

The impact of infection with COVID-19 on the respiratory microbiome: A narrative review

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ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has affected millions of individuals with various implications. Consistent with the crucial role of the microbiome in determining health and disease in humans, various studies have investigated the gut and respiratory microbiome effect on the COVID-19. Microbiota dysbiosis might support the entry, replication, and establishment of SARS-CoV-2 infection by modulating various mechanisms. One of the main mechanisms that the modulation of respiratory microbiota composition during the COVID-19 infection affects the magnitude of the disease is changes in innate and acquired immune responses, including inflammatory markers and cytokines and B- and T-cells. The diversity of respiratory microbiota in COVID-19 patients is controversial; some studies reported low microbial diversity, while others found high diversity, suggesting the role of respiratory microbiota in this disease. Modulating microbiota diversity and profile by supplementations and nutrients can be applied prophylactic and therapeutic in combating COVID-19. Here, we discussed the lung microbiome dysbiosis during various lung diseases and its interaction with immune cells, focusing on COVID-19.

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Introduction

Although severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in China's Hubei province in November 2019, it rapidly spread globally and caused Coronavirus Disease 2019 (COVID-19) pandemic disease [1]. Despite the approval of some preventive vaccines and the availability of vaccination programs, COVID-19 has affected around 311 million people worldwide by now (11 January 2022). Mild fever, fatigue, dry cough, sore throat, diarrhoea, and anosmia are the most common symptoms in SARS-CoV-2-infected patients [2]. Many infected people remain asymptomatic or have only mild upper respiratory tract symptoms, but others exhibit acute respiratory distress syndrome (ARDS) as well as pneumonia, necessitating intubation that can experience fatal complications [3]. Alongside respiratory complications, infection with SARS-CoV-2 can cause acute kidney injury, vascular thrombosis, endothelial sloughing, and shock [4]. Thus, multi-organ failures and complications imposition great stress for healthcare systems and prolonged hospitalization [5].

The microbiome is an aggregate of microorganisms, including bacteria, fungi, viruses, and protozoans, that reside in a particular microenvironment on or within the human body and are involved actively in cellular metabolisms and functions [6]. There is evidence that the interaction between microbes and humans plays a decisive role in determining the health or disease status in the human body owing to the contribution of microbiomes to the improvement or impairment of immune and metabolic functions [7]. Thus, understanding host-microbe interactions and dysbiosis of the microbiome could be useful in diagnosing and introducing appropriate treatment for diseases. Due to the high expression of the angiotensin-converting enzyme 2 (ACE2), a receptor of the SARS-CoV-2 virus, in respiratory and gastrointestinal tracts [8], some emerging scientific and clinical investigations have indicated the dysbiosis of the gut and respiratory microbiome during COVID-19 infection [9,10]. For example, the analyses of stool microbiome from 15 patients during hospitalization in Hong Kong revealed

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that COVID-19 patients exhibited remarkably reduced bacterial diversity and increased abundance of opportunistic ones, such as *Rothia*, *Streptococcus*, *Actinomyces*, and *Veillonella* [11]. On the other hand, the invasion of the SARS-CoV-2 virus towards the lungs for beginning COVID-19 disease is related to the lung infection and subsequent immune responses in which the lung microbiome might play an essential role in the initiation, development, and response to therapeutic agents. This review will summarize the lung microbiome dysbiosis during various lung diseases and its interaction with immune cells and responses. We focused on the alterations in the lung microbiome in patients infected with COVID-19 and how these alterations can be used to improve treatment outcomes.

Lung microbiome dysbiosis during diseases

The studies based on the traditional culture systems have reported that the bronchial tree in healthy individuals is sterile [12,13]. Nowadays, the advent of high-throughput sequencing technologies and culture-independent techniques, such as sequencing the 16S rRNA gene, revealed that the bronchial tree is not sterile, even in healthy people. The lungs are a microbial ecosystem with a collection of microorganisms, both viable and non-viable, colonizing in the bronchial tree and parenchymal tissues, and are essential for health, including immunoregulation, epithelial integrity, and colonization resistance [14]. The main genera have been identified in healthy lungs are *Streptococcus*, *Prevotella*, *Fusobacterium*, *Haemophilus*, and *Veillonella* [15,16]. Compared to the gastrointestinal (GI) tract, which contains 10^{11} - 10^{12} bacteria per gram of tissue, the microbial population in the lungs of healthy individuals is relatively lower (about 10^3 - 10^5 bacteria per gram of tissue) [17]. It is reported that there are 10–100 bacteria per 1000 human cells in the lung tissue samples [18], and three factors determine its composition: 1) microbial immigration, such as direct mucosal dispersion, bacteria inhalation, and microaspiration; 2) microbial elimination, such as host defence responses, mucociliary clearance, and cough; and 3) regional growth conditions, such as pH, temperature, and nutrient availability [19,20].

It has been shown that both acute and chronic respiratory diseases dramatically alter the lung microbiome composition (Table 1). For instance, patients with idiopathic pulmonary fibrosis (IPF) [35], cystic fibrosis (CF) [36] and bronchiectasis [37] have exhibited an increase in bacterial burden in their lower airways. In addition to changes in the load of bacteria, some specific bacteria are frequently identified in the

airways of unhealthy people, such as *Streptococcus*, *Veillonella*, *Neisseria*, and *Haemophilus* (in IPF); *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Burkholderia* spp. (in CF); and *Haemophilus*, *P. aeruginosa*, *Prevotella*, and *Veillonella* (in bronchiectasis) [38]. In asthma, bacterial composition and dysbiosis in the lung airway contribute to the severity of the disease and its pathogenesis. Using 16S rRNA-based methods, Huang *et al.* indicated airway dysbiosis in patients with the severe asthmatic condition compared with milder asthma which promotes inflammatory responses by recruiting neutrophils in a Th17-dependent manner. They also found that the lungs of patients with severe asthma were remarkably enriched with *Actinobacteria* and *Klebsiella* [39]. In patients with chronic obstructive pulmonary disease (COPD), the microbiome analysis revealed a reduction in microbial diversity associated with remodelling of the alveolar and bronchiolar tissue, the infiltration of CD4+ T-cells, and emphysematous destruction. The COPD patients exhibited an increase in *Actinobacteria* and *Proteobacteria* and reduced *Bacteroidetes* and *Firmicutes*. This alteration in microbiota composition was associated with the infiltration of neutrophils, B-cells, and eosinophils, suggesting the correlation between host immune responses and lung dysbiosis [40]. Furthermore, numerous studies showed that the gut microbiota composition in lung cancer patients is significantly different from healthy control ones, and the respiratory microbiome dysbiosis contributes to inflammation responses and tumorigenesis of lung cancer [41]. A systematic review conducted on 41 studies indicated that pre-existing *Mycobacterium tuberculosis* is associated with the risk of lung cancer [42]. The examination of bronchoalveolar lavage fluid (BALF) samples from 28 patients (20 with lung cancer and 8 at the benign stage) revealed the increase of genera *Megasphaera* and *Veillonella* in patients with lung cancer and TM7 and Firmicutes phyla compared with patients at the benign stage [31].

In addition to the mentioned pathological complications, there is evidence that smoking is another condition that affects the lung microbiota. Smoking and exposure to tobacco could increase the risk of infection with bacteria by increasing the microbial diversity at the lower respiratory tract [43]. Cigarette smoking increases the risk of ARDS in patients following severe trauma. The lung of these patients with smoking status was enriched with pathogenic bacteria, including *Haemophilus*, *Fusobacterium*, *Streptococcus*, *Prevotella*, and *Treponema* [44]. Also, cigarette smoking could alter virus populations in the lungs. Gregory *et al.* indicated that the abundance of bacteriophages infecting *Actinomyces*, *Haemophilus*, *Xanthomonas*, *Rodofexax*,

Table 1. Alteration of lung microbiome during respiratory diseases.

Disease	Sample type	Sample size	Microbiome alteration	Ref
CF	BAL	95	↑ <i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Pseudomonas</i>	[21]
CF	Sputum	17	↑ <i>Staphylococcus</i> , <i>Pseudomonas</i> , <i>Achromobacter</i> , <i>Stenotrophomonas</i>	[22]
CF	BAL	12	↑ <i>Burkholderia</i> , <i>Streptococcus</i> , <i>Prevotella</i> , <i>Porphyromonas</i> , <i>Haemophilus</i> , <i>Veillonella</i>	[23]
COPD	Sputum	16	↑ <i>Pseudomonas</i> , <i>Moraxella</i> , <i>Corynebacterium</i>	[24]
COPD	Lung tissue	24	↑ <i>Firmicutes</i> , <i>Lactobacillus</i>	[18]
COPD	Sputum	8	↑ <i>Fusobacterium</i> , <i>Bacteroidetes</i> , <i>Actinobacteria</i> , <i>Firmicutes</i> , <i>Streptococcus</i> , ↓ <i>Proteobacteria</i>	[25]
Asthma	Nasopharyngeal	234	↑ <i>Proteobacteria</i> , <i>Streptococcus</i>	[26]
Asthma	Bronchoscopy	42	↑ <i>Neisseria</i> , <i>Haemophilus</i> , <i>Porphyromonas</i> , <i>Fusobacterium</i>	[27]
Asthma	BAL and endobronchial brushing	58	↑ <i>Pseudomonas</i> , <i>Lactobacillus</i> , <i>Rickettsia</i>	[28]
Lung cancer	Saliva	30	↑ <i>Selenomonas</i> , <i>Veillonella</i> , <i>Capnocytophaga</i> , ↓ <i>Streptococcus</i> , <i>Neisseria</i>	[29]
Lung cancer	Lung tissues and bronchoscopy	42	↑ <i>Streptococcus</i> , ↓ <i>Staphylococcus</i>	[30]
Lung cancer	BAL	28	↑ <i>Veillonella</i> , <i>Megasphaera</i>	[31]
IPF	BAL	55	↑ <i>Streptococcus</i> , <i>Staphylococcus</i>	[32]
IPF	BAL	65	↑ <i>Streptococcus</i> , <i>Veillonella</i> , <i>Haemophilus</i> , <i>Neisseria</i>	[33]
IPF	Bronchoscopy	35	↑ <i>Pseudomonas</i> , <i>Streptococcus</i> , <i>Haemophilus</i> , <i>Prevotella</i> , <i>Veillonella</i>	[34]

CF, cystic fibrosis; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis.

Aeromonas, *Prevotella*, and *Capnocytophaga* was increased in smokers, whereas *Morganella* and *Enhydrobacter* bacteriophages were prevailing in non-smokers [45].

Immune responses against SARS-CoV-2 infection

The intracellular adherence and tight junctions act as connectors of adjacent cells as well as barriers to regulate paracellular permeability. The impairment of barrier function during diseases increases epithelial permeability and, subsequently, pathogen entry [46]. During infection with viruses, they can bind to their cognate cellular receptors and induce the stimulation of pattern recognition receptors (PRRs) in the epithelial cells, resulting in the secretion of molecules, enzymes, peptides, reactive oxygen species (ROs), and chemokines with anti-microbial activities. Collectively, the released molecules recruit immune cells and facilitate their communication with each other, contributing to the immune responses that are essential for controlling the infection [47]. In the case of COVID-19, the infection of alveolar type II cells with higher expression of ACE2 receptor through SARS-CoV-2 disrupts the critical functions of these cells, including stabilization of airway epithelial barrier, airway regeneration following injury, and production of pulmonary surfactant [48]. These cells also are involved in the immune responses against pathogens and alveolar damage by producing cytokines to stimulate the recruitment and activation of immune cells, especially macrophages, in defence of the alveolus [49]. Following the binding of the S1 subunit in the viral spike (S) protein, through its receptor-binding domain (RBD), to the receptor, the S protein undergoes protease cleavage by serine protease transmembrane protease serine 2 (TMPRSS2), resulting in uncovering the S2 site and subsequent membrane fusion between the virus and alveolar type II cells, endocytosis of

the virus, and release of viral compartments into the cell cytoplasm [50,51]. Neuropilin 1 (NRP1) and Furin also have been identified as co-factors of virus internalization [51,52]. The infected cells release damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), for-example viral RNA, which are recognized by various intracellular PRRs, like RIG-I/MDA5/MAVS/TRAF3/IRF3/IRF7/type I IFNs and TLRs/TRIF/MyD88/TRAF6/p65/p50/TNF pathways (Figure 1). Certainly, targeting these pathways could be beneficial therapeutic strategies in virus-induced diseases. For example, targeting TNF- and IFN-mediated responses during the respiratory syncytial virus infection protects lung cells against the harmful effects of the viral infection [53,54].

The involvement of the host immune system is characterized by the contribution of the innate immune system in the first phase. A typical alveolar immune landscape in the healthy lung is composed mainly of alveolar macrophages located at the air-liquid interface and a few granulocytes, including eosinophils and basophils [55]. Triggering PRR signalling within cells involved in the innate immune responses elevates the levels of pro-inflammatory factors, including tumour necrosis factor (TNF)- α and granulocyte macrophage-colony stimulating factor (GM-CSF) in the plasma of COVID-19 patients as well as interleukin (IL)-1 β , IL-6, and IL-8, in their BALF and plasma [56–59]. Additionally, PRR signalling could promote the recruitment and infiltration of neutrophils, monocytes, and T-cells to the infection site by upregulating CXCL8, CCL2, and CCL7 [57,60]. Importantly, the signalling of PRR activates the production of anti-viral type I IFNs within plasmacytoid dendritic cells (DCs) [61]. IFNs could stimulate phagocytosis in macrophages and the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway, which directs the polarization of anti-viral helper T-cells [62].

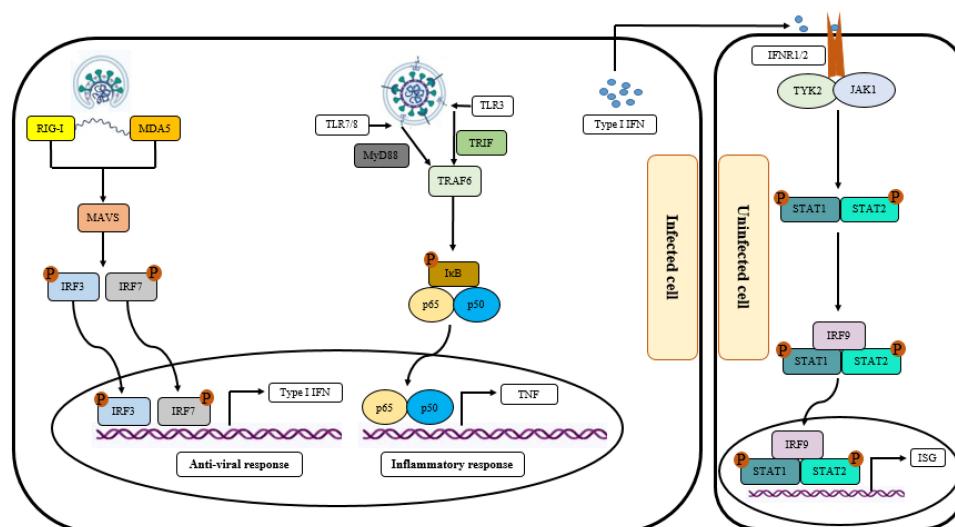


Figure 1. The effect of infection with SARS-CoV-2 on the innate immune response. Following infecting the target cells, the RNA of the virus was recognized by the cytosolic pattern recognition receptors (PRRs), such as the melanoma differentiation-associated protein (MDA5) and the retinoic acid-inducible gene 1 (RIG-I), which recruit the mitochondrial antiviral signaling protein (MAVS), followed by the phosphorylation and activation of interferon regulatory factor 3 and 7 (IRF3 and IRF7). The phosphorylated form of IRF3 and IRF7 upregulates the expression of type I interferons, as the antiviral response. In addition to the cytosolic receptors, the infection with SARS-CoV-2 is recognized by the endosomal receptors, such as toll-like receptors 3, 7, and 8 (TLR3, TLR7, and TLR8), leading to trigger a cascade to upregulate inflammatory responses. The secreted type I IFNs from the infected cells are recognized through their cognate receptors on the uninfected cells, resulting in the activation of IRF9, STAT1, and STAT2, and subsequently, upregulation of interferon-stimulated gene (ISG).

Besides the innate immune system, adaptive immune responses in COVID-19 patients contribute to managing and controlling viral infection. The lysed and damaged epithelium cells in the lung and sub-epithelial DCs present virus antigens to CD8+ and CD4+ T-cells, leading to cytotoxicity activation against virus-infected cells and induction of apoptosis as well as promoting differentiation of CD4+ T-cells towards Th1, Th2, Th17, and T follicular helper (FH) [63]. Recently, Pavel *et al.* indicated that the imbalance in Th2/Th1 and Th17/Th1 cytokines could affect the outcomes in patients with SARS-CoV2 infection and is related to their mortality risk [64]. Also, T_{FH} cells help the development of B-cells into plasma cells, promoting the production of virus-specific IgG, IgM, and IgA [63]. Mazzoni *et al.* exhibited that asymptomatic patients showed lower proportions of the viral-specific CD4+ T-cells and lower humoral response, suggesting that the frequency of SARS-CoV-2-reactive T-cells is associated with the disease severity. Furthermore, they revealed that asymptomatic individuals show lower multifunctionality of antigen-specific T-cells and lower expression of TIGIT and PD-1 immune-checkpoints compared with symptomatic patients. This indicates that immune responses in symptomatic patients against SARS-CoV-2 can not eliminate the virus rapidly, thus, resulting in repeated activation of

immune system cells [65]. In another study, Qin *et al.* found that the population of memory and regulatory T-cells were lessened in COVID-19 patients, whereas naïve T-cell counts were increased compared to healthy ones [66]. Single-cell analysis of T-cells from COVID-19 patients revealed that CD4+ T-cells were activated with high expression of regulatory responses and CD25 as well as suppression of FOXP3 expression in severe COVID-19. These cells showed unique differentiation pathways in the patient's lungs with both Th1 and Th2 characteristics. In addition, highly activated CD25-expressing CD4+ T-cells facilitated SARS-CoV-2 entry by producing the protease Furin [67].

Infection with SARS-CoV-2 and microbiome dysbiosis

Since super-infection or co-infection with bacteria partially attributed to the worth outcome and mortality of virus pandemics, including the 1918 H1N1 and 2009 H1N1 influenza, various studies examined this in the infection with COVID-19. The microbiome in humans differs across sex, age, ethnicity, and race, suggesting the unique and specific profiles of the microbial population [68]. The infection of the GI tract by SARS-CoV-2 could disrupt gut microbiota and lead to dysbiosis, GI symptoms, and intestinal inflammation. This

imbalance can not be fully restored after even three months of recovery [69]. Also, there is evidence that gut microbiota dysbiosis during infection with SARS-CoV-2 could affect the severity of COVID-19 [70]. It has been shown that gut microbiota could regulate immune responses and modulation of their composition had preventive and therapeutic benefits [71]. For instance, beneficial bacteria *Bifidobacteria* and *Lactobacilli* and butyrate-producing bacteria *Eubacterium rectale* and *Faecalibacterium prausnitzii* were decreased in patients with COVID-19 [72,73], whereas the population of opportunistic bacteria such as *Clostridium hathewayi* and *Clostridium ramosum* were increased and positively correlated with COVID-19 severity [73]. Zuo *et al.* indicated that the faecal samples from patients with COVID-19 were enriched with *Collinsella tanakaei*, *Collinsella aerofaciens*, *Morganella morganii*, and *Streptococcus infantis*, whereas the samples from none-to-low SARS-CoV-2 infectivity displayed a higher abundance of *Lachnospiraceae bacterium 1_1_57FAA*, *Alistipes onderdonkii*, and *Bacteroides stercoris* [74]. Furthermore, there is a negative correlation between the diversity of gut microbiota and COVID-19 severity levels; the diversity decreases with increasing the symptoms of severity in which the “severe” patients show the least diversity [75]. Khan *et al.* demonstrated that the severely COVID-19 patients had elevated levels of IL-21 in comparison with mildly ill and healthy participants, while there were no remarkable differences in the levels of INF- γ and TNF- α among the groups [75]. The investigation of alterations in the gut microbiota of 115 patients and its influence on COVID-19 severity revealed that moderate and severe patients were enriched with *Proteobacteria* compared with mild ones. In contrast, the abundance of butyrate-producing bacteria, including *Lachnospira* and *Roseburia*, and the *Firmicutes/Bacteroidetes* ratio was lower in moderate and severe patients [76]. The produced butyrate, short-chain fatty acids (SCFAs), and propionate with gut microbiota could increase the expression of Tc17 cells and CD8+ cytotoxic T lymphocytes (CTLs). For instance, butyrate could increase granzyme B and IFN- γ expression on CTLs as well as promote the Tc17 cells switch towards cytotoxic phenotypes [77]. In a study, Zhou *et al.* compared the gut microbiota composition and immune responses in moderate COVID-19 patients with and without fever. They found that opportunistic pathogens, including *Saccharomyces cerevisiae* and *Enterococcus faecalis*, were enriched in COVID-19 patients with fever. *S. cerevisiae* was positively associated with diarrhoea symptoms in these patients, whereas *E. faecalis* was

positively associated with D-dimer and lactate dehydrogenase (LDH) and negatively correlated with IL-4 and CD8+ T-cells. On the other hand, the proportion of species with protective and anti-inflammatory effects, including *Eubacterium ramulus* and *Bacteroides fragilis*, was increased in non-fever patients. Also, there was a negative correlation between enrichment with *E. ramulus* with aspartate aminotransferase (AST), LDH, and IL-6. This study suggested that the gut microbiota contributes to the induction of fever in COVID-19 patients by increasing the pathogenic bacteria in the GI tract, which stimulate the secretion of inflammatory cytokines, including IL-6 [78]. Tao *et al.* found that the changes in the gut microbiota composition is positively correlated with the higher expression of the pro-inflammatory factor IL-18 [79]. Thus, gut microbiota diversity and composition could be act as prognostic biomarkers for COVID-19 severity and immune responses.

Profiling of the respiratory microbiome in COVID-19 patients

Besides the gut microbiota, infection with SARS-CoV-2 could affect the population and metabolism of respiratory microbiota. The profiling microbiomes of the COVID-19 respiratory tract by Haiminen *et al.* revealed that metabolic pathways are modulated, including a decrease in lipid metabolism (such as sphingolipid metabolism) and glycan metabolism pathways (such as glycan degradation), and an increase in carbohydrate metabolism pathways (such as glycolysis and gluconeogenesis) [80]. It has been shown that sphingosine, as a part of sphingolipids, hinders the interaction of the S subunit of SARS-CoV-2 with ACE2 and reduces the virus infectivity [81]. Also, the changes in glucose metabolism in both innate immune cells and pulmonary epithelial cells contribute to cytokine synthesis and inflammatory responses [82]. Under high glucose conditions, viral replication and cytokine production increase, leading to lung epithelial cell death [83]. Thus, lung microbiome alterations could affect the metabolism in immune cells and lung epithelial cells, affecting the SARS-CoV-2 life cycle. Because bacterial dysbiosis is discussed in detail throughout the article, Table 2. summarizes the dysbiosis of other respiratory microbiota in COVID-19 patients. There is evidence that respiratory viral co-infection in respiratory diseases is 3–68.0% [84]. For instance, Lin *et al.* stated that viral co-infections in SARS-CoV-2 patients were 3.2% [85]. Furthermore, a metagenomics analysis revealed that in addition to betacoronavirus, COVID-19 samples were associated with other viral co-infections, such as

Table 2. Dysbiosis of respiratory microbiota in COVID-19 patients.

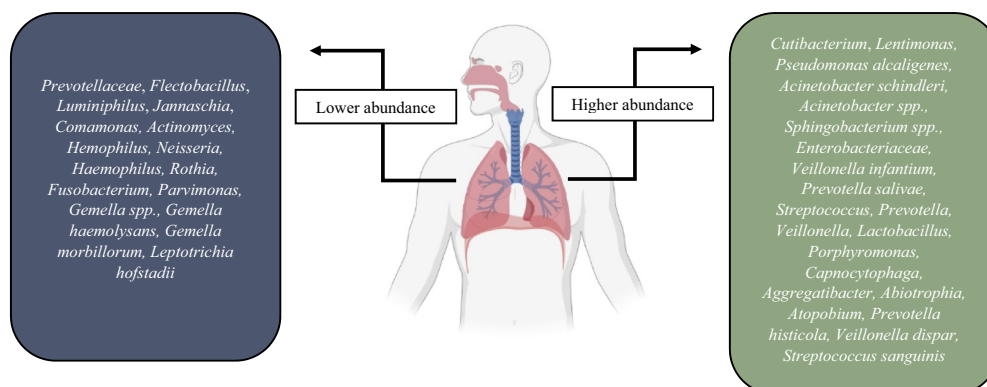
Microorganism	Sample type	Sample size	Dysbiosis	Ref
Fungi	BAL	26	- ↑ <i>Candida</i> spp. - ↑Ascomycota in patients with COVID-19 not colonized with <i>Candida</i> spp.	[87]
Fungi	BAS, BAL	90	↑ <i>Candida albicans</i>	[88]
Fungi	Sputum, EA	99	- ↑ <i>Candida glabrata</i> - ↑ <i>Candida albicans</i>	[3]
Virus	Throat swab, Nasal swab, Sputum	23	- ↑ <i>Human alphaherpesvirus 1</i> - ↑ <i>Rhinovirus B</i> - ↑ <i>Human orthopneumovirus</i>	[89]
Virus	Nasopharyngeal swabs	8	- ↑ <i>Betacoronavirus</i> - ↓ <i>Alphacoronavirus</i> - ↓ <i>Cystovirus</i> - ↑ <i>Macavirus</i> - ↓ <i>Myovirus</i>	[90]
Virus	Nasopharyngeal swabs	10	- ↑ <i>Betacoronavirus</i> - ↓ <i>Siphovirus</i> - ↓ <i>Alphapapillomavirus</i> - ↓ <i>Myovirus</i>	[86]
Archaea	Nasopharyngeal swabs	8	- ↑ <i>Halogeometricum</i> - ↑ <i>Haloquadratum</i> - ↑ <i>Natrialba</i> - ↓ <i>Methanospirillum</i> - ↓ <i>Methanoregula</i> - ↓ <i>Methanocaldococcus</i>	[90]
Archaea	Nasopharyngeal swabs	10	- ↑ <i>Methanosarcina</i> - ↑ <i>Methanocaldococcus</i> - ↑ <i>Thermococcus</i> - ↑ <i>Haloarcula</i> - ↓ <i>Methanobrevibacter</i> - ↓ <i>Methanococcus</i> - ↓ <i>Methanocorpusculum</i>	[86]

BAL, bronchoalveolar lavage; BAS, bronchial aspirates; EA, endotracheal aspirates.

Tombusvirus, Partitivirus, Victorivirus, Totivirus, and Chrysovirus [86].

It has been stated that the lung microbiota composition of healthy individuals is mainly enriched with commensal bacteria, including *Tropheryma whippelii*, *Prevotella* spp., *Streptococcus* spp., and *Veillonella* spp., that maintain the immune homeostasis [91]. The bacterial diversity in COVID-19 patients is controversial; some studies found low microbial diversity [92,93], while others reported a high diversity [94]. These paradoxical results could be owing to the patients' severity, treatment, disease stage, and differences in sampling location of the respiratory tract. Figure 2 represents

the alteration in the respiratory bacteria during infection with COVID-19. To identify the association between upper and lower respiratory tract microbiota and the COVID-19 severity, Lloréns-Rico *et al.* used nasopharyngeal swabs and BAL samples. They explained that the microbiome variation within the upper respiratory tract could be affected by the type of oxygen support (predominantly mechanical ventilation), time in ICU, and treatments (such as antibiotics), while viral load showed a reduced impact [95]. For instance, COVID-19 patients who develop ventilator-associated pneumonia have an impaired innate immune defence that leads to their susceptibility to secondary

**Figure 2.** The higher and lower abundance of bacteria in the respiratory tract of COVID-19 patients.

infection as well as impaired clearance of SARS-CoV-2 infection [96]. Otherwise, there is evidence that the lung microbiome composition between COVID-19 negative and COVID-19 positive patients who developed ventilator-associated pneumonia was similar [97]. Another study revealed that COVID-19 symptomatic patients exhibited low nasopharyngeal bacteria diversity compared with asymptomatic and COVID-19 negative ones. They explained that running sneezing and nose could wash off the nasal microbiota of the symptomatic patients. In the symptomatic patients, nasal microbiota dysbiosis led to high levels of *Cutibacterium* and *Lentimonas*, while reducing the abundance of *Prevotellaceae*, *Flectobacillus*, *Luminiphilus*, *Jannaschia*, and *Comamonas* [98]. Liu *et al.* conducted an investigation to identify metabolome features and nasopharyngeal bacteria of COVID-19 patients. The sera examination of COVID-19 patients revealed that the levels of chlorogenic acid methyl ester (CME), L-proline, and lactic acid were notably reduced compared with COVID-19 negative individuals. Also, the pharynges of these patients were depleted from *Gemella haemolysans*, *Gemella morbillorum*, and *Leptotrichia hofstadii*. In contrast, the population of *Prevotella histicola*, *Veillonella dispar*, and *Streptococcus sanguinis* were increased. They found that the abundance of *L. hofstadii* and *G. haemolysans* were remarkably associated with the serum levels of CME [99]. CME could reduce the expression of inflammatory factors and alleviate the pathological impairment of lung tissue [100]. Gaibani *et al.* characterized the diversity of different bacteria in critically ill COVID-19 patients and healthy subjects using a 16S rRNA profiling on BAL samples. They found that the lung of COVID-19 patients enriched with *Pseudomonas alcaligenes*, *Acinetobacter schindleri*, *Acinetobacter* spp., *Sphingobacterium* spp., and *Enterobacteriaceae*. In contrast, *Veillonella dispar*, *Haemophilus influenzae*, *Granulicatella* spp., *Streptococcus* spp., and *Porphyromonas* spp., characterized in the lung of COVID-19-negative participants [101]. In another study, Merenstein *et al.* indicated the lower abundance of *Actinomyces*, *Hemophilus*, and *Neisseria* in the oropharyngeal of severe COVID-19 patients in comparison with normal ones. They also showed that the lower lymphocyte/neutrophil ratio was associated with the lower microbiome composition and diversity and inversely associated with the disease severity [102]. Iebba *et al.* identified *Veillonella infantium* and *Prevotella salivae* as predominant in patients suffering COVID-19, while *Rothia mucilaginosa* and *Neisseria perflava* were abundant in controls. Furthermore, the levels of cytokines IL-2, IL-5, IL-6, INF- γ , G-CSF, GM-CSF, and TNF- α were augmented

in the COVID-19 patients, whereas only IL-12p70 was elevated in control subjects [103]. In a cross-sectional study, Soffritti *et al.* demonstrated that oral microbiome dysbiosis is inversely correlated with the COVID-19 severity. They reported the higher abundance of *Streptococcus*, *Prevotella*, *Veillonella*, *Lactobacillus*, *Porphyromonas*, *Capnocytophaga*, *Aggregatibacter*, *Abiotrophia*, and *Atopobium* in COVID-19 patients, whereas *Haemophilus*, *Rothia*, *Fusobacterium*, *Parvimonas*, and *Gemella* spp. showed lower frequency. Interestingly, the oral fungal and virome in COVID-19 patients were increased in comparison with controls. While *Saccharomyces* spp. and *Candida* were more abundant fungi in control participants, *Nakaseomyces*, *Aspergillus*, and *Malassezia* spp. were high in the COVID-19 patients. Bacteriophages targeted towards *Streptococcus* (phage PH10 and phage EJ-1), *Lactobacillus* (phage phiadh), and *Staphylococcus* (phage ROSA) as well as herpes simplex virus type 1 (HSV-1) were abundant in the COVID-19 patients. Furthermore, a reduction in mucosal sIgA responses was exhibited in more severely COVID-19 patients, suggesting the importance of local immune response in controlling virus infection at the early phase. Among the pro-inflammatory cytokines/chemokines, the levels of IL-6 and IL-17 were meaningfully higher in the oral of COVID-19 patients, without remarkable changes in GM-CSF and TNF- α [104].

There is evidence reporting bacterial, fungal, and viral co-infection with COVID-19. Two meta-analysis studies showed bacterial co-infection in 7% and 14% of hospitalized and critically ill patients, respectively [105,106]. The most commonly reported bacterial co-infection with COVID-19 patients are *Enterobacteriaceae*, *H. influenzae*, and *P. aeruginosa* [106,107]. Gaibani *et al.* found that infection with carbapenem-resistant *Acinetobacter baumannii* elevated during COVID-19 [101]. In addition, *Redondoviridae* and *Anelloviridae*, airway commensal DNA viruses, exhibited more colonization frequency in COVID-19 patients and were completely linked to the severity of the disease [102].

The microbiome and treatments for COVID-19

Due to the undeniable role of microbiota in the COVID-19 disease, management of microbiota to shift its composition towards a healthy state could be effective in controlling SARS-CoV-2 infection and preventing the disease severity. Patients suffering from asymptomatic or mild COVID-19 infections are advised to eat a balanced, healthy, and anti-inflammatory diet, including legumes, fruits, grains, and vegetables. It has been reported that there is

Table 3. The effect of nutrients and probiotics in microbiota modulation of COVID-19 under clinical trials.

Intervention/treatment	Participants (n)	Goal	ClinicalTrials.gov Identifier
Probiorinse of <i>Lactococcus Lactis</i> W136 NSS using <i>Saccharomyces boulardii</i>	23	Reduction of the severity of COVID-19 symptoms	NCT04458519
	80	Reduction of complications in COVID-19 patients and comorbidities	NCT04507867
Combination of <i>Pediococcus acidilactici</i> CECT7483, <i>Lactobacillus plantarum</i> CECT7484, <i>L. plantarum</i> CECT30292, and <i>L. plantarum</i> CECT7485 <i>Lactobacillus</i>	300	How does the combination of the probiotics reduce the risk of progression of COVID-19 to moderate or severe	NCT04517422
	201	Effects on the incidence and severity of COVID-19 in the elderly population living in a nursing home	NCT04756466
NBT-NM108	100	Evaluating the effectiveness and feasibility of NBT-NM108, as a treatment, in modulating gut microbiota of COVID-19.	NCT04540406
Dry extract of polyphenols (tannins) from quebracho and chestnut <i>Lactobacillus Coryniformis</i> K8	124	Evaluating the efficacy of Tannin against COVID-19 infection	NCT04403646
	314	Evaluating the protective effect against COVID-19 in healthcare workers	NCT04366180
Probiotic strains with maltodextrin as excipient <i>Lactobacillus salivarius</i> with Vit D and Zinc <i>Lactobacillus rhamnosus</i> GG	41	Improvement of symptoms in COVID-19 patients and reduction of hospitalization days	NCT04390477
	60	Effects on immune responses in COVID-19 patients	NCT04937556
<i>Lactobacilli</i> and <i>Bifidobacteria</i>	182	Effects on the microbiome of household contacts exposed to COVID-19	NCT04399252
	300	Facilitating faster recovery from COVID-19 and enhancing immune responses	NCT04907877

NSS, Nutritional support system.

a reverse correlation between the amount of fibre intake with inflammatory markers, for example TNF- α , C-reactive protein, IL-6, and IL-18. Furthermore, high-fibre diets are associated with higher adiponectin and lower glycaemia, which have positive anti-inflammatory effects. Also, the anti-inflammatory activities of dietary fibre could support the functions of immunosuppressive and anti-viral drugs [108]. Various studies, including clinical trials, have been directed to investigate the preventive and therapeutic efficacy of microbiota modulation on COVID-19 using probiotics, synbiotics, prebiotics, nutraceuticals, and trace elements. For example, probiotics such as *Paenibacillus* and *Lactobacilli* produce peptides with the binding ability to ACE2, inhibiting the binding of the SARS-CoV-2 to its targeted cells [109,110]. Ceccarelli *et al.* concluded that treatment of patients suffering severe COVID-19 pneumonia with probiotics was correlated with a reduced risk of death [111]. Probiotics also promote anti-viral immune responses by activating toll-like receptor 4 (TLR4) signalling and inducing the secretion of type I and II IFNs [112,113]. Liu *et al.* indicated that orally receiving capsules as faecal microbiota transplantation (FMT) in COVID-19 patients could improve GI symptoms and alter B-cell populations, in which naïve B-cells were decreased and memory and non-switched B-cells were augmented. In discharged patients after FMT administration, the abundance of *Actinobacteria* was increased from 4% to 15%, and the proportion of *Proteobacteria* was reduced from 9.2% to 2.8%. However, they reported abdominal pain and diarrhoea as side effects

of FMT administration [114]. In a case report study with two patients, Biliński *et al.* showed that FMT administration to treat concomitant COVID-19 and *C. difficile* infection led to rapid resolution of COVID-19 [115]. A clinical trial is investigating the impact of FMT, as immunomodulatory intervention, on reduced risk of progression of COVID-19 disease with increased inflammatory parameters and cytokine storm (NCT04824222). A meta-analysis study demonstrated that vitamin D deficiency is associated with COVID-19 severity; lower levels in poor prognosis patients [116]. It has been reported that vitamin D could increase the production of butyrate, which improves the gut barrier, exerts anti-inflammatory activities, and promotes anti-viral effects [71]. Table 3. summarizes the clinical trials conducted to evaluate microbiota modulation's efficacy on COVID-19.

Conclusion

Human microbiota can affect immune responses, thus influencing disease progression and prevention. In the case of COVID-19, respiratory microbiota dysbiosis could be associated with underactive and overactive immune responses, which results in various clinical complications. Currently, the respiratory microbiome alteration is effect or cause of the COVID-19 is not clearly distinguished. However, the composition of microbiota differs from person to person and may

explain the inter-individual variation in response to COVID-19. Due to the indispensable roles of microbiota in response to COVID-19, the assumption of inexpensive and safe supplementations and nutrients, such as probiotics, can be considered a preventive strategy for COVID-19 negative individuals or an adjunctive for treatment strategies to limit the progression of COVID-19 infection in the suffering patients. Furthermore, personalized diet regimens could direct the therapeutic strategies towards personalized medicine. In the sampling, it should be considered that sampling the lungs for microbial flora sequencing is technically demanding due to the relatively low biomass. In addition, lower respiratory tract sampling by bronchoscopy requires the instrument to be passed through the oral or nasal route, which may lead to the contaminations of samples.

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