Effectiveness of Probiotics for Treatment of COVID-19: A Systematic Review and Meta-analysis

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Abstract: Background: Recently specific interactions and crosslinks between the gut microbiota and the lungs have been recognized, particularly with regard to respiratory immune and anti-microbial reactions. This is often known as the "gut-lung axis" or "a common mucosal immunological system". **Objective:** The aim of the current systematic review was to evaluate evidence, from published clinical trials and cohort studies, if probiotics may have an effect in improving and managing COVID-19 symptoms. **Materials and methods:** The available studies were searched through a comprehensive search of electronic databases that included PubMed, Science Direct, Scirus, ISI Web of Knowledge, Google Scholar and CENTRAL (Cochrane Central Register of Controlled Trials), using a combination of the following keywords: "COVID-19" OR "SARS-CoV-2" AND "Microbiota" OR "Probiotics" OR "Gut Lung Axis". The literature was reviewed until August 31, 2022. **Results:** Only 3 studies were included. One of them evaluated the efficacy of probiotics in COVID-19 patients to obtain complete remission of all signs and symptoms. The clinical trial proves that probiotics have a significant effect on complete remission of all signs and symptoms of COVID-19 patients with statistical significant difference. Only one clinical trial out of the 3 included studies had evaluated the need for O2 therapy during the study between the probiotics and control groups, but without statistical significant difference. No statistical significant difference between the probiotics group and placebo group was observed regarding fatal prognosis during the only clinical trial that measured death as an outcome. **Conclusion:** We couldn't judge on these results as they are insufficient data for pooling and meta-analysis. However, what we can say is "Most probably Probiotics have no role in treatment of COVID-19 infection".

Keywords: COVID-19, Probiotics, Management, Meta-analysis, Systematic Review

1. Introduction

In January 2020, the World Health Organization announced the global coronavirus disease 2019 (COVID-19) Pandemic. Since then, the researchers have focused their efforts to form a complete picture of the new viral infection, its clinical symptoms, prevention, and treatment. It was noticed that the clinical symptoms varied a lot including respiratory symptoms, GIT symptoms, hypercoagulability symptoms, and even dermatological symptoms. The spectrum of the clinical course of the disease may range from asymptomatic cases to severe pneumonia resulting in acute respiratory distress syndrome and sometimes leading to multi-organ failure (MOF). Therefore, COVID-19 has been known to be a multi-system and multi-organ disorder that affects all age groups especially the elderly with comorbidities who showed a high probability of developing severe illness [1].

In several recent studies, COVID-19 severity has been associated with increased levels of inflammatory cytokines, accompanied by multi-organ dysfunction, respiratory failure, and a clinically distinct hypercoagulable state. So, the immune state of the patient has a great effect on the progression of the disease. Controlling the exaggerated immune reaction can protect the lungs, but the infection needs a completely intact immune system. So, maintaining the host-microbiota balance may be useful in COVID-19 [2].

It is well known that the main function of the microbiota of different mucosal surfaces is in colonization resistance, i.e., the reduction in the probability of developing infections either by the direct action of microbiota upon microbes or indirectly (mainly by interactions with the host immune system). However, recently specific interactions and crosslinks between the gut microbiota and the lungs have been recognized, particularly with regard to respiratory immune and anti-microbial reactions. This is often known as the "gut-lung axis" or "a common mucosal immunological system".

By extension, modulation of the gut microbiota by influencing the gut-lung axis has been an area of interest, with probiotics being one of the main interventions that have been explored in human trials. Probiotics are living microorganisms that, when taken in adequate doses, grant a great benefit to the host (FAO and WHO, UN). Probiotics' mode of action for preventive and therapeutic purposes consists of modifying the intestinal microbial communities, immunomodulation, reducing the pathogens, and protecting the intestinal barrier. Different studies have explored the use of probiotics to reduce the risk of respiratory infections, with a focus on viral upper respiratory tract infections (URTI) in particular (90% of URTIs are viral in nature) [3].

Colonization of probiotic bacteria in the intestinal epithelium has been proven to decrease the risk and symptoms of viral respiratory infections. This is achieved by the boosting of IgA-expressing B cells in the colon and lymph nodes in conjunction with the increasing population of the T follicular helper cells and IL-23–expressing dendritic cells. Moreover, probiotics also include immunostimulatory constituents such as peptidoglycan, lipoteichoic acid, Toll-like receptor (TLR) ligands, and muramyl dipeptide, which augment their immunomodulatory potency [4].

On the other hand, the administration of probiotics has been proven to increase the release of short-chain fatty acid (SCFA) producing gut microbiota which consequently up-regulates the production of interferon β; In addition to the increase of *Corynebacterium* and *Lactobacillus* species in the lung due to higher SCFA production, leading to the production of interferon β in alveolar macrophages.

COVID-19 infection affects the lung tissues and gut, activating the inflammatory reaction. It raises the proinflammatory cytokines (IFN-γ, TNF-α) which leads to the cytokine storm. This response is due to the activation of T helper cells (Th1) cell response in the lung tissue [5]. In the case of the human gut environment, dysbiosis in the gut microbiota results in the imbalance of Th1 and Th2 which leads to the activation of proinflammatory cytokine and the cytokine storm in the lungs as well. Upon supplementation of probiotics, colonization of what's called "good bacteria" in the gut occurs which results in an imbalance between Th1/ Th2 cells that decrease the cytokine storm and reduce the severity of diseases [6]. Recently it has been proved that medication with probiotic bacteria using *Bifidobacteria* and *Lactobacillus* gives a significant opportunity for recovery against COVID-19 [7]. Administration and consumption of probiotics advance the immune system by boosting the level of type I interferons, antigen-presenting cells (APC), Natural Killer cells (NK cells), and B and T cells of the lungs [8]. Probiotic supplementation can also improve the pro-and anti-inflammatory cytokines, leading to recovery by reducing the cell damage in the lungs [9].

The aim of the current systematic review was to evaluate evidence, from published clinical trials and cohort studies, if probiotics may have an effect in improving and managing COVID-19 symptoms.

2. Materials and Methods

2.1. Study design: Systematic Review and Meta-analysis

2.1.1. Methods

Review of literature: Available studies and abstracts concerning Probiotics in the treatment and prevention of COVID-19 were identified. The available studies were searched through a comprehensive search of electronic databases that included PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Science Direct (www.sciencedirect.com), Scirus (www.scirus.com/ srsapp), ISI Web of Knowledge (http://www.isiwebof knowledge.com), Google Scholar (http://scholar. google.com) and CENTRAL (Cochrane Central Register of Controlled Trials (http://www.mrw.interscience. wiley.com/cochrane/cochrane_clcentral_articles_fs.htm), using a combination of the following keywords: "COVID-19" OR "SARS-CoV-2" AND "Microbiota" OR "Probiotics" OR "Gut Lung Axis". The study was limited to articles published in English, French, German, Italian, Spanish and Arabic languages. The literature was reviewed until August 31, 2022 and three studies were found that met our inclusion criteria. All were in English, no available studies were published in French, German, Italian, Spanish or Arabic languages.

The following outcomes were assessed in the study: Complete remission of COVID 19 after Probiotics administration and duration of diarrhea.

Screening and evaluation of articles: The following screening was used to screen the articles yielded by reviewing the literature (total 260 articles) after ranking the articles according to authors and year of publication. Only articles fulfilling the inclusion criteria were included for further steps of data collection, analysis and reporting.

Inclusion criteria: Randomized controlled trials and cohort studies. Studies were published till the date of August 31, 2022. Studies concerned with the treatment of COVID-19. Studies conducted on patients diagnosed with COVID-19 confirmed (positive polymerase chain reaction) and Hospitalized. Moderate and severe COVID-19 pneumonia (via computed tomography and or X-ray imaging), requires hospitalization. Studies were conducted on human subjects.

Exclusion criteria: Studies other than randomized controlled trials and cohort studies. Opinion/review study. Studies were conducted on animals.

Data collection sheet: There were 3 articles included in our meta-analysis.

Data Extraction: A copy of each study identified was obtained, and the first author abstracted relevant data for a quantitative overview. The type of risk estimate (i.e., relative risk) was extracted from the published manuscript. In case of discrepancies or when the information presented in a study is unclear, abstraction by a second reviewer (MFA) was sought to resolve the discrepancy. Data were abstracted from every study in the form of a risk estimate and its 95% confidence interval. When a risk estimate and its 95% confidence interval were not available from the article, unadjusted values from the published data of the article were calculated using the Epi Info 6 computer program version 6.04d.

Statistical Methods: Statistical analysis was done using Comprehensive Meta-Analysis© version 2.2.064 (Biostat™, NJ, USA). A two-sided P-value ≤0.05 denoted statistical significance.

3. Results

In six databases, we identified 27 articles, of them 3 duplicates were removed. Out of 24-screened abstracts, we did not exclude any article. Thus, 24 full-text studies were

assessed for eligibility and 21 were excluded. Finally, 3 studies were included for further qualitative and quantitative analyses (Figure 1).
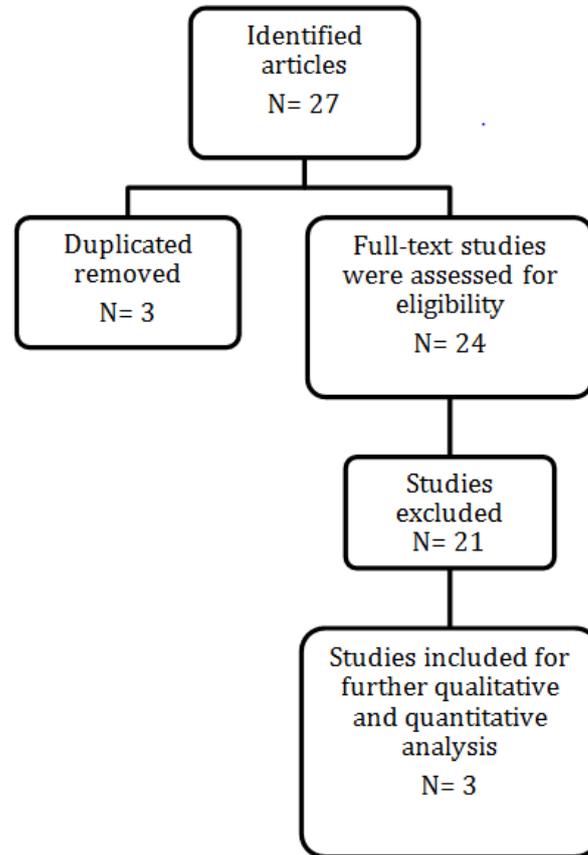

Figure 1. PRISMA flow diagram showing selection of studies. PRISMA; Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

3.1. Duration of COVID-19 illness till recovery

Only one clinical trial compared COVID-19 patients who received probiotics as an adjunctive treatment with patients who received standard treatment only, regarding the total duration of COVID-19 illness till recovery. Total duration of disease in days was nearly similar in both groups with no statistical significant difference (Table 1).

Table 1. Efficacy of Probiotics on the total duration illness till recovery in COVID-19 Patients.

N.	Authors	Year of publication	Type of study	Mean	Range	P value
3	Ivashkin et al. [10]	2021	RCT	Probiotics: 20 Control: 21	18 – 24 18 – 25	0.471

Only one clinical trial evaluated the efficacy of probiotics in COVID-19 patients to obtain complete remission of all signs and symptoms. The clinical trial of Gutiérrez-Castrellón and collaborators (2022) **[11]** proves that probiotics have a significant effect on complete remission of all signs and symptoms of COVID-19 patients with statistical significant difference (Table 2).

Table 2. Efficacy of Probiotics on complete remission in Patients with COVID-19.

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
1	Gutiérrez-Castrellón et al. [11]	2022	RCT	1.89	1.40 – 2.55	<0.001

Only 2 clinical trials compared COVID-19 patients who received probiotics as an adjunctive treatment with patients who received standard treatment only, regarding the duration of diarrhea. The duration of diarrhea in the probiotics group was significantly shorter than that in the control group (P<0.05). However, the clinical trial of Ivashkin and collaborators (2021) found no significant difference between probiotics and control groups regarding the duration of the diarrhea (P=0.785), but this was conducted on patients with diarrhea on admission, so it couldn't be considered as an outcome in our systematic review (Table 3).

Table 3. Efficacy of Probiotics on the duration of diarrhea in COVID-19 Patients.

N.	Authors	Year of publication	Type of study	Average value	Standard deviation	P value
2	Wang et al. [12]	2021	RCT	Probiotics: 8.60 Control: 11.50	2.31 2.35	0.030

3.2. Need for hospitalization

All cohort studies and clinical trials that evaluated the effectiveness of probiotics as an adjunctive treatment for COVID-19 were conducted in hospitalized patients, except for the study of Gutiérrez-Castrellón and collaborators (2022) **[11]**, which was conducted on outpatient clinics and reported that there were no hospitalization or ICU admission occurred during the study in probiotics and control groups.

3.3. Need for O$_2$ therapy

Only one clinical trial out of the 3 included studies had evaluated the need for O2 therapy during the study between the probiotics and control groups, but without statistical significant difference (Table 4).

Table 4. Efficacy of Probiotics on oxygen support in COVID-19 Patients

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
3	Ivashkin et al. [10]	2021	RCT	1.09	0.80 – 1.48	0.579

3.4. Need for ICU admission.

Ivashkin and collaborator (2021) **[10]** was the only study that discussed the need for ICU admission as an outcome measure, and it revealed no significant difference between the probiotics and control groups (Table 5).

Table 5. Efficacy of Probiotics on admission to intensive care unit in COVID-19 Patients

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
3	Ivashkin et al. [10]	2021	RCT	0.73	0.24 – 2.22	0.576

3.5. Need artificial ventilation

This outcome was measured by only one clinical trial which showed no significant difference between the probiotics and control groups (Table 6).

Table 6. Efficacy of Probiotics on the need for mechanical ventilation in COVID-19 Patients

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
3	Ivashkin et al. [10]	2021	RCT	0.82	0.23 – 2.96	0.976

3.6. Fatal prognosis

No statistical significant difference between the probiotics group and placebo group was observed regarding fatal prognosis during the only clinical trial that measured death as an outcome (Table 7).

Table 7. Efficacy of Probiotics on death in COVID-19 Patients.

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
3	Ivashkin et al. [10]	2021	RCT	1.02	0.26 – 3.97	0.491

3.7. Other outcomes reported in the identified studies include

Only one clinical trial evaluated the efficacy of probiotics as an adjunctive treatment for preventing diarrhea during hospitalization of COVID-19 patients. The results of the study showed that COVID-19 hospitalized patients were less likely to develop diarrhea if they received probiotics as an adjunctive treatment, but without statistical significant difference (Table 8).

Table 8. Efficacy of Probiotics on hospital-acquired diarrhea in COVID-19 Patients

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
3	Ivashkin et al. [10]	2021	RCT	0.41	0.13 – 1.26	0.109

COVID-19 patients who received probiotics were highly protected against adverse events by 65%, confirmed by 95% confidence interval with highly significant difference. Unfortunately the study didn't specify the adverse events that they observed during their study (Table 9).

Table 9. Efficacy of Probiotics on developing adverse events in outpatients with COVID-19

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
1	Gutiérrez-Castrellón et al. [11]	2022	RCT	0.65	0.47 – 0.90	0.008

Only one clinical trial evaluated the efficacy of probiotics as an adjunctive treatment for reducing the number of medications in COVID-19 outpatients. The results of the study showed that COVID-19 outpatients were less likely to need more than two medications if they received probiotics as an adjunctive treatment, but without statistical significant difference (Table 10).

Table 10. Efficacy of Probiotics on taking more than two medications in outpatients with COVID-19

N	Authors	Year of publication	Type of study	Relative Risk	95% CI	P value
1	Gutiérrez-Castrellón et al. [11]	2022	RCT	0.66	0.29 – 1.48	0.312

4. Discussion

Many Studies discussed the effect of probiotics on human immunity and how it enhances the immune response. Most of these studies highlighted the link between the gut, lungs, and brain. Since COVID-19 decreases the immune response and affects the lungs and even the brain in some cases of long COVID, probiotics will be beneficial. Although we searched a lot for clinical trials and cohort studies that can prove this theory and we actually found 24 RCT studies only but unfortunately, only three of them published results. However, these three studies do not have the same outcome measured so we could not compare them.

Helen Tang and collaborators (2021) [13] conducted a clinical trial on 566 COVID-19 patients and used specific strains from Probiotics for the treatment of patients. It was expected reliable results from this huge effort but their results have not been published yet and the other 20 studies are the same. The studies found to have results published were all in the English language which could introduce bias.

The first study was conducted by *Gutiérrez-Castrellón et al.* [11] where they had 147 patients in the intervention group took a single daily dose of oral probiotics and 146 control group who took the placebo. The authors followed up with both groups and evaluated the effect of probiotics in different aspects. The main aspects were complete remission (*i.e.* complete symptomatic and viral clearance) which was 53.1% in the probiotic group and 28.1% in the control group (P<*0.001).* Development of more than the adverse event was 27.3% in the probiotics group and 42.0% in the placebo group with a P-value of *0.008,* and the need of taking more than two medications was 29.2% in the probiotics group and 44.4% in the placebo group with P-value of 0.312. This highly significant P-value proves that probiotics have a positive effect on the three main aspects evaluated. They also measured the difference in the duration of some symptoms between the two groups (like fever, cough, headache, shortness of breath, boney ache, nausea, diarrhea, and abdominal pain) which all were less in the probiotics group. Nevertheless, unfortunately, the authors mentioned the duration in interquartile range so we could not calculate the Relative risk. Multiple graphs showing different measurements on day 15 and 30 of the intervention including (viral load, lung infiltration, level of D-dimer and CRP) but no detailed data. They also reported no hospitalizations, ICU admissions, or deaths occurred during the study.

The second clinical trial [12] was discussed in a confusing way; 23 intervention cases and 35 controls. The authors included 156 patients and then divided them according to the severity of the disease. 98 cases have mild to moderate symptoms while 58 cases have severe and critical disease. Therefore, they decided to focus on the critically ill patients and give 23 patients probiotics and placebo to 35 patients from 58. After which they measured the duration of the diarrhea only and mentioned other lab measurements with incomplete data.

The third one was conducted by *Ivashkin and collaborators* (2021) [10]. The good point in this study is that it excluded patients consuming probiotics 3 months prior admission. They had many outcomes including (liver injuries, different biomarkers, duration of diarrhea, renal functions and lung injuries). Their results states that probiotics have no significant effect on treatment and mortality rate of COVID-19 patients but it may be a treatment for diarrhea as a symptom if it occurs. The authors also believe that probiotics

may have a tendency towards a decrease in mortality rate in patients with cardiovascular diseases. Therefore, they suggest more studies on this point. We believe that if they have included more sample size in both groups, they could have obtained different results.

The three included clinical trials didn't have the same outcomes measured but among the different outcomes we noticed that there was one outcome common between the first and third study discussing duration of COVID-19 illness till recovery but the results were controversial. *Gutiérrez-Castrellón and collaborators* (2022) showed that there is a high statistical significant difference between probiotics group and placebo group regarding complete remission of all signs and symptoms of COVID-19 patients. On the other hand, Ivashkin and collaborators (2021) had found no statistical significant difference between probiotics group and control group regarding the duration of illness. Besides, we also noticed that both *Wang and collaborators* (2021) [12] and *Ivashkin and collaborators* (2021) [10] had measured the duration of diarrhea in COVID-19 patients. *Wang and collaborators* (2021) [12] found a significant difference, but *Ivashkin and collaborators* (2021) [10] did not; but this was conducted on patients with diarrhea on admission, so it could not be considered as an outcome in our systematic review.

Another outcome that has a significant difference was development of adverse events, which was revealed by *Gutiérrez-Castrellón and collaborators* (2022) [11].

The remaining outcomes measured by the three included studies had no statistical significant difference. From the data we have from the three clinical trials we cannot rely on probiotics in clinical practice in the treatment of COVID-19. Hence we recommend more clinical trials, especially multicenter clinical trials with large sample sizes, are needed to confirm or invalidate the effectiveness of Probiotics in the therapeutic protocol of COVID-19.

5. Conclusion

A conclusive judgment couldn't be obtained because of insufficient data that hindered pooling and meta-analysis. However, Probiotics have a significant effect on complete remission of all signs and symptoms of COVID-19 patients with statistical significant difference, as proved by only one study. There are controversial results between studies regarding the effectiveness of Probiotics on the duration of the diarrhea in COVID patients with GI symptoms. Probiotics were protective against adverse events (RR 0.65) proved by one study. Only one study proved that Probiotics has no effect on the following outcomes: Need for O2 therapy, need for ICU admission, need for artificial ventilation and fatal prognosis.

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