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The effect of probiotics on respiratory tract infection with special emphasis on COVID-19: Systematic review 2010–20

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ARTICLE INFO

Article history:
Received 16 November 2020
Received in revised form 2 February 2021
Accepted 2 February 2021

Keywords:
Probiotic
COVID-19
Respiratory tract infection
Influenza vaccines
Randomized controlled trials

ABSTRACT

To evaluate the effects of probiotics on respiratory tract infection (RTI) a systematic review of randomized controlled trials (RCTs) from January 2010 to January 2020 was conducted. The PubMed, Google Scholar, Embase, Scopus, ClinicalTrials.gov, and International Clinical Trials Registry Platform databases were systematically searched for the following keywords: respiratory tract infection, probiotics, viral infection, COVID-19, and clinical trial. A total of 27 clinical trials conducted on 9433 patients with RTI plus 10 ongoing clinical studies of probiotics intervention in Coronavirus disease 2019 (COVID-19) were reviewed. The review looked at the potency of probiotics for the hindrance and/or treatment of RTI diseases, this may also apply to COVID-19. The review found that probiotics could significantly increase the plasma levels of cytokines, the effect of influenza vaccine and quality of life, as well as reducing the titer of viruses and the incidence and duration of respiratory infections. These antiviral and immune-modulating activities and their ability to stimulate interferon production recommend the use of probiotics as an adjunctive therapy to prevent COVID-19.

Based on this extensive review of RCTs we suggest that probiotics are a rational complementary treatment for RTI diseases and a viable option to support faster recovery.

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Introduction

Respiratory tract infection (RTI) is one of the most common infectious diseases of viral or bacterial origin. The disease is divided into upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI). The upper respiratory tract includes the nose, sinuses, pharynx, and larynx. Common upper respiratory tract infections include tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, certain types of influenza, and the common cold (Eccles et al., 2007). Symptoms of URTIs include cough, sore throat, runny nose, nasal congestion, headache, low-grade fever, facial pressure, and sneezing. The lower respiratory tract consists of the trachea (windpipe), bronchial tubes, bronchioles, and lungs. Medically, lower respiratory tract infections are more serious and important than upper respiratory tract infections (Van Riel et al., 2006).

Viral pathogens are the most common cause of RTIs; these include rhinovirus, respiratory syncytial virus, influenza virus, human parainfluenza virus, human metapneumovirus, measles, mumps, adenovirus, and coronavirus. Bacterial pathogens causing RTI are less common than viral pathogens but can also cause outbreaks and sporadic cases of respiratory illness; these include Streptococcus pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Chlamydia pneumoniae, Coxiella burnetti, and Legionella pneumophila. Bacterial sinusitis, bronchitis or pneumonia may occur as secondary infections after a viral respiratory infection.

Influenza affects both the upper and lower respiratory tracts and can produce a variety of symptoms including high fever, chills, sore throat, headache, runny or blocked nose, weakness, muscle pain, and diarrhea (Barik, 2012). More than 200 serologically different virus types are responsible for human URTIs, among

https://doi.org/10.1016/j.ijid.2021.02.011
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which rhinoviruses are the most common cause (Wang and Liu, 2014).

Coronavirus disease 2019 (COVID-19) is a new pandemic disease caused by a novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]). Zoonotic coronaviruses have emerged in recent years and caused human outbreaks such as severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). The virus enters cells via spike proteins (S) and angiotensin-converting enzyme 2 (ACE2) receptor proteins on host cells. It can affect both upper (sinuses, nose, and throat) and lower (windpipe and lungs) respiratory tracts and cause problems such as acute respiratory distress syndrome (ARDS), respiratory failure, multiple organ failure, or even death (~10%); it represents a global health challenge (Serratoso et al., 2012).

Probiotics have shown a positive response in clinical treatment for several diseases. Inhibition of gastric coronavirus, rotavirus, hemagglutinin type 1 and neuraminidase type 1 (H1N1) influenza virus, and HIV in vitro and reduction of viral load in vivo, using Lactobacillus has been well established (Anwar et al., 2020). Probiotics are defined as live microorganisms that have health benefits for the host and contain immunostimulatory substances such as lipoteichoic acid, peptidoglycan and nucleic acid, which are Toll-like receptor (TLR) ligands, and muramyl dipeptide, which is a Nod-like receptor ligand (Jensen and Thomsen, 2012). Studies have shown antiviral activities of probiotic strains against common respiratory viruses, such as rhinovirus, influenza and respiratory syncytial virus (Luoto et al., 2014). Probiotics affect both the acquired and innate immune systems and reduce the severity of infections in the upper respiratory and gastrointestinal tracts (Khani et al., 2012). Probiotics increase the level of interferons; the number and activity of natural killer (NK) cells, T cells and antigen-presenting cells; and the level of specific antibodies in the lungs (de Vrese et al., 2005). Probiotic strains regulate the dynamic balance between immunoregulatory and pro-inflammatory cytokines.

RTIs are the result of an imbalance in the microbial population of the respiratory tract and gastrointestinal tract affecting the lungs mucosa (Kumar et al., 2018). This dysbiosis may subsequently alter immune function and predispose the patient to secondary bacterial infection (Getahun et al., 2010). The gut microbiome has a critical impact on inducing immune responses at distant mucosal sites including the lungs (Abt et al., 2012). Studies have shown that the administration of certain Bifidobacteria or Lactobacillus has a beneficial impact on RTIs (Zelaya et al., 2016).

In a study the frequency and severity of common cold symptoms in patients with rhinovirus infection were shown to be lower in the Lactobacillus rhamnosus (LGG) treated group than in the control group (Kumpu et al., 2015). Lactobacillus delbrueckii subsp. bulgaricus OLL0173R-1 has been shown to augment NK cell activity and reduce common cold symptoms (Makino et al., 2010). Lactobacillus paracasei subsp. paracasei (L. casei 431) shortened the duration of upper respiratory infection symptoms (Nagai et al., 2011). Lactobacillus plantarum L-137 augmented the innate and acquired immune responses in mice and humans and reduced RTIs in the treatment group compared to the control group (Merenstein et al., 2010). Bifidobacterium animalis subsp. lactis BI-04 (BI-04) was shown to decrease the risk of upper respiratory illness in a natural setting (Turner et al., 2017).

Given the results of these studies on the use of probiotics, their use may also play a role in reducing the severity of ARDS, a major complication of COVID-19. The effect of probiotics against several
Table 1  
The outcomes of different clinical trials assessing the efficacy of probiotics on respiratory tract infections.

| Outcomes                                                                 | Intervention | Dose                                                                 | Probiotics                                      | No. of Participants (Mean age ± SD) | Participants Characteristics | Study Design | Country   | Reference                  |
|--------------------------------------------------------------------------|--------------|----------------------------------------------------------------------|------------------------------------------------|-------------------------------------|-------------------------------|--------------|-----------|---------------------------|
| Probiotic was beneficial for children and reduced the severity of common colds | 1 sachet/ o. d/ 12wks | $1 \times 10^9$ CFU | *L. plantarum* HEALS, *L. paracasei* 8700:2 | 131 (3.1 ± 1.4) | URTI                          | RDBPCT | Sweden   | Alrén et al. (2021)     |
| Prebiotics affected intestinal microbiota and maintained antibody titers in elderly individuals | Doses of GOS and BGS used in group F were 4.0 and 0.4 g/day, respectively. | Not reported | Prebiotic | 43 (84.5 ± 7.5) | Influenza vaccine             | RCT          | Nagoya   | Akatsu et al. (2016)     |
| Prebiotics alleviated the symptoms of URTI by improving inflammatory parameters and enhancing immunomodulatory properties. | O.d/ 12 wks | $1 \times 10^7$ CFU | *L. plantarum* HEALS 8700:2 | 318 (46.5) | Common cold                   | RDBPC | Sweden   | Berggren et al. (2011)   |
| Probiotics alleviated the symptoms of URTI by improving inflammatory parameters and enhancing immunomodulatory properties. | 1 sachet /12 wk | $1 \times 10^9$ CFU | *L. plantarum* DR7 | 109(≤ 60) | URTI                          | RDBPCT | Malaysia | Chong et al. (2019)      |
| Lactobacillus GG was as a potentially important adjuvant to improve influenza vaccine immunogenicity | 1 capsule /b. L.d/ 4wks | $1 \times 10^{10}$ CFU | Lactobacillus GG | 42 (33.5) | Live-attenuated influenza vaccine (LAIV) | RDBPCT | USA      | Davidson et al. (2011)   |
| Antigen-specific B and T cell activation following an in vitro recall challenge with the influenza vaccine was not affected by a synbiotic | 1 sachet /o. d/ 8 wks | $1 \times 10^9$ CFU in 1 g skim milk powder | *B. longum*, *B. infantis* CCUG 52486 | 63 older cohorts: (60-85 y) 62 younger cohort: (18-35 y) | Influenza vaccine | RDBPCT | U.K      | Enani et al. (2018)      |
| Probiotics increased the immune response against the influenza vaccine and decreased symptoms associated with respiratory infection | 1 capsule /d/ 2 wks | $3 \times 10^6$ CFU | *L. coryniformis* K8 CECT5711 | 98 (83.79 ± 6.5) | Inactivated trivalent influenza vaccine | RDBPCT | Spain    | Fonollá et al. (2019)    |
| *L. salivarius* did not reduce the frequency of URTI and did not affect the levels of salivary antimicrobial proteins or blood leukocyte and lymphocyte subsets counts during a spring period | 1 sachet /d/ 16 wks | $2 \times 10^6$ CFU | *L. salivarius* | 66 (23.9 ± 4.7) | URTI                          | RDBPCT | UK       | Gleeson et al. (2012)    |
| Probiotics reduced plasma CMV and EBV antibody titers as well as URS episode incidence | 65 ml milk pot / b.i.d/ 20 wks | $6.5 \times 10^9$ CFU | *L. casei* strain Shirotai | 268 (21 ± 3) | URS (CMV, EBV)                | RDBPCT | UK       | Gleeson et al. (2016)    |
| Probiotics reduced the risk of common infections in stressed individuals such as shift workers | 2 bottles/b.i.d/ 12 wks | $1 \times 10^8$ CFU | *L. casei* DN-114 001, *S. thermophiles*, *L. delbrueckii* subsp. *bulgaricus* Prebiotic | 1000 (32) | RGCID                        | RDBRCT | France   | Guillemaud et al. (2010) |
| Probiotics reduced stress-induced gastrointestinal dysfunction and the number of days with common cold or influenza | 1 packet /o.d/ 8 wks | 2.5 g and 5 g of galactooligosaccharides | *L. paracasei* subsp. *paracasei*, *L. casei* 431 | 1104 (31.6) | Influenza vaccine             | RDBPCT-parallel-group study | Denmark   | Jespersen et al. (2015)  |
| *L. casei* 431 dose did not affect the immune response to influenza vaccination but reduced the duration of upper respiratory symptoms | 100 mL acidified milk drink / o.d/ 6 wks | $1 \times 10^8$ CFU | *L. paracasei* subsp. *paracasei*, *L. casei* 431 | 1104 (31.6) | Influenza vaccine             | RDBPCT-parallel-group study | Denmark   | Jespersen et al. (2015)  |
| Probiotics had no significant effect on preventing influenza or enhancing NK cells activity but increased IFN-γ | 112 ml yogurt drink fermented /o.d/ 16 wks | $1.12 \times 10^9$ CFU | *L. delbrueckii* subsp. *bulgaricus* OLL1073R-1 | 961 (39.3) | Influenza and the common cold | Randomized, controlled, open-labeled | Japan     | Kinoshita et al. (2019)  |
| The occurrence and severity of cold symptoms and the number of subjects with symptoms | o.d / 6 wks | $1 \times 10^9$ CFU | *L. rhamnosus* *GG* | 60 (24.3) | Rhinovirus                    | RDBPCT | Finland | Kumpu et al. (2015)      |
| Outcomes                                                                 | Intervention | Dose                | Probiotics | No. of Participants (Mean age ± SD) | Participants Characteristics | Study Design | Country | Reference                                |
|-------------------------------------------------------------------------|--------------|---------------------|------------|------------------------------------|-----------------------------|--------------|---------|------------------------------------------|
| Rhinovirus infection was reduced in the group receiving live L. rhamnosus GG but not significantly compared to the group receiving inactivated strain, Probiotics played a role against viruses causing common cold, but did not reduce viral occurrence in symptomatic conscripts Rิดีโอบางเรียก benefit related to cold/flu outcomes during acute stress Gut microbiota modification with specific prebiotics and probiotics reduce the risk of rhinovirus infections | 1 tablet/ b.i. d/ 1/2 and 21wks | $5 \times 10^8$ CFU | L. rhamnosus GG, B. animalis subsp. lactis BB-12 | 982 (19.3) | URTI | RDBPCT | Finland | Lehtoranta et al. (2014) |
|                                                                         | 1 capsule/ o. d/ 5 wks | $3 \times 10^8$ CFU | L. helveticus R0052, B. longum subsp. infantis R0033, B. bifidum R0071 | 583 (19.8 ± 0.1) | Cold/flu symptoms | RDBPCT | USA | Langkamp–Henken et al. (2015) |
|                                                                         | Mix with 10 mL of breast milk or formula/o.d /0 to 4 wks Mix with 10 mL of breast milk or formula/o.d /4 to 8 wks | $1 \times 600$ mg | | | | | | |
|                                                                         | Mix with 10 mL of breast milk or formula/o.d /0 to 4 wks Mix with 10 mL of breast milk or formula/o.d /4 to 8 wks | $2 \times 600$ mg | | | | | | |
|                                                                         | 1 capsule/ o. d/ 14 weeks | $1 \times 10^7$ CFU | B. longum BB536 | 94 (32 ± 0 to 36 ± 6 weeks) | RVIs | RDBPCT | Finland | Luoto et al. (2014) |
| L. bulgaricus augmented natural killer cell activity and reduced the risk of catching the common cold in elderly individuals Non-viable L. paracasei MCC1849 had no significant effect on immune parameters probiotics reduces the incidence of influenza and fever, probably by potentiating innate immunity Probiotics combination reduced the symptoms of the common cold and school absenteeism Probiotics improved immune function by augmenting systemic and mucosal immune responses to challenge L. pentosus b240 reduced the incidence rate of the common cold in elderly adults and improved resistance against infection by mucosal immunity Probiotics reduced influenza-like illness via the enhancement of an IFN-α-mediated response to the influenza virus Probiotics affected the innate immunity in the elderly | 1 sachet /o. d/12 wks | $1 \times 10^7$ CFU | B. acidophilus, B. bifidum | 80 (12) | Common cold | RDBPCT | Thailand | (Rerksuppaphol and Rerksuppaphol, 2012) Rizzardi et al. (2012) |
|                                                                         | 1 capsule/o. d/ 6 wks | $1 \times 10^9$ CFU | B. animalis subsp. lactis, BB-12 L. paracasei ssp. paracasei, L. casei | 211(37.3 ± 13.9) | Influenza vaccination | RDBPCT | Italy | |
|                                                                         | 1 tablet/ o. d/20 wks | Low dose: $2 \times 10^2$ CFU High dose: $2 \times 10^4$ CFU | L. pentosus strain b240 | 300 (70.8 ± 3.4) | Common cold | RDBPCT | Japan | Shinkai et al. (2013) |
|                                                                         | 100 mL of JCM5805 yogurt/o.d/ 10 wks | $1 \times 10^3$CFU | L. lactis subsp. lactis JCM5805 | 92 (46) | Influenza-like illness | RDBPCT | Japan | Sugimura et al. (2015) |
|                                                                         | 1 sachet/ o. d/ 4 wks | $2 \times 10^9$ CFU | B. animalis subsp. lactis BI-04 | 190 (22 ± 6) | Rhinovirus- A39 | RDBPCT | USA | Turner et al. (2017) |
coronavirus species has been reported in various studies, however, these effects and mechanisms have not yet been examined for the new SARS-CoV-2 (Din et al., 2020).

Data show that SARS-CoV-2 causes gastrointestinal complications and probiotics appear to be able to reduce the dissemination of coronavirus via the gut, however, these probiotic strains have not yet been administered to the respiratory tract (Seo et al., 2010). Direct inhibition seems to be impossible at this site. Host-microbe, microbe-microbe and immune-microbe interactions could influence the course of respiratory diseases (Gonzalez-Ochoa et al., 2017). An imbalance between anti-inflammatory and pro-inflammatory cytokines, leading to a cytokine storm, contributes to the development of COVID-19 (Rizzardini et al., 2012). Some probiotic components could bind to spike proteins (S) and ACE2 receptor proteins and prevent the virus from entering the host body (Anwar et al., 2020). The use of probiotics could be effective in reducing other respiratory infections as well as COVID-19.

This systematic review aimed to evaluate the outcomes of clinical trials assessing the efficacy of probiotics in the treatment of respiratory infections over the past 10 years.

Materials and methods

Guidelines

This systematic review followed the guidelines established by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (Moher et al., 2009).

The keywords, including respiratory tract infection, probiotics, viral infection, COVID-19, and clinical trial, were searched in the PubMed, Google Scholar, Embase Scopus, Clinicaltrials.gov, and International Clinical Trials Registry Platform databases. Papers published in English from January 2010 to January 2020 were further assessed based on their title, abstract and main text to ensure their relevance to the present study. Data extraction was conducted by 2 independent researchers. Papers indexed in 2 or more databases were considered only once. The reference lists of the selected papers were investigated to identify any further relevant papers. A third researcher checked the results to ensure that all the eligible papers were evaluated.

The extracted data were organized based on the name of authors, country, date of publication, type of clinical trial, size of the sample, diagnostic criteria, characteristics of patients, the period of study, genus and species of probiotics used, dose of probiotics used, side effects of probiotics, and outcomes of treatment. A Chi-square test was used to analyze the qualitative variables. Data were analyzed using SPSS software Version 24.0 (IBM, NY), and a P value ≤ 0.05 was considered statistically significant.

The inclusion criteria for considering full-text publications were: (i) papers published during the last 10 years; (ii) clinical trial studies; and (iii) clinical trial studies conducted on patients with RTI.

The exclusion criteria were: (i) animal experiments; (ii) congress papers; (iii) reviews, meta-analysis, case reports, letters to the editor, and correspondence; (iv) clinical feature summary; (v) non-English papers; and (vi) studies with no clear information.

Results

A total of 1268 papers were retrieved from the Google Scholar, Medline, Embase, and Scopus databases. Figure 1 shows a schematic representation of the search method used in this review. In the second phase, after removing duplicates, 77 papers remained, of which 50 were excluded based on their title and abstract, and 27 selected for detailed full-text evaluation and analysis. The outcomes of the clinical trials that assessed the efficacy of the probiotics in the treatment of RTIs are shown in Table 1. The largest number of studies were conducted in Japan (7–27 studies, 3293/9433 patients). In total 73% of the patients surveyed were women (Figure 2) with an age range of infant to 89 years. The specimens included blood, serum and nasopharyngeal swab.

Of the 27 clinical trials, 23 were conducted on the efficacy of probiotics in the treatment of RTI, 1 on symbiotic efficacy, 2 on prebiotics efficacy, and 1 on probiotic and prebiotic efficacy separately. Of the 15 different probiotic strains studied, Lactobacillus species were studied more than Bifidobacteria, and L. paracasei and L. rhamnosus (15%) were the most common probiotic strains used. The frequency of the different types of probiotic species studied is shown in Figure 3. The prescribed doses of these strains were between 1 × 10⁹ and 1 × 10¹¹ (average dose: 5.05 × 10⁹) colony-forming units (CFU) for 6 and 24 weeks.

Of the 27 clinical trials reviewed, 9 examined a combination of multi-strain probiotic bacteria, 15 examined mono-strain probiotic bacteria and, as mentioned above, 2 trials were conducted on prebiotics efficacy and 1 on probiotic and prebiotic cocktails. In 9 studies, a combination of 2 probiotic bacteria was used, while in 2 studies, a combination of 3 probiotic bacteria was used.

Total effects of probiotics on the immune response

Of the 27 clinical trials, 3 showed that probiotics did not play a significant role in boosting the immune system and improving the
body’s defenses against diseases (Enani et al., 2018; Gleeson et al., 2012; Lehtoranta et al., 2014).

In 1 study, the probiotic intake was reported to increase the population of NK T cells compared to the control group (40.9 vs. 24.1) as well as the expression of the memory marker CD45RO on the surface of CD8+ lymphocytes. The plasma levels of cytokines in various categories, anaphylatoxins, pro-inflammation and other cases, were also studied. The plasma concentrations of complement C4a and C5a, cytokines (interleukin-(IL)1β, IL-6, IL-8, IL-12, IL-5, and IL-10), and tumor necrosis factor α (TNF-α) were evaluated in the probiotic and control groups. In general, the level of cytokines increased in the probiotic groups due to the use of probiotics. However, the IL-6 level in the probiotic groups was higher than in the control groups.

Effects of probiotics on the immune response to influenza vaccination

Of the 27 clinical trials, 7 were conducted on the effects of probiotics and 1 on the effects of prebiotic compounds to improve vaccine performance. The findings on the effects of probiotics on the immune response to influenza vaccination are shown in Table 2. Patients in these studies were distributed into control and probiotic groups. The mean age of the patients was 59.7 years, ranging from 18 to 99 years; 60% of patients were female. The most common probiotic species studied was Lactobacillus, 2 studies evaluated Bifidobacterium longum, and 1 evaluated a combination of Lactobacillus and Bifidobacterium.

On average, the probiotic groups consumed capsules containing $1 \times 10^3$ to $1 \times 10^{11}$ CFU of probiotic strains daily for 28 to 98 days. The vaccination was conducted by medical services with a vaccine containing inactivated trivalent (H1N1, H3N2, and B) influenza virus. The antibody titers against the 3 viral antigens included in the vaccine were evaluated at baseline and 2, 6, 8, and 10 weeks after influenza vaccination. Seroconversion was defined as the proportion of vaccinated individuals achieving a haemagglutination inhibition titer of >1:40 against at least 1 of the viral subtypes included in the vaccine. The present study results showed a significant increase in total plasma immunoglobulin (Ig) G titer in the probiotic groups compared to the control groups (14.27 and 10.67, respectively). The seroconversion against at least 1 of the antigens in the vaccine was 4.94 times higher in the probiotic groups than in the control groups (Table 2).

Evaluation of the immune benefits of 2 probiotic strains of B. animalis subsp. lactis BB-12 and L. paracasei subsp. paracasei, L. casei 431 in an influenza vaccination model indicated that an increase in specific IgG was greater in both probiotic groups vs. the control (Rizzardini et al., 2012).

Adverse events (AEs)

There was no significant difference between the 2 study groups (probiotic groups vs. control groups) in terms of the incidence of AEs (Turner et al., 2017; Wang et al., 2018). However, there were more gastrointestinal-related AEs in the control groups (41.8%) compared to the probiotic groups (32.25%). The most common gastrointestinal-related AEs in the control groups were nausea (16.74%), diarrhea (3.59%), vomiting (16.9%), abdominal pain (3.83%), then flatulence (3.62%), while in the probiotic groups the incidence of AEs was nausea (5.72%), diarrhea (7.93%), vomiting (3.9%), abdominal pain (2.85%), and flatulence (2.07%). Non-gastrointestinal AEs including rhinorrhea, headache, cough, muscle aches, sore throat weakness, chills, and fever were lower in the probiotic groups (11.87%) compared to the control groups (22.5%).

Microbiota

The review of fecal recovery results of various studies indicated that the count of 2 important Lactobacillus species significantly increased in the probiotic groups (Akatsu et al., 2016; Berggren et al., 2011). A significant increase was reported in the number of L.
Table 2
Effects of probiotics on the immune response to influenza vaccination.

| Reference       | Vaccine Content | Probiotics & Dosage | Probiotic & Vaccination time | Immunological Parameters | Effects of Probiotics on Response to Vaccine |
|-----------------|-----------------|---------------------|-----------------------------|--------------------------|---------------------------------------------|
| Namba et al. (2010) | A/ [H1N1] B    | $1 \times 10^{11}$ CFU daily for 5 weeks | 2 w before vaccination | IgA (mg/dL): 419 IgG (mg/dL): 1630 IgM (mg/dL): 88 NK cell activity (%): 26 Neutrophil bactericidal activity (%): 88.9 A/H1N1 antibody titer (log): 0.8 A/H3N2 antibody titer (log): 15 B antibody titer (log): 0.5 | The proportion of subjects who contracted influenza was significantly lower in the BBS36 group than in the placebo group. The proportion of subjects with fever was also significantly lower in the BBS36 group than in the placebo group. In the BBS36 group, the NK cell activity and the bactericidal activity of the neutrophils were significantly higher at week 5 than before BBS36 administration. |
| Fonollí et al. (2019) | A/ [H1N1] A/ [H3N2] B | L. casei 3 \times 10^6 CFU | 2 w before vaccination | IgA (mg/dL): 4.13 IgG (mg/dL): 10.5 IL-4 (pg/ml): 0.47 TNF-α (pg/ml): 7.07 | Increase in hemagglutinin titters in response to H3N2 strain 4 w after vaccination (p = .048). |
| Akatsu et al. (2016) | A/ [H1N1] B    | Prebiotics, GOS and BGS | The doses of GOS and BGS were 4.0 g/day. Influenza vaccines were given at week 4 | IgG (g/dl): 1.5 IgA (g/dl): 374 IgM (mg/dl): 105 H3N2: 7 | There was no difference in seroconversion rates due to H1N1 and B strains between the treatment and placebo groups from baseline. |
| Davidson et al. (2011) | A/ [H1N1] A/ [H3N2] B | LGG. 1 \times 10^9 CFU | Immediately after probiotics | IgA (mg/dl): 1.5 IgA (mg/dl): 367 IgM (mg/dl): 108 H3N2: 1 | There was no difference in H1N1, A/H3N2, and B strains-specific IgG levels 3 w after vaccination (p-values NS). There was no difference in seroconversion rates 3 w after vaccination (p-values NS). |
| Jespersen et al. (2015) | A/ [H1N1] A/ [H3N2] B | L. paracasei subsp. paracasei, L. casei 431 1 \times 10^9 CFU | 3 w after probiotics | H1N1: 448 H3N2: 536 B: 475 | There was no difference in A/H1N1, A/H3N2, and B strains-specific IgG levels 3 w after vaccination (values NS). |
| Maruyama et al. (2016) | A/ [H1N1] A/ [H3N2] B | L. paracasei 1 \times 10^8 CFU | 3 w after probiotics | IgG (g/l): 14.4 IgA (g/l): 285 IgM (g/l): 0.59 NK activity (%): 31.8 Neutrophil bactericidal activity (%): 99.1 Neutrophil phagocytic activity (%): 93.4 | There was no difference in A/H1N1, A/H3N2, and B strains-specific IgG levels 6 w after vaccination (p = .643, .767, .828). There was no difference in total IgA, IgG, IgM levels 6 w after vaccination (p = .632, .821, .329). There was no difference in NK-cell activity, neutrophil bactericidal and phagocytic activity 6 w after vaccination (p = .814, .217, .560) |
| Rizzardi et al. (2012) | A/ [H1N1] A/ [H3N2] B | BB-12, L. paracasei subsp. paracasei, L. casei 1 \times 10^9 CFU | 2 w after probiotics | IgA (BB-12): 81.1 IgG (L. casei 431): 28.6 | Vaccine-specific plasma IgG in both probiotics was significantly greater, salivary vaccine-specific IgA was greater but not significant. No difference was found for salivary vaccine-specific IgA or IgM but was significantly greater in total Vaccination (time effect) increased the number of memory IgA + memory, IgG + memory, NCS memory, and total IgG + B cells in young subjects |
| Enani et al. (2018) | A/ [H1N1] A/ [H3N2] B | B. longum bv. infantis 1 \times 10^9 CFU with a prebiotic | 4 w after probiotics | IgA memory: 14.3 IgG memory: 13 NCS memory: 46.6 Total IgA: 22.4 Total IgG: 20.6 | Clostridium leptum, Enterobacteriaceae, and Enterococcus between the control and probiotic groups (Berggren et al., 2011). |

Plantarum (6.9 \times 10^5 and 6.4 \times 10^5 cells/g in the probiotic and control group, respectively) and L. paracasei (from 9.7 \times 10^4 to 1.4 \times 10^6 and 1.8 \times 10^5 cells/g in the probiotic and control groups, respectively) after 2 and 12 weeks.

The Lactobacillus and Bifidobacterium count in the probiotic group increased (≥10-fold) while the Bacteroides count in the probiotic group after 8 weeks was significantly lower than in the control group. There was no significant difference in the count of Clostridium leptum, Enterobacteriaceae, and Enterococcus between the control and probiotic groups (Berggren et al., 2011).

Clinical and respiratory symptoms

Incidence, prevalence and duration of respiratory infection symptoms were significantly lower in the probiotic groups, especially sore throat and cough. The incidence of local respiratory
| Study Start Date/Reference | Country   | Study Design | Participants Characteristics | Participants (age) | Probiotics                      | Days | Dose (CFU) | Comparators   | Outcomes                                                                                                                                                                                                                                                                                                                                 |
|---------------------------|-----------|--------------|------------------------------|-------------------|---------------------------------|------|------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| November 16, 2020/ NCT04621071 | Canada    | RDBPCT       | COVID-19 patients that Self-caring at home | 84(≥18) | 2 strains of probiotics | 25   | 1 × 10¹⁰  | Oral, o.d /capsule | placebo Duration and severity of symptoms, effect on oral and fecal microbiota Cases with discharge to ICU or home, evolution of mortality and safety of treatment, detection of new cases of SARS-CoV-2 infection among healthcare personnel Change in severity of COVID-19 infection, number of days with any symptom of anosmia, number of days where rescue medication is required Incidence of SARS CoV-2 infection in healthcare workers, evaluation of clinical symptoms and medical treatment Viral load during the period of admission to the nasopharyngeal smear, evaluation of clinical indicator, analytical parameters, mobility, microbiome analysis in feces Severity progression of COVID-19, stay at ICU, mortality ratio, lung abnormalities, viral load, levels of immunoglobulins, gastrointestinal manifestations, fecal microbiome, adverse events, change in serum biomarkers Delta in the number of patients requiring orotracheal intubation despite treatment, delta of crude mortality and mobility, delta in the value of interleukin (IL)-1, IL-6, IL-10, TNF-alpha, cluster of differentiation, fecal calprotectin, lipopolysaccharide, zonulin, alpha 1-antitrypsin Change in Shannon diversity, change in |
| May 4, 2020/ NCT043960477 | Spain     | OLRCT        | COVID-19 patients requiring hospitalization | 40(≥18) | Probiotics | 30   | 1 × 1₀⁴  | Oral, o.d/ 1capsule | No Intervention                                                                                                                                                                                                                                                                                                                     |
| July 16, 2020/ NCT0438519 | Canada    | Single-blinded, randomized, prospective trial | COVID-19 patients that Self-caring at home | 40(18–59) | Probiorinse of lactobacillus Lactis W136 | 14   | 2.4 × 1₀⁹  | Nasal irrigation/ b.i.d | Saline solution                                                                                                                                                                                                                                                                                                                   |
| April 24, 2020/ NCT04366180 | Spain     | RDBPCT       | Active healthcare personnel without COVID-19 | 314(≥20) | Lactobacillus K8 | 60   | 3 × 1₀⁶  | Oral/o.d/ 1 capsule | placebo                                                                                                                                                                                                                                                                                                                        |
| April 1, 2020/ NCT04666116 | Spain     | SBRCT        | COVID-19 patients admitted with infection secondary | 96(18–99) | Probiotics | 365  | N. D      | N. D | No dietary supplementation                                                                                                                                                                                                                                                                                                           |
| August 19, 2020/ NCT04517422 | Mexico    | RDBPCT       | COVID-19 patients requiring hospitalization | 300(18–60) | L. plantarum, L. plantarum CECT7481 L. plantarum CECT 7484 CECT 7485 P. acidilactici CECT 7483 | 30   | N. D      | Oral/ capsule | Placebo                                                                                                                                                                                                                                                                                                         |
| March 26, 2020/ NCT04366089 | Italy     | SBRCT        | COVID-19 patients requiring hospitalization | 152(≥18) | S. thermophilus DSM322245 E. lactis DSM 32246 B. lactis DSM 32247 L. acidophilus DSM 32241 L. helveticus DSM 32242 L. paracasei DSM 32243 L. plantarum DSM 32244 L. brevis DSM 2796 | 21   | 2 × 1₀¹³  | Oral/ b.i.d/ 6 sachets | Standard of care                                                                                                                                                                                                                                                                                                                                                                           |
| June 24, 2020/ NCT04399252  | United States | RDBPCT   | People with household | 1000(≥1) | L. rhamnosus GG | 28   | Oral/o.d/ 2 capsule | Placebo | Change in Shannon diversity, change in |
symptoms (sore throat, cough, and/or nasal congestion) was approximately 42% lower in the probiotic groups compared to the control groups (P = 0.007) (Wang et al., 2018).

Quality of life (QOL)

Patients’ QOL was assessed by interview with questions associated with lifestyle and health status. Questions covered the following items were asked twice, once before the start and once after the end of the treatment course: physical functioning, group performance, body pain, general health perception, vitality, social functioning, the role of emotion, and mental health. The post-intervention score of the general health perception subscale in the probiotic groups was significantly improved compared with the control groups (King et al., 2014).

Virological findings and probiotic intervention

Of the 27 clinical trials reviewed, 4 detected effects of probiotics on reducing viral load (Gleeson et al., 2016; Kumpu et al., 2015; Lehtoranta et al., 2014; Turner et al., 2017). The immune response to viral infections was significantly increased in the probiotic-treated groups. Probiotics also decreased viral titer in the respiratory system and the proportion of subjects shedding the virus in nasal secretions (56% in the probiotic groups, 91% in the control groups).

In a trial by Lehtoranta et al. fewer viruses were detected in the probiotic group than in the control group, including picornavirus, influenza A and B viruses, respiratory syncytial virus (A and B), human parainfluenza viruses type 1 to 4, adenovirus, and human metapneumovirus. The number of picornaviruses (mainly respiratory viral) in the probiotic group was 3 times lower than in the control group (P = 0.0069) of the study (Lehtoranta et al., 2014).

Many studies have shown the role of probiotics in reducing respiratory infections, however, there are also some studies suggesting the opposite. For example, Gleeson et al. showed that the incidence of URTI symptoms was unexpectedly low (mean 0.6 per individual), and there was no significant difference between the probiotic and control groups. The duration and severity of URTI symptoms were not influenced by probiotics (Gleeson et al., 2012).

Turner et al. (2017) indicated that there was no significant difference between the probiotic (B. animalis subsp. lactis BI-04) and control groups in terms of the serum level of CXCL10, IL-6, GCSF, CXCL8, IL-1p, and CCL2; decrease in viral titers (RV-A39); and shedding of virus in nasal secretions (P = 0.02) (Turner et al., 2017).

Ongoing clinical studies of probiotics effects in COVID-19

Probiotics have been considered in clinical trials to reduce the clinical presentation and severity of COVID-19. To the best of the authors’ knowledge, no published studies have so far reported the use of probiotics as a supportive treatment for the management of COVID-19.

There were 10 ongoing clinical studies (commencing between January 2020 and January 2021) identified on probiotic intervention in COVID-19, these are shown in Table 3. Some of these studies did not specify the exact type of probiotic being studied but most examined lactobacilli. Scientists in Canada are exploring whether probiotics could potentially reduce the duration and severity of symptoms and rebalance oral and fecal microbiota. Also in Canada, a study on the effect of Lactobacillus lactis W136 on the severity of COVID-19 infection is considering the number of days with any symptom of anosmia and the number of days where rescue medication is required. Researchers from Spain are conducting 3 separate research studies to determine the effect of probiotics on: (i) incidence of SARS-CoV-2 infection in healthcare workers; (ii) patient viral load, clinical indicators, mobility, and microbiome analysis in feces; and (iii) patient mortality and safety of treatment. A study in Mexico is looking at the combined effect of L. plantarum CECT7481, L. plantarum CECT 7484, L. plantarum CECT 7485, and Pediococcus acidilactici CECT 7483 on the severity and progression of COVID-19, including duration of stay in the intensive care unit, mortality ratio, lung abnormalities, viral load, levels of immunoglobulins, gastrointestinal manifestations, fecal microbiome, AEs and serum biomarkers. Scientists in Italy are exploring whether specific strains of Streptococcus thermophilus DSM322245, Bifidobacterium lactis DSM 32246, B. lactis DSM 32247, Lactobacillus acidophilus DSM 32241, Lactobacillus helveticus DSM 32242, L. paracasei DSM 32243, and L. plantarum DSM 32244 could affect the number of patients requiring orotracheal-intubation despite

| Table 3 (Continued) |
|---------------------|
| Study Start Date/Reference | Country | Study Design | Participants Characteristics | No. of Participants (age) | Probiotics | Days | Dose (CFU) | Comparators | Outcomes |
|--------------------------|---------|--------------|-----------------------------|--------------------------|------------|------|-----------|------------|----------|
| 2020-07-18/IRCT20110200049765 | Iran | RDBPCT | contact of COVID-19 | 80(≥18) | Lactocare® probiotic | 30 | N. D | Oral, o.d/1 capsule | placebo | Shannon diversity in patients that develop COVID-19 fever, findings of CT scan and CXR, number of lymphocytes, WBC and infection by COVID-19, cough and sore throat, nausea, vomiting and diarrhea. Cough, all-cause mortality, time to effervescence, time to clinical recovery, Gastrointestinal symptom, chest CT, mechanical ventilation, SARS-CoV-2 RT-PCR |
| CHICTR2000029974 | China | OLRCT | Mild or moderate novel coronavirus pneumonia (NCP) patients | 300(≥18) | probiotics | N. D | N. D | N. D | standard treatment | | |
treatment; mortality and mobility; values of IL-1, IL-6, IL-10, TNF-α, cluster of differentiation, fecal calprotectin, lipopolysaccharide, zonulin and alpha-1-antitrypsin. Researchers from Iran are investigating the effect of Lactocare® symbiotic on fever, findings of computed tomography scan and chest x-ray, number of lymphocytes, white blood cell count and infection by COVID-19, cough and sore throat, nausea, vomiting, and diarrhea. Researchers in Belgium, the United States and China are also looking at probiotics for improving treatment for COVID-19 patients.

Discussion

Previous studies have reported that the total annual cost of non-influenza-related viral respiratory tract infections approaches US $40 billion (Berggren et al., 2011). One of the most important causes of respiratory infections is influenza, which is the leading cause of morbidity and mortality in the USA among VRTIs, leading to approximately 19 000–36 000 deaths and 200 000 surplus hospitalizations per year (Davidson et al., 2011; Fiore et al., 2010). COVID-19, a respiratory viral infection, has swiftly extended into a global pandemic with significant health and economic burden. To date, there is no approved remedy or prophylactic remedial strategy for COVID-19. The overall cost of SARS-CoV-2 to the global economy has been estimated to be between US$30 billion and US $100 billion. Treatment of this respiratory disease is often symptomatic and oxygen therapy is the mainstay of treatment for patients with severe infections. In cases of oxygen-resistant respiratory failure, for controlling septic shock, mechanical ventilation may be necessary (Lythgoe and Middleton, 2020).

Other COVID-19 treatments used so far include systemic corticosteroids not recommended for the treatment of viral pneumonia or ARDS. Methods such as lopinavir/ritonavir (100/400 mg every 12 h), chloroquine (500 mg every 12 h), and hydroxychloroquine (200 mg every 12 h) have been suggested for treatment. Treatments reducing the period of infection, the rate of infection, the severity of symptoms, and the mortality rate are of chief interest for individuals, the entire society, and treatment staff. Unreflective or inappropriate administration of antibiotics should be avoided. Professionals are proposing diverse alternative drugs like Ayurveda, Siddha, herbal medicines, and other related therapeutic approaches to control COVID-19 (Hu et al., 2020).

This review aimed to comprehensively evaluate the effectiveness of probiotics in the control, prevention, and/or treatment of RTIs, which may also be effective against COVID-19. Most applications and studies on probiotics are confined to the gastrointestinal tract because of their significance and traditional use in this area. However, recent RCTs on the use of probiotics on RTIs have emerged because of their broad range of applications and their potential effectiveness in the control or therapy of some infections, including allergic and atopic dermatitis (Prince, 2020). Our review of previous studies showed that probiotics’ impact on common URTIs or viral infections was widely evaluated in RCTs and in numerous reviews and meta-analyses (e.g. King et al., 2014; Prince, 2020; Shin et al., 2015). This systematic review aimed to evaluate the outcomes of clinical trials assessing the efficacy of the probiotic in RTI treatment over the past 10 years.

Of the 27 clinical trials reviewed, 3 studies showed that probiotics did not play a significant role in boosting the immune system and improving the body’s defenses against diseases. The discrepancies in response to probiotic treatment in different studies might be due to the differences in the characteristics of the host (age, sex and lifestyle), differences in URTI incidence, insufficient number of subjects, the season of study, the dose of regimens, duration of treatment, severity of the disease, the single or multi-strain formulation used, delivery modes, and so on, which require further detailed investigation.

The exact mechanisms of action of probiotics on RTI are not yet known. The action of probiotics seems to be specific to particular species and strains. There appear to be several mechanisms in probiotic function, depending on the probiotic’s specifications and target diseases. However, it seems that probiotics have a similar effect in general so that many of the mechanisms of anti-inflammatory actions of probiotics in the gut system might be applied to the respiratory tract (Prince, 2020). Inflammation plays a fundamental role in the pathogenesis of COVID-19; an imbalance between pro-inflammatory and anti-inflammatory cytokines, resulting in a cytokine storm, is currently considered a major factor in the expansion and development of COVID-19 (Hao et al., 2015). Thus, the homeostatic balance between Th1/Th10 and Th17 (IL-17) cells is disturbed in COVID-19. Intestinal epithelial cells could secrete and reply to different cytokines via the presentation of CD1d, as an MHC-like molecule producing anti-inflammatory cytokine IL-10 since the activation of STAT3 (Hao et al., 2015).

Dendritic cells (DCs), a heterogeneous family of immune cells, are one of the key factors in regulating immune responses by linking innate and acquired immune responses through accurately recognizing pathogenic and endogenous inflammatory signals. They are subdivided into plasmacytoid DCs (pDCs), myeloid DCs (mDCs) and CD8+ dendritic cells. pDCs are a rare and essential subset acting as a “control tower” in viral infections by producing a large number of interferons (IFNs) (Lythgoe and Middleton, 2020). In macrophage cell culture L. rhamnosus LC705 hinders influenza A viral replication and viral protein production by inducing type I interferon-related gene activation (Lythgoe and Middleton, 2020). In some studies, the probiotic L. lactis JC5805 has been reported to decrease common cold symptoms and activate human pDCs among peripheral blood mononuclear cells, especially in a subgroup of healthy candidates initially demonstrating low pDC activity (Rizzardini et al., 2012).

The role of pDCs and type 1 IFNs in viral and bacterial infections is complex: pDCs use specific TLRs to detect bacteria and viruses. Among TLRs, TLR9 diagnoses microbial nucleic acids through detecting unmethylated CpG motifs of DNA, and TLR7 recognizes microbial RNA or synthetic guanosine analogs. The activation of pDCs by TLR ligand binding contributes to the production of type 1 IFNs as the first-line defense against infection, which acts through blocking viral replication. The production of type 1 IFNs is often associated with viral infections, and it is well known that pathogenic bacteria stimulate IFN-α production. However, non-pathogenic bacteria including probiotics used in food preparation, have been less intensively studied regarding their potency to stimulate DC-mediated IFNs induction. In a study, various lactic acid bacteria were examined for their ability to stimulate pDCs-mediated IFN-α production, leading to the identification of L. lactis JCM 5805 probiotic as a strong stimulator of type 1 IFN production (Kawai and Akira, 2011; Prince, 2020; Swiecik and Colonna, 2015).

Another important factor playing an important role in regulating the antiviral immune responses is NK cells; the cytotoxic activity of NK cells involved in host defense against viral infections is stimulated by IFN-α. Rask et al. showed that the use of L. paracasei increased the count of NK T cells (P = 0.06) as well as the expression of the memory marker CD45RO on the surface of CD8+ lymphocytes. In this study, an increase was observed in the phagocytic activity of polymorphonuclear leukocytes and monocytes isolated from patients treated by either L. paracasei (P = 0.05) or L. plantarum strains (P = 0.06) compared to placebo (Rask et al., 2013). Charlson et al. injected L. rhamnosus GG strains to mice nasally for 3 days to provoke the cytotoxic activity of pulmonary NK cells and then infected them with influenza virus H1N1, resulting in a significant increase in the secretion of IL-1 and TNF-α. They concluded that these effects could be the cause of better
survival of treated mice (60%) after 15 days compared with the control mice (20%) (Charlson et al., 2011). Several RCTs have shown different effects of probiotic strains on different innate immune markers (Cox et al., 2010; Olivares et al., 2007; Rautava et al., 2006; Schiffrin et al., 1995). However, a typical viral infection challenge in the immune system increases the cascade of immune functions by increasing cytokines, NK activity and phagocytosis in the early days of infection, which are gradually replaced by viral antigen-specific T cells responses (Burleson and Burleson, 2007; Calder, 2007). The liberation of soluble agents by probiotics could increase the processes of immune cells or epithelial cells that subsequently affect immune cells. Also, probiotics could regulate the activity of the immune system near or far from the target organ (Darbandi et al., 2019; Shin et al., 2015).

Many clinical trials have shown that probiotics stimulate the antibody response to viral vaccination (Aubin et al., 2008; Boge et al., 2009; Davidson et al., 2011; Namba et al., 2010). In this review, the most common probiotic species studied in vaccination was found to be Lactobacillus, while 1 study examined B. longum, and 1 study examined the effect of a combination of Lactobacillus and Bifidobacterium. Namba et al. used B. longum BB 536 strain as an adjuvant to enhance the immune response to the influenza vaccine, which resulted in the enhancement of antibody titers and cell-mediated immunity when administered to the elderly (Namba et al., 2010). De Vrese et al. also reported similar results, indicating an enhancement in poliovirus-neutralizing antibody and poliovirus-specific IgA and IgG titers when LGG was administered 1 week before oral administration of the polo booster (de Vrese et al., 2005). Evaluation of the immune benefits of 2 probiotic strains of B. animalis subsp. lactis BB-12 and L. paracasei subsp. paracasei, L. casei 431 in an influenza vaccination model by Rizzardin et al. indicated that an increase in specific IgG was significantly greater in both probiotic groups compared to the placebo group. A significant increase in both IgG1 and IgG3 after probiotic supplementation in this research suggests that the activities of both T-helper (Th1 and Th2) lymphocytes are boosted, as IgG1 and IgG3 are considered to be more representative of Th2 and Th1 responses, respectively. In addition to being preferentially associated with Th1 and Th2 T-cell subsets, IgG1 and IgG3 are also correlated with optimal activation of supplement and phagocytosis by macrophages, respectively (Rizzardin et al., 2012).

The results of the previous studies on humans and animals support the function of different Lactobacillus strains in the prevention of influenza infection. In a study on a mouse model of H1N1 influenza infection, intranasal disposal to LGG for 3 days was significantly associated with a lower frequency of accumulated symptoms and also a higher survival rate compared to control mice (Harata et al., 2010). In another study on the same mouse model of influenza infection, oral administration of LGG or Lactobacillus TMC0356 for 19 days resulted in lower clinical symptom scores and pulmonary viral titters in comparison with control mice (Kawase et al., 2010).

Based on the safety measures of the US Centers for Disease Control and Prevention’s Advisory Committee on Immunization Practices, the annual influenza vaccination is currently recommended for all adults and children >6 months (Fiore et al., 2010). Although the highest morbidity and mortality rates are among young children and the elderly, studies have shown that the influenza vaccination in healthy adults reduces both direct and indirect medical costs such as visiting a doctor, prevents co-infections with bacteria and viruses, and use of antibiotics. Therefore, probiotic use is recommended to further increase immunization of vaccination (Nichol et al., 1999; Wilde et al., 1999). A study by the North Carolina State University on Coronavirus disease is engineering L. acidophilus probiotics expressing SARS-CoV-2 proteins to be tested as potential vaccines (Hu et al., 2020).

The discussion over the superiority of different species/strains of probiotics is very complex. Because of the strain-specific abilities of probiotics and the different categories of patients, a specific probiotic might not be suitable for all patients (Zocco et al., 2006). Most probiotic products include species of Lactobacillus and Bifidobacterium genera, which modulate the gut microbial population and enhance intestinal barrier function (Kleerebezem and Vaughan, 2009; Li et al., 2016). It is believed that probiotics could modulate intestinal microbiota and stabilize the beneficial microbial population by competing with pathogenic bacteria for nutrients and adhesion sites and producing metabolites. Yoshi-matsu et al. showed that probiotic therapy was most beneficial for patients who initially had cluster-I microflora rather than cluster-II flora (Yoshimatsu et al., 2015). In a study, the effects of LGG on the prevention of experimental rhinovirus infections in healthy volunteers were investigated. The severity of cold symptoms and the frequency and number of patients with rhinovirus infection in the LGG group were lower than in the control group, although there was no significant difference between the study groups (Kumpu et al., 2015; Van Puyenbroeck et al., 2012). A placebo-controlled phase 2 trial at Duke University will measure if the probiotic L. rhamnosus GG has a function in hindrance and cure of COVID-19 infection (Prince, 2020). In another study, children with cryptosporidium diarrhea infection given LGG showed a significant improvement in intestinal permeability (Hu et al., 2020). The exact mechanism of action of probiotics on the immune system is not fully understood. However, LGG has been shown to modulate innate and adaptive immune responses by increasing serum IgG and IgA levels and targeting intestinal pathogens such as rotavirus (Kumpu et al., 2015; Prince, 2020).

Most studies on microbiota in the human body have been conducted on the intestinal tract, however, studies on the respiratory tract are expanding and advancing owing to novel biotechnologies (Gollwitzer and Marsland, 2014). It is hard to assure that respiratory microbiota constituents and mechanisms are analogous to intestinal microbiota in expanding and protecting the immune activities of the lung. There is some evidence to suggest that there are relationships between the respiratory microbiota and many lung diseases and that changes in respiratory microbiota could cause illness in a human. Thus, it could be suggested that probiotic bacteria potentially control the intestinal inflammatory responses by confirming the gut mucosal barrier and the microbial environment as well as by degrading enteral antigens and changing their immunogenicity (Darbandi et al., 2019). The results of another study showed that inter-personal diversity in the gut microbiome will affect the effectiveness of suggested microbiota interventions for SARS-CoV-2 infection. Since the onset of COVID-19 different antiviral drugs have been studied to treat the SARS-CoV-2 virus; on 1 May 2020, an antiviral drug called Remdesivir became the first US Food and Drug Administration-approved emergency drug to treat COVID-19 (Grein et al., 2020). Although the results of the observations do not provide any direct evidence of the interaction of the intestinal microbiome with Remdesivir, some observe that there is a history of modification of the intestinal microbiome by other antiviral drugs (Dominguez-Díaz et al., 2019).

No published studies have reported the use of probiotics as a supportive treatment for the management of COVID-19 (Table 3). However, the International Scientific Association of Probiotics and Prebiotics has highlighted that scientists and clinicians globally are investigating the relationship between the gut microbiome and susceptibility to COVID-19 and assessing the role of various probiotics strains to lower viral load via different mechanisms (Hu et al., 2020).
Most of the properties of probiotics are strain-specific and multi-species probiotics may be more efficient than mono-species probiotics in the treatment of certain clinical conditions. For example, Rerkasattrakul et al. reported that administration of a 2-strain probiotic combination (L. acidophilus 10^9 CFU capsule and B. bifidum 10^9 CFU capsule) to school children aged 8–13 twice daily for 3 months during winter resulted in a significant decrease in respiratory symptoms including fever and cough as well as the length of absenteeism from school compared to placebo (Rerkasattrakul and Rerkasattrakul, 2012). Although there are conflicting data regarding the effects of probiotics on RTI treatment (Jespersen et al., 2015; Kinoshita et al., 2019; Kumpu et al., 2015), 8 extensive studies reviewed in the current research showed that the probiotic cocktail VSL#3 could successfully induce RTI remission among patients.

The results of many studies suggest that probiotics as health supplements and treatment agents are safe (Ahren et al., 2021). However, the side effects of probiotics should be controlled when used in a specific population. According to the literature search, 2 trials showed side effects of probiotic consumption. Ahren et al. reported that there was no significant difference between the study groups in the incidence and the overall number of side effects reported by children. However, more gastrointestinal side effects in the probiotic group compared to the control group seem to be explained by a higher frequency of vomiting; an AE was also reported in other studies following the administration of probiotics to young children (Ahren et al., 2021).

Ongoing studies in different countries are looking at whether probiotic strains, especially lactobacilli, in the respiratory tract can decrease viral activity through multifactorial function, barrier and anti-inflammatory, and the risk of secondary bacterial infections for COVID-19 patients. Scientists are also developing special strains of lactobacilli with immunostimulants to support the intranasal SARS-CoV-2 immunization or potentially for a genetically engineered antigen-producing organism for vaccine transfer. Another RCT showed the role of the gut microbiota in rendering a good antibody response against influenza, demonstrating that gut microbiota could change the response for vaccines (Hagan et al., 2019).

Many studies have shown that a mixture of probiotics or prebiotic-synbiotics combinations could improve RTIs and clinical symptoms in treated patients in all age groups. For example, a clinical study by Sazawal et al. on 1–4 year-old children reported the beneficial effect of adding prebiotics oligosaccharides and B. lactis HNO19 to milk in preventing and controlling severe respiratory infections and preventing diarrhea in this group (Sazawal et al., 2010). Similarly, a RCT showed that consumption of a probiotics-containing dairy drink was able to reduce the occurrence of dysentery episodes by 21% (95% CI: 0–38%; P = 0.05); pneumonia by 24% (95% CI: 0–42%; P = 0.05); severe acute lower respiratory infection by 35% (95% CI: 0–58%; P = 0.05); and the duration of disease severity and high fever by 16% (95% CI: 5–26%; P = 0.004) and 5% (95% CI: 0–10%; P = 0.05), respectively (Guillemard et al., 2010; Makino et al., 2010).

The optimal combination and dose of probiotics for the treatment of diseases has not yet been determined. However, it is commonly asserted that 10^9–10^10 CFU/g of probiotic should be used daily to deliver a minimum concentration of 10^9 viable cells into the intestine to exert positive effects on the host (Knorr, 1998; Neffe-Skocinska et al., 2018). In the RCTs assessed in this review, probiotics were administered at doses between 1 × 10^9 to 1 × 10^11 CFU for 6 and 12 weeks. From treatment results of patients with RTI, the best-recommended dose was 5.05 × 10^9 CFU/g an average, showing good efficacy in reducing respiratory symptoms, reducing the duration of the disease, and increasing immunity. Some studies have shown that a probiotic dose higher or lower than 5.05 × 10^9 CFU/g is only effective in increasing QOL and response to general symptoms (Lorenzo-Zúñiga et al., 2014). Not all the probiotic effects on human health seem to be related to the viability of bacteria since even heat-killed probiotic bacteria or probiotic-derived DNA were shown to have the potency to improve significant health problems (Arimori et al., 2012; Hirose et al., 2006; Merenstein et al., 2010).

Conclusion

We conclude from the literature review that the benefit of probiotics and synbiotics as prophylactic and complementary treatment in patients with RTI is a promising preventive strategy to reduce the severity of respiratory infection symptoms, reduce the duration of disease, improve QOL, and induce and maintain remission in patients with RTI. Therapeutic strategies such as the optimal dose, duration or specific probiotic species to be used are yet to be agreed upon.

This systematic review of broad clinical-based studies with multi-center activities from different countries will progress our understanding for determining the potential use of probiotics and/or prebiotics in the context of SARS-CoV-2.

Author Contributions

Atieh Darbandi, Malike Talebi, and Maryam Kakanji initiated the idea of this study. Arezoo Asadi and Roya Ghanavati contributed to data collection, interpretation and final approval of data for the work. Atieh Darbandi and Roghayeh Affifrad developed the first and final draft of the manuscript. Amir Darb Emamie developed the second draft of the manuscript. All figures and tables were designed and checked by Atieh Darbandi and Arezoo Asadi. Malike Talebi and Maryam Kakanji critically reviewed and revised the manuscript. All authors reviewed and contributed to the revisions and finalized the drafts.

Conflict of interest

The authors report no conflict of interest.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

The ethical committee of Iran University of Medical Sciences provided and endorsed the ethical approval for this study (IR.IUMS.REC.1399.1046).

Acknowledgement

This study was supported by research committee of Iran University of Medical Sciences (Registration No. 99-3-73-18757).

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