Gut Microbiome and COVID-19: Role of Probiotics on Gut Lung Axis

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has caused the greatest worldwide pandemic called Coronavirus-2019 (COVID-19) disease. The SARS-CoV-2 virus primarily attacks the respiratory tract, but it also disturbs the gastrointestinal system (GIT). The presence of the angiotensin-converting enzyme-2 (ACE-2) receptor in the intestinal epithelial cells, suggest the transmission of SARS-CoV-2 viruses from lungs to gut through systemic circulation. The virus detected in fecal samples of COVID-19 patients causes several gastrointestinal maladies including vomiting, diarrhea, and pain in abdomen. The gastrointestinal symptoms are associated with alterations in gut microbial composition, an increase in inflammatory cytokines and delayed virus clearance. Several studies demonstrated a decreased abundance of beneficial microbial species and increased opportunistic pathogens in the fecal samples of COVID-19 patients. The gut and lungs, share a bi-directional relationship called the "gut-lung axis" which is modulated by imbalanced gut microbiota. Since the gut microbes are suggested to play a vital role in health and disease by maintaining homeostasis of the immune system, therefore targeting the intestinal dysbiosis with beneficial microbial species, seems plausible to eventually diminish the effects of pulmonary infections and diseases. In this review, we have summarized studies demonstrating the gut-lung axis in association with gut dysbiosis in COVID-19 patients. In addition, the review also highlights the studies showing the potential role of probiotic supplementation in the amelioration of various respiratory infections and diseases. Data demonstrate that the restoration of gut microbial communities by probiotic supplementation can enhance lung capacity to combat respiratory viral infections including SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2; Gut lung axis; Gut microbiota; Gastrointestinal symptoms; Probiotics

1. INTRODUCTION

December 2019, witnessed the emergence of a new virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) from Wuhan city (Hubei province), China which is responsible for COVID-19 disease. Due to its sudden surge in every corner of the world, mainly because of increased globalisation, World Health Organization (WHO) declared the outbreak as a pandemic[1]. SARS-CoV-2 markedly posed a worldwide threat by causing thousands of mortalities and millions of morbidities across the globe. As of now, the virus has infected nearly 420 million people worldwide and the number continues to increase. The pandemic revealed an unprecedented challenge to public health that led to a dramatic loss of human life. Moreover, the implementation of nationwide lockdown resulted in devastating economic disruption with millions of people at risk of falling into extreme poverty, unemployment, and an increased number of undernourished people[2].

In addition to common clinical manifestations, gastrointestinal symptoms are very common among COVID-19 patients[3]. The world already experienced two more epidemics in the year 2002 and 2011 i.e, Severe Acute Respiratory Syndrome (SARS) and Middle-East Respiratory Syndrome (MERS) respectively[4]. The phylogenetic analysis also showed sequence similarity of 79.5 per cent between SARS-CoV-2 and SARS-CoV[7]. SARS-CoV-2 is a single-stranded positive-sense RNA (+ssRNA) virus, which includes non-structural proteins (nsp1-16) and several structural proteins such as nucleocapsid (N), spike (S), envelope (E) and membrane (M) protein[6].

The entry point of both SARS-CoV and SARS-CoV-2 is common, i.e., via binding of S protein to ACE-2 receptor[5]. Almost every human organ and tissue including oral and nasal mucosa, nasopharynx, lymph nodes, GIT, thymus, skin, spleen, liver, kidney, brain, heart and blood vessels express ACE-2 receptor[7]. Moreover, a recent study has shown increased expression of ACE-2 in the small intestine enterocytes indicating its role in

mediating the entry and amplification of virus which results in inflammation in the gastrointestinal tract[8]. ACE-2 receptor, a homolog of ACE, forms converts Ang (1-7) from Angiotensin-II (Ang-II) and counteracts the negative part of the renin-angiotensin system (RAS) in many diseases by exhibiting anti-inflammatory effects[9]. Studies conducted in ACE-2 knockout mice showed decreased uptake of ingested tryptophan-2, increased inflammatory cytokine production[10] and compromised intestinal permeability by disrupted tight junction proteins (ZO-1, claudin and occludin)[10,11] leading to gut dysbiosis[10]. The gastrointestinal tract contains a dynamic and diverse community of commensal microorganisms, collectively known as "gut microbiota". The gut microbiota regulates energy metabolism, preclusion of detrimental microbes, maintenance of intestinal mucosal integrity and development and modulation of immune system[12-13]. Recent studies have shown a gut-lung axis (Fig. 1), crosstalk between gut microbiota and lung which is mediated by metabolites secreted from microbiota, endotoxins, cytokines, and hormones[14]. However, the alteration in gut microbial diversity and function leads to dysbiosis that may affect immunity to viral infections including SARS-CoV-2[15].

Due to the lack of a precise drug regimen, the COVID-19 pandemic has resulted in increased mortality in various countries. Many viral infections including COVID-19 are linked to microbial dysbiosis which can lead to serious gastrointestinal illnesses. The most effective way of reducing the severity of such viral infections is to improve/strengthen host immunity. The probiotic intervention has shown to exert both prophylactic and therapeutic, including regulating the diversity of human gut bacteria, improving gut barrier integrity and enhancing protective immune responses. In this context, the present review sheds light on the activation of the gut-lung axis through gut microbiota and their secretory metabolites to the lungs via the lymphatic system, as well as the alteration of gut microbiota and mycobiota in SARS-CoV-2 patients. In addition, we also discussed the potential roles of probiotic strains in disease mitigation and ongoing clinical trials in COVID-19 patients.

2. GUT-LUNG AXIS : A BIDIRECTIONAL CROSSTALK

The gut and lungs are important body compartments that are inhabited by microbiota and share a mutualistic bond with the host through the mesenteric lymphatic system and systemic circulation. Gut microbes have the potential to stimulate distal mucosal sites like lungs via release of short-chain fatty acids (SCFAs) and exerts regulation of the immune system. Several studies state that gut dysbiosis occurs during various respiratory infection[16]. Similarly, the respiratory microbiome also plays a vital role in host metabolic homeostasis and various diseases[17]. At the phylum level, a healthy lung microbiota is characterized by Proteobacteria, Firmicutes, and Bacteroidetes while at the genus level *Veillonella*, *Prevotella*, *Acinetobacter*, *Streptococcus*, *Neisseria, Fusobacteria*, and *Porphyromonas* are the most prominent[18, 19]. Sepsis and acute respiratory

distress syndrome (ARDS) are associated with pulmonary tract dysbiosis with enrichment of gut bacteria such as *Bacteroides* and *Enterobacteriaceae*[17,20] correlated with significantly increased expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-8[21]. Altered gut microbial composition contributes to inflamed intestine, compromised epithelial integrity, and reduced production of anti-microbial peptides (AMPs) and thereby increasing susceptibility to secondary infections[22-24].The leaky epithelial membrane enhances the translocation of bacteria and its contents to distal organs through interconnected mucosal tissues and triggers systemic inflammation. This bidirectional communication between the gut and the lung is known as the"gut-lung axis" Fig. 1[30] which allows migration of cytokines, endotoxins (lipopolysaccharides) and bacterial metabolites via systemic circulation[24].

Wang, *et al*., 2014 found that lung disease caused by a respiratory influenza virus also triggered intestinal injury and altered intestinal microbial compositions with a decrease in *Lactobacillus*, *Lactococcus* and an increase in *Enterobacteriaceae* with markedly higher Th17 (T helper 17) cells in the small intestine leading to gastro enteritis-like symptoms such as diarrhea[25]. It has been also noted that *Bifidobacterium breves*, a gut commensal bacterium which can repress the inflammatory responses of the host by causing an increase in the Treg cell population that suppresses the production of Th17 cells in lung and intestinal tissues[26].

A recent study in the murine model revealed that Influenza A virus altered gut microbiota which make lungs more susceptible to pneumococcal infection and highlights the importance of SCFAs on the host's pulmonary defense system against other secondary common bacterial infections[27]. Studies carried out with COVID-19 patients demonstrate that in addition to intestinal dysbiosis, patients may also have perturbed pharyngeal and pulmonary microbiota. Additionally, several studies have highlighted the anti-viral functions of acetate (SCFA) derived from gut microbiota that produces the type I IFNs by G-protein coupled receptor-43 (GPR43) signalling[28, 29] at mucosal sites of the lungs. In view of this, microbial metabolites such as desaminotyrosine and acetate have been shown to be critical in protecting from influenza infection via augmenting type I IFN signaling (IFN-α and IFN-β) and promoting the synthesis of IFN stimulated genes in lungs and pulmonary phagocytes[29]. Intestinal dysbiosis is associated with increased severity including death in patients related to respiratory tract infections, probably because of increased secretion of pro-inflammatory chemokines and cytokines such as CCL2, IFN-γ, IL-6 and decreased Treg cells in the lung as well as in GIT. Therefore, it can be hypothesized that intestinal microbial communities and/or their by-products have the ability to enhance the lung capacity to combat respiratory infections induced by different types of viruses.

3. CLINICAL MANIFESTATIONS OF COVID-19

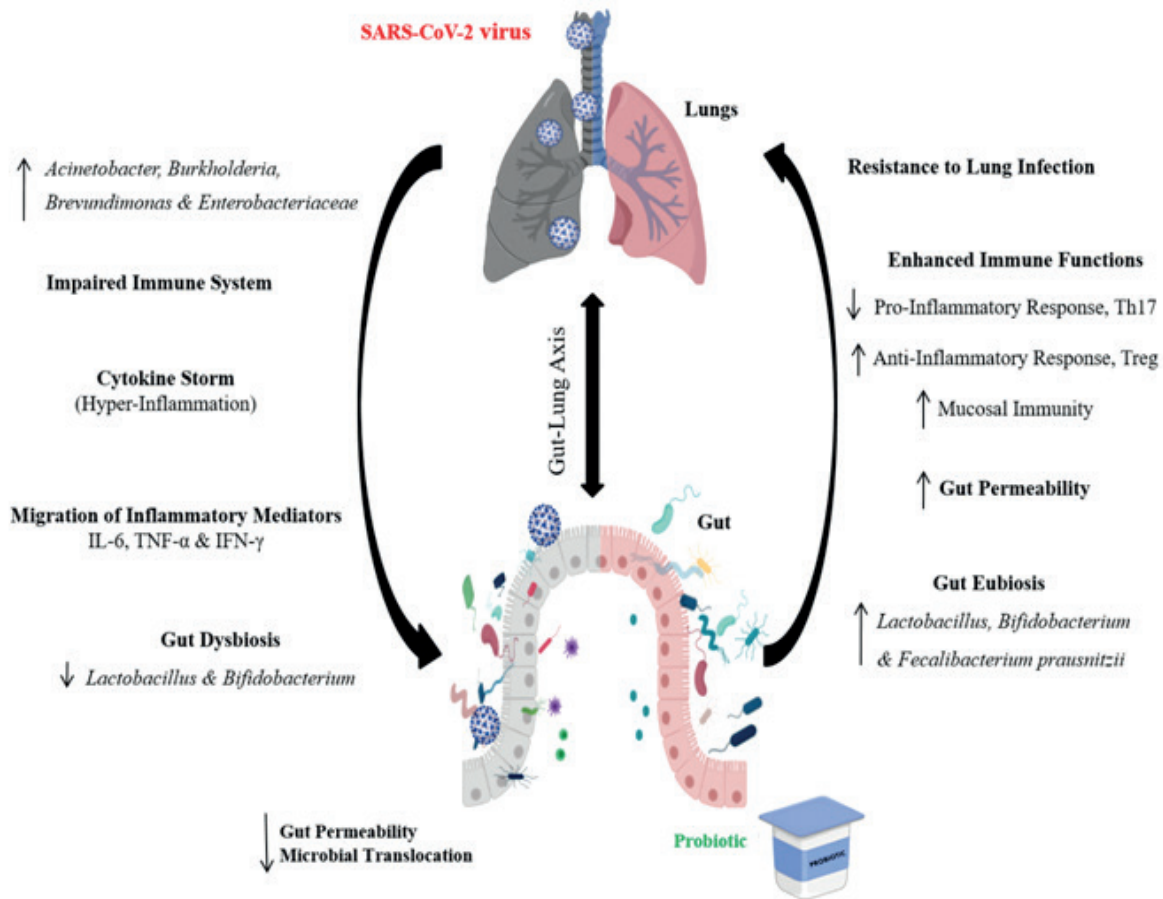It is well known that virus-induced infections are

Figure 1. Gut-Lung axis, SARS-CoV-2 infection30, and effect of probiotics.

transmitted primarily through the respiratory route by exposure to respirational droplets from asymptomatic or symptomatic individuals harboring the viruses[31]. Interestingly, a very recent study demonstrated the presence of SARS-CoV-2 receptors in the main fetal organs and maternal-fetal interface indicated vertical transmission of SARS-CoV-2 in humans[32]. The virus infection leads to symptoms ranging from fever, nasal congestion, runny nose, cough and expectoration to critical cases with severe pneumonia, chest tightness, abdominal distension, acute respiratory distress[33]. In addition to these common clinical manifestations, gastrointestinal symptoms are very common among COVID-19 patients[3]. The fundamental reason for chronic inflammation during COVID-19 is the imbalance between redox equilibrium and inflammatory response, increasing in free radicals and cytokine storm. Furthermore, SARS-CoV-2 infection is also associated with gut dysbiosis, leading to the abundance of pathogenic bacteria in the host[34].

3.1 Drugs and Co-morbidities in COVID-19 Disease: Impact on Gut Microbiota

The majority of COVID-19 severe clinical conditions and mortalities were observed in the elderly or patients with co-morbidities such as hypertension, hyperlipidemia, cardiovascular diseases, diabetes, and cancer[35] which required chronic and extensive pharmacological regimens. Multiple medication usage invariably results in a corresponding increase in side effects of drugs and adverse responses. In addition, polypharmacy can also alter microbiome integrity and as a result, it also damages the host's capacity to combat viral infections, including SARS-CoV-2[36]. The gut microbiota of the elderly is known to be "fragile" with reduced diversity characterized by an increased relative abundance of pathogenic bacteria such as *Helicobacter* which has numerous extra-gastric pathological implications. Moreover, reduction of families such as *Lachnospiraceae* and *Succinivibrionaceae*, essential for maintaining host cardiorespiratory and gastrointestinal health, can leads to serious complication in diseases such as COVID-19 if their abundance exacerbates[36]. Furthermore, medicines that are routinely used to treat COVID-19 have been proven to exacerbate a variety of interactions with gut microbiota. Prescription of non-steroidal anti-inflammatory drugs during COVID-19, drastically altered gut microbial composition while dysregulating the inflammatory responses, especially in elderly patients[37]. Langford, B. J *et al;* 2021, found that

74.6 per cent of COVID-19 patients received antibiotic therapy in a meta-analysis study[38]. Antibiotic therapy can indiscriminately kill pathogenic as well as commensal bacteria, resulting in microbial dysbiosis and antimicrobial resistance[39]. Glucocorticoids, particularly dexamethasone, were also recommended during COVID-19, as the most effective medication for controlling and reversing hyper-inflammation. According to a recent study, it was found that glucocorticoids alter gut microbial abundance that including an increase in Firmicutes and a decrease in Bacteroidetes[40]. The current epidemic has exposed everyone to extremely high levels of psychological stress, including persistent dread of infection, social stigmatization, restrictions, and isolation, as well as depression, despair and frustration. Studies have shown that stressful experiences have a significant influence on the composition and functional capabilities of the host gut microbiota[41-43]. A new study shows how stressful events alter the gut microbial profiling longitudinally over the period in front-line and healthcare workers, which underpins a dynamic shift in their mental state[44].

3.2 Altered Gut Microbiota During COVID-19 Infection

As discussed above, the gut bacterial composition modulated in SARS-CoV-2 infection results in depletion of the commensal bacterial population. Interestingly, it was reported that nucleic acid of SARS-CoV-2 was found in the stool samples of infected patients suggesting that the virus may be harbored in the digestive tract of patients and further affecting the GIT health including intestinal microflora[45-46]. Further, a study evaluated the intestinal microbiota of 24 H1N1 patients, 30 COVID-19 patients and 30 healthy individuals as control. It revealed altered gut microbial diversity in COVID-19 patients with a higher rate of increase in opportunistic bacteria (such as *Actinomyces, Rothia, Streptococcus*, and *Veillonella)* along with a reduced relative abundance of beneficial microbe (*Bifidobacterium*) (Fig. 1). While in H1N1 patients, a reduction in the relative abundance of *Actinobacteria*, *Erysipelotrichia*, *Clostridia*, *Lachnospiraceae* and *Ruminococcaceae* were observed[47]. Moreover, alteration in gut bacterial diversity has also been implicated in several other diseases such as obesity, diabetes mellitus, cardiovascular and other several age-related disorders[48]. A study by Yeoh YK *et al*; 2021 suggested a strong association between gut microbiota and COVID-19 disease severity. Additionally, the study found a lower abundance of several commensals with immunomodulatory properties such as *Faecalibacterium prausnitzii* (butyrate-producing bacteria) and *Eubacterium rectale* in samples collected after 30 days of disease recovered patients[49] (Fig. 1). The shifting in the bacterial community may help in discriminating critical patients from others, suggest that the potential of the above bacterial groups and further they can be used as diagnostic biomarkers for COVID-19 disease[20].

Another study, utilizing RNA shotgun metagenome sequencing data revealed the presence of opportunistic bacterial pathogens including *Collinsella aerofaciens* , *Morganella morganii* and *Streptococcus infantis* in stool samples of severe patients. In addition, *Parabacteroides merdae* , *Lachnospiraceae bacterium 1_1_57FAA* and *Alistipes onderdonkii* were also found in very low abundance[50]. However, in a murine model, *Bacteroidesstercoris* which is known to suppress the colonic expression of ACE-2 suggesting its high abundance further contributes to the transmission of viral infection[51]. The study performed by Yu L *et al*; 2020 on the gut microbiome of COVID-19 patients also found a lower abundance of probiotic strains such as *Bifidobacterium*, *Lactobacillus* and *Eubacterium* while the population of pathogenic bacteria e.g., *Corynebacterium* and *Ruthenibacterium* were increased significantly in diseased patients[52] (Fig. 1).

3.3 Altered Lung Mycobiota: Fungal Co-infection in COVID-19 Patients

Additionally, fungi have also been recognized as an integral part of our commensal microflora[53]. The interaction between bacteria and fungi involves competition for the niche, nutrients and secreting molecules to promote or inhibit growth and modulate the host immune response. From April to June 2021, the second wave of COVID-19 affected India to a great extent which was accompanied by fungal co-infections including invasive pulmonary aspergillosis (IPA) and mucormycosis (popularly known as black fungus) a mold infection, which are ubiquitously present in humans[54,55]. These fungal infections have been recognized as secondary complications of COVID-19 disease mostly among critically ill patients which were associated with high morbidity and mortality[56]. The major risk factors associated with IPA and mucormycosis include uncontrolled diabetes mellitus, SARS-CoV-2 infection and immuno-suppressed patients receiving extensive treatment with corticosteroids for long duration[57,58].

A study analyzed the fungal community of lung tissue in COVID-19 patients and the composition was dominated by Cryptococcus followed by Issatchenkia, Cladosporium, Aspergillus, Naganishia, Diutina and Candida[48]. The species belonging to the above-mentioned genus have been found to cause fungal co-infection in COVID-19 patients, particularly in immune-compromised patients. For instance, Cryptococcus infections were related to high mortality rates[59] and opportunistic species of the Issatchenkia, Cladosporium and Candida were involved in mycosis in immuno-compromised patients[48]. It is well known that SARS-CoV-2 infection damages ciliary activity of the mucous pulmonary epithelium, immune system and promotes uncontrolled colonization of Aspergillus, Rhizopus and Candida species in the lungs exacerbate into overt fungal diseases[57,60]. Results from a recent systematic review revealed that the most affected organs by mucormold are nose and sinus (88.9%) followed by rhino-orbital (56.7%). The co-morbidity, diabetes mellitus was present in 80 per cent of cases while extensive corticosteroid intake was recorded in 76.3 per cent cases[61]. A recent study revealed that altered

gut mycobiota during SARS-CoV-2 infection resulted in decreased abundance of gut fungi[59]. Since, the gut microbiota is playing a critical role as a regulator of type 1 IFN production and alters the Th2 responses during pulmonary viral infections, suggesting that the gut microbiota has indeed the potential to influence anti-fungal immunity[62-63]. The above findings suggest that dysbiosis due to antibiotic or immuno-suppression due to COVID-19 disease influences lung microbiome and modulation of immune responses in the lungs (Fig. 1).

4. PROBIOTICS AS AN ANTIVIRAL AND PREVENTIVE MEASURE FOR COVID-19 DISEASE MANAGEMENT

In the current pandemic situation, regulating immune responses and boosting host immunity is critical, as vaccines and treatment regimens to combat COVID-19 are still being developed. Consumption of probiotics improves immunological protection in humans by balancing immune responses (Th1/Th2) and may help to prevent or alleviate a variety of disease pathologies[64]. Thus, probiotics supplementation in the management of viral infections like COVID-19 is plausible. Probiotics can be defined as live, commensal microorganisms consumed in an adequate amount which gives bolstering health benefits including improved gut homeostasis[64-65]. It has already been reported that COVID-19 disease negatively affects the GIT homeostasis by attacking the gut microbiota leading to dysbiosis and eventually gastrointestinal symptoms and disorders[20-21]. It was also reported that patients with SARS-CoV-2 infection had decreased levels of probiotic bacteria having the potential of modulating the immune system i.e., Lactobacillus and Bifidobacterium along with gut commensals such as Faecalibacterium prausnitzii , Eubacterium rectale (in rectale), and Clostridium species, however, the abundance of opportunistic pathogens were increased[20,27]. Studies have been demonstrated astounding antiviral effects of probiotic strains against common respiratory viruses including rhinovirus, influenza and common cold[65,70,72] (Fig.1).

Table 1 describes the clinical trials carried out to investigate the potential impact of probiotics on viral infections. Probiotics as a dietary supplement containing a mixture of three strains of *Lactobacillus* were found to be significantly alleviated the episodes of upper respiratory tract infection (URTI) and flu like symptoms in adults (Table 1). After 12 weeks, it was found that treated subjects had significantly increased levels of IFN-γ in serum and secretory IgA in the gut compared with untreated subjects or their baseline results[64]. Another strain, *Lactobacillus rhamnosus* GG reduced the incidences of rhinovirus-induced infections in infants, however, the study did not determine the immunomodulatory effects of the strain[65]. In a separate study, administration of *Lactobacillus plantarum* DR7 for 12 weeks marginally reduced the nasopharyngeal and flu-like symptoms in subjects, accompanied by increased levels of IL-4 and IL-10 (anti-inflammatory cytokines) and decreased

expression of IFN-γ and TNF-α (pro-inflammatory cytokines) indicating that *Lactobacillus plantarum* DR7 attenuated inflammatory parameters during viral infections[66]. IL-1 and TNF-α cytokines are known to stimulate the expression of chemokines such as CCL2-5 and CXCL8, which exhibited a vital role against viral infections by enhanced recruitment of cytotoxic innate and adaptive immune cells[67]. Consuming probiotic-containing *Bifidobacterium animalis sub* spp. *Lactis* Bl-04 significantly reduced the CXCL8 response and eventually decreased viral load in nasal lavage, indicating its beneficial effects in ameliorating the risk of upper respiratory tract illnesses[68] (Fig. 1).

Elderly people have always been susceptible to seasonal influenza and other infections due to their compromised immune systems and reduced microbial diversity. Therefore, vaccination is strongly recommended to attenuate the negative effect of infectious diseases. However, owing to co-morbidities/or immune dysfunction, vaccine efficacy is also low. Two randomized controlled trials demonstrated that *Lactobacillus coryniformis* K8 CECT5711 and *Lactobacillus delbrueckii*s sp. B*ulgaricus* OLL1073R-1 decreased the incidences of gastrointestinal symptoms, influenza-like illnesses, and respiratory symptoms[70,73]. Another multicenter clinical trial used *Lactiplantibacillus plantarum* HEAL9 combined with *LLactiplantibacillus plantarum* in subjects who were susceptible to common cold. The trial showed reduced incidences of common cold in subjects belonging to the probiotic group with a significant increase in the percentage of memory CD8+ cells (CD45R0-memory marker) which elucidated priming of the immune system[72].

5. PROBABLE MECHANISM OF ACTION OFPROBIOTICS FOR MANAGEMENT OF COVID-19 DISEASE

Above evidences have clearly illustrated the favorable impact of probiotic supplementation. Generally, probiotics confounds a similar effect that includes enhancement of mucosal barrier functions, restriction of binding sites for pathogens, releasing of anti-microbial compounds and eventually leading to modulation of the immune system. The exact mechanisms of action of probiotics on RTI are not fully understood, but a probable mechanism by which probiotics may help in eliciting an anti-viral activity and further participating in the management of respiratory viral infections including COVID-19 disease is summarised below.

5.1 Reinforcement of Mucosal Barrier Function

Intestinal mucosal barrier consists of a mucous layer, anti-microbial peptides, secretory IgA and tight junctions in epithelial cells. Administration of probiotic strains such as *Lactobacillus plantarum* WCFS1 in human subjects has shown increased expression of ZO-1 and occluding(tight junction proteins) and mucous glycoproteins, thereby improving epithelial integrity and preventing the translocation of the intestinal microbes and their endotoxins[74] (Fig.2).

Table 1. List of clinical trials and potential impact of probiotics on viral respiratory infections

S No.	Viral Infection	Probiotic bacteria	Dose in (CFU)	Reported effects	Reference
1.	URTI[a]	Lactobacillus paracasei	3×10^7	Reduced rate of upper respiratory tract infection and flu likesymptoms	64
		Lactobacillus casei 431®	3×10^7		
		Lactobacillus fermentium PCC®	3×10^6		
2.	Rhinovirus[a]	Lactobacillus rhamnosus GG	1×10^9	Lower incidence of rhinovirus induced infection in probiotic group	65
3.	URTI[a]	Lactobacillus plantarum DR7	1×10^9	Alleviated the symptoms of URTI by improving Immune- modulatory activities of immune system	66
4.	Respiratory illness[a]	Lactobacillus rhamnosus GG	6.7×10^5- 1.9×10^6	Significant reduction in respiratory manifestations	68
5.	Common cold[a]	Lactobacillus plantarum HEAL9, Lactobacillus paracasei 8700:2	1×10^9	Reduced the severity, episodes and common cold in children	72
6.	Rhinovirus[b]	Bifidobacteriumanimalissubsp. lactis Bl-04	2×10^9	Reduced virus shedding in nasal secretions and thereby decreased viral load	69
7.	Influenza[b]	Lactobacillus coryniformisK8 CECT5711	3×10^9	Improved the immunological response to the influenza vaccination and reduced the symptoms of respiratory illnesses.	70
8.	Common cold[c]	Lactobacillus bulgaricus OLL1073R-1	$2 \cdot 0 - 3 \cdot 5 \times 10^8$	Diminished the risk of catching the common cold in the elderly people	73
9.	Influenza[d]	Lactobacillus delbrueckii sub sp. Bulgaricus OLL1073R-1	1.12×10^9	Probiotic had no significant effect on preventing influenza but found to improve the gastro-intestinal symptoms like constipation	71

5.2 Inhibition of Viral Attachment

During SARS-CoV-2 infection, the virus first attaches itself to the host cell using the ACE-2 receptor as described above[5]. Colonization and persistence of probiotic bacteria in the GIT is the preliminary step required for interaction with host, stimulate the immune response and competitive exclusion of enteric pathogens to intestinal cells. In addition to common surface molecules, *Lactobacillus* and *Bifidobacterium* species have mucin binding proteins that play an important role in the interaction with components of the mucus layer[75]. Strains or species with excellent adhesion capabilities protects the gut against enteric infections by competitive exclusion[76] (Fig. 2). Probiotic species such as *Lactobacillus* and *Bifidobacterium* in non-tumorigenic porcine intestinal epithelial cells (IECs)

show blockage of vesicular stomatitis virus into cells, possibly by hindering the adsorption and internalization[77], thereby inhibiting attachment of the virus to the host cells.

5.3 Release of Anti-microbial Compounds

Probiotic strains such as *Lactobacillus* produce a wide range of AMP like hydrogen peroxide, SCFA, lactic acid, bacteriocin-like inhibitory substances (BLIS) and bacteriocins[22] which have the potential to decrease the viral loads[23]. A study reported that *Lactobacillus spp.*, produced a bacteriocin which specifically inhibited virus multiplication[78]. Enterocin ST4V inhibited the late stages of viral replication of herpes simplex viruses HSV-1, HSV-2, measles virus and polio viruses[81]. Plantiricin compound, a metabolic product of *Lactobacillus plantarum* blocks
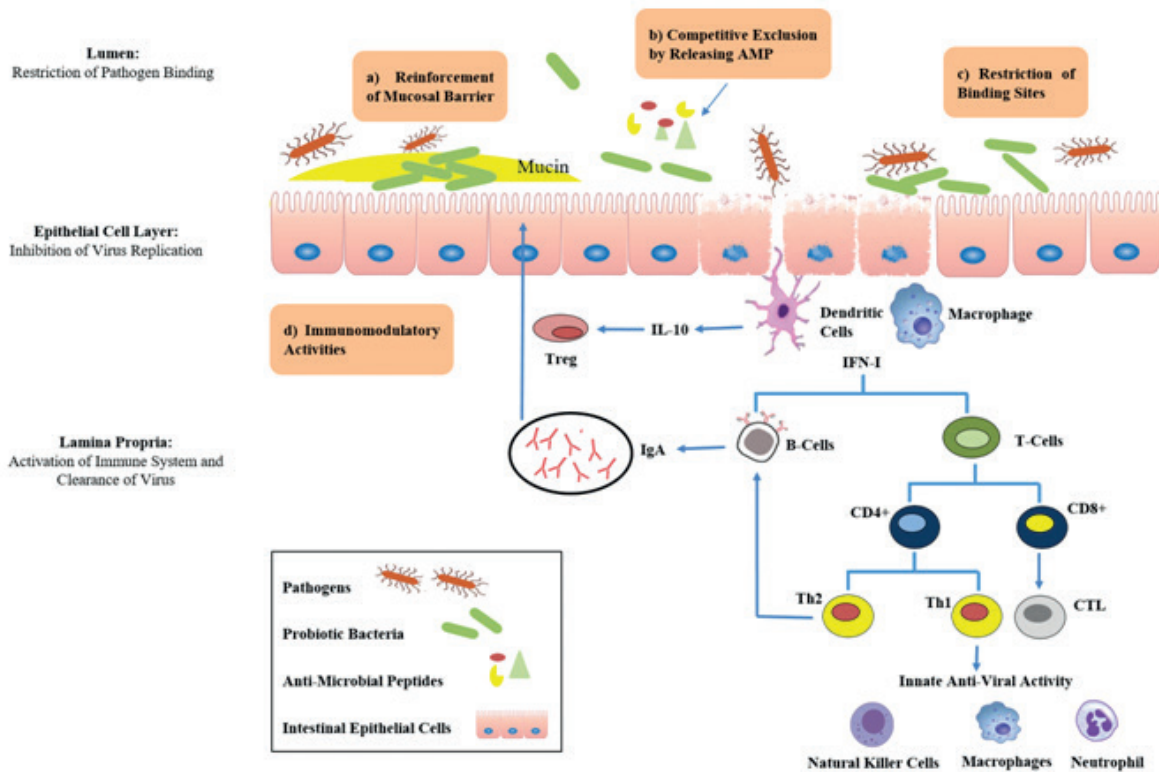
Figure 2. Probable mechanism of action of probiotics in management of SARS-CoV-2 infection.

the entry via binding with spike proteins and ACE-2 receptor[79]. Mechanism of action of AMP includes blocking of cell surface molecules such as heparan sulfate which acts as a binding site for viruses thereby reducing the viral infection[80] (Fig.2).

5.4 Modulation of the Immune System

During a virus attack, probiotic bacteria have a vital role in the activation of anti-viral immunity via modulation of host immunological responses. The Genetic material of viruses is sensed by dendritic and macrophage cells via pattern recognition receptors (PRRs). Detection of PRRs by pathogen-associated molecular patterns (PAMPs) further activates the antigen-presenting cells (APCs). Upon activation, dendritic cells initiate a suitable response by secreting IL-10 mediated differentiation of Th0 to Treg which is responsible for maintaining a balance between inflammatory and regulatory responses. *Lactobacillus reut*eri and *Lactobacillus casei*, probiotic candidates can stimulate the production of IFN-γ and activates pro-inflammatory Th1 cells which are required for virus elimination[82-84]. Activated APCs further prime T and B cells where T cells are responsible for the production of pro-inflammatory and regulatory mediators, which kill infected cells and B cells stimulate and produce antibodies in the gut, particularly IgA which inhibits proliferation of coronavirus[85] (Fig.2).

6. CLINICAL TRIALS EVALUATING PROBIOTIC INTERVENTION IN COVID-19 PATIENTS

In addition, several clinical trials (registered at ClinicalTrials.gov) are ongoing around the world and are currently in progress to assess the effect of probiotics or synbiotics for COVID-19 disease pathophysiology and management, a few of which are compiled in (Table 2). The most common probiotic species studied were *Lactobacillus* (6 studies), a combination of *Lactobacillus* with other probiotic strains *i.e., Bifidobacterium* (4 studies) or *Pediococcus acidilactici* (1study). A single trial used *Saccharomyces cerevisiae* and few studies did not reveal the name of strains used. A randomized, single-blinded clinical trial (NCT04666116) aim to evaluate the efficacy of dietary supplementation of probiotics in reduction of viral load in COVID-19 hospitalized patients. Another randomized single-blinded trial (NCT0448519) examining the effect of *Lactobacillus lactis* W136 by administering intranasally in symptom improvement in non-hospitalized COVID-19 patients. Strains of *Lactobacillus* have shown promising results in URTI, rhinovirus and influenza in reducing symptoms and incidences by modulating immune response[50,59]. Using these strains in the ongoing clinical trials might have a positive influence in improving COVID-19 disease pathophysiology. Other clinical studies (NCT04621071, NCT04390477, NCT05080244,

Table 2. Clinical trials investigating potential effect of probiotic intervention in COVID-19 disease

S. No	Description and Trial Identifier	Title of the study	Probiotic bacteria (strain)	Dose (in CFU)	Administration route* and Duration
1.	RSBCT NCT04666116	Changes in viral load in COVID-19 after probiotics	*Bifidobacterium longum, Bifidobacterium animalis subspp, Lactis* and *Lactobacillus rhamnosus*	Not revealed	Not revealed
2.	RSBCT NCT04458519	Efficacy of intranasal probiotic treatment to reduce severity of symptoms in COVID-19 infection	*Lactococcus lactis* W136	2.4×10^9	14 days (Twice a day) Nasal irrigation with Probiorinse
3.	RDBCT NCT04621071	Efficacy of probiotics in reducing duration and symptoms of COVID-19 (PROVID-19)	Not revealed	10×10^9	2 capsules a day for 25 days
4.	RCT NCT04877704	Symprove (Probiotic) as an add-on to COVID-19 management	Not revealed	Not revealed	3 months water based formula
5.	RQBCT NCT04734886	The effect of probiotic supplementation on SARS-CoV-2 antibody response after COVID-19	*Lactobacillus reuteri*DSM 17938	1×10^8	2 capsules per days for 6 weeks
6.	Randomized open label case control study NCT04390477	Study to evaluate the effect of a probiotic in COVID-19	Not revealed	1×10^9	1 capsule daily for 30 days. Dietary supplement
7.	Multicenter randomized quadruple blinded NCT04366180	Evaluation of the probiotic *Lactobacillus Coryniformi*s K8 on COVID-19 prevention in healthcare workers	*Lactobacillus K8*	3×10^9	1 capsule daily for 2 months
8.	RDBPCT NCT04937556	Evaluation of a probiotic supplementation in the immune response of participants with COVID-19	*Lactobacillus salivarius* PS7	1×10^9	1 capsule for 28 Days
9.	RQBCT NCT04907877	Bifido- and Lactobacilli in symptomatic adult COVID- 19 outpatients (ProCOVID)	*Bifidobacteria* and *Lactobacteria*	5×10^9	Once in a day before breakfast for 30 days
10.	Randomized open label clinical trial NCT04854941	Efficacy of probiotics in the treatment of hospitalised patients with novel coronavirus infection	*Lactobacillus rhamnosus PDV 1705, Bifidobacterium bifidum PDV 0903, Bifidobacterium longum sub spp infantis PDV 1911 and Bifidobacterium longum PDV 2301*	1×10^9 of each strain	3 times per day for 2 weeks

S. No	Description and Trial Identifier	Title of the study	Probiotic bacteria (strain)	Dose (in CFU)	Administration route* and Duration
11.	RDBPCT NCT04798677	Efficacy and tolerability of ABBC1 in volunteers receiving the influenza or Covid-19 vaccine	beta-1,3/1,6-glucan with inactivated *Saccharomyces cerevisiae*	Not revealed	30-35 days
12.	RDBCPS NCT04847349	Live microbials to boost anti-severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) immunity clinical trial	Not revealed	Not revealed	1 capsule per day with breakfast for 21 days
13.	RDBCPS NCT04756466	Effect of the consumption of a *Lactobacillus* strain on the incidence of covid-19 in the elderly.	*Lactobacillus*	3×10^9	1 capsule daily for 3 months
14.	Open label NCT04922918	*Ligilactobacillus Salivarius MP101* for elderly in a nursing home (PROBELDERLY)	*Ligilactobacillussalivarius MP101*	1×10^9	Fermented milk for 4 months
15.	RCT NCT04517422	Efficacy of *Lactobacillus Plantarum* and *P. acidilactici in* adults with SARS-CoV-2 and COVID19	*Lactobacillus plantarum* CECT 30292, *Lactobacillus plantarum* CECT 7484, *Lactobacillus plantarum* CECT 7485 and *Pediococcus Acidilactici*	Not revealed	30 days
16	RCT NCT05080244	Evaluation of the efficacy of probiotics to reduce the occurrence of long COVID (PROVID-LD)	Not revealed	10×10^9 of each strain	Two capsules per day for 25 days
17	Randomized open label controlled clinical trial NCT05043376	Study to investigate the treatment benefits of probiotic Streptococcus Salivarius K12 for mild-tomoderate COVID-19	*Streptococcus salivarius K12*	Not revealed	2 tablets daily for 14 days
18	RCT NCT04950803	A randomised-controlled trial of an oral microbiome immunity formula in recovered COVID-19 patients	*Bifidobacteria*	10×10^9	1 sachet daily for 3 months
19	Pilot Study NCT04813718	Post COVID-19 syndrome and the Gut-lung Axis	Not revealed	Not revealed	Not revealed

and NCT05043376) are evaluating the effectiveness of probiotics in symptomatic COVID-19 patients to see whether they may reduce the period of severity and symptoms (https://clinicaltrials.gov/). These trials will also include, analysis and evaluation of oral and fecalmicrobiota after probiotic administration (Table 2). Some of the proposed clinical trials (NCT04734886 and NCT04907877) will be evaluated the SARS-CoV-2 specific IgG/IgM antibody upon probiotic administration containing strains of *Lactobacillus* and *Bifidobacterium*. Clinical studies (NCT04854941 and NCT04366180) are being initiated to understand how effective probiotics are in mitigating the impact and occurrence of COVID-19 (https://clinicaltrials.gov/). Researchers across the world, hypothesised that a symbiotic composition helps regulate seasonal influenza viruses or COVID-19 by enhancing immune responses in people who get influenza or COVID-19 vaccinations (NCT04798677). COVID-19 symptoms do not conclude at the time of discharge from the hospital, leading to "Post-Covid" syndrome with a broad range of manifestations. Therefore, two clinical trials aimed at the gut-lung axis as a potential therapeutic target for comprehensive care of recovered COVID-19 patients (NCT04950803 and NCT04813718) (Table 2).

7. CONCLUSION AND FUTURE PERSPECTIVE

The COVID-19 pandemic has taught us the utmost importance of a strong immune system. The SARS-CoV-2 virus affects mucosal surfaces of both the respiratory and gastrointestinal tract, leading to a significant alteration in local microflora and eventually hyper-inflammation. As exemplified by the cases of upper respiratory tract infections, the positive potential of gut microbiota in ameliorating lung infections against respiratory infections can be deciphered. Considering the gut-lung axis, re-establishment of eubiosis via probiotic intervention as a therapeutic modality can be a promising strategy to reduce the severity, incidences, and duration of COVID-19 disease. Evidence from previous clinical trials where probiotics have been used to treat respiratory infection has shown positive results. Currently, several clinical trials are also under process using probiotics as a therapeutic modality to treat or manage COVID-19 manifestation. However, the optimal dose, duration, and probiotic strains to be used are some of the questions which remain to be investigated.

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