

# Targeting Microbiome: An Alternative Strategy for Fighting SARS-CoV-2 Infection

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## Keywords

Dysbiosis · COVID-19 · Gut-lung axis · Probiotics · Systemic cytokine storm

## Abstract

Respiratory and gastrointestinal symptoms are the predominant clinical manifestations of the coronavirus disease 2019 (COVID-19). Infecting intestinal epithelial cells, the severe acute respiratory syndrome coronavirus-2 may impact on host’s microbiota and gut inflammation. It is well established that an imbalanced intestinal microbiome can affect pulmonary function, modulating the host immune response (“gut-lung axis”). While effective vaccines and targeted drugs are being tested, alternative pathophysiology-based options to prevent and treat COVID-19 infection must be considered on top of the limited evidence-based therapy currently available. Addressing intestinal dysbiosis with a probiotic supplement may, therefore, be a sensible option to be evaluated, in addition to current best available medical treatments.

Herein, we summed up pathophysiologic assumptions and current evidence regarding bacteriotherapy administration in preventing and treating COVID-19 pneumonia.

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## Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been more than a disease outbreak. Aside from putting an unprecedented strain on health-care systems, the global economy, and society, it boosted the research community worldwide toward new therapeutic options as never before. Surprisingly, among all treatment strategies tested in Randomized Controlled Studies

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(RCTs) so far, the most effective resulted in being the simplest and mostly available at times [1]. While over the first hit of SARS-CoV-2 outbreak, multiple drugs that had already been tested in other conditions with similar infection patterns (i.e., Ebola virus, MERS-CoV) were deployed at this stage of the pandemic specific methods based on physiopathology to prevent and treat COVID-19 are expected to emerge.

Angiotensin-converting enzyme 2 (ACE2) acts as a functional receptor for SARS-CoV-2 [2]. As the alveolar epithelial cells, enterocytes equally express ACE2 receptors in the brush border membrane [3], representing, therefore, an entry point and reservoir for the virus [4].

A growing body of evidence suggests that alteration of intestinal flora composition, the so-called dysbiosis, which was observed in COVID-19 patients, may play a relevant role in determining the course of the disease by increasing systemic pro-inflammatory cytokine production [5, 6]. Since oral bacteriotherapy is able to restore the composition of intestinal flora and therefore modulate pro-inflammatory cytokine production [7], its potential clinical impact in COVID-19 patients should be thoroughly evaluated.

### **SARS-CoV-2 and GUT: An Undervalued Relationship**

In addition to fever and typical pulmonary infection manifestations, an increasing number of patients with COVID-19 reported gastrointestinal symptoms such as diarrhea, anorexia, nausea, vomiting, stomach discomfort, and gastrointestinal bleeding [4, 8–10]. Interestingly, the COVID-19 patients experiencing gastrointestinal symptoms had a more severe respiratory disease to the extent that these symptoms could be used to predict ventilatory support requirement and ICU admission.

It is now well-recognized that ACE2 receptor is equally expressed in the lung and the intestinal epithelium. In particular, studies involving immunofluorescence techniques showed that this protein is largely expressed in gastric, duodenal, and rectal epithelial glandular cells, representing a possible gateway for SARS-CoV-2 [11]. In this context, SARS-CoV-2 could be responsible for gastrointestinal inflammation [12] leading to malabsorption, intestinal disorders, activation of the enteric nervous system, and, ultimately, diarrhea.

Indeed, the interaction of specific SARS-CoV-2 spike proteins with ACE2 receptor could induce pro-inflammatory chemokine and cytokine excessive release. This massive release, a hallmark of COVID-19 patients, leads

to an acute intestinal inflammatory response, confirmed by raised levels of fecal calprotectin and serum IL-6, and to multi-organ damage consequent to systemic cytokine storm [5, 6].

In addition to that, gut ACE2 is also a relevant regulator of amino acid transport, being a chaperone for the membrane trafficking of neutral amino acid transporter (B0AT1), which is expressed both in the proximal kidney tubule and the small intestine [13, 14]. Considering that ACE2 deficient mice were found to have low plasma levels of tryptophan, increased susceptibility to ulcerative colitis, and severe diarrhea [15], it was postulated that SARS-CoV-2 might alter intestinal microbiome and inflammatory response affecting local amino acid metabolism [13, 15, 16].

Moreover, this alternative mechanism could also promote an excessive gut permeability through epithelial tight junctions' alterations affecting the intestinal barrier function in COVID-19 patients. This was associated with a profound increase of Zonulin, a well-known physiological regulator of tight junction complex in the digestive tract, which was also found to be correlated with higher mortality in COVID-19 patients [17].

These pieces of evidence point out that the gut may represent a route of infection and a SARS-CoV-2 reservoir [4, 18]. Indeed, up to 50% of patients released SARS-CoV-2 and its nucleic acid in the stool samples during the disease's acute phase. The infection itself generally lasted longer in COVID-19 patients who had previously experienced gastrointestinal symptoms.

### **Microbiome and Gut-Lung Axis**

Nobel et al. [19] reported that SARS-CoV-2 infection generally lasted longer in COVID-19 patients who had previously experienced gastrointestinal problems. Besides, subjects with diabetes mellitus, hypertension, cerebrovascular disease, and chronic obstructive pulmonary disease experience a more severe course of COVID-19 disease. All these co-morbidities have a common denominator: gut dysbiosis.

Not surprisingly, compared to healthy controls, some COVID-19 patients showed microbial dysbiosis with decreased levels of beneficial bacteria as *Lactobacillus* and *Bifidobacterium*, lower bacterial diversity, and higher relative abundance of opportunistic pathogens including *Streptococcus* spp., *Rothia* spp., *Veillonella* spp., and *Actinomyces* spp [18]. It is becoming increasingly clear that the loss of certain intestinal bacterial strains might be re-

sponsible for a dysregulated immune response to SARS-CoV-2 [20]. Far away from being considered just part of the digestive tract, the gut is crucially involved in the immune system, hosting a large microbial population that can affect the body's respiratory tract through immune regulation [21, 22].

The "gut-lung axis" refers to the bidirectional cross-talk between the intestinal tract and the lungs, whose ultimate scope is to modulate the immune response in both compartments as a result of their respective microbial composition and related patterns [23]. This gut-lung interplay embraces multiple anatomical communications and complex pathways [24]. The mesenteric lymphatic system is one of those. Using this route, intact bacteria, their fragments, or metabolites can cross the intestinal barrier and reach the circulatory system influencing other organs' immune response, among these the lung [25–27]. However, although the gut's impact on respiratory function is well reckoned, the available evidence of the other way round is still sparse. Nevertheless, postulated apoptosis dysfunction in the intestine tract related to concurrent respiratory infections [28] may account for COVID-19-associated gastrointestinal symptoms.

On the contrary, some COVID-19 patients who resolved SARS-CoV-2 infection in their respiratory tract exhibited the presence of SARS-CoV-2 RNA in their fecal samples, suggesting that the virus replication in the gastrointestinal tract may be independent of the respiratory compartment [11, 29, 30]. This hypothesis is endorsed by recent studies suggesting that gut involvement in COVID-19 is even more severe and prolonged compared with the respiratory tract [31].

Gut microbiome components have significant microbial inhibitory properties toward lung tissues, accomplished through alveolar macrophage, neutrophils, and natural killer cell activity [32, 33]. To a greater extent, bacterial metabolites, as short-chain fatty acids (SCFAs), are proved to act in the lungs attenuating inflammatory responses. Moreover, some bacterial strains could enhance the release of molecules with antiviral activity like the nuclear factor erythroid 2p45-related factor 2 (Nrf2) and its target Heme oxygenase-1 (HO-1) [34–38].

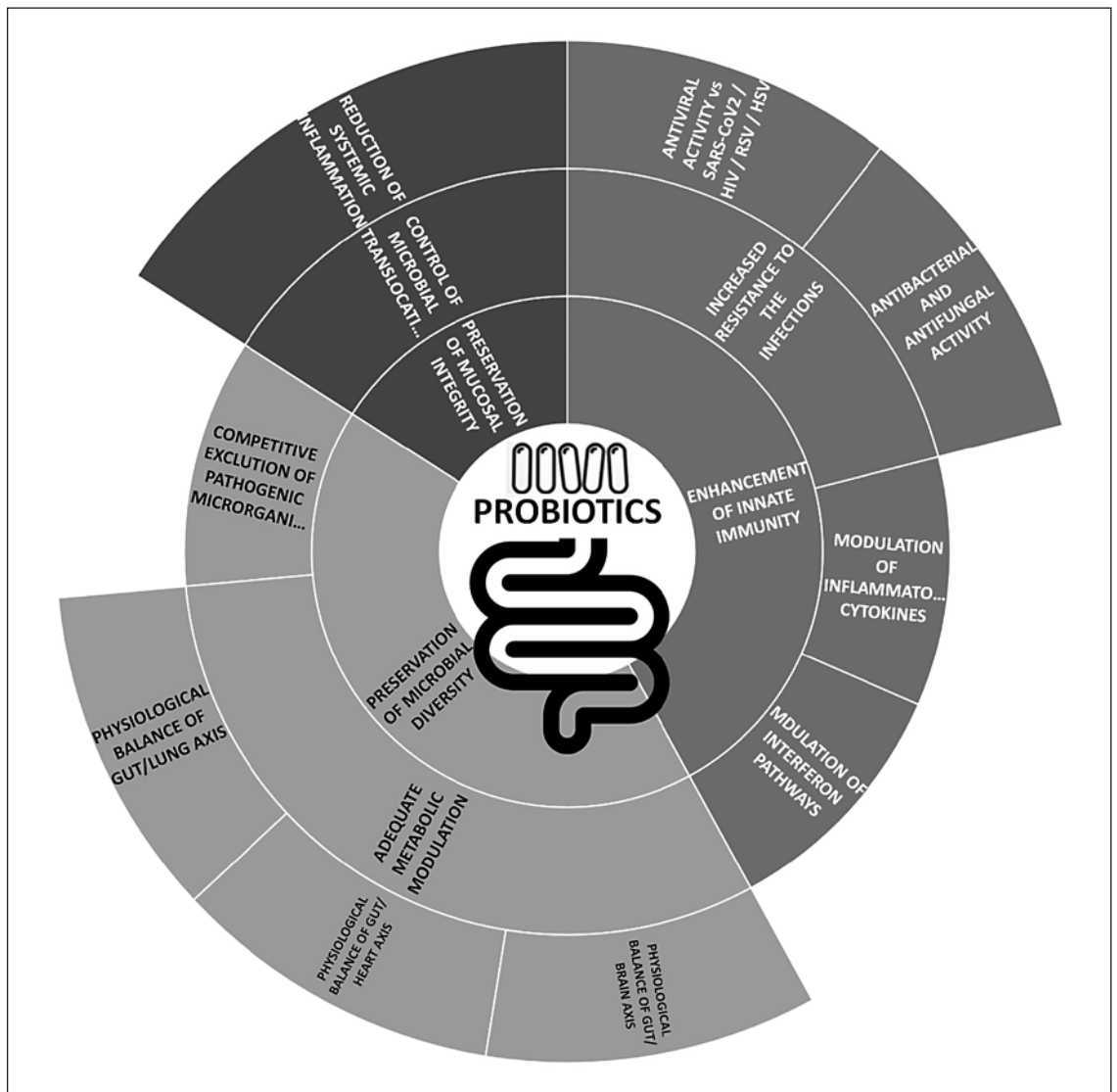
Although the evidence in COVID-19 is still sparse, bacteriotherapy could represent a potential strategy to counteract SARS-CoV-2 infection. Given the microbiome's crucial role in modulating host immune and inflammatory response, bacteriotherapy may minimize gastrointestinal symptoms and shield the respiratory tract.

## Targeting Microbiome to Prevent SARS-CoV-2

As stated by the WHO, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [39]. Historically, the concept of probiotics began around 1,900 by the Nobel laureate Elie Metchnikoff, who discovered that the consumption of live bacteria (*Lactobacillus bulgaricus*) in yogurt or fermented milk improved some biological features of the gastrointestinal tract [40]. Probiotics are now widely available, generally in dairy products, such as yogurt, dessert, ice cream, juices, and capsules, drops, sachets, etc. The most common strains commercially available belong to the *Lactobacillus* and *Bifidobacterium* species, which proved some beneficial effects to the human body when administered in adequate amounts. These mentioned bacterial species are known to be involved in some essential physiological functions such as stimulation of immune response, prevention of pathogenic and opportunistic microbial colonization, production of SCFA, catabolism of carcinogenic substances, and synthesis of vitamins such as B and K [41–43]. In this regard, medical data showed that particular strains of probiotics facilitate the prevention of viral and bacterial infections (such as sepsis, gastroenteritis, and respiratory tract syndrome [4]), improve the intestinal epithelial barrier function, and compete with disease-causing agents for nutrients. Similar to other SARS coronaviruses, SARS-CoV-2 interacts with ACE2 receptor for gut and lung intracellular invasion [2, 24] (Fig. 1). For these reasons, some researchers suggested that ACE inhibitors might benefit patients with COVID-19 by reducing pulmonary inflammation [44] although others argued that ACE inhibitors might enhance viral entry regulating ACE2 levels.

The potential interaction between probiotics and ACE enzymes was suggested in the previous studies addressing the probiotics' potential antihypertensive effect [45]. Indeed, during food fermentation, probiotics release bioactive peptides able to inhibit the ACE enzymes by blocking the active sites [46, 47]. The debris of the dead probiotic cells also acted as ACE inhibitors [48].

In this respect, Anwar et al. [49] demonstrated in a computational docking study that 3 metabolites of *Lactobacillus plantarum* (*Plantaricin W*, *Plantaricin JLA-9*, and *Plantaricin D*) prevent the binding of SARS-CoV-2 with ACE2 receptors suggesting therefore antiviral property of *L. plantarum* against SARS-CoV-2. Taken all together, these findings stress the assumption that probiotics could compete with ACE2 receptors paving the way for their potential use to prevent SARS-CoV-2 infection.



**Fig. 1.** Mechanisms of action of probiotic supplementation. Legend: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

Bacteriotherapy was found to reduce both upper and lower respiratory tract infections [50]. Probiotic lactic acid bacteria were administered directly in the respiratory tract or as oral supplements to improve the immune response and prevent viral infections [51]. As an alternative mechanism of action, probiotics were also found to inhibit viruses by interacting directly with them with a mechanism similar to phagocytosis.

More recently, lactobacilli isolated from healthy human noses showed probiotic effects in the form of nasal spray [52], by avoiding the attack of viral particles to mu-

cosal cells. These findings also open the chance to deploy probiotics in a nasal spray to boost the immune system and avoid respiratory tract infections.

To the best of our knowledge, no clinical trial has formally investigated the role of probiotics in preventing COVID-19 so far. However, a multicentric RCT is currently evaluating the effects of a 2-month probiotic supplement on the incidence and severity of COVID-19 among health-care workers exposed to SARS-CoV-2 (Table 1) [53]. The trial was completed in October 2020, and results are expected soon.

**Table 1.** Summary of studies and trials addressing the role of probiotics in the prevention and treatment of COVID-19 [54–64]

Reference	Methodology	Primary outcome	Probiotic strains	Probiotic supplementation strategy	Results
Blanque [57] (NCT043566180)	RCT, multicenter	Prevention Incidence and severity of SARS-CoV-2 infection in health-care workers	<i>Lactobacillus coryniformis</i> K8	3×10 <sup>9</sup> UFC/day Duration: 2 months	Ongoing
d'Ettoire et al. [54]	Open-label, parallel-group trial, single center	Treatment Comparing respiratory failure incidence and symptoms control	<i>Streptococcus thermophilus</i> DSM 32345, <i>L. acidophilus</i> DSM 32241, <i>L. helveticus</i> DSM 32242, <i>L. paracasei</i> DSM 32243, <i>L. plantarum</i> DSM 32244, <i>L. brevis</i> DSM 27961, <i>B. lactis</i> DSM 32246, <i>B. lactis</i> DSM 32247 (Sivomixx <sup>®</sup> , SivobioMe <sup>®</sup> )	2.4×10 <sup>6</sup> UFC/day in 3 equal doses Duration: 14 days	Probiotic administration is associated with a lower risk of respiratory failure and a faster control of COVID-19-related symptoms (in particular diarrhea)
Ceccarelli et al. [55]	Retrospective, observational, single center	Treatment Comparing mortality, length of hospitalization, need of intensive care treatment	<i>Streptococcus thermophilus</i> DSM 32245, <i>Bifidobacterium lactis</i> DSM 32246, <i>Bifidobacterium lactis</i> DSM 32247, <i>L. acidophilus</i> DSM 32241, <i>L. helveticus</i> DSM 32242, <i>L. paracasei</i> DSM 32243, <i>L. plantarum</i> DSM 32244, and <i>L. brevis</i> DSM 27961 (Sivomixx)	2.4×10 <sup>6</sup> UFC/day in 3 equal doses Duration: not available	Oral bacteriotherapy is associated with a lower mortality but a longer length of hospitalization
Sung et al. [58] (NCT04399252)	RCT	Basic Science Impact on microbiome in patients that develop COVID-19	<i>Lactobacillus rhamnosus</i> GG	2 caps/day Duration: 28 days	Ongoing
Pugliese [56, 59] (NCT043566089)	RCT	Treatment Delta in the number of patients requiring orotracheal intubation despite treatment (ozone therapy-based intervention accompanied by supplementation with probiotics vs. standard of care)	<i>Streptococcus thermophilus</i> DSM 32245, <i>Bifidobacterium lactis</i> DSM 32246, <i>Bifidobacterium lactis</i> DSM 32247, <i>L. acidophilus</i> DSM 32241, <i>L. helveticus</i> DSM 32242, <i>L. paracasei</i> DSM 32243, <i>L. plantarum</i> DSM 32244, and <i>L. brevis</i> DSM 27961 (Sivomixx)	200 billion – 6 sachets twice a day Duration: 7 days	Ongoing
Pasquier [60] (NCT04621071)	RCT	Treatment Duration of COVID-19 symptoms	Not available	2 strains 10×10 <sup>9</sup> CFU Duration: 25 days	Ongoing
Desrosiers [61] (NCT04458519)	RCT	Treatment Change in severity of COVID-19 infection	Nasal irrigations with <i>Lactococcus Lactis</i> W136 (probiotrise)	2.4 billion CFU Nasal irrigation twice a day Duration: 14 days	Ongoing
Navarro [62] (NCT04390477)	RCT	Treatment Percentage of patients with discharge in ICU	Not available	1×10 <sup>9</sup> CFU/day Duration: 30 days	Ongoing
Gea Gonzalez [63] (NCT04517422)	RCT	Treatment Severity progression of COVID-19, length of stay at ICU, mortality ratio	<i>L. plantarum</i> CECT7481, <i>L. plantarum</i> CECT7484, <i>L. plantarum</i> CECT7485, and <i>Pediococcus acidilactici</i> CECT7483	Once a day Duration: 30 days	Ongoing
Stadlbauer [64] (NCT04420676)	RCT	Treatment Duration of diarrhea in COVID-19 patients	<i>Bifidobacterium bifidum</i> W23, <i>Bifidobacterium lactis</i> W51, <i>Enterococcus faecium</i> W54, <i>L. acidophilus</i> W37, <i>L. acidophilus</i> W55, <i>L. paracasei</i> W20, <i>L. plantarum</i> W1, <i>L. plantarum</i> W62, <i>L. rhamnosus</i> W71, and <i>L. salivarius</i> W24 (Omni-Biotic 10.AAD)	Twice a day Duration: 30 days	Ongoing
RCT, randomized controlled study; ICU, intensive care unit; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.					

In summary, probiotics may act as antiviral agents interfering with viral entry into cells and/or inhibiting virus replication. This may lead to a limitation in the spread of SARS-CoV-2 in the gut and respiratory tract, as a result of the restoration of the gut and respiratory microbiota.

### Targeting Microbiome to ht SARS-CoV2

Since the gut microbiome is altered in COVID-19 patients [18], probiotics supplementation may help restore the gut microbiota and, therefore, maintain a healthy gut-lung axis and minimize translocation of pathogenic bacteria across the gut barrier as well as the chances of secondary bacterial infections. As cytokines storm occurs in patients with severe COVID-19, immune-modulatory effects of probiotics might be relevant to prevent acute lung injury, acute respiratory distress syndrome, and multiple organ failure, which are life-threatening complications of COVID-19 [54].

Boosting immune responses during the incubation and nonsevere stages of COVID-19 infection are perceived as crucial to eliminate the virus and prevent disease progression to severe stages. Administration of certain *Bifidobacteria* or *Lactobacilli* has a beneficial impact on influenza virus clearance from the respiratory tract [65]. Besides, probiotic strains increase the levels of type I interferons, the number and activity of antigen-presenting cells, NK cells, T cells, and the levels of systemic and mucosal-specific antibodies in the lungs [65].

Another relevant effect of probiotics is to enforce and maintain the integrity of tight junctions between enterocytes: Hummel et al. [66] showed that gram-positive probiotic lactobacilli modulate epithelial barrier function via their effect on adherence junction protein expression and complex formation [66]. Also, incubation with lactobacilli differentially influences the phosphorylation of adherence junction proteins and the abundance of protein kinase C (PKC) isoforms such as PKC $\delta$  that positively modulates epithelial barrier function.

To this extent, in our previous work, HIV-1-infected patients receiving oral bacteriotherapy exhibited histomorphological and ultrastructural changes in their gut mucosa, characterized by an improvement of epithelial integrity, a reduction of inflammatory infiltrate and enterocyte apoptosis in the terminal ileum, cecum, ascending, transverse, and descending colon [67].

Consistent with previous findings, these immunomodulatory benefits seem to be equally crucial in COVID-19 patients. Based on these shreds of evidence, our group ad-

ressed the topic over the past year, carrying out 2 retrospective observational studies including adults with severe COVID-19 pneumonia to investigate the role of oral bacteriotherapy on the top of best available therapy [54, 55].

In the first piece of work, we compared respiratory failure incidence and symptoms control in patients with COVID-19 pneumonia receiving a probiotic multistrain formulation (Sivomixx<sup>®</sup>, SivoBiome<sup>®</sup>) in adjunction to standard medical therapy [54]. Out of 70 patients enrolled in the study, 28 received oral bacteriotherapy for 14 days. According to our results, 92.9% of the intervention group achieved diarrhea and other symptoms control within 72 h from study inclusion (vs. less than half in 7 days in the not supplemented group). Moreover, the risk of developing respiratory failure was 8-fold lower in patients receiving oral bacteriotherapy. Both the prevalence of ICU admission and mortality were lower in the intervention group [54].

In the second study, we extended our sample size. We focused our observation on mortality, ICU admission, and length of hospital staying of patients with COVID-19 pneumonia receiving probiotics as complementary therapy [55]. Out of 200 patients enrolled, 88 received oral bacteriotherapy (Sivomixx<sup>®</sup>). Results concerning mortality were quite encouraging: we found a significant reduction in the intervention group (11 vs. 30%;  $p < 0.001$ ). In addition to that, by multivariate analysis, bacteriotherapy emerged as an independent variable associated with a reduced risk for death. In terms of ICU admission, no significant difference among the 2 groups was found. By contrast, we found a longer length of hospitalization in patients receiving bacteriotherapy. We interpreted this data in line with the lower mortality rate of this group [55].

Interestingly, no adverse reactions in patients treated with oral bacteriotherapy were recorded in both studies. In conclusion, for the first time, we determined the role of probiotics in treating patients with COVID-19 pneumonia, providing positive evidence in favor of their implementation in addition to the best available therapy. To the best of our knowledge, 7 RCTs that may soon replicate this insight are currently ongoing (Table 1).

### The New Frontier: Are Distinct Microbiome Patterns Associated with Different Risks of COVID-19 Progression?

Several studies showed that dietary habits and the amount of food consumed could shape human microbiome [68]. Diet in developing countries usually con-

sists of food containing more fiber than a modern Western diet consisting of food often processed and kept in cold storage [69]. It is now well reckoned that different populations with different diets could have distinct microbiome patterns. Generally, *Firmicutes* are dominant in people with animal-based diets, whereas *Bacteroidetes* are dominant in people with a vegetarian diet [70]. Similarly, molecules involved in the digestion of fiber and concentration of SCFAs are differently represented in the microbiome of populations with different diets [71, 72]. Interestingly, a plant food-based diet maintains a more stable microbiota diversity and eubiosis and promotes the microbes that ensue anti-inflammatory response [73]. Preliminary reports suggested that different progression rates to severe disease and fatality rates observed for SARS-CoV-2 in diverse populations could be related to distinct microbiome patterns. For example, it was observed that India reported a fatality rate caused by SARS-CoV-2 lower if compared to other regions consuming meat-rich diet and saturated fatty acids, such as the USA, Brazil, and European countries [74]. Currently, it is not possible to strongly support this hypothesis, but nevertheless, it could be a clue to understand the variations observed in the impact of COVID-19 in populations residing in different geographical areas.

### Expert Opinion

Probiotics may play a beneficial role even though there is much to be discovered on their specific mechanisms of action against SARS-CoV-2. The damage of the gut barrier integrity associated with microbial translocation along with the dysregulated inflammatory response explains why probiotics could represent a valuable therapeutic tool in COVID-19 patients [56, 69, 75–79].

However, clinicians should be mindful that probiotics' clinical benefit depends upon several factors, such as the bacterial composition of different commercial products, manufacturing processes, dose regimen, etc. Several studies had addressed the impact of probiotics in treating many gastrointestinal disorders, such as *Clostridium difficile* colitis, inflammatory bowel disease, *Helicobacter pylori* infection, etc. [70, 71]. Moreover, in recent studies, probiotics were found to restore gut barrier integrity and, therefore, the gut-brain axis in HIV patients [72]. In conclusion, further understanding of gut microbiome modulation on host health is expected to expand probiotic clinical applications soon.

### Conclusion

The “gut-lung axis” pathophysiology suggests that the intestinal microbiota may play a role in counteracting the “cytokine storm,” which is now clearly being the cornerstone of COVID-19 disease [80]. Even though evidence coming from clinical trials is still on the way, we showed for the first time a consistent reduction in mortality and more successful symptoms control in patients with COVID-19 pneumonia receiving oral bacteriotherapy as a complementary therapy.

Therefore, we suggest physicians consider the early administration of oral bacteriotherapy on the top of best available treatment while dealing with patients with COVID-19 pneumonia, especially in those experiencing gastrointestinal symptoms. This alternative option has multiple advantages, indeed: it is mostly freely available, cheap, and with limited/no adverse effects.

### Statement of Ethics

Not applicable

### Conflict of Interest Statement

All the authors declare no competing interests.

### Funding Sources

The authors did not receive any financial support (funding, grants, and sponsorship) to be acknowledged.

### Author Contributions

All the authors equally contributed to the literature search and manuscript drafting.

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