

Probiotics: An Alternative Therapeutic Strategy For COVID-19

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The COVID-19 pandemic has made the scientists today all around the globe to look for its specific prevention and treatment modalities. The disease presents itself as asymptomatic to mild to severe respiratory symptoms along with lesser common gastrointestinal symptoms of diarrhoea, nausea and vomiting. The situation worsens due to lack of precise treatment strategy. Healthcare system is being overwhelmed, emphasizing on the need to look for alternate supportive therapy which can not only enhance the immune status of people worldwide but at the same time, ensure better prognosis. The relationship between the gut microbiota and upper and lower respiratory tract viral infections are well studied. Oral administration of probiotic microorganisms of genera *Lactobacillus*, *Bifidobacterium* and *Bacillus* in mice suffering from influenza infection have shown increased TNF α , IFN- γ and NK cell responses along with production of anti-Influenza IgG. At the same time they have shown immunomodulation by producing anti-inflammatory cytokines as well as cytotoxic T-cells and T-suppressor cells. Hence, probiotic strains of genera *Lactobacillus*, *Bifidobacterium* and *Bacillus* have shown a probability to be used as preventive and therapeutic agents for SARS-CoV-2.

Keywords: COVID-19, Dysbiosis, Gut microbiota, Probiotics, Respiratory tract infections.

The world today is facing the havoc of SARS-CoV-2 also known as COVID-19. The disease which reportedly was first seen in Wuhan city of China in December 2019 has now spread to nearly 190 countries of the world. It is a zoonotic disease primarily affecting a species of bats but due to close proximity of animals and humans in the present world it has mutated and attacked humans. The spread of this disease has overwhelmed the healthcare systems of developing as well as developed countries. Various continuously evolving treatment strategies are being currently used ranging from the use of hydroxychloroquine to plasma therapy. Simultaneously several vaccine

candidates are in I or II phase of clinical trials which means that currently no specific treatment or preventive vaccine is available. This has greatly emphasized on the need of some alternate therapy not only to combat the pathogen but also to enhance the immunity of the person to make him less prone to the infection as well as with better chances of recovery.

Various studies have shown that gastrointestinal tract is also involved in the spread of the virus as the virus has been isolated from stool samples of the patients¹⁻⁶. Further, the study has revealed that corona virus including the novel one can invade enterocytes⁷ causing severe distress in

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patients. Probiotic bacteria are well documented for their role in correcting gut dysbiosis^{8,9}, reducing pro inflammatory reactions increasing defense against various pathogens¹⁰ and upgrading mucosal immunity¹¹. They are useful in treating gut dysbiosis¹², antibiotic associated diarrhea^{13,14}, irritating bowel syndrome¹⁵ etc. Latest studies have shown their impact in treating disorders caused due to gut dysbiosis such as obesity and atopic dermatitis¹⁶. This review tries to assess the potentiality of the probiotic strains as an alternate or supplementary therapeutic option for treating the COVID-19 disease.

Gut microbiota and Viral respiratory tract infections

Numerous studies have focussed on the importance of gut microbiota in defending or providing better immune response against various diseases¹⁷⁻²¹ and respiratory viral infections are no exception. In a study, when mice were treated with antibiotics to deplete gut microbiota, a decrease in antibody production and reduction in influenza virus specific T-cells was observed²², along with increased morbidity and mortality^{22,23}. This occurred due to decreased T- cell numbers and migration rate of dendritic cells. These mice were also unable to stimulate the response of CD4⁺ T-cell mediated to PR8 antigen. A decrease in number of influenza specific CD8⁺ T- cells was also observed.

Gut microbes also modulate the macrophage response during viral pulmonary infection. According to a study, the ability of macrophages to limit viral replication was impaired in mice treated with antibiotics along with reduced response to type I and type II IFNs²³. The study highlighted the importance of gut microbiota in strengthening the immune system against viral infections.

Conversely, different studies have reported that viral respiratory infections, like influenza and Respiratory Syncytial Virus (RSV) causing common cold, have an impact on the gut microbiota²⁴⁻²⁸. These infections caused a disbalance of gut microbiota by increasing the phylum Bacteroidetes and leading to decrease in phylum Firmicutes²⁸, especially *Lactobacillus*^{24,29}. This change in gut microbiota composition after influenza infection is due to type- 2 IFN which is produced by T- cells derived in the lung and recruited to the intestine²⁸.

COVID-19 and Gut

Till 2019, there were six known species of coronavirus which caused human diseases. These include severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) causing severe respiratory tract infections and had high mortality rate. SARS-CoV-2, or COVID-19 as it is widely known, is the seventh to be added in this category³⁰.

According to the document published by MoHFW³¹, Govt. of India in coherence with WHO guidelines, the disease presents itself in asymptomatic, mild to severe forms. The common symptoms range from flu like cough, cold, fever to non-specific flu like pneumonia to life threatening severe acute respiratory illness (SARI). It's a droplet borne infection spreading through direct contact with the patient or through fomites. The lesser common symptoms includes production of sputum, headache, GI symptoms like diarrhoea, nausea, vomiting^{1,5}, thus exhibiting gut- lung crosstalk. Some studies have reported diarrhoea in the range of 1-3.8% patients^{1,32-34} and other has observed a greater incidence of these symptoms i.e. nausea in 10.1% and vomiting in 3.6%⁵. These reports highlight the occurrence of gastrointestinal disorders in COVID-19 patients. This was proved by observing viral nucleic acid in anals swabs and fecal samples of COVID-19 patients^{2,3,6} as well as in fecal samples of patients who had recovered from COVID-19 infections, 11 days ago³⁵.

Earlier studies have reported angiotensin-converting enzyme (ACE-2) as the receptor for binding of SARS-CoV in lung epithelia^{36,37}. This receptor is also found on the cholangiocytes in liver and patients of COVID-19 have shown presence of SARS-CoV-2 in liver³⁸ indicating that this virus is also using ACE-2 as receptor for binding host cell³⁹⁻⁴¹. As this receptor is found in intestinal epithelia as well^{2,3,42-46}, thus increasing the possibility of its infection by COVID-19. ACE-2 mutants have shown a decrease in expression of antimicrobial peptides in gut and have shown dysbiosis. Thus, it can be inferred that COVID-19 infection may be related to gut microbiota and its dysbiosis⁴⁷.

Yet another study found that cellular serine protease known as transmembrane protease serine 2 (TMPRSS-2) is also necessary for entry of COVID -19 in cells along with ACE- 2 receptor⁴⁸.

This expression of TMPRSS- 2 was found in lung alveolar type 2 cells, ileum, oesophageal upper epithelial cells and colon. Therefore, COVID-19 can easily gain entry into these enterocytes and cause infection.

Probiotics and Viral Respiratory Tract Infections

According to FAO/WHO⁴⁹, probiotics are defined as “live microorganisms, which when administered in adequate amounts confer a health benefit to the host”. Probiotic microorganisms suppress pathogens in host and stimulate the proliferation of epithelial cells and hence provide health benefits to the host. They also regulate the gut microbiota and play an important role in immunomodulation⁵⁰. Relation between gut microbiota dysbiosis and metabolic disorders and chronic low-grade inflammation has been established in different studies^{51,52}. Beneficial effect of probiotics in alleviating gastrointestinal diseases has been reported by different randomized clinical trials^{53,50}. These benefits may be due to alteration of diversity of gut microbiota by modulating the intestinal immunity, producing growth substrates and by competing for nutrients⁵⁴.

Viral respiratory tract infections are a major cause of severe morbidity and mortality in human adults worldwide. Both innate^{55,56} and adaptive²² immune response play an important role for defence against influenza virus infection. Under *in vivo* conditions, the innate system recognizes the influenza virus through pattern recognition receptors (PRRs), and lead to generation of adaptive immune response. Ichinohe *et al*⁵⁷ reported reduced influenza virus-specific antibody titre and CD4 T-cell response in antibiotic treated mice along with diminished cytokine secretion and influenza virus specific CTLs. Synthesis of pro IL-1b and pro IL-18 and NLRPs was also found to be diminished.

Oral administration of probiotic microorganisms have been shown to reduce duration and severity of viral infections^{58,59}. During influenza infection in mice *L. plantarum* has shown to stimulate type-I IFN responses and reduced viral titers in lung⁶⁰. In another study, *Lactobacillus* spp. stimulated production of TNF- α and IFN- γ in nasal lymphocytes during influenza infection has been reported⁶¹. Ingestion of probiotic cocktail containing *Lactobacillus* has been shown to

stimulate the signal pathways when infected with single-stranded RNA virus⁶².

Similarly, *Bifidobacterium breve* YIT 4064 stimulates production of anti- influenza IgG in children⁶³. *Bifidobacterium* spp. has also lead to enhanced B-cell and T-cell immunity, IFN release and NK cells response⁶⁴. Even *L. rhamnosus* GG (ATCC 53103) consumption in children decreased incidence of respiratory tract infections during winter season⁶⁵. In a study by de Vrese *et al*⁶⁶ inclusion of *L. gasseri* PA 16/8, *B. longum* SP 07/3, *B. bifidum* MF 20/5 in daily diet reduced the duration of common cold infection. This may be due to immunomodulation by the test organism owing to release of anti-inflammatory cytokines⁶⁷⁻⁶⁹ and stimulation of cytotoxic T cells and T-suppressor cells (CD8⁺)⁶⁶.

Amongst the elderly, the fourth most common cause of death is influenza and pneumonia^{70,71}, while 77% deaths are due to infection in gastrointestinal tract⁷² whereas as reportedly ninety percent due to respiratory tract infections⁷³. This age-related diminution of innate and acquired immune system can be controlled by inclusion of functional foods like probiotics in daily diet. *L. casei* DN 114001 provided through a fermented dairy product reduced the duration of infection of gastrointestinal tract and respiratory tract, especially for upper respiratory tract infection like rhinopharyngitis. Others like *L. paracasei* and *L. johnsonii* reduced incidence of infection and improved systems of symptoms of respiratory tract infections^{74,75}.

Probiotics can also regulate the immune system of the intestine as they produce many factors and metabolites which aid in the immunomodulation of the intestine⁵³. Under *in vitro* conditions, *L. reuteri* 100-23 has been shown to reduce IL-2 production in bone marrow derived dendritic cells (BMDCs) and increase production of transforming growth factor beta (TGF-b) in mice⁷⁶. *L. reuteri* 100-23 colonization has shown to increase number of FoxP3 positive cells in spleen and mesenteric lymph nodes. This suggests that *L. reuteri* 100-23 regulates inflammation of the gastrointestinal tract by recruiting immune cells and it also regulates recruitment of T-cells to gut epithelium.

Bacteria of genus *Bacillus* are one of the largest known producers of antimicrobials, out of which more than 795 have been identified⁷⁷. These peptides have shown antibacterial⁷⁸, antifungal⁷⁹ and antiviral properties⁸⁰. Some species of this genus have also shown probiotic properties⁸¹⁻⁸³. In one such study, probiotic *B. subtilis* 3 was able to inhibit influenza virus replication under *in vitro* conditions and saved 30% of mice from death due to the same virus¹². It also produced a peptide, P18 which showed homology to Influenza A neutralizing antibody. It had the ability to inhibit the virus completely *in vitro* from concentrations ranging from 12.5- 100 µg/ml. In mice, it proved as a better treatment by preventing 80% of mice from death due to Influenza as compared to oseltamivir phosphate treatment i.e. Tamiflu, which provided protection to 70% of mice. Overall, it was found more effective than oseltamivir phosphate for elimination of virus after its infection in mice.

Probiotics and COVID-19

Many different studies have shown that ventilator-associated pneumonia and enteritis can be reduced by modulating the gut microbiota⁴⁸. As of now there is absence of data on whether gut microbiota modulation can help in alleviating symptoms of COVID-19. However, according to guideline of China's National Health Commission and National Administration of Traditional Chinese Medicine, probiotics may prove useful in treating dysbiosis and preventing secondary bacterial infections and thus treating COVID-19 patients successfully⁸⁴.

Lactobacillus rhamnosus GG, a probiotic bacterium, has shown its role in improving gut/lung barrier and intestinal homeostasis. It has further shown to down-regulate pro-inflammatory cytokines, up-regulate regulatory T cells and also to better anti-viral defense in respiratory infections^{85,86}. These immunomodulatory actions of *Lactobacillus rhamnosus* GG can certainly help individuals with COVID-19, or persons at risk of contracting the disease.

CONCLUSION

The review of literature done suggests that probiotic bacterial genera *Lactobacillus*, *Bifidobacterium* and *Bacillus* have shown great impact on the reinforcement of the immune system

and at the same time have balanced the intestinal dysbiosis to ensure improved gut-lung crosstalk. The use of these probiotics for prevention and treatment of COVID-19 requires in-depth scientific study using metaanalysis and randomized clinical trials. At the same time, they can be used as supportive or alternative therapeutic agents that can build good immune responses for better prognosis of COVID-19.

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