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Comparison of microbial diversity of respiratory tract between COVID-19 patients and healthy population

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Since its outbreak in late 2019, the SARS-CoV-2 virus has been the main subject of interest for a number of studies. Clinical manifestations are ranging from asymptomatic to mild and severe. Major risk factors for developing severe COVID-19 are age and comorbidities, although younger people suffer from severe COVID-19 as well. One of the explanations for why can be the composition of respiratory tract microbiota. In this article, we review studies linking respiratory tract microbiome and its changes during COVID-19 infection. The respiratory tract microbiome helps shape immunity and it is assumed that it can affect the outcome of several viral infections. Several studies show differences in the microbial composition of the respiratory tract between COVID-19 patients and healthy individuals. The diversity of the respiratory tract microbiome is reduced with increasing severity of COVID-19.

KEYWORDS

SARS-CoV-2, microbiome, respiratory tract, COVID-19, microbial diversity

SARS-CoV-2

In late December 2019, several patients with symptoms of novel pneumonia were reported to health authorities in Wuhan, Hubei Province, China (Zhou et al., 2020). The majority of the initial patients were associated with the Huanan seafood and wildlife market in Wuhan City. Through retrospective analysis, they were able to identify a patient whose symptoms began as early as 1 December (Zhang and Holmes, 2020). Isolation of viral RNA and next-generation sequencing identified this viral pathogen as a novel coronavirus (Zhou et al., 2020). The International Committee on Taxonomy of Viruses named this novel coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Gorbalenya et al., 2020).

SARS-CoV-2 belongs to the viral family *Coronaviridae*. Members of the family *Coronaviridae* are single-stranded positive-sense RNA viruses up to 32 kb in length. In addition, this family can be divided into two subfamilies: *Coronavirinae* and

Torovirinae. Subfamily *Coronavirinae* is divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, *Deltacoronavirus* and subfamily *Torovirinae*, which consists of the genus *Torovirus* (Payne, 2017). *Alphacoronavirus* and *Betacoronavirus* can generally infect mammals, whereas *Gammacoronavirus* and *Deltacoronavirus* mainly infect birds (Ludwig and Zarbock, 2020). In humans, they are the cause of mild to severe respiratory infections. By 2019, in the 21st century, humanity experienced two spreads of novel coronaviruses: severe acute respiratory syndrome (SARS) in 2002 and Middle East respiratory syndrome (MERS) in 2012 (de Wit et al., 2016; Hu et al., 2020). SARS occurred in China in 2002, sickening nearly 8,000 people and killing 774. The natural reservoir for SARS-CoV was Chinese horseshoe bats (Lau et al., 2005), but transmission to humans occurred via intermediate hosts, civets (Kan et al., 2005). MERS-CoV emerged in Saudi Arabia in 2012 and infected approximately 2,500 people with a mortality rate of 30% (Lu et al., 2020). As in the case of SARS, the MERS-CoV virus persists in nature in bats and as an intermediate host serve dromedaries (Sabir et al., 2016). A study by Zhou et al. (2020) showed that SARS-CoV-2 is 96.2% identical to bat coronavirus at the whole genome level, further supporting the theory that bats are natural reservoirs for coronaviruses. It is unknown whether SARS-CoV-2 was transmitted directly from a bat to a human or whether there is another intermediate host. Recent studies speculated that intermediate hosts could be rodents (Huang et al., 2021), pangolins (Liu et al., 2020) and raccoon dogs (Wang et al., 2022), but there could be multiple intermediate hosts.

Next-generation sequencing revealed 79% nucleotide-level identity between SARS-CoV-1 and SARS-CoV-2 and 50% between SARS-CoV-2 and MERS-CoV (Lu et al., 2020). The genome of SARS-CoV-2 resembles that of a typical coronavirus genome and contains at least nine open reading frames and four structural proteins with the gene sequence 5′-ORF1ab-S-E-M-N-3′ (S-spike, E-envelope, M-membrane, N-nucleocapsid protein) (Figure 1) (Li et al., 2020). ORF1a and ORF1b are two overlapping ORFs encoding polyproteins pp1a and pp1ab, which are cleaved into 16 non-structural proteins (NSP) (Ziebuhr and Siddell, 1999; Abu Turab Naqvi et al., 2020; Dutartre et al., 2020; Yadav et al., 2021). These NSPs (1-16) are responsible for viral replication (Yadav et al., 2021). The spike (S) protein ensures the entry of the virus into the target cell. The S protein consists of two subunits, the N-terminal S1 subunit and the C-terminal S2 subunit. The S1 unit recognizes and binds to the cell surface receptor angiotensin-converting enzyme-2 (ACE-2), the same one that binds the spike protein of SARS-CoV-1, followed by fusion of viral and host cellular membrane. Binding to the ACE-2 receptor requires S-protein priming by the host cellular serine TMPRSS2 protease. This priming involves cleavage at the S1/S2 furin cleavage site, making the closed form of S1 open and accessible to the ACE-2 receptor (Benton et al., 2020; Hoffmann et al., 2020; Guruprasad, 2021). ACE-2 is expressed in epithelial tissues that form protective barriers. ACE-2 is localized in many cells and tissues such as the heart, kidneys, intestinal tract, gallbladder, testis, and most importantly, respiratory tract epithelium in the nose, mouth, and lungs. In the lungs, it is strongly expressed in the pneumocytes in the alveoli (Hikmet et al., 2020; Wang et al., 2020). Proteins M and E form the viral envelope, while protein N protects viral RNA (Kirtipal et al., 2020).

SARS-CoV-2 has various manifestations in patients, from mild to severe. Unlike SARS and MERS, the mortality rate of SARS-CoV-2 is significantly lower, with most patients having mild symptoms or being asymptomatic. Symptoms vary from patient to patient and depend on various factors such as age, gender, blood type, and other comorbidities (diabetes, hypertension, cardiovascular disease, etc.) (Huang et al., 2020). The most common symptoms are fever, dry cough, fatigue, sputum production, and loss of taste and smell. Less common symptoms include headache, sore throat, diarrhea, chills, nausea, and vomiting (Hu et al., 2020; Ludwig and Zarbock, 2020).

Respiratory tract

The respiratory tract (RT) is a part of the respiratory system, whose main function is the exchange of oxygen and carbon dioxide. It is structurally divided into the upper respiratory tract (URT) and the lower respiratory tract (LRT). The UTR consists of the nostrils, nasopharynx, oropharynx, and the portion of the larynx above the vocal cords. The LRT, on the other hand, includes the portion of the larynx below the vocal cords, the trachea, bronchi, bronchioles, and lungs with alveoli (Robinson and Furlow, 2007). The entire surface is covered by bacterial communities, with the UTR having the highest density of bacterial communities. Different anatomical structures of the respiratory tract contain specialized bacterial communities. The human microbiome is the collection of all microorganisms that live in association with the human body. The microbiome includes eukaryotes, archaea, bacteria, and viruses (https://www.hmpdacc.org/overview/). The most studied parts of the microbiome are bacteria, commensal or pathogenic. The RT microbiota is thought to play a role in respiratory development and in shaping local immunity (Olszak et al., 2012). During bacterial or viral infection of the human respiratory tract, the first step for these pathogens is usually colonization of the UTR before causing infection of the UTR and LTR. The process of inhibiting this first step is also referred to as colonization resistance and its mechanisms involve competition for the attachment site, nutrition, or production of antibacterial peptides (Stadio et al., 2020).

Composition of RT microbiota

Colonization of the URT begins at birth and is influenced by the mode of delivery. During vaginal delivery, the child is

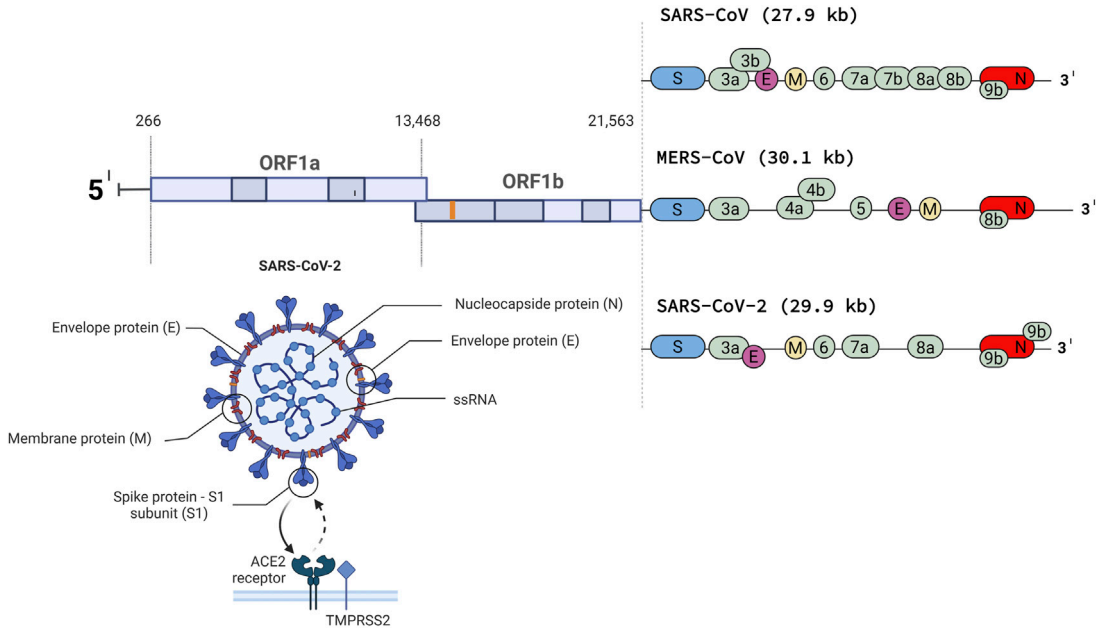

FIGURE 1
The genome of SARS-CoV, MERS-CoV and SARSCoV- 2 (Created with BioRender.com).

exposed to the maternal vaginal microbiome, and during cesarean delivery, the infant is exposed to the maternal skin and environmental microbiome. During the first week of life, the nasopharynx is colonized by *Staphylococcus aureus* regardless of the mode of delivery. Although *S. aureus* is primarily known as a pathogenic bacterium, it may be an important commensal bacterium in the nasopharynx during early life. Wang et al. (2013) showed that *S. aureus* significantly attenuates influenza-mediated immune injury of the lung in mice by inducing alveolar macrophages. In later weeks, the abundance of *S. aureus* decreases and species such as *Corynebacterium*, *Dolosigranulum*, and *Moraxella* increase. Increased abundance of these three species is important for the healthy development of the nasopharyngeal microbiota in later stages of life. In the first months of life, infants born by cesarean section exhibited a variable microbial profile and a loss of abundance of *Corynebacterium* and *Dolosigranulum*, resulting in an increase in respiratory infections. The abundance of *S. aureus* is not significantly reduced, while *Prevotella*, *Veillonela*, and *Porphyromonas* begin to emerge (Bosch et al., 2017). In contrast, infants born naturally and breastfed showed a higher abundance of beneficial bacteria (Bosch et al., 2016). This could also be due to the transfer of beneficial microbiota, such as *Lactobacillus* and *Bifidobacterium*, in milk during breastfeeding (Biesbroek et al., 2014). Breastfed children also have a higher abundance of *Corynebacterium* and *Dolosigranulum* and a lower incidence of respiratory diseases compared to formula-fed children. The microbiota of children can be influenced by many different aspects, such as the type of birth and feeding mentioned above, presence of siblings, previous infections, use of antibiotics, vaccinations, season, exposure to different environments (home, kindergarten, park), etc. In the first year of life, exposure to microbial communities is crucial for the formation of the immune system (Shukla et al., 2017).

The human body is colonized by five phyla of bacteria: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria* (Ibironke et al., 2020). In healthy adults, the nasal cavity microbiome consists of bacteria associated with the skin, mainly *Actinobacteria* with the most abundant families being *Corynebacteriaceae* and *Propionibacteriaceae*, followed by *Firmicutes* and *Proteobacteria* (Bassis et al., 2014). The nasopharynx differs from the oral cavity in terms of bacterial niche composition. In spring, the dominant phyla in the nasopharynx are *Bacteroidetes*, and in fall, *Proteobacteria* and *Firmicutes*. At the genus level, *Moraxella* is the most widespread (Bogaert et al., 2011). *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are represented in the oropharynx. The oropharynx has a greater bacterial diversity compared to the nasopharynx (Charlson et al., 2010). Commonly known pathogenic bacteria such as *Pneumococcus*, *Haemophilus influenzae*, and *Neisseria meningitidis* can be considered commensal bacteria of the URT (Table 1). Despite the dogma that the lung is sterile, it has quite a dynamic microbiome. The microbiome of the lung is similar to the microbiome of the nasopharynx in terms of ecological composition (Dickson et al., 2014). The most dominant phyla in the lung are *Firmicutes* and

TABLE 1 Composition of microbial communities in various anatomical structures in different stages of life.

		Child	Adult	Elderly
UTR	Nasal cavity	*Streptococcus, Corynebacterium, Dolosigranulum*	*Corynebacterium, Propionibacterium, Staphylococcus aureus,*	*Streptococcus, Prevotella, Veillonella, Staphylococcus*
	Nasopharynx	*Staphylococcus aureus, Corynebacterium, Dolosigranulum, Moraxella, Propionibacterium, Bifidobacterium, Streptococcus, Haemophilus, Enterococcus*	*Streptococcus, Haemophilus influenzae, Moraxella cat., Corynebacterium, Dolosigranulum, Neisseria, Staphylococcus, Cutibacterium*	*Streptococcus, Prevotella, Veillonella*
	Oropharynx	*Rothia, Corynebacterium, Prevotella, Porphyromonas, Streptococcus, Veillonella, Haemophilus, Moraxella*	*Prevotella, Leptotrichia, Veillonella, Streptococcus, Rothia, Neisseria, Haemophilus, Porphyromonas*	*Propionibacterium, Corynebacterium, Bifidobacterium, Prevotella, Streptococcus (44%), Staphylococcus, Veillonella, Moraxella, Pseudomonas*
LTR	Trachea Lungs	*Veillonella, Prevotella, Fusobacterium*	Low biomass: *Prevotella, Veillonella, Streptococcus*	

Bacteroidetes with a low biomass of *Prevotella*, *Veillonella*, and *Streptococcus*. Bacteria enter the lungs mainly through the UTR (Cui et al., 2014). However, *Tropheryma whipplei* was found in the lung as part of the microbiota, but not in the nasopharynx or oropharynx (Charlson et al., 2011). When comparing the microbiota of middle-aged people and seniors, seniors had the lowest abundance, especially in the anterior nares. In the anterior nares of elderly people, the relative abundance of *Propionibacterium* and *Corynebacterium* decreases and *Streptococcus* increases. The genus *Streptococcus* was depleted in the oropharynx. The anterior nares are barriers between the external environment and the respiratory tract, so changes in their microbiome may be one of the causes of the higher incidence of respiratory infections in seniors (Whelan et al., 2014).

The nasal mucosa and its microbiota are the first to come into contact with the infection. Viral infection of the respiratory tract alters the composition of the microbiota in RT, mainly through the decrease of alpha diversity (the average species diversity within a given area) and the loss of beneficial microbiota, mainly anaerobes and the genus *Prevotella*. On the other hand, pathogenic bacteria such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Corynebacterium propinquum* and *Moraxella catarrhalis* are enriched (Edouard et al., 2018). These changes contribute to the ease of shifting infection from the UTR to LTR and allow bacterial pathogens to translocate as well, leading to secondary bacterial infection (Cyprian et al., 2021) and drastically altering the mortality of viral infections (Gupta et al., 2008).

The microbiota can reduce pathogens by alerting the host immune system or directly inhibiting or killing pathogens. A study by Larsen et al. (2012) showed that *Prevotella* can reduce *Haemophilus*-induced production of proinflammatory cytokines in dendritic cells by ~50% (Larsen et al., 2012). The signaling pathway, by which the commensal activates the host immune response is unclear, but it activates cells of host immunity, for example, macrophages, mucosa-associated invariant T cells, group 3 innate lymphoid cells, and natural killer cells. Murine studies have shown that mice with a lowered abundance of microbiota reduced by antibiotic use had lower expression of macrophage-induced viral genes upon influenza virus infection than the control group (Abt et al., 2012). In addition, *Staphylococcus epidermidis* activates the production of interferon in the nasal epithelium, increasing resistance to influenza A in mice (Kim et al., 2019). Two main ways in which bacteria compete with each other are exploitation and interference. In exploitation, bacteria extract nutrients from the environment. *Corynebacterium* produces siderophores and inhibits *Staphylococcus* growth due to decreased bioavailability of iron as a result of the chelation of iron by siderophores *in vitro* (Stubbendieck et al., 2019). In contrast, competing bacteria produce molecules during interference that inhibit growth or kill competitors. Bacteria can produce bacteriocins and ribosomally synthesized antimicrobial peptides that can inhibit growth or kill other bacteria, but do not harm the bacteria themselves through specific immunity proteins (Benítez-Chao et al., 2021; Soltani et al., 2021). *Staphylococcus salivarius* produces bacteriocins and thus inhibits *Staphylococcus pneumoniae* (Santagati et al., 2012). Other mechanisms of interference include biofilm disruption, enzyme secretion, hydrogen peroxide production, and virulence gene downregulation (Khan et al., 2019).

COVID-19 and respiratory microbiome

As mentioned earlier, the microbiota of the respiratory tract plays a crucial role in maintaining respiratory health. Multiple studies have shown that the composition of respiratory microbiota can affect susceptibility to respiratory infections including COVID-19. Understanding interactions between the

host, virus and respiratory microbiome may provide insight into new potential therapeutic targets in the prevention and treatment of those infections. SARS-CoV-2 enters the human body primarily through the respiratory tract, oral cavity, and nose. The microbiome is the first entity to confront infection. It plays an important role in stimulating the immune system and protecting against pathogens. When microbiome homeostasis is disturbed (microbial dysbiosis), pathogens overgrow and colonize the respiratory tract, eventually leading to infection of the LTR. In samples from patients with severe COVID-19 bacterial alpha diversity decreased. Furthermore, alpha diversity is reduced in a severity-dependent fashion—the lower diversity, the higher severity. As with most infections, the trend of beneficial bacteria decreasing and pathogens increasing can be seen in SARS-CoV-2 as well.

The severity of COVID-19 is related to age and comorbidities. Since the elderly have low diversity of the microbiome in RT, this may be the reason for their high susceptibility to SARS-CoV-2 and their higher probability of ending up in the intensive care unit (ICU). At the same time, young patients with no apparent risk factors suffer complications and end up in the ICU on automatic ventilation. The role of the microbiome in the host immune response suggests that a healthy microbiome may positively influence the progression of COVID-19. On the other hand, dysbiosis may lead to a higher rate of severe cases in individuals without obvious risk factors. The URT microbiome may impact the symptoms experienced during SARS-CoV-2 infection by modulating the host immune response. For example, the ACE-2 receptor, as an entry site for SARS-CoV-2, is stimulated by interferon and therefore may be affected by the microbiome. Respiratory tract viral infections may predispose patients to bacterial superinfections. One of the most important mechanisms is the structural and functional disruption of the mucosal epithelium (Bakaletz, 1995). In previous studies, coronavirus enhanced streptococcal attachment to epithelial cells, resulting in pulmonary infections (Golda et al., 2011). Patients with additional coinfection have a higher risk of severe COVID-19 and a higher mortality rate. In the study by Zhou et al., 50% of COVID-19 patients had a secondary bacterial infection. The three most common bacteria in patients' lungs were *Klebsiella pneumoniae* (9.9%), *Streptococcus pneumoniae* (8.2%), and *Staphylococcus aureus* (7.7%) (Tang et al., 2022).

In a study in which Gupta et al., 2022 analyzed microbial composition at the bacterial strain level, there was an increased abundance of *Proteobacteria* and decreased abundance of *Bacteroidetes*. In mice, *Bacteroidetes* can downregulate ACE-2 receptor expression, this reduction can promote severe SARS-CoV-2 infection. COVID-19 patients' nasal microbiome is enriched in pathogenic bacteria, *Acinetobacter*, *Rothia*, *Moraxella*, *Haemophilus*, *Stenotrophomonas*, and *Pseudomonas* (Rhoades et al., 2021; Gupta et al., 2022).

In the nasopharynx, *Corynebacterium* and *Dolosigranulum* are negatively correlated with COVID-19 severity (Smith et al., 2021). *Corynebacterium* and *Dolosigranulum* are among the most important commensal bacteria in healthy RT (Shilts et al., 2022). *Corynebacterium accolens* can inhibit the growth of *Staphylococcus aureus* and *Staphylococcus pneumoniae* (Bomar et al., 2016). In addition, COVID-19 patients show a significant reduction in abundance of *Proteobacteria* (Kolhe et al., 2021) and *Fusobacteria* at the phylum level compared to controls (Nardelli et al., 2021). *Leptotrichia* and *Haemophilus* are significantly reduced and species such as *Streptococcus*, *Prevotella* and *Campylobacter* are enriched (Nardelli et al., 2021; Xiong et al., 2021). Overexpressed *Prevotella* proteins can increase COVID-19 severity by interacting with NF-kB (Khan and Khan, 2020; Ventero et al., 2021). The study showed that two *Streptococcus strains*, *S. suis* and *S. agalactiae*, can stimulate the expression of ACE-2 in VERO cells (Xiong et al., 2021), which could be another reason for severe COVID-19. *Campylobacter* is known to cause inflammation and diarrhea, one of the symptoms of COVID-19. In COVID-19 patients, several pathways were significantly downregulated in the nasopharyngeal metabolome, including platelet activation pathway (Liu et al., 2021).

The bacterial communities in the oropharynx show a higher relative abundance of *Streptococcus*, *Gemella*, *Haemophilus*, and *Neisseria*, and a lower relative abundance of the phylum *Proteobacteria* (Liu et al., 2021). In addition, a study by Shi et al. presented three predominant bacteria in the oropharynx in COVID-19 patients with mild symptoms: *Streptococcus*, *Veillonella*, and *Haemophilus* (~60%). In addition, *Campylobacter* (including *C. rectus* and *C. fetus*) and *Roseburia* were limited at the genus level in patients with a higher viral load of SARS-CoV-2 (Shi et al., 2022). SARS-CoV-2 intensive care patients showed complete depletion of *Clostridium* and *Bifidobacterium*. In addition, the *Pseudomonadaceae* family was found exclusively in ICU patients compared with hospitalized patients and healthy controls (Rueca et al., 2021). Members of the *Pseudomonadaceae* family are known to cause a pathological condition and reduce microbial diversity (Borges et al., 2018) In their study Ren et al. identified *Streptococcus* as the most enriched microbiome at the time of admission in the oropharynx of a patient recovered from COVID-19 (Ren et al., 2021). In the oropharynx of COVID-19 patients, specific amino acid metabolic pathways were enriched compared to healthy control and influenza patients. Tyrosine, phenylalanine, beta-alanine, phosphonate, and phosphinate metabolic pathways were those enriched in COVID-19 patients, suggesting that specific amino acids are metabolized by the microbiome in the oropharynx of COVID-19 patients (Ma et al., 2021). The amino acid imbalance was the reason for increased intestinal inflammation via ACE2-dependent changes in epithelial immunity (Hashimoto et al.,

2012). Moreover, the oropharyngeal microbiome of COVID-19 and flu patients showed relatively depleted nucleotide metabolism, replication and repair, and greatly depleted membrane transport and cell motility. These findings suggest a lowered ability to process genetic information and transport ions, lipids, sterols, peptides, proteins and carbohydrates (Ma et al., 2021).

In the lungs of deceased patients with COVID-19, the most common bacteria were *Actinobacter* (Fan et al., 2020). The difference in lung microbiome composition between healthy individuals and patients with COVID-19 suggests lung dysbiosis during infection with SARS-CoV-2. Intubated patients have lower microbiome diversity, respiratory pathogens such as *Staphylococcus*, *Klebsiella*, and *Stenotrophemonas* and typical bacterial communities for UTR such as *Corynebacterium* and *Prevotella* are present. *Prevotella* is known for its ability to promote viral infections by interacting with NF-κB (Bertelsen et al., 2020). Some patients had a high abundance of *Enterococcus*, which causes bloodstream infection in critically ill COVID-19 patients (Merenstein et al., 2021). Patients on automatic ventilation have elevated levels of *Serratia*, *Streptococcus*, *Enterobacter*, *Veillonella*, *Prevotella*, and *Rothia* (Feehan et al., 2021), but it is not known whether this is directly from COVID-19 or from intubation and pulmonary ventilation. *Serratia marcescens* is known to cause pneumonia (Goldstein et al., 1982). A study by Haiminen et al. (2021) focused on altered lung microbiome pathways; patients with COVID-19 had downregulated pathways related to glycan biosynthesis and metabolism and lipid metabolism and increased carbohydrate metabolism (Haiminen et al., 2021).

During infection, the LRT produces mucus by activating various immune cells (Li and Tang, 2021). The presence of mucus in LTR promotes the growth of anaerobic bacteria and, at the same time, reduces the growth of aerobic bacteria. This supports the findings that *Staphylococcus*, *Prevotella*, and *Peptostreptococcus* are increased in COVID-19 patients in critical condition (Smith et al., 2021). *Prevotella* and *Peptostreptococcus* are anaerobic bacteria (Brook, 2001), *Staphylococcus* grows best in the presence of oxygen but can live in anaerobic conditions as well.

Changes in UTR microbiota during SARS-CoV-2 infection are extensively studied, but little is known about changes in metabolome in COVID-19 patients. The main target of metabolomic studies is the focus on the gut metabolome. Recent studies suggest that changes in the respiratory tract microbiome and its metabolites may be a contributing factor to the severity of COVID-19. A study by Clausen et al. (2020) shows that heparan sulfate (HS) is needed for the binding of SARS-CoV-2 to the ACE2 receptor. SARS-CoV-2 infection and binding of the virus to the receptor can be eliminated by the removal of HS from the cell surface (Clausen et al., 2020). Bacteria have co-localized set of genes, called polysaccharide utilization locus (PUL), that are responsible for the degradation of complex carbohydrates, including HS (Terrapon et al., 2015; Martino et al., 2020). PULs are prevalent in Bacteroidetes, the incidence of which is lower in patients with COVID-19.

In a study by Liu et al. (2008) in a macrophage-derived cell line, proinflammatory cytokine production was induced by histone-like DNA binding protein produced by *Streptococcus intermedius*. Therefore, elevated levels of *Streptococcus* can be one of the reasons for higher inflammation in COVID-19 patients.

Another metabolite produced by respiratory microbiota such as *Lactobacillus* spp. is lactic acid (Abedi et al., 2017). Lactic acid can inhibit the growth of pathogenic bacteria by maintaining low pH in the respiratory tract and enhancing the activity of immune cells. Lactic acid-producing bacteria can regulate the population of natural killer cells and thereby inhibiting the influenza A virus (Miyazaki, 2017). Natural killer cells are one of the first components of immunity to respond also to SARS-CoV-2 infection and are important in controlling the first stages of the infection.

Nitric oxide (NO) has an important role in modulating immune response through various mechanisms. NO is produced by cells of innate immunity (macrophages, neutrophils and natural killer cells). Macrophages activated by pathogen release NO, therefore, which inhibits the replication of the pathogen (Tripathi et al., 2007; Wink et al., 2011; Rosier et al., 2020). In their study, Åkerström et al. (2005) infected cells with the SARS-CoV virus and then the cells were treated with different concentrations of the NO donors S-nitroso-N-acetylpenicillamine (SNAP). SNAP inhibited viral replication of SARS-CoV in a dose-dependent manner. When SNAP was used on cells infected with SARS-CoV-2, the results show the same SNAP dose-dependent reduction of SARS-CoV-2 viral RNA copy numbers. The same was seen when observing the correlation between SNAP and the development of viral cytopathic effect. However, if noninfected control cells were treated with 400 μM SNAP, SNAP exhibited a cytotoxic effect. However, cells treated with 200 μM SNAP showed no decrease in viability (Akaberi et al., 2020). Reactive nitrogen species (RNS) can be formed if NO interacts with reactive oxygen species (ROS). When cells are exposed to oxidative stress, RNS may have a cytotoxic effect (Ricciardolo et al., 2004). Several bacteria can produce NO by denitrification of nitrate to nitrite and to NO (Zumft, 1993). Human saliva contains nitrate and oral microbiota (*Veillonella* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Nocardia* spp., *Corynebacterium* spp., *Neisseria* spp., *Actinomyces* spp., *Haemophilus* spp.) is responsible for its conversion to nitrite (Macknight et al., 1997; Hyde et al., 2014).

Conclusion

Respiratory microbiota is a powerful tool in the defense system against viruses. Recent studies show different RT microbial compositions in patients infected with coronavirus SARS-CoV-2, for example, reduced or increased prevalence of bacteria involved in mechanisms that are able to change the progression of COVID-19 disease. Results from these studies are ambiguous, with conflicting conclusions. The composition of the microbiota can be influenced by many factors, including the method of sampling. COVID-19 outbreak as a global pandemic is studied around the globe, but the microbiota of healthy individuals is altered between individuals from different environments. So, studies from different countries with different conditions will not be coherent. Moreover, some studies did not conduct a thorough background investigation, so they do not have information about previous infections or antibiotic use prior to the infection with SARS-CoV-2. Antibiotic use changes the microbiome 4 weeks after administration of the last dose. The sample size was small in most studies as well.

However, the most important finding is that the microbiome is altered in patients infected with COVID-19. Changes in bacterial composition may be the cause of a wide variety of COVID-19 manifestations. It is unknown whether this change contributes to SARS-CoV-2 infection or is the consequence of COVID-19. Nevertheless, alpha diversity is reduced as well as the number of commensal bacteria, while the relative abundance of pathogens increases. Pathogens that are not fully controlled by commensals exacerbate the manifestation of COVID-19.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

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