still largely unknown as most published studies so far have focused on earlier variants.

Studies in children. The gut microbiota in early childhood is less mature and more vulnerable to environmental exposures compared with that in adults[40]; hence, microbiome alterations during early life, whereby children's immune, metabolic and cognition systems are under development, have been associated with subsequent risks of several childhood illnesses, including infections, asthma, allergy and diabetes[41–44]. Preliminary data showed that an altered gut microbiome in nine children with COVID-19 aged between 7 and 139 months was dominated by the genus *Pseudomonas*, and this change persisted for weeks after hospital discharge[45]. In 12 asymptomatic young children aged 0–24 months with SARS-CoV-2 infection, depletion of *Bifidobacterium bifidum* and *Akkermansia muciniphila*, which are linked to protection against inflammation, were observed in SARS-CoV-2-positive faecal samples[46]. Multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19 has been increasingly recognized[47]. Interestingly, substantial changes in the composition of the gut microbiota were found in children with MIS-C ($n = 25$) and COVID-19 ($n = 20$), including a reduction of *Faecalibacterium prausnitzii*[48]. Nevertheless, whether the development of MIS-C might, in part, be attributed to COVID-19-related microbial dysbiosis remains unclear.

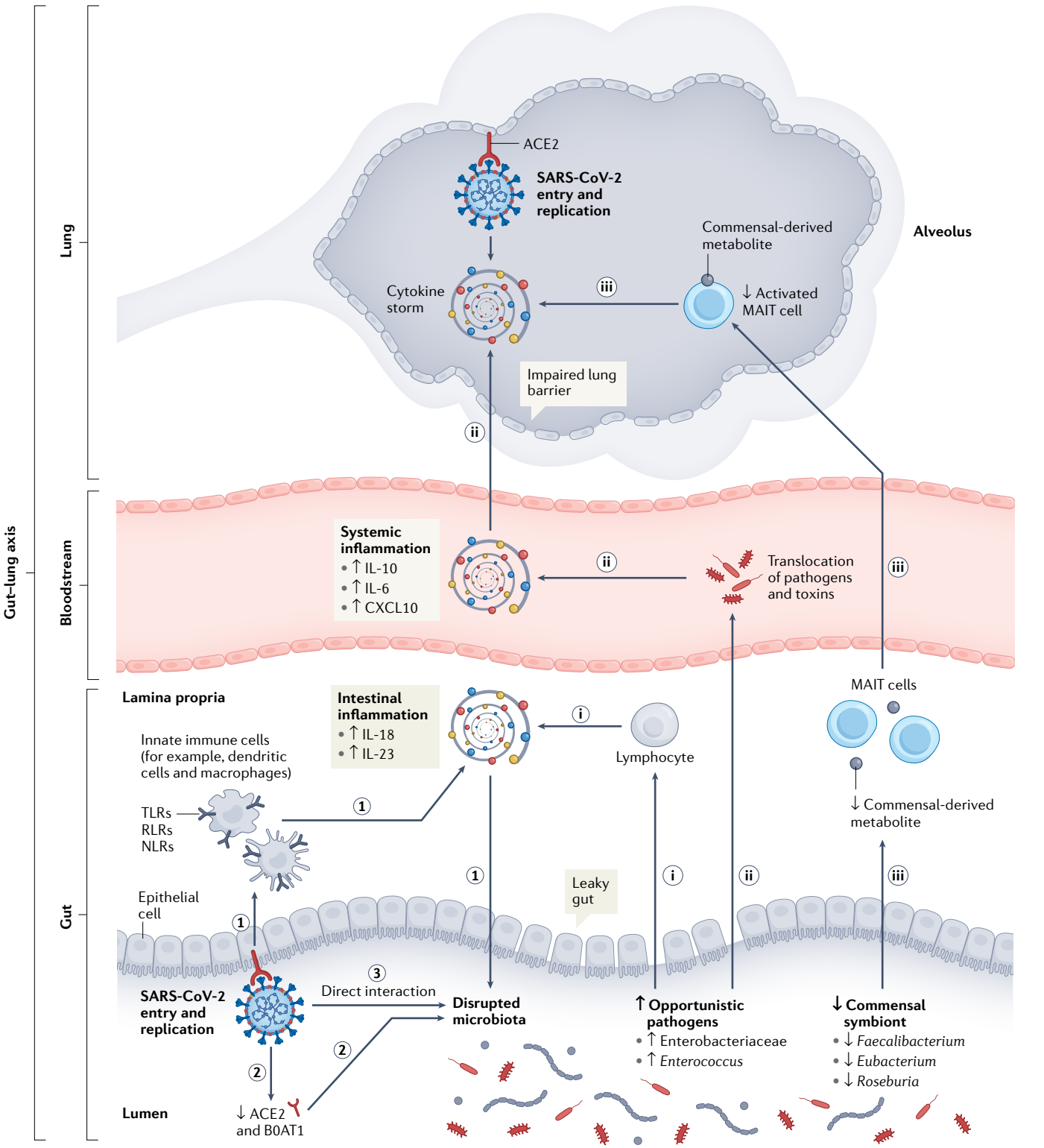
Beyond bacteria. Beyond bacteria, the gastrointestinal tract is also home to a large number of fungi, viruses and archaea, which regulate host homeostasis, pathophysiological processes, immune regulation and assembly of the co-residing gut bacterial microbiome[49,50]. Two observational studies reported that SARS-CoV-2 infection was linked to altered composition of fungal microbiota[51,52]. Both patients with COVID-19 ($n = 67$) and patients with H1N1 infection ($n = 35$) were found to have an increased fungal load and enrichment of *Aspergillus* and *Penicillium* in their faecal samples compared with 48 healthy individuals as controls[51]. Patients with COVID-19 also harboured highly heterogeneous mycobiome configurations and a higher abundance of opportunistic fungal pathogens, including *Candida albicans*, *Candida auris* and *Aspergillus flavus*[52]. Fungal infection caused by *Candida* spp. has been reported as a major complication in severe COVID-19, with the prevalence ranging from 0.7% to 23.5%[53,54]. This co-infection was associated with a high mortality rate due to a longer intensive care unit stay, catheterization and broad-spectrum antibiotic use[53]. Additionally, two studies showed the distinct composition of gut viral community in patients with SARS-CoV-2 infection compared with that of healthy individuals[55,56]. Metagenomic profiling of RNA and DNA viruses of faecal samples from 100 patients with COVID-19 and 78 individuals without COVID-19 found that faecal viruses in COVID-19 were characterized by the depletion of multiple bacteriophage lineages (DNA viruses)[55]. Interestingly, the abundance of four gut viruses (*Myxococcus* phage, *Bacteroides* phage, Murmansk poxvirus and *Sphaerotilus* phage) inversely correlated with both severity of COVID-19 and host age[55]. This observation could partly explain why older individuals (aged above 65 years) were more susceptible to severe and worse COVID-19 outcomes; however, this aspect needs to be further explored in animal studies. In a mouse model, SARS-CoV-2 infection can cause a shift in the composition of gut viruses with altered expression of immune-related or infection-related genes in gut epithelial cells, including host–virus or host–bacteria crosstalk genes *IL15*, *C3* and *MUC2* (ref.[56]). Mechanistic studies will be necessary to explore the contribution of specific microbial communities (such as bacteria, fungi and viruses) to SARS-CoV-2 pathogenesis and clinical outcomes.

Severity of COVID-19

In SARS-CoV-2 infection, increased severity of the disease is attributed not only to the virus itself but also to an aggressive immune response marked by a cytokine storm leading to systemic inflammation and tissue damage[57] (Fig. 1). It is believed that an 'abnormal' community of gut microorganisms is associated with hyperactive inflammatory responses in severe COVID-19 as well as with autoinflammatory and autoimmune disorders that occur during or after the infection[58]. It is assumed that, during the infection, commensal bacteria are replaced by opportunistic pathogens[39,58,59]. It remains unclear, however, whether it is the absence of beneficial bacteria or an active role played by dysbiotic bacteria that triggers and leads to sustained and excessive inflammation during severe COVID-19. It has been hypothesized that damage to the gut epithelium caused by SARS-CoV-2 infection leads to translocation of opportunistic pathogens and endotoxins in the gut, which could further exacerbate the systemic inflammatory response and predispose the individual to secondary infections, acute respiratory distress syndrome, multi-organ failure and even death[20,21]. Dysbiosis could also negatively influence the balance and recruitment of immune cells, such as mucosal-associated T cells, in the lungs through the gut–lung axis, which contributes to the development of respiratory tract infections[22–24].

In patients with COVID-19, studies (including a preprint) have shown a higher level of several plasma markers of gut permeability, including fatty acid-binding protein 2 (FABP2), peptidoglycan, lipopolysaccharide-binding protein (LBP) and faecal calprotectin, compared with healthy controls, indicating the disruption of gut barrier integrity during SARS-CoV-2 infection[60,61]. Interestingly, in a cohort of 60 patients with COVID-19, elevated levels of zonulin, a protein that modulates tight junctions of the gastrointestinal tract, were found to be associated with higher mortality, more severe illness and increased levels of markers of systemic inflammation (IL-6), highlighting the relationship between impaired intestinal barrier function and COVID-19 severity[62]. Furthermore, opportunistic pathogens from the Enterobacteriaceae family (such as *Escherichia coli* and *Klebsiella pneumoniae*) and the *Enterococcus* genus (such as *Enterococcus faecalis*) were found to be over-represented in the gastrointestinal tract of patients with COVID-19 who have critical illness[27,34]. Increased permeability and expansion of opportunistic pathogenic microorganisms in the gut might contribute to a high risk of bloodstream infections in COVID-19 (ref.[63]). Co-infections have been identified in 3–25% of patients in various studies[64–67], including *Klebsiella* spp., *Enterococcus* spp. and *E. coli* infections, which generally accounted for up to 50% of death in patients with critical illness[68].

In addition, several human studies reported that the abundance of butyrate-producing genera *Faecalibacterium*[33,37] and *Roseburia*[33,38,69] negatively correlated with disease severity. In a prospective study of 117 patients with COVID-19 from Germany, the abundance of *Faecalibacterium* and *Roseburia* negatively correlated with disease severity[33]. It remains unclear whether these microbial signals precede the infection or represent a consequence of the disease as only a few studies had included asymptomatic individuals. The relative abundance of a specific bacterial species, *Spirochaetes*, was higher in asymptomatic individuals with SARS-CoV-2 infection than in healthy individuals[70]. A study from Hong Kong of 100 patients with COVID-19 and 78 individuals without COVID-19 as controls showed that changes in the composition of the gut microbiota correlated with severity of COVID-19 and



altered levels of blood inflammatory markers[37]. Depletion of bacterial species with immunomodulatory potential, including *F. prausnitzii* and *Eubacterium rectale*, was linked to increased serum levels of pro-inflammatory mediators, plasma TNF, IL-10, C-X-C motif ligand 10 (CXCL10) and C-X-C motif ligand 2 (CXCL2)[37]. These data suggest that some microorganisms, such as *F. prausnitzii* and *E. rectale*, which are commonly abundant in healthy individuals, might have a role in limiting or preventing inflammation[37]. Intriguingly, altered gut microbiota

Fig. 1 | Possible roles of the gut microbiota in dysfunctional immune responses and COVID-19 severity. We propose potential mechanisms by which the gut microbiota can contribute to dysfunctional immune responses and coronavirus disease 2019 (COVID-19) severity. The first part hypothesizes that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could lead to gut dysbiosis by several possible mechanisms: (1) invasion of SARS-CoV-2 can activate pattern-recognition receptors (Toll-like receptors (TLRs), RLRs, NLRs), which are recognized by innate immune cells, resulting in the release of various pro-inflammatory cytokines[109]. These activated immune responses could impair gut permeability, disrupt gut microbiota equilibrium and result in an increased abundance of opportunistic pathogens (for example, Enterobacteriaceae and *Enterococcus*) and decreased abundance of commensal symbionts (for example, *Faecalibacterium*, *Eubacterium* and *Roseburia*). (2) SARS-CoV-2 infection was found to downregulate the expression of angiotensin-converting enzyme 2 (ACE2) and B0AT1 (a molecular ACE2 chaperone) on the luminal surfaces of intestinal epithelial cells, which may facilitate the growth of the pathogens[25,113,114]. (3) An in vitro study found that SARS-CoV-2 might directly infect bacteria[115]. In the second part, we propose that specific intrinsic 'microbiome signatures' at the point of SARS-CoV-2 infection could influence the severity of infection and host immune response by several putative mechanisms: (i) increased opportunistic pathogens might be further recognized by innate lymphocytes and intensify gut pro-inflammatory responses[185]. (ii) Opportunistic pathogens and toxins could translocate into the circulatory system, causing bacteraemia and exacerbating systematic inflammation and disease severity[20,21]. (iii) Depleted commensal symbionts could negatively influence the recruitment of immune cells, such as activated mucosal-associated invariant T (MAIT) cells, to affect susceptibility and severity of respiratory tract infections[22–24]. CXCL10, C-X-C motif ligand 10.

composition persisted for at least 30 days after SARS-CoV-2 infection had been cleared[37], and the changes seen, such as depletion of *F. prausnitzii*, were similar to those reported in other chronic inflammatory conditions[58]. Others have reported that the abundance of *Eubacterium ramulus* showed a negative correlation with IL-6, and *E. faecalis* had a negative correlation with IL-4 and plasma $CD8^+$ T cells in patients with COVID-19 (ref.[71]). These cytokines and chemokines are associated with an interferon-driven T helper 1 (T_H1) response[72], indicating that the gut microbiota might modulate the systemic T_H1 response in COVID-19. Moreover, faecal levels of IL-18, which contributed to the breakdown of barrier integrity, showed a positive correlation with the abundance of *Peptostreptococcus*, *Citrobacter* and *Fusobacterium*, highlighting a possible role of the gut microbiota in affecting gut permeability and inflammation during SARS-CoV-2 infection[35,73]. Further studies to dissect the relationship between cytokine release and shifts in gut microbiota will facilitate our understanding of whether these microbial changes directly contribute to cytokine storms in patients with COVID-19 or represent a consequence of critical disease.

The oral cavity has been shown to be an important site for SARS-CoV-2 infection and human saliva could be a potential route of SARS-CoV-2 transmission[74]. Accumulating data have reported associations between the oral microbiome and COVID-19 severity, host immune response, and SARS-CoV-2 viral load[36,75–78]. Similar to the gastrointestinal tract, the oral microbiome of patients with COVID-19 was characterized by reduced diversity of bacteria[36,75]. Interestingly, some bacterial genera and species, such as *Rothia mucilaginosa*[36], *Granulicatella*[36], *Veillonella*[28,36] and *Campylobacter*[36,79], were found to be significantly (all $P < 0.05$) enriched in both oral and faecal samples of patients with COVID-19. The oral microbiome of patients with COVID-19 also showed elevations in xenobiotic biodegradation and amino acid metabolism[78]. Importantly, a number of bacteria of the genus *Veillonella* that were enriched in the oral cavity of 31 patients with COVID-19 were reported to be over-represented in the bronchoalveolar lavage fluid (BALF) of a patient with COVID-19 (ref.[78]). These data suggest that the oral cavity might serve as a possible reservoir for microorganisms to induce co-infections in the lungs and as a potential source through which translocated species contribute to gut dysbiosis in COVID-19.

Post-acute COVID-19 syndrome

Features. Following acute infection with SARS-CoV-2, a systematic review of 45 studies including 9,751 participants with COVID-19 concluded that 73% of patients develop persisting debilitating symptoms, a condition termed post-acute COVID-19 syndrome (PACS), commonly known as 'long COVID'[80]. PACS can affect multiple organs, including persistent respiratory symptoms (such as coughing or shortness of breath), cardiovascular symptoms (such as chest pain or palpitations), gastrointestinal symptoms (such as loss of appetite or diarrhoea), neuropsychiatric symptoms (such as anxiety or insomnia), musculoskeletal symptoms (such as joint pain or muscle weakness) and dermatological symptoms (such as skin rash or hair loss)[81–83] (Fig. 2). Patients with PACS had a higher risk of prolonged health impairments 6 months after the acute infection, and PACS is associated with reduced health-related quality of life and physical functions[81,84]. Although the exact cause of PACS is not clear, it has been hypothesized that several mechanisms, either alone or in combination, could contribute to this syndrome, including the persistence of residual viral structures, perturbations of inflammatory and immune responses, and tissue or organ damage[85]. Some of these potential mechanisms resemble that of post-infectious irritable bowel syndrome (PI-IBS), a comparable condition whereby long-term gastrointestinal symptoms persist following acute gastroenteritis[86,87]. It was reported that between 1.02% (15 of 1,475) and 7.14% (20 of 280) of patients with COVID-19 developed symptoms of IBS after acute SARS-CoV-2 infection[86,87]. We hypothesize that these symptoms might be part of an emerging phenomenon known as 'post-COVID IBS'[88,89]. Interestingly, gastrointestinal sequelae were reported to be greater in patients with acute gastrointestinal symptoms during the acute phase of SARS-CoV-2 infection than in patients without acute gastrointestinal symptoms[88]. The roles of the gut microbiota and host immune response that underlie the physiological manifestations of PI-IBS could also be a factor and warrant further investigations. Vaccination against SARS-CoV-2 only conferred limited protection against PACS when comparing 6-month risks of incident post-acute sequelae in 16,035 patients with COVID-19 with prior vaccination and 48,536 patients with COVID-19 without prior vaccination[90]. Thus, reliance on vaccination as a sole alleviation approach might only partially reduce the long-term health consequences of SARS-CoV-2 infection. As the human gut microbiota has a crucial role in the maturation and modulation of the immune system[91], an aberrant immune response to SARS-CoV-2 infection induced by resident microorganisms could also affect the convalescent phase (Fig. 2).

Gut microbiome analysis. Several groups have reported differences in the gut microbiome in individuals who developed PACS compared with those who made a complete recovery from COVID-19 (refs.[83,92–95]). Microbial differences were detected at the time of diagnosis but were exaggerated after 6 months[83,92]. In particular, microbiota richness was not

restored to normal conditions after discharge in patients with PACS. Persistent symptoms can be associated with the presence of a small quantity of residual virus in immunopriviledged tissues, especially regions of the body such as the gut that are not directly protected by antibodies[96,97]. A striking observation was that dysbiosis persisted for months after the clearance of the virus. Compared with healthy individuals as controls,

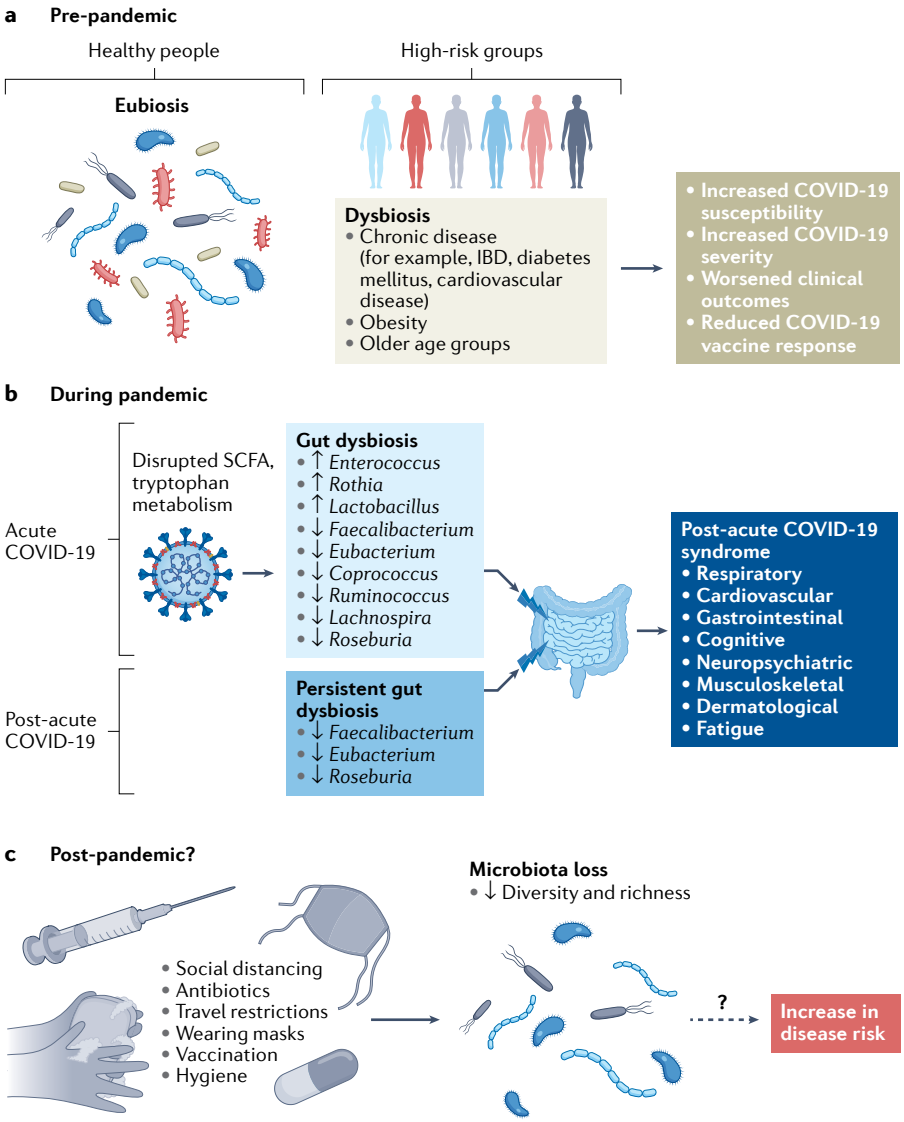


Fig. 2 | Proposed model of gut microbiome changes pre-pandemic, during pandemic, and post-pandemic and how COVID-19 measures influence microbiota diversity during an individual's lifetime. a, Prior to the coronavirus disease 2019 (COVID-19) pandemic, the gut microbiota in a healthy individual was characterized by 'eubiosis', a balanced gut ecosystem with rich microbial diversity, whilst certain individuals, including older age groups and those with chronic diseases such as inflammatory bowel disease (IBD), diabetes mellitus, cardiovascular disease and obesity, had an altered gut ecosystem with reduced microbial diversity and altered gut microbial composition[104,105]. 'Dysbiosis' could contribute to increased susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, increased COVID-19 severity, worsened clinical outcomes and/or decreased COVID-19 vaccine response. **b**, During the pandemic, acute COVID-19 infection was associated with consistent gut microbiota composition changes and impaired short-chain fatty acid (SCFA) biosynthesis[20,120,124] and disrupted tryptophan metabolism[127–130]. Dysbiosis seen in the initial infection was also associated with post-acute COVID-19 syndrome, including chronic respiratory symptoms (for example, coughing or shortness of breath), cardiovascular symptoms (for example, chest pain or palpitation), gastrointestinal symptoms (for example, loss of appetite or diarrhoea), neuropsychiatric symptoms (for example, anxiety or insomnia), musculoskeletal symptoms (for example, joint pain or muscle weakness), and dermatological symptoms (for example, skin rash or hair loss)[81–83]. In the post-acute COVID-19 phase, the gut microbiota remained persistently disrupted, characterized by persistent depletion of SCFA-producing bacteria *Faecalibacterium*, *Eubacterium* and *Roseburia*[83,93]. Alterations of gut microbiota composition in the post-acute stage were also associated with multi-organ post-acute COVID-19 syndrome[83]. **c**, Beyond the pandemic, existing pandemic control practices with strict implementation of social distancing, extensive hygiene measures, regular vaccination and restricted travel could negatively affect microbiome diversity in infants and have substantial effects on early-life bacterial colonization in the gut, with unknown consequences for disease risk. The key microbial changes consistently reported in multiple human studies are presented.

those recovered from COVID-19 had reduced bacterial diversity and richness at 3 months, accompanied by a lower abundance of beneficial commensals and a higher abundance of opportunistic pathogens[83,98]. The relative abundance of members of *Bifidobacterium* and *Ruminococcus* remained significantly ($P < 0.001$) depleted in patients with COVID-19 compared with controls at 6-month follow-up[83].

An observational study showed that respiratory dysfunction after discharge in 85 patients with COVID-19 was associated with dysbiosis based on 16S ribosomal RNA sequencing of faecal samples as well as raised plasma LBP levels[93]. Respiratory dysfunction assessed by diffusing capacity of the lungs for carbon monoxide (DL_{CO}) after 3 months of discharge from hospital was linked to increased abundance of the genera *Flavonifractor* and *Veillonella* (potentially linked to fibrosis) and reduced abundance of several members of the Lachnospiraceae family (Lachnospiraceae FCS020 group, *Eubacterium ventriosum* group, *Fusicatenibacter*, Lachnospiraceae ND3007 group) and Ruminococcaceae family (*Ruminococcus*, *Subdoligranulum*); the latter microbial communities were butyrate producers. These data point to the potential involvement of gut–lung crosstalk in relation to long-term pulmonary dysfunction and long COVID symptoms.

In a prospective study that tracked longitudinal dynamics of gut microbiota of 106 hospitalized patients with COVID-19 in Hong Kong, approximately three-quarters of patients developed PACS (commonly fatigue, poor memory and anxiety) 6 months after the infection[83]. Shotgun metagenomic analysis of faecal samples showed significantly lower microbial diversity and reduced types of bacteria compared with individuals without PACS and healthy individuals as controls[83]. Patients with PACS also had an increased abundance of *Bacteroides vulgatus* and *Ruminococcus gnavus* and a reduced abundance of *F. prausnitzii*. Interestingly, *B. vulgatus* was also shown to be sixfold elevated in the faecal samples of patients ($n = 11$) with PI-IBS compared with healthy individuals ($n = 11$)[86,99]; this finding suggests that *B. vulgatus* is potentially linked to the pathogenesis of both PACS and PI-IBS. Moreover, having respiratory symptoms at 6 months post-SARS-CoV-2 infection was associated with increased levels of opportunistic pathogenic species such as *Streptococcus vestibularis* and *Streptococcus anginosus*, whereas fatigue and neuropsychiatric symptoms were associated with nosocomial pathogens such as *Clostridium innocuum* and *Actinomyces naeslundii*[83]. Butyrate-producing bacteria were substantially decreased in patients with hair loss and certain bacteria, such as *Bifidobacterium pseudocatenulatum* and *F. prausnitzii*, had the largest inverse correlations with PACS development[83]. Bacterial species, including *B. longum* and *Blautia wexlerae*, at admission were negatively associated with the development of PACS at 6 months, implying a potential protective role of these species in the convalescent phase[83]. By contrast, *Atopobium parvulum*, *Actinomyces johnsonii* and *Actinomyces sp.* S6 Spd3 were enriched in patients with PACS. These findings suggest that a person's gut microbiome configuration at the time of infection might affect their susceptibility to long-term complications of COVID-19. Nonetheless, these changes could represent reactive changes to PACS, and future research needs to include prospective longitudinal studies of non-hospitalized patients followed from the time of infection until symptom development to delineate the exact contribution of dysbiosis to PACS symptoms.

Mechanistic links

ACE2 and the gut microbiota

ACE2, an entry receptor for SARS-CoV-2, is highly expressed in small bowel enterocytes[100]. ACE2 can regulate microbial ecology, innate immunity and dietary amino acid homeostasis by maintaining the balance of the renin–angiotensin system and amino acid transporter B0AT1 (also known as SLC6A19)–ACE2 complex[25,26]. Several animal studies have explored the effect of gut microbiota on intestinal ACE2 expression. Intestinal and lung ACE2 expression levels were markedly higher in germ-free mice than in conventional mice[101]. Gnotobiotic mice colonized with different microbiota showed variability in intestinal ACE2 expression, which could partially be attributable to differences in types of microbiome-encoded proteases and peptidases[101]. A study published in 2021 identified transcriptional factors regulating ACE2 expression in the gut[102], including GATA4, which was known to be regulated by the gut microbiota[70]. These data suggest that the gut microbiota might play a part in regulating ACE2 expression.

Moreover, specific bacterial species, such as *Bacteroides dorei* and *Bifidobacterium longum*, were shown to inhibit colonic ACE2 expression in mouse models[21,103]. In addition, four *Bacteroides* species associated with downregulation of ACE2 expression in the mouse colon[103] showed significant ($P < 0.05$) inverse correlations with faecal SARS-CoV-2 load[39]. High-risk groups succumbing to COVID-19 were often those with comorbid conditions, such as diabetes, cardiovascular disease and obesity, which were also associated with microbiome abnormalities, characterized by reduced bacterial diversity[104,105]. This finding suggests that gut microbiota prior to infection might contribute to host susceptibility to SARS-CoV-2 and ACE expression[106,107] (Fig. 2). In addition, other SARS-CoV-2 entry factors, such as host glycosaminoglycan heparan sulfate necessary for virus cellular binding, can be modified by bacteria such as *Bacteroides*[108].

SARS-CoV-2 infection and dysbiosis

To date, the majority of clinical studies have shown associations between SARS-CoV-2 infection and an altered gut microbiome, although it remains unclear whether changes were a cause or effect of the infection. We hypothesize several putative mechanisms by which SARS-CoV-2 can result in gut dysbiosis. Invasion of SARS-CoV-2 into the lungs could cause tissue damage and activate strong pro-inflammatory pathways characterized by upregulation of NF-κB and TNF pathways to induce the cytokine storm[57,109,110]. Alternatively, gut infection could cause direct impairment of intestinal structure and breakdown of the intestinal epithelial barrier and promote intestinal inflammation[111]. Activated systematic and intestinal inflammation might contribute to changes in gut microbiota[112]. SARS-CoV-2 infection could downregulate the expression of ACE2 and B0AT1 (a molecular ACE2 chaperone) on the luminal surfaces of intestinal epithelial cells, which might then facilitate pathogen growth[25,113,114]. An in vitro study observed that SARS-CoV-2 might function like bacteriophages and directly infect bacteria[115], revealing another possible mechanism of SARS-CoV-2 affecting the gut microbiota.

Four animal studies have provided evidence that SARS-CoV-2 infection might play a part in driving alterations in gut microbiota ecology[56,116–118]. When a non-human primate model of rhesus macaques and cynomolgus macaques was challenged with SARS-CoV-2, the gut microbiome changed gradually from day 0 until day 13 post-infection[117]. An increase in abundance of *Acinetobacter* and genera of the Ruminococcaceae family was associated with the presence of SARS-CoV-2 in the upper respiratory tract, whereas a decrease in SCFA levels and changes in the levels of tryptophan and several bile acid metabolites were observed in infected animals[117]. In addition, hamster models were able to recapitulate some of the hallmark features of severe COVID-19 seen in humans. SARS-CoV-2 infection resulted in an over-representation of deleterious bacterial taxa, such as Desulfovibrionaceae and

Enterobacteriaceae, a lower abundance of SCFA-producing bacteria, and faecal SCFA, in line with findings seen in humans[118]. However, the infectious virus has not been detected in the gut of hamsters suggesting that alterations of gut microbiota could be, in part, secondary to systematic inflammation caused by SARS-CoV-2 infection in the lungs[118]. Supplementation of a combination of sodium acetate, propionate and butyrate in the hamster model challenged by SARS-CoV-2 failed to interfere with SARS-CoV-2 replication in the lungs or ameliorate gut inflammation at the gene expression level[118]. In a study using human colon biopsy samples obtained from healthy individuals, the addition of SCFAs (including acetate, propionate and butyrate) had minimal effects on the expression of antiviral and anti-inflammatory-related genes or viral load in intestinal cells[119]. Whether changes in the gut microbiota can directly affect the clinical manifestations of COVID-19 remains largely unknown although strong associations between COVID-19 severity and specific bacteria and metabolites have been consistently observed in human studies.

Microbial metabolites in COVID-19

Emerging human studies determining the functional capacity of gut microbiota and the faecal metabolomic and proteomic profile in patients with COVID-19 have provided deeper insights into understanding microbiota–host interactions and their consequences on disease outcomes. SARS-CoV-2 infection was associated with alterations in carbohydrate, lipid and amino acid metabolism of the gut microbiota[38,45,120–123]. Several studies have shown impaired SCFA biosynthesis in faecal samples of patients with COVID-19 (refs.[20,120,124]). In a metagenomic analysis of 66 antibiotics-naive patients with COVID-19 and 70 individuals without COVID-19 (ref.[124]), patients with SARS-CoV-2 infection showed a reduced capacity of their gut microbiota for SCFA biosynthesis, which negatively correlated with disease severity and elevated plasma concentrations of the pro-inflammatory cytokine IL-10 and the chemokine CXCL10 (ref.[124]). Consistently, 19 patients with COVID-19-related severe and/or critical illness showed reduced faecal concentrations of SCFAs, including acetate, propionate, butyrate, valeric acid and caproic acid, through measurement of faecal metabolites[124]. SCFAs can activate anti-inflammatory responses of immune cells, inhibit inflammatory signalling pathways[125] and maintain the integrity of the gut barrier to prevent translocation of gut endotoxins and bacteria into the circulation, thereby alleviating local and systemic inflammatory responses[126]. Given the importance of SCFAs in regulating host immune response, the deficiency of SCFA biosynthesis in COVID-19 could be associated with disease pathogenesis and severity. However, whether SCFA depletion is a cause or consequence of COVID-19 infection remains to be elucidated.

Several studies measuring plasma metabolites of patients with COVID-19 have shown disrupted tryptophan metabolism and augmented activation of the kynurenine pathway, which is involved in tryptophan metabolism, when compared with healthy individuals as controls[127–130]. Tryptophan metabolism is linked to autoimmunity, viral infections and gut health through regulation of the ratio of regulatory T cells to T_H17 cells and B cell activity[131]. Increased metabolites of the kynurenine pathway transported into the brain could trigger symptoms such as fatigue, poor memory and depression in both human and animal studies[130,132], which are common symptoms of 'long COVID'[130]. Importantly, tryptophan metabolites are key mediators of the host–microbiota interface. The gut microbiota can directly use tryptophan as a substrate and influence host tryptophan absorption and metabolism to regulate host physiological and immune responses according to evidence from both human and animal studies[133]. Endogenous host tryptophan metabolites can profoundly influence gut microbiota composition and functions such as *Akkermansia* and *Lactobacillus*[134,135]. Taken together, these data suggest tryptophan metabolism as a possible mechanism by which gut microbiota is involved in COVID-19.

A reduced concentration of sphingolipids in sera[136] and faeces[121] and altered gut microbial sphingolipid metabolism[120] were reported in patients with COVID-19. Sphingolipids are components of biomembranes, mediating signal transduction and immune activation[137]. Sphingolipids produced by *Bacteroides* can increase exogenous sphingolipids[138] and thus enhance differentiation of regulatory T cells as observed in in vitro or in vivo studies[139], which could inhibit replication of coronaviruses. This observation supports the hypothesis that gut microbiota-derived sphingolipids might regulate host defence against SARS-CoV-2 infection.

Additionally, increased faecal sucrose and depleted faecal glucose levels were reported in 56 patients with COVID-19 compared with 47 healthy individuals as controls[122]. Abnormal levels of sucrose and glucose might be associated with impaired sucrase-isomaltase activity[140,141]. This change could be associated with common intestinal symptoms, such as diarrhoea, vomiting, flatulence and abdominal pain, in COVID-19 (ref.[140]). Flatulence is typically caused by the fermentation of unabsorbed carbohydrates in the intestine by bacteria[142]. Increased levels of sucrose have been associated with enriched levels of *Actinomyces* and *Streptococcus parasanguinis*[122], implying that dysbiosis in COVID-19 might disrupt intestinal fermentation and contribute to gastrointestinal symptoms.

Vaccine immune response and dysbiosis

As of the end of August 2022, over 12.4 billion doses of SARS-CoV-2 vaccines have been administered (156 vaccines per 100 people) worldwide according to the World Health Organization. Different types of vaccines developed to elicit effective immune responses to protect from COVID-19 or to reduce disease severity if infected have changed the course of the pandemic[143,144]. Yet, there is a great variation in vaccine response among different individuals in different populations. A growing number of animal and clinical studies have shown the critical role of gut microbiota composition and function in modulating responses to vaccines such as trivalent influenza vaccine and oral rotavirus vaccine[145]. The gut microbiota can influence vaccine immunogenicity, including the activation of antigen-presenting cells through pattern-recognition receptors by bacteria-derived molecules, such as flagellin and peptidoglycan, that act as potent natural adjuvants to enhance immune response[146]. In particular, bacterial flagellin was shown to be a critical contributor to the antibody response to the flu vaccine through Toll-like receptor 5 (TLR5) sensing[147]. In germ-free and antibiotic-treated mice, NOD2-mediated sensing of bacterial peptidoglycan enhanced immune responses to intranasal cholera toxin vaccine[148]. The gut microbiota could also modulate vaccine response via bacteria-derived metabolites such as SCFAs, which upregulate gene expression for antibody response and promote plasma B cell differentiation according to in vivo evidence[149].

A prospective study published in 2022 characterized the gut microbiome associated with immune responses against an inactivated vaccine (CoronaVac, Sinovac) or an mRNA vaccine (BNT162b2, BioNTech, Comirnaty) and associated adverse events in 138 individuals who had received a COVID-19 vaccine[150]. The relative abundance of *Bifidobacterium adolescentis* in high responders of the CoronaVac vaccine was significantly ($P = 0.023$) higher than that of

low responders prior to vaccination, with enrichment of carbohydrate pathways related to *B. adolescentis*[150]. One month following the second dose of CoronaVac vaccination, *B. adolescentis* remained more abundant in high responders[150]. Furthermore, the neutralizing antibody response was positively correlated with the abundance of butyrate-producing bacteria with flagella and fimbriae, including *Roseburia faecis*[150]. Interestingly, the abundance of *Prevotella copri* and two *Megamonas* species before COVID-19 vaccination were associated with fewer adverse events among both groups of vaccinees, implying that these bacteria might play anti-inflammatory roles in immune response[150]. A prospective study in 43 patients with inflammatory bowel disease also showed associations between gut dysbiosis and serological response against BNT162b2 or ChAdOx1 nCoV-19 (Vaxzevria, AstraZeneca) vaccines[151]. *Bilophila* abundance and levels of the faecal metabolite trimethylamine were positively associated with vaccine response, whereas *Streptococcus* abundance and levels of metabolites, including phenylalanine, phenylacetate and succinate, showed a negative association[151]. These findings indicate the importance of SCFAs, pattern-recognition receptor-mediated sensing and disease-associated gut dysbiosis in modulating COVID-19 vaccine immunogenicity. A study also showed a slight shift in the gut microbiota after the first dose of vaccine, although it is unclear if microbial changes were caused by vaccination or due to COVID-19-related measures as a control group who did not receive vaccines was not studied[150]. Others have shown that an mRNA vaccine led to enriched oral microbial diversity, decreased abundance of *Bacteroides* and changes in the gut microbiome during the course of SinoVac vaccine in 40 healthy individuals[152].

To date, one of the main challenges regarding COVID-19 vaccination is the waning of protection over time, even for booster doses[153,154]. Those aged 60 years or above, those who are immunocompromised, or those with comorbidities, such as obesity, are highly susceptible to the rapid waning of vaccine immunogenecity[154,155]. It has been hypothesized that gut dysbiosis could be one of the mechanisms by which these risk factors cause weakened defences against SARS-CoV-2 infection following vaccination. In young children aged 3–5 years, COVID-19 vaccine (CoronaVac, Sinovac) effectiveness has been shown to be suboptimal[156]. Thus, these vulnerable individuals or those with contraindications to COVID-19 vaccines might potentially benefit from microbiota-based interventions in complementing the effectiveness of COVID-19 vaccines. Clinical trials on the efficacy of microbiota-based interventions in improving COVID-19 vaccine immunogenicity, reducing vaccine-associated adverse events and prolonging vaccine effectiveness over time are warranted.

Consequences on the human microbiome

Current pandemic control measures have been proposed to have large, uneven and potentially long-term detrimental effects on the human microbiome globally with strict implementation of social distancing, extensive hygiene measures, restricted travel and other measures that influence overall microbial loss[157]. A small study comparing pre-pandemic gut microbiota of 23 healthy individuals from Buenos Aires, Argentina, and its variation during social isolation found a significant ($P < 0.05$) decrease in phylogenetic diversity and in the phylum Verrucomicrobiota, which contributes to intestinal health and glucose homeostasis, during isolation[158]. The COVID-19 pandemic has been associated with a markedly lower Actinobacteria (Actinomycetota) richness, higher Bacteroidetes (now known as Bacteroidota) richness, and an altered abundance of antibiotic-resistance genes in faecal samples collected from 32 healthy individuals before and during the first wave of the pandemic[159].

Birth and early infancy are critical windows for microbiome establishment and development, and microbial colonization in early life can influence subsequent risks of common illnesses, including eczema, asthma, autoimmunity, allergy and diabetes[41–44]. It has been hypothesized that the pandemic could have disrupted the gut bacterial composition of infants and created other long-term complications in their later life due to increased hygiene, restricted social exposure and decreased travel, which are essential factors affecting early-life bacterial colonization in the gut[160]. Preliminary analyses of faecal microbiome ($n = 307$) collected from infants born before and during the pandemic in a neonatal intensive care unit in the USA showed marked differences in microbiome diversity, highlighting that the COVID-19 pandemic might cause a loss of microbial diversity and richness in infants[161]. Studies have demonstrated that the pandemic increased the risk of childhood diseases, including obesity, myopia and mental health disorders with prolonged home confinement[160,162–165]. More work is needed to investigate and confirm potential links between the pandemic, microbiota loss and future disease risk. Although our knowledge of COVID-19 and the microbiome is incomplete, future infection control measures that result in microbial loss need to be balanced with strategies that promote microbial diversity to ensure health benefits for future generations (Fig. 2).

Microbiota-based interventions

Clinical trials using dietary modification, probiotics, prebiotics and faecal microbiota transplantation (FMT) are under way to determine their effectiveness in the treatment of acute COVID-19 and in enhancing the effectiveness of the SARS-CoV-2 vaccine. Supplementary Table 2 summarizes clinical trials involving modulation of the gut microbiome for COVID-19 management.

Antibiotic stewardship

In the early pandemic, antibiotics were commonly used. A meta-analysis estimated that three in four patients with COVID-19 globally had been prescribed antibiotics, and such proportion is markedly above the estimated incidence of bacterial co-infections in COVID-19, which stands at only 8.6%[166]. Clinical outcomes in 45 patients with moderate COVID-19 showed no difference between those who had and had not received antibiotics, suggesting that antibiotics were not beneficial in improving clinical outcomes of COVID-19 (ref.[37]). Marked gut dysbiosis has been reported in patients with COVID-19 who have received antibiotics during hospitalization[37], and antibiotic-induced gut dysbiosis impaired immune responses to seasonal influenza vaccines in humans[167]. A longitudinal study of 200 patients with COVID-19 showed that less intake of antibiotics in the year prior to COVID-19 was associated with milder disease severity and more rapid clearance of SARS-CoV-2 (ref.[168]). Thus, antimicrobial stewardship is critical to preventing antibiotic-induced dysbiosis, severe COVID-19 and the risk of antimicrobial resistance in patients with COVID-19.

Dietary modification

Dietary modification has been proposed as a means to modulate the gut microbiota and improve the clinical outcomes of COVID-19 (Fig. 3). Several population studies have provided epidemiological evidence that specific dietary habits were associated with COVID-19-related mortality and disease outcomes. In a large prospective survey of 592,571 participants in the UK and USA, a diet rich in plant food was

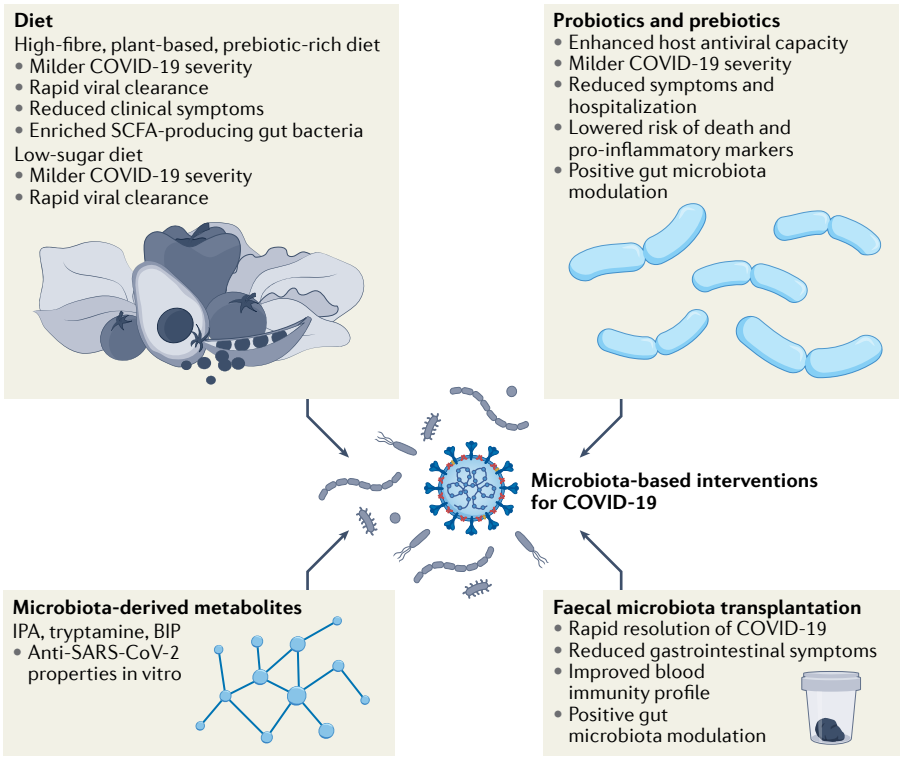


Fig. 3 | Microbiota-based interventions in COVID-19. Multiple studies have suggested the role of diet, probiotics, and microbiota-derived metabolites and faecal microbiota transplantation in enhancing antiviral capacity and improving clinical outcomes of coronavirus disease 2019 (COVID-19), such as severity and symptoms, potentially through the modulation of gut microbiota. Nevertheless, many of these preliminary conclusions were drawn based on association and retrospective analyses. Further animal and clinical studies are warranted to elucidate the mechanistic links underlying the therapeutic effects of these microbiota-based interventions. BIP, 2,5-bis(3-indolylmethyl)pyrazine; IPA, N6-(Δ2-isopentenyl)adenosine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SCFA, short-chain fatty acid.

linked to reduced risk and severity of COVID-19 (ref.[169]). A retrospective study of 509 patients with COVID-19 found statistically significant associations between a vegetarian diet and milder COVID-19 severity in people aged >65 years, whilst non-vegetarian individuals were found to be more prone to developing critically severe COVID-19 (ref.[170]). Higher intake of prebiotic-rich food and less intake of sugar in the year prior to COVID-19 was also associated with milder disease severity and faster clearance of SARS-CoV-2 in a cohort of 200 people[168]. A multicentre study of 7,337 individuals showed that, in patients with COVID-19 with type 2 diabetes mellitus, clinical outcomes could be improved by well-controlled blood glucose, highlighting the potential of low glycaemic index dietary modification with increased intake of vegetables and fruit in improving outcomes among hospitalized patients[171]. Dietary patterns across European countries were found to be associated with ACE2 activity and death rate[172], suggesting the possible mechanistic link between diet and COVID-19 mortality. A case report revealed improvement in severe post-acute gastrointestinal symptoms in a patient with COVID-19 with high-fibre intervention containing brans, inulin and Fibersol-2, which was associated with gut bacteria compositional shifts with increased SCFA-producing bacteria, including *Oscillibacter* sp., *Anaerofustis* sp., *Blautia* spp. and *Eubacterium hallii*[173]. Understanding the effect of diet on microbial changes in response to coronavirus will pave novel dietary interventions during the COVID-19 pandemic[174].

Probiotics and prebiotics

Several small to medium retrospective studies and clinical trials with sample sizes ranging from 55 to 411 have demonstrated that administration of oral probiotics and prebiotics induced antiviral activity and had positive effects on gut microbial composition and diversity and was associated with improved clinical outcomes in patients with COVID-19 (refs.[175–180]). Two retrospective studies including 200 and 411 hospitalized patients with COVID-19 have shown that use of the probiotic species *Bifidobacterium*, *Enterococcus*, *Lactobacillus* and *Streptococcus* was associated with more rapid clinical improvement by 3 days, decreased hospitalization length and reduced risk of death in patients with severe pneumonia[175,176]. Results of a randomized controlled trial involving 200 patients with COVID-19 showed that a 14-day probiotic supplementation of *Bacillus coagulans*, *Bacillus subtilis* and *Bacillus clausii* combined with systemic enzymes with immunomodulatory potential (serratiopeptidase, bromelain, amylase, lysozyme, peptidase, catalase, papain, glucoamylase and lactoferrin) improved physical and mental fatigue in individuals recovered from COVID-19 (ref.[177]). Another randomized controlled trial of 300 participants also found that supplementation of a probiotic formula consisting of *Lactiplantibacillus plantarum* and *Pediococcus acidilactici* in patients with COVID-19 was associated with a reduction in nasopharyngeal viral load, lung infiltrates, shortened duration of clinical symptoms by 2–7 days and improved immune response

in the intervention group compared with the control group receiving placebo[178]. Mechanisms of probiotics were related to improved immune response and positive compositional shifts of the gut microbiota with an increased similarity to the microbial composition of the gut microbiota in healthy individuals as well as a decrease in COVID-19-associated bacteria as shown in pilot studies[179,180]. In an open-label study of 55 hospitalized patients with COVID-19, a higher proportion of individuals (88% versus 63.3%) who received 4 weeks of a synbiotic formula consisting of *Bifidobacterium* strains and prebiotics (SIM01) achieved resolution of clinical symptoms, increased IgG antibodies against SARS-CoV-2, and reduced blood pro-inflammatory markers such as IL-6, CCL2, M-CSF, TNF and IL-1RA, than individuals in the standard treatment arm[180]. There was also an increase in the abundance of commensal bacteria, such as *Bifidobacterium*, *Eubacterium* and *Faecalibacterium* species, and a decrease of opportunistic pathogens, such as *E. coli* and *Bacteroides* species, in the gut microbiota of individuals who received SIM01 (ref.[180]). A longitudinal cohort study of 200 individuals showed that regular intake of probiotic yoghurt in the year prior to COVID-19 was associated with milder disease severity[168].

As of August 2022, 31 clinical studies have been registered on ClinicalTrials.gov and Supplementary Table 2 summarizes all current clinical trials evaluating the efficacy of probiotics or synbiotics in treating and preventing COVID-19. The majority of clinical trials focused on the use of probiotic species in the *Lactobacillus*, *Bifidobacterium* and *Enterococcus* genera for COVID-19 treatment or prevention. Live biotherapeutic products, regarded as biological medicinal products that contain live microorganisms for human therapeutic use (such as a *Lactococcus lactis* engineered to secrete IL-10)[181], have not been studied for COVID-19 but represent a promising avenue because of a multifactorial mode of action. An in vitro study using bacterial extracts obtained from human samples has identified three novel anti-SARS-CoV-2 metabolites, N6-(Δ2-isopentenyl)adenosine, tryptamine and 2,5-bis(3-indolylmethyl)pyrazine[182], which are structurally or functionally comparable to synthetic COVID-19 drugs, including remdesivir, fluvoxamine and favipiravir. Further in vivo investigations and clinical trials are needed to determine the safety and efficacy of live biotherapeutic products and microbiota-derived metabolites for COVID-19.

Faecal microbiota transplantation

FMT aims to restore microbial dysbiosis via the transfer of a healthy microbiome to an individual with a disease. Two case reports have shown the use of FMT in COVID-19 (refs.[183,184]). Improved gastrointestinal symptoms were reported in 5 of 11 individuals with COVID-19 (ref.[183]), and favourable improvement was observed in blood immunity markers and gut microbiota composition with increased abundance of *Bifidobacterium* and *Faecalibacterium*. In two patients with COVID-19

Box 1

Future research and directions

Prevention of COVID-19

- To determine microbial risk factors for susceptibility to SARS-CoV-2 infection
- To understand the interplay between keystone species with SARS-CoV-2 viral entry factors, including ACE2 and heparan sulfate
- To understand the role of the gut microbiota as a potential reservoir for recurrent or breakthrough SARS-CoV-2 infection and continuous immune cell stimulation
- To develop dietary and microbiota-based interventions to reduce host susceptibility to SARS-CoV-2 infections
- To evaluate the efficacy of microbiota-based interventions in improving vaccine immunogenicity, prolonging vaccine effectiveness and reducing vaccine-associated adverse events

Acute COVID-19

- To explore mechanisms of host–microorganism interactions in response to SARS-CoV-2 infection and severity
- To determine the effects of SARS-CoV-2 infection on other microbial components such as fungi, viruses and archaea
- To understand how the gut virome and mycobiome calibrate host immunity and regulate severity of SARS-CoV-2 infection
- To identify keystone bacteria and mechanisms specific to COVID-19 in different populations
- To explore the effect of different variants of concern (delta, omicron) on the gut microbiota
- To develop novel oral live biotherapeutics in improving COVID-19 outcomes in hospitalized patients

Post-acute COVID-19

- To elucidate how gut microbiota dysbiosis contributes to PACS and persistent symptoms affecting different organs
- To define microbial enterotypes for predicting the risk of developing post-acute complications
- To evaluate the effects of residual SARS-CoV-2 viral particles on gut microbiota and PACS
- To develop microbial therapeutic approaches for the prevention and treatment of PACS

Beyond COVID-19 pandemic

- To track changes in the gut microbiome during COVID-19 and its effect on long-term health
- To understand the effects of hygienic measures during the pandemic on microbiome acquisition, microbiome loss and reinoculation
- To study the effect of SARS-CoV-2 infection on the newborns' gut microbiome and their long-term health consequences

ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; PACS; post-acute COVID-19 syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

and concurrent recurrent *Clostridioides difficile* infection, FMT treatment seemed safe and COVID-19-related respiratory symptoms rapidly resolved within 1 month after FMT[184]. Nonetheless, many of these preliminary conclusions regarding the use of FMT and probiotics were drawn based on association and retrospective analyses. Further animal and clinical studies are warranted to elucidate the mechanistic links underlying the therapeutic effects of these microbiota-based interventions.

Conclusions

Rapidly expanding knowledge of the importance of the gut microbiota in COVID-19 represents a paradigm shift in understanding the contribution of specific microorganisms to host susceptibility and immune response to SARS-CoV-2 infection. Observational studies have provided important insights into the role of dysbiosis in acute and post-acute COVID-19 conditions and their relationship with disease severity and vaccine immunogenicity. Preliminary clinical studies have revealed potential modulatory effects of probiotic bacteria, mainly *Bifidobacterium* and *Lactobacillus* species, against SARS-CoV-2 infection. Although there are limited large-scale clinical trials, we believe this field is growing with tremendous opportunities, and future research and directions have been illustrated in Box 1. Collectively, these data have provided an unprecedented opportunity to drive microbiota discoveries towards clinical applications during the different waves of the pandemic. With more breakthroughs in metagenomics, metabolomics, viral immunology and novel microbiome therapeutics, these microbiota discoveries will hopefully take us into a new paradigm to combat COVID-19 in the near future.

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Author contributions

F.Z. and R.I.L. researched data for the article. S.C.N., F.Z., R.I.L. and Q.L. contributed substantially to discussion of the content. F.Z., R.I.L., Q.L. and Q.S. wrote the article. S.C.N., F.Z., R.I.L. and F.K.L.C. reviewed and/or edited the manuscript before submission.

Competing interests

S.C.N. and F.K.L.C. are the scientific co-founders and sit on the board of Directors of GenieBiome. S.C.N. has served as an advisory board member for Pfizer, Ferring, Janssen and Abbvie, and as a speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie and Takeda. She has received research grants from Olympus, Ferring and Abbvie. F.K.L.C. has served as an adviser and lecture speaker for Eisai, AstraZeneca, Pfizer, Takeda Pharmaceutical, and Takeda (China) Holdings. The other authors declare no competing interests.

Additional information

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Correspondence should be addressed to Siew C. Ng.

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