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# Gut microbiota and COVID-19: an intriguing pediatric perspective

Gut microbiota and COVID-19 in childhood

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**Abstract**

Gastrointestinal involvement has been reported in approximately 50% of patients with Coronavirus disease 2019 (COVID-19), since the pathogenic role of inflammation and the intestinal function of the angiotensin-converting enzyme 2 (ACE2) and its receptor. Accumulating adult data have pointed out that gut dysbiosis might occur in these patients with a potential impact on the severity of the disease, but the role of gut microbiota in susceptibility and severity of COVID-19 disease in children is still poorly known. During the last decades, the crosstalk between gut and lung has been largely recognized resulting in the concept of "gut-lung axis" as a central player in modulating the development of several diseases. Both organs are involved in the common mucosal immune system (including bronchus associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT)) and their homeostasis is crucial for human health. In this framework, a role for gastrointestinal dysbiosis in affecting the homeostasis of the gut-liver axis has been found. Of note, a gut microbiome imbalance has been linked to COVID-19 severity in adult subjects, but it remains to be clarified. Based on the increased risk of inflammatory diseases in children with COVID-19, the potential correlation between gut microbiota dysfunction and COVID-19 needs to be studied in this population. We aimed to summarize the most recent evidence on this striking aspect of COVID-19 in childhood.

**Key Words:** Gut; Microbiota; Dysbiosis; Microbiome; Coronavirus disease 2019; Children

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**Core Tip:** Growing evidence has shown that severe acute respiratory syndrome coronavirus 2 (SARS- CoV- 2) exerted a role behind the respiratory system. Due to the

release of inflammatory cytokines, it might play a "pleiotropic" effect by modulating also the course of several diseases. In particular, recent adult data supported a bidirectional relationship between gut microbiota changes and Coronavirus disease 2019 (COVID-19) infection. However, similar evidence in childhood is less defined. We aimed to provide a comprehensive pediatric overview in this intriguing field.

## INTRODUCTION

Since its first description in China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) has rapidly spread worldwide being declared a pandemic by the World Health Organization in March 2020 [1]. Accumulating data have showed a different course (including severity, hospitalisation, and mortality) of the Coronavirus disease (COVID-19) infection across different ages. In fact, a more severe form of the disease increasing the age has been reported, while a milder course of the infection and a relatively lower rate of death have been observed in children and young adults[2-5]. Of note, these findings have been supported by additional studies demonstrating remarkably low rates of vertical virus transmission (as from mother to offspring) and self-limited symptoms in most cases of horizontal transmission (as transmitted among individuals of the same generation) [6,7]. Nevertheless, the pandemic had a significant impact behind the respiratory tract, by affecting also cardiovascular and gastrointestinal systems both in children and adults [8,9]. In particular, different clinical gastrointestinal features COVID-19 related have been reported in the affected subjects, ranging from vomiting, diarrhoea, liver injury to gut microbial impairments. Noteworthy, a potential role for gut dysbiosis COVID-19 induced in modulating the course of the disease has been recently suggested [10,11].

More, lifestyle changes caused by the COVID-19 pandemic have been supposed to modify microbiota composition [10].

Recent intriguing findings suggested a potential interaction between SARS-CoV2 and microbiome[11]. As its role in immune response regulation, some authors focused on modifications of microbiome composition during COVID-19 infection, by supposing

potential different patterns in adults and children and a possible link with disease severity [10,11].

On this ground, we aimed to summarize the most recent evidence regarding the tangled relationship between gut microbiota and COVID-19 in children.

**The pleiotropic effect of gut microbiota in pediatric diseases**

Microbiota refers to all the commensal microorganisms (more of 100 trillion) hosted by the human body, mainly located in the gastrointestinal (GI) tract but also in the respiratory and skin system. Robust evidence has supported its pivotal role in the development of innate and acquired immune system [12,13] and numerous factors such as delivery mode, nutrition, lifestyle and living environment have been found to influence both its composition and diversity in children [13]. Remarkably, gut microbiota abnormalities have been linked to a wide spectrum of non-communicable diseases [14] including metabolic derangements [15] (e.g. obesity, metabolic syndrome, type 2 diabetes, and Non Alcoholic fatty liver disease (NAFLD)), cardiovascular disease [16], rheumatic disease [17], and celiac disease[18] both in adults and children[15-18] (Figure 1), although no specific microbiome signature has been currently demonstrated[18,19]. Noteworthy, evidence has supported a bidirectional influence of SARS-coV-2 on the host microbiome through the well-known immune dysregulation driven by the virus[19,20]. As recently reported in adult and pediatric studies[20-22], both interaction with host microbiome and immunity dysregulation have been implied in the persistence of symptoms related to COVID-19 infection (also known as long COVID-19 syndrome) as potential pathogenic contributors[20,21].

**The gut-lung axis in COVID-19 infection**

The concept of "gut-lung axis" refers to the crosstalk between gut and respiratory tracts immune systems classically mediated by microbiota, microbiota metabolites, microbial dysbiosis and common mucosal immunity[23] (Table 1). Indeed, bidirectional interactions between the gut microbiota[24-28] and the respiratory mucosa [29-31] have been

supposed to be involved in the response to SARS-CoV2. Changes in the taxonomic composition and decreased diversity and function of the gut microbiota, known as dysbiosis, might affect the lung immunity status[23, 30-31]. Conversely, the respiratory tract has its own microbiota and lung inflammation may lead to intestinal dysbiosis [23]. Since the common coexistence of gastrointestinal and respiratory disorders in COVID-19 infection [9,32] and the potential detection of SARS-CoV2 RNA in both oral and rectal swabs[33,34], Zhou *et al* [35] suggested a possible involvement of the axis in COVID-19 pathogenesis (Figure 2). As a consequence, the COVID-19 infection might act as a trigger for cytokine storm leading to multiorgan dysfunction including gut. Therefore, this process might lead to gut microbiota composition changes with a dysfunctional immune modulation potentially influencing a more aggressive course of the disease[35]. From a pathogenic point of view, the sensitized immune cells switch from gut-associated lymphoid tissue (GALT) to bronchial-associated lymphoid tissue (BALT) might enhance the lung immune response leading to a considerable increase of inflammation and subsequent organ injury. In addition, the potential role of the angiotensin-converting enzyme 2 (ACE2) receptor (expressed both in respiratory and gastrointestinal tracts) as a main route for SARS-CoV2 invasion, its involvement in gut tryptophan homeostasis, and its downregulation virus-mediated might contribute to gut dysbiosis[35].

On the other hand, this latter might result in a decreased production of some metabolites such as short-chain fatty acids (SCFAs) included butyrate, propionate and acetate [30,33]. In murine studies [36], the depletion of these metabolites has been related to an increased susceptibility to pulmonary viral infections [36].

**Gut microbiota changes COVID-19 induced: evidence from adulthood to childhood**

Although the respiratory system is the main target of COVID-19 infection, GI tract has been found to be largely involved in the disease [37]. Indeed, it has been demonstrated that SARS-CoV-2 can infect and replicate in human small intestine enterocytes [38] and virus RNA can be detected in fecal samples [33,34]. Given the well-known role of the GI

tract as the largest human immunological organ and of its resident microbiota in modulating host immune responses [39], changes in fecal microbiomes of hospitalized patients with SARS- CoV-2 infection and their potential link with severity and fecal shedding of virus were explored [25]. Authors performed metagenomic sequencing analyses of fecal samples from 15 patients with COVID-19 from February through March 2020 and compared microbiome data with those from 6 subjects with community- acquired pneumonia and 15 healthy individuals, by assessing also gut microbiome profiles according to disease severity and changes in fecal shedding of SARS-CoV-2[25]. Patients with COVID-19 had significant alterations in fecal microbiomes than controls, characterized by an overall enrichment of opportunistic pathogens and depletion of beneficial commensals, even after SARS-CoV-2 clearance (determined from throat swabs) and resolution of respiratory symptoms. The baseline abundance of Coprobacillus, Clostridium ramosum, and Clostridium hathewayi correlated with COVID-19 severity. An inverse correlation between Faecalibacterium prausnitzii (an anti-inflammatory bacterium) and disease severity was reported[25]. During hospitalization, different Bacteroides species (including B.dorei, B. thetaiotaomicron, B. massiliensis, and B. ovatus) determining downregulation of ACE2 and ACE2 receptor expression in murine gut were found to be associated with SARS-CoV-2 Load in fecal samples of affected patients [25].

Similarly, Yeoh *et al* [37] obtained blood, stool, and patient records from 100 patients with laboratory-confirmed SARS-CoV-2 infection. Serial stool samples were collected from 27 of the 100 patients up to 30 days after SARS-CoV-2 clearance. Gut microbiome composition was characterized by shotgun sequencing total DNA extracted from stools. Moreover, inflammatory cytokines and blood markers levels were assessed. Gut microbiome composition was significantly altered in patients with COVID-19 compared to non-COVID-19 individuals. In particular, several gut commensals with a well-known immunomodulatory potential such as Faecalibacterium prausnitzii, Eubacterium rectale and Bifidobacteria were underrepresented in patients and remained low in samples collected up to 30 days after disease resolution. More, the altered composition in

COVID-19 hospitalized patients was correlated with plasma concentrations of several cytokines, chemokines and inflammation markers, suggesting that the gut microbiota might play a role in modulating host immune response and potentially influence disease course. Specifically, the depletion of several bacterial species in the COVID-19 cohort was linked to increased concentrations of TNF-a, CXCL10, CCL2 and IL-10, indicating that these depleted taxa may have a role in preventing overaggressive inflammation [37].

Unlike adults, pediatric evidence in this field is still limited as the common asymptomatic course of the disease at this stage (Table 2).

Nashed *et al* [40] performed a case-control study by comparing microbiomes of 595 affected children aged 0-24 mo. Findings revealed in affected patients a decreased abundance of Bifidobacterium bifidum and Akkermansia muciniphila, both commonly exerting a protective effect against inflammation [41,42]. Of note, reduced levels of anti-inflammatory taxa were also detectable in asymptomatic infected infants, as described in symptomatic adults [37].

In another case-control study [43], nine COVID-19 children aged between 7 and 139 mo were studied for 25-28 days after symptom onset and their microbiome composition was compared to that of 14 age-matched healthy control children. Microbiome patterns were significantly different between the two tested groups in the various human body tracts. Particularly, the microbiome composition in throat and nasal swabs had significantly lower richness in COVID-19 children than healthy controls. At phylum level, Bacteroidetes and Firmicutes were predominant in the gut of COVID-19 patients, while Proteobacteria were enriched in the gut of healthy controls. On the contrary, higher Bacteroidetes and Firmicutes concentrations were found in in the upper respiratory tract of healthy controls, while in the same site of COVID-19 patients Proteobacteria levels were predominant. Compared to COVID-19 patients, both gut and upper respiratory tract of healthy controls were found to be mainly colonized by resident commensals, while some opportunistic pathogenic and environmental bacteria such as Pseudomonas, Herbaspirillum, and Burkholderia were significantly

predominant in the gut and the upper respiratory tract of the affected subjects. Notably, data supported the persistence (up to 25-52 days after the onset of symptoms) of dysbiosis in COVID-19 children mainly in the upper respiratory tract. However, dynamic microbiome changes were divergent between the upper respiratory tract and the gut, by showing a nearly-full gut microbiome restoration at 50-55 days after the onset of symptoms. Based on these findings, it could be supposed that the "gut-lung axis" is still not established during the childhood [28,43].

To sum up, current evidence suggests that COVID-19 infection might affect both gut and upper respiratory tract microbiomes in children, resulting in a persistent dysbiosis as a potential risk factor for short and long-term adverse health outcomes.

**Infant microbiota and COVID-19 infection**

The impact of COVID-19 infection has been explored from the earliest ages [1,6] (Table 3). In a single-centre observational cohort study, Romano-Keeler *et al* [44] examined 21 deliveries of COVID-19 positive mothers between March and August 2020. A higher rate of Caesarean section emerged in the study population compared to institutional (29% in 2019) and national rates (31.9% in 2018) [44]. To prevent the virus transmission, mother-infant contact was minimized, delayed cord clamping and skin-to-skin were avoided, and infants were admitted to the Neonatal Intensive Care Unit (NICU). No COVID-19 infection was detected in all the enrolled infants at 24 and 48 h and their average hospitalization time was of nine days. As these measures may decrease virus transmission, a potential impact on the neonatal microbiome has been described [44]. Compared to the colonization of Lactobacillus after a vaginal delivery, the C-section delivery represents a well-known risk factor for early life intestinal dysbiosis due to colonization of the newborn with potentially skin or hospital pathogenic organisms [6,45]. Indeed, there are several evidences linking C-sections delivery to an increased incidence of atopic disorders [46-48] and autoimmune diseases [49].

More, the infant feeding pattern has been found to play a crucial role in the microbiota composition in the first year of life [10,50]. Of note, breastfeeding exerts an important influence on the gut microbiome compared to the formula feeding. Jost *et al*[51] examined mother-infant faecal samples and maternal breast milk collected from seven mothers-newborn dyads. Authors identified a shared gut microbiota composition including obligate anaerobic genera such as *Bifidobacterium*, *Bacteroides*, *Parabacteroides,* and members of the Clostridia (*Blautia*, *Clostridium*, *Collinsella* and *Veillonella*). Notably, a viable strain of *Bifidobacterium breve* was shown to be shared among all three ecosystems within one dyad. Furthermore, pyrosequencing revealed that several butyrate-producing members of Clostridia (e.g. *Coprococcus*, *Faecalibacterium*, *Roseburia,* and *Subdoligranulum*) were shared between maternal feces and breast milk. Of note, this latter as feeding mode has been previously linked to a reduced risk of type 1 [52] and type 2 diabetes development [53]. In addition, further evidence pointed out the association between breastfeeding and a lower risk for multiple sclerosis in two case-control studies [54,55]. Since there is no evidence about the presence of SARS-CoV-2 in the breast milk of infected mothers and its transmission through breastfeeding [56,57], this feeding pattern has been recommended even for suspicious or infected new mothers [10,58].

Owing to the pandemic, the higher attention to hygiene resulting in an increased use of detergents as a further preventive measure has been experienced also at very early age. Gerasimidis *et al* [59] investigated the effect of food additives, artificial sweeteners, and domestic hygiene products on the gut microbiome and fibre fermentation capacity. The use of dishwashing detergent was associated with an altered microbiota pattern including a decreased concentration of Firmicutes. As previously reported, metabolites of Firmicutes (e.g. *Faecalibacterium* and *Subdoligranulum)* as butyric acid-producing bacteria

and other short-chain fatty acids (SCFAs) have been linked to reduced incidence of atopic disorders [60], multiple sclerosis [61], and type 1 diabetes [62,63].

Besides, social habits COVID-19 related such as sedentariness and increased domestic contacts with pets should be considered. Geography and ethnicity are well-known critical determinants of microbial composition, including differences in the incidence of obesity, gastric cancer, and chronic liver diseases [6,64-66], while it has been observed that living with pets increases the richness and diversity of infant gut microbiota. Azad *et al*[67] found that infants living with pets have significant over-representation of Clostridiaceae, Veillonella, Peptostreptococcaceae and Coprococcus, while Bifidobacteriaceae are under-represented. Moreover, interaction with pets within the first year of life has been associated with a decreased prevalence of allergic disesases [10, 68].

Since numerous studies have shown the essential role of a healthy micriobiota, the changes and the subsequent dysbiosis caused by the COVID-19 pandemic might increase the incidence of many disorders later in life, such as allergic, metabolic, and autoimmune diseases [6,10]. However, the exact impact of this condition on newborns cannot be currently established. Given the paucity of data in this field, more epidemiological studies are needed to better clarify this relationship and its implications.

**Gut microbiota, immune response, and vaccine response: is there a link?**

During the past years, several studies have examined the impact of the microbiota on the innate and adaptive immunity [1], by demonstrating over time a dynamic equilibrium between microbes and host [69]. In addition to defective production of immunoglobulin A, dysbiosis has been associated with an abnormal development of lymphoid tissues and intestinal T cells [70-73].

Nevertheless, microbiota plays also a role in the relationships between host and viral infections [70-73]. Indeed, microbiota composition has been found to influence vaccine responses both in adults and children [74]. Pediatric data found a positive association between Actinobacteria phylum and humoral and cellular responses both to oral and

parenteral vaccines [75], while an inverse correlation of the phylum Proteobacteria with the responses to the same vaccines and of Bacteroidetes with humoral responses to oral vaccines have been reported [75,76]. Moreover, both in children and adults a prevalence of the phylum Firmicutes has been associated to higher humoral and cellular responses to oral vaccines [1,76,77].

Regarding SARS- CoV-2 vaccines, in a prospective observational study on adults receiving either the inactivated vaccine (CoronaVac; Sinovac) or the mRNA vaccine (BNT162b2; BioNTech; Comirnaty), Ng *et al* [78] found that Bifidobacterium adolescents was found to be persistently higher in subjects with high neutralizing antibodies to CoronaVac vaccine, while neutralizing antibodies in BNT162b2 vaccines showed a positive correlation with the total abundance of bacteria with flagella and fimbriae including Roseburia faecis. In individuals with fewer adverse events following either of the vaccines a higher prevalence of Prevotella copri and two Megamonas species was detected, indicating that these bacteria may play an anti-inflammatory role in host immune response [67].

Given the potential influence of microbiota composition on vaccine responses especially in children and its changes in different age groups [58], it could be also supposed a similar role in modulating immune responses to viral infections.

In the context of COVID-19 infection, there are no pediatric studies evaluating this tangled relationship. Further studies are needed to clarify the potential influence of the microbiota age-related differences on the disease severity and COVID-19 vaccine response in the pediatric population [1].

## CONCLUSION

The occurrence of gut dysbiosis as disruptor of the gut-lung axis homeostasis and its potential correlation with disease severity has been largely described in COVID-19 adult patients, while there is paucity of similar data in childhood. As observed in adults, changes in gut microbiota composition seemed to negatively affect the course of the infection even in very young children. Given also the higher risk of autoimmune

and autoinflammatory diseases development in children with COVID-19, a deeper dissection of the role of gut microbiota might provide insightful therapeutic perspectives in this field.

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