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Antiviral Activity of Probiotics in the Prophylaxis and Therapy of Respiratory Infections Associated with Coronavirus (COVID-19): Meta-Analysis of Randomized Controlled Trials

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Abstract

Introduction: Probiotics, living microorganisms administered in sufficient quantities, exert beneficial effects on host health. Given the high prevalence of SARS-CoV-2, recent studies suggest potential positive impacts of probiotics on COVID-19 patients.

Methods: A predetermined search strategy encompassing seven databases: NCBI, PubMed, Science Direct, Springer Link, Embase, CNKI, and Cochrane Library Databases, was implemented. Human RCTs studies were scrutinized independently, involving data extraction, quality and risk of bias assessment, and statistical analysis. Pooled data, employing the random-effects model were expressed as Standardized Mean Differences (SMD) with a 95% Confidence Interval (CI). Assessments of (p) value and heterogeneity (I^2) were conducted and quantified.

Results: Five studies, comprising 282 out of 375 participants, were included. Meta-analysis revealed effects on various parameters: CRP (SMD=0.26 MG/L, 95% CI (0.10, 0.43), p=0.002, (I^2=67%, p=0.03)), BMI (SMD=0.28 KG/m², 95% CI (0.07, 0.50), p=0.01, (I^2=67%, p=0.40)), T-cells (SMD=0.09 G/L, 95% CI (-0.07, 0.26), p=0.26, (I^2=0%,p=0.73)), Albumin (SMD=0.28 G/DL, 95% CI (0.04, 0.52), p=0.02, (I^2=7%, p=0.34)), IL-6 (SMD=0.67, 95% CI (0.45, 0.90), p=0.00001, (I^2=94%, p=0.0001)), LDH (SMD=0.12 mmol/L, 95% CI (-0.05, 0.30), p=0.17, (I^2=55%, p=0.13)), and Ferritin (SMD=0.19 mmol/L, 95% CI (-0.27, 0.66), p=0.41, (I^2=77%, p=0.04)).

Conclusion: This meta-analysis suggests significant positive effects of probiotics on various measures for COVID-19 treatment.

Keywords: Probiotics; Probiotics intervention; Probiotics treatment; Respiratory infections; COVID-19; SARS-CoV-2

Abbreviations: BMI: Body Mass Index; CD: Cluster of Differentiation; CI: Confidence Interval; COVID-19: Coronavirus Disease of 2019; CRP: C-Reactive Protein; FAO: Food and Agriculture Organization; IL-6: Interleukin-6; WHO: World Health Organization; LDH: Lactate Dehydrogenase; NCBI: National Center for Biotechnology Information; CFU: Colony Forming Unit; ORF: Open Reading Frames; RCTs: Randomized Controlled Trials; CNKI: China National Knowledge Infrastructure; RNA: Ribonucleic Acid; ROS: Reactive Oxygen Species; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SD: Standard Deviation; SMD: Standardized Mean Difference

Introduction

In late December, 2020, a novel and unfamiliar virus within the coronavirus family emerged in Wuhan, China, triggering a global health crisis [1]. This led to a state of emergency, overwhelming hospital facilities due to the rapid spread of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). The WHO officially declared this new disease a pandemic in March, 2020 [1]. SARS-CoV-2 is an enveloped, single-stranded, positive RNA virus with a genome size of approximately 29.9 KB [2]. The genome consists of two main coding sequences, ORF1a and ORF1b, comprising 22 KB. These sequences are responsible for translating two polyproteins, which are then cleaved into 16 non-structural proteins are important for viral replication [3]. The remaining 7.9 KB of the genome encode four membrane glycoproteins, which form the virus's structure [2,3]. The virus spreads primarily through respiratory droplets, causing severe lung inflammation, cell destruction, and ultimately, death, particularly in adults [4]. Efforts to control the spread of the disease have led to the development of several vaccines, including the British "AstraZeneca", the Russian "Sputnik V", the Chinese "Sinovac" the European "Pfizer" and the American "Johnson & Johnson" [5]. While these vaccines have shown safety and efficacy to some extent, the virus's continuous mutations pose challenges. Variants such as delta and omicron necessitate ongoing research and adaptation of preventive measures [6].

There is a growing concern that current vaccines may become ineffective against future mutations, highlighting the need for alternative treatments [7]. Probiotics have emerged as a potential therapeutic option due to their antiviral activity [8]. Defined by the FAO/WHO in 2001 as live microorganisms providing health benefits when administered in adequate quantities [9], probiotics offer a promising avenue for combating infections. Originating from various sources such as *Lactobacillus* spp, *Bifidobacterium* spp, and *Saccharomyces cerevisiae*, probiotics have a long history dating back to the early 1900s with Elie Metchnikoff's research [10]. These microorganisms can be incorporated into various products, including yogurt and pharmaceuticals, and have been studied extensively for their role in preventing infectious diseases and modulating immune function [11-15]. Clinical studies, including randomized controlled trials, have demonstrated the efficacy of probiotics in mitigating COVID-19 symptoms and improving immune responses [16,17]. Probiotics have shown comparable or even superior outcomes compared to traditional treatments in some cases. This underscores their potential as a complementary approach to managing the disease. Additionally, concepts such as prebiotics, symbiotics, postbiotics, and parabiotics have expanded our understanding of probiotics' therapeutic applications, offering further avenues for research and development [16].

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Literature review

Study design

This meta-analysis, a quantitative analysis, utilizes statistical analyses to screen data sets, employing one or more representative schemes. Data entry is manually performed on Cochrane Review, Revman version 5.4.1, yielding analyzed data for scientific publication. Qualitative analysis is based on multiple scientific publications, presented as studies of human randomized controlled trials.

Subject and objective of the study

This meta-analysis aims to investigate the effects of probiotics on prophylaxis and treatment of coronavirus-related respiratory infections. Clinical research studies aim to reach conclusions, such as evaluating probiotics' effectiveness in combating respiratory infections. The study aims to:

- Determine if probiotics are more effective in prophylaxis compared to other agents.
- Assess if efficacy varies based on dosage (treatment duration, age group) or species (*Lactobacillus* spp, *Bifidobacterium* spp, etc.).
- The research questions are as follows:
- Do different probiotic species exhibit varying effectiveness in preventing respiratory infections?

Research Protocol

Is there a disparity in effect between drugs proven effective against COVID-19 and probiotics?

For the identification of relevant or similar studies, a search strategy was chosen, utilizing the following databases: PubMed, NCBI (The National Center for Biotechnology Information), Science Direct, Springer link, EMBASE, CNKI (China National of Knowledge Infrastructure), and Cochrane Library databases.

Search criteria: A search for relevant articles was conducted in the aforementioned databases between January 2020 and 2023. The search utilized keywords such as "Probiotic", "Probiotic treatment", "Probiotic intervention", "Probiotic prevention", "COVID-19 Disease"," SARS-CoV-2", "Viral respiratory infection", "Randomized clinical trials", and "Randomized controlled trials". In the Chinese language on the CNKI database, the keywords used were "Beneficial born bacteria", "Beneficial born bacteria rule therapy", "Beneficial born bacteria Dry pre", "Beneficial born bacteria pre Defend", "COVID-19 disease", "SARS-CoV-2", "Follow machine Pro bed try test" and " Follow machine right According to try test" [18-21].

Inclusion criteria: After reviewing the literature, titles, and abstracts, the inclusion criteria were established as follows:

- Studies and articles available in English, French, and Chinese, with full text accessible.
- Human randomized controlled trials with intervention and control groups, or pre-and post-intervention study groups, demonstrating clear outcomes.
- Studies examining the effects of probiotics on COVID-19 patients, regardless of demographic characteristics or health status.
- Use of probiotics from genera such as *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Propionibacterium*, and *Streptococcus*, whether alone or in combination with prebiotics, postbiotics, or symbiotics.

Exclusion Criteria: Following literature review, titles, and abstracts, exclusion criteria were defined as follows:

- Studies lacking relevant outcomes or not meeting RCT criteria.
- Studies in the form of conference abstracts, case reports, editorials, book chapters, reviews, commentaries, letters, and similar articles with no relevant data.
- Trials with insufficient, unclear, incomplete, or inaccessible data.

Quality assessment

Quality assessment was conducted independently for each study based on Cochrane risk of bias manual guidelines. Egger's tests were performed using STATA software, with a p-value<0.05 indicating small study effects. Quality assessment considered seven elements: Random sequence generation, allocation concealment, blinding of participants and assessors, incomplete outcome data, selective reporting, and other biases, categorized as (Low risk), (High risk) or (Unclear risk), Over all study quality was rated as good, fair, or poor [22].

Data extraction

From each eligible RCT study, the necessary data and information were extracted, including author(s), year of publication, study country, design, participant characteristics (total number, age, gender), sample size of intervention and control groups, probiotic source and type, dosage, duration of treatment, and outcome precision. Descriptive and statistical data for each outcome were collected.

Statistical analysis

Data from included studies were analyzed using Review Manager 5.4.1 and STATA version 12.0. Mean (standard deviation) meta-analysis was performed, with Standardized Mean Differences (SMD) calculated. Heterogeneity between study-specific estimates was assessed using the I^2 statistic and Cochran Q test, with I^2>50% or p-value<0.05 indicating significant heterogeneity. Cochran Q test results were presented as I^2, defining heterogeneity as low (I^2<25%), moderate (25%<I^2<75%), or high (I^2>75%). Risk of Bias Assessment, Risk of bias was evaluated using the Cochrane Rev Man risk of bias tool. Publication bias was assessed visually through funnel plots and quantitatively using Egger's tests performed *via* STATA software, with a p-value<0.05 indicating small study effects [21].

Results

Results of the selection studies, data extraction and risk bias assessment

The assessment of quality and risk of bias in the included studies is based on seven key areas: Random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases [22-26]. This assessment relies on established criteria outlined in the "Cochrane Handbook for Systematic Reviews of Interventions" to evaluate the likelihood of bias in each domain. The nature of risk is categorized as "High risk," "Low risk," or "Unclear risk" for each study, considering its methodological quality and potential biases (Table 1).

Study (Author, year)	Country	Study design	Participants	Intervention	Duration	Outcomes
Li, Q et al., [22]	China	Single-centre retrospective study	311 adult patients (≥ 18 years). 123 patients were treated with probiotics, 67 males and 56 females. The remaining 188 patients, 83 males and 105 females, received other drugs, for example, Chloroquine Phosphate.	4g of oral probiotics contain strains of: • 1.5 grams of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Bacillus* tablet, and *Bifidobacterium* infants. • 2 grams of *Bifidobacterium* and *Lactobacillus* tablet (*Bifidobacterium* longum, *Lactobacillus bulgaricus*, and Streptococcus thermophiles). • And finally 0.5 grams of *Bacillus subtilis* and *Enterococcus Faecium* soluble capsules (*Enterococcus faecium*, *Bacillus subtilis*).	26 Days	08 selected laboratory parameters: (IL-6, CRP, LDH, total T cells, NK cells, B cells, $CD4^+$ T cells, $CD8^+$ T cells, and CD4/CD8 ratio).
Ceccarelli G et al., [23]	Italy	Observational and retrospective cohort study	The study involved 200 adult patients (≥ 18 years), consisting of 113 males and 87 females. • 112 patients received the BAT protocol without oral bacteriotherapy (probiotic). • 88 patients received the BAT protocol with oral bacteriotherapy.	Treatment by oral administration of SivoMixx formulations includes the probiotics: *Lactobacillus* brevis DSM 27961, Streptococcus thermophilus DSM32245, *Bifidobacterium* lactis DSM 32246, *Bifidobacterium* lactis DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus* paracasei DSM 32243, *Lactobacillus plantarum* DSM 32244, The formulation was administered in three equal doses per day, for a total of 24×1011 CFU per day.	51 Days	(CRP, LDH, T-cells, Albumin, Monocytes, BMI, PSI, Lymphocytes, White blood cells, Neutrophils, Duration of hospital stays (days), Deaths).
Gobbi M et al., [24]	Italy	Observational study	48 patients (26 males/22 females), 29 patients were treated with a nutritional intervention containing probiotics.	An oral intervention of fat-free milk powder contains vitamins, amino acids, minerals, and probiotics: *Streptococcus thermophilus*, *Bifidobacterium* breve, *Bifidobacterium* longum, *Bifidobacterium* infantis, *Lactobacillus* acidophilus, *Lactobacillus* plantarum, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*. A minimum of 1 to 1.3g/day/kg of bodyweight. An optimal fat-to-carbohydrate energy ratio was 30:70, 50:50.	25 Days	This study included laboratory parameters measured from day 1 today 7 of administration: (IL-6, CRP, T-cells, albumin, ferritin, BMI, creatinine, serum iron), muscle strength (HG), performance test (TUG).
Araimo F et al., [25]	Italy	An interventional, non-pharmacological, open-label, randomized, prospective, double-blind study	85 patients (≥ 18years) confirmed with COVID-19; only 28 patients were admitted to the study. • The first experimental group of 14 patients, known as the ozone group, was treated with the BAT protocol + O_3-AHT therapy through the administration of oxygen-ozone, followed by duration of a probiotic mixture. • The second groupof14 patients was treated with the therapeutic protocol only.	Intervention treatment of the administered SivoMixx product sachets contains probiotics: *Streptococcus thermophilus* DSM 32345, L. acidophilus DSM 32241, *L.helveticus* DSM 32242, *L. paracasei*, DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *B. lactis* DSM 32246, and *B. lactis* DSM 32247. • Administration was only to the 14 patients, with one sachet every 12 hours for 7 days. • Each sachet of SivoMixx in this study contained a high concentration of 8×109 CFU, administered every 12 hours.	07 Days	This study included laboratory parameters measured from day 1 today 7 of administration: (IL-6, CRP, T-cells, albumin, ferritin, BMI, creatinine, serum iron), muscle strength (HG), performance test (TUG).
d' Ettorre G et al., [26]	Italy	A retrospective cohort study	70 COVID-19 positive patients • The first group of 48 patients received a BAT treatment protocol often containing hydroxychloroquine, antibiotics, and tocilizumab. • The second group of 28 patients received oral bacteriotherapy (probiotic formulation).	The SivoMixx formulation administered in this study contained: *Streptococcus thermophilus* DSM 32345, *L. acidophilus* DSM 32241, *L. helveticus* DSM 32242, *L. paracasei* DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *B. lactis* DSM 32246, and *B. lactis* DSM 32247. • This oral bacteriotherapy involved the use of 24×1011 CFU per day. • The formulation was administered in three equal doses per day.	17 Days	This study includes respiratory parameters: (FiO_2, SO_2, pH, HCO_3, PO_2, PCO_2), and BMI.

The rationale for the application of probiotics as a potential prevention and alternative treatment strategy against COVID-19

The meta-analysis was conducted to analyze existing studies in the literature. Our focus was on probiotic treatment for respiratory infections. Q-statistics were employed to evaluate whether effect sizes across individual studies were homogeneous, indicating the degree of heterogeneity. Additionally, heterogeneity of individual outcome estimates in the meta-analysis was assessed using forest plots. The efficacy of probiotics varies based on specific strains, age groups, clinical dosages, and mode of administration. Clinical studies, including randomized, double-blind, intervention-controlled, or experimental human trials of probiotics, were planned. The overall effects of probiotics were quantified using values such as Standardized Mean Differences (SMD) and Confidence Intervals (CI) [27].

Selection of parameters or outcomes in the analysis

The five included studies comprise human clinical trials involving COVID-19 patients experiencing respiratory difficulties due to inflammation. A total of 657 patients were divided into two groups, each following specific therapeutic protocols that included probiotics of various strains (such as *Bifidobacterium*, *Lactobacillus*, *Streptococcus*) sourced from products like SivoMixx capsules, and milk powder. Following the treatment period in each trial, post-treatment monitoring of patients' immune and health status was conducted. This assessment involved various parameters, including blood tests and respiratory status evaluations, administered by specialists across all studies. Among these parameters, CRP, BMI, T Cells, Albumin, IL-6, LDH, and Ferritin were identified as common outcomes across all five studies for subsequent analysis.

Probiotics and C-Reactive Protein (CRP)

The CRP level serves as an indicator of inflammation, commonly associated with respiratory infections caused by COVID-19 [28]. It is a protein of the acute phase of inflammation, whose levels rise in serum or plasma during responses to infections or early inflammatory events [29]. In regard to this parameter, a meta-analysis of four studies based on five RCTs [22-25] involving 587 participants (254 in the experimental group and 333 in the control group) revealed an overall significant effect (p=0.002) with a Standardized Mean Difference (SMD) of 0.26 and a Confidence Interval (CI) of 0.10 to 0.43. The analysis showed a statistically significant increase in CRP levels (p=0.002<0.05), indicating significant heterogeneity.

Probiotics and Body Mass Index (BMI)

The Body Mass Index (BMI) is a widely used measure for assessing individuals' corpulence and tracking changes in body composition over time. It is calculated based on height and body mass, serving as an indicator of overweight and obesity according to WHO guidelines, with the formula $BMI=P/T^2$ or $BMI=m\times h^{-2}$ [30].

In the analysis of BMI across four studies from five RCTs [23-26], involving 346 patients (159 in the test group and 187 in the control group), there was an overall indicative increase in BMI levels (SMD 0.28; CI 0.07 to 0.50; p=0.01). This indicates a significant increase (p=0.01<0.05) in BMI. Notably, the included studies exhibited low heterogeneity, which was not statistically significant (I^2=0%; p=0.40>0.05).

Probiotics and t-cells

T cell count serves as an indicator of the adaptive immune response, offering insight into the cellular immunity status, particularly in coronavirus-affected lung cells [31]. T cells, including subsets such as $CD4^+$ T cells, $CD8^+$ T cells, B cells, Natural Killer (NK) cells, are important for maintaining immune system function post-viral infection [32]. In the context of T-cell analysis across four studies from five RCTs [22-25], involving 587 participants (254 in the experimental group and 333 in the control group) included in this meta-analysis, each individual received probiotic intake. The overall effect revealed a non-significant increase (p=0.26>0.05) with a Standardized Mean Difference (SMD) of 0.09 and a Confidence Interval (CI) of -0.07 to 0.26 (p=0.26). Notably, the included studies exhibited complete lack of heterogeneity, indicating a low and statistically non-significant level (I^2=0%; p=0.73).

Probiotics and albumin

Albumin levels serve as an indicator of enzyme, drug, and hormone transport, as well as blood circulation stabilization [33]. As an acute phase reactant with antioxidant properties, plasma albumin is important in scavenging Reactive Oxygen Species (ROS) under normal physiological conditions [34]. In cases of oxidation, ROS accumulation in neutrophils triggers extra cellular neutrophil traps, which can accumulate in the lungs, as seen in COVID-19 cases [34]. Regarding albumin levels, analysis of three studies from five RCTs [23-25], involving a total of 276 participants (131 in the experimental group and 145 in the control group), showed an increase in albumin levels following probiotic supplementation. The overall effect of this increase was significant (p=0.02), with a Standardized Mean Difference (SMD) of 0.28 and a Confidence Interval (CI) of 0.04 to 0.52, as the p-value (p=0.02<0.34) indicated significance.

Probiotics and IL-6

Only two out of the five RCTs [22,25], involving 339 patients (137 in the first group and 202 in the second group), demonstrated a significant increase in interleukin-6 levels following the administration of probiotic formulas. Interleukin-6 is produced by the immune system as part of the defensive response against SARS-CoV-2, involving the intervention of various white blood cells, including macrophages, T cells, and B cells [35].

The meta-analysis revealed a pooled effect of a significant increase (p=0.00001) (SMD 0.67; CI, 0.45 to 0.90), indicating statistical significance (p=0.00001<0.05). Furthermore, a high level of heterogeneity (I^2=94%) was observed, which is considered significant, given the p-value (p=0.0001) being less than 0.05.

Probiotics and LDH

LDH levels serve as a non-specific indicator of cell death in various respiratory diseases, including COVID-19 [36]. Lactate Dehydrogenase (LDH) is an enzyme important for the conversion of lactate to pyruvate in most body tissues, and its levels typically rise following tissue breakdown, reflecting elevated serum LDH in numerous clinical conditions [37].

In this context, only two out of five RCTs [22,23] involving 511 confirmed COVID-19 participants (211 in one group and 300 in another) included in the meta-analysis demonstrated a decrease in LDH levels among patients receiving probiotics. However, the overall association between probiotic intervention and LDH levels was non-significant, with a p-value of 0.17, indicating no statistical significance (SMD 0.12; CI -0.05 to 0.30; p=0.17). Additionally, the included studies exhibited moderate heterogeneity (I^2=55%; p=0.13), which was not statistically significant (p=0.13<0.05).

Probiotics and ferritin

Ferritin serves as a vital protein for iron storage, facilitating iron availability for important cellular processes while safeguarding DNA, lipids, and proteins from the potential toxic effects of iron. Monitoring ferritin levels through blood sampling is essential, given its involvement in various diseases, including inflammatory, neurodegenerative, and malignant conditions [38].

In this analysis, only two out of five RCTs [24,25] involving a total of 76 participants contributed to the meta-analysis, revealing an increase in ferritin levels following probiotic intake. However, the overall association between probiotic usage and ferritin levels did not demonstrate significance, with a p-value of 0.41 (SMD 0.19; CI -0.27 to 0.66; p=0.41<0.05). Notably, the included studies exhibited a higher level of heterogeneity, which was statistically significant (I^2=77%; p=0.04).

Result of the publication bias assessment

The funnel plot, depicted in Figures 1-3, illustrates minimal indications of publication bias across the five selected studies. The plot's funnel traces graphically represent the curves constructed for the analyzed parameters: CRP, BMI, T-cells, Albumin, IL-6, LDH, and ferritin, facilitating the assessment of bias in each included study. Of particular interest are the p-values obtained from the Egger test, which were 0.0001, 0.04, 0.13, 0.34, 0.40, 0.40, and 0.73 for the respective parameters. Notably, most of these values were statistically insignificant, as they exceeded the threshold of 0.05, except for IL-6 (p=0.0001), indicating a small study effect. Consequently, only one parameter, IL-6, exhibited bias, while the remaining six parameters showed no signs of publication bias.

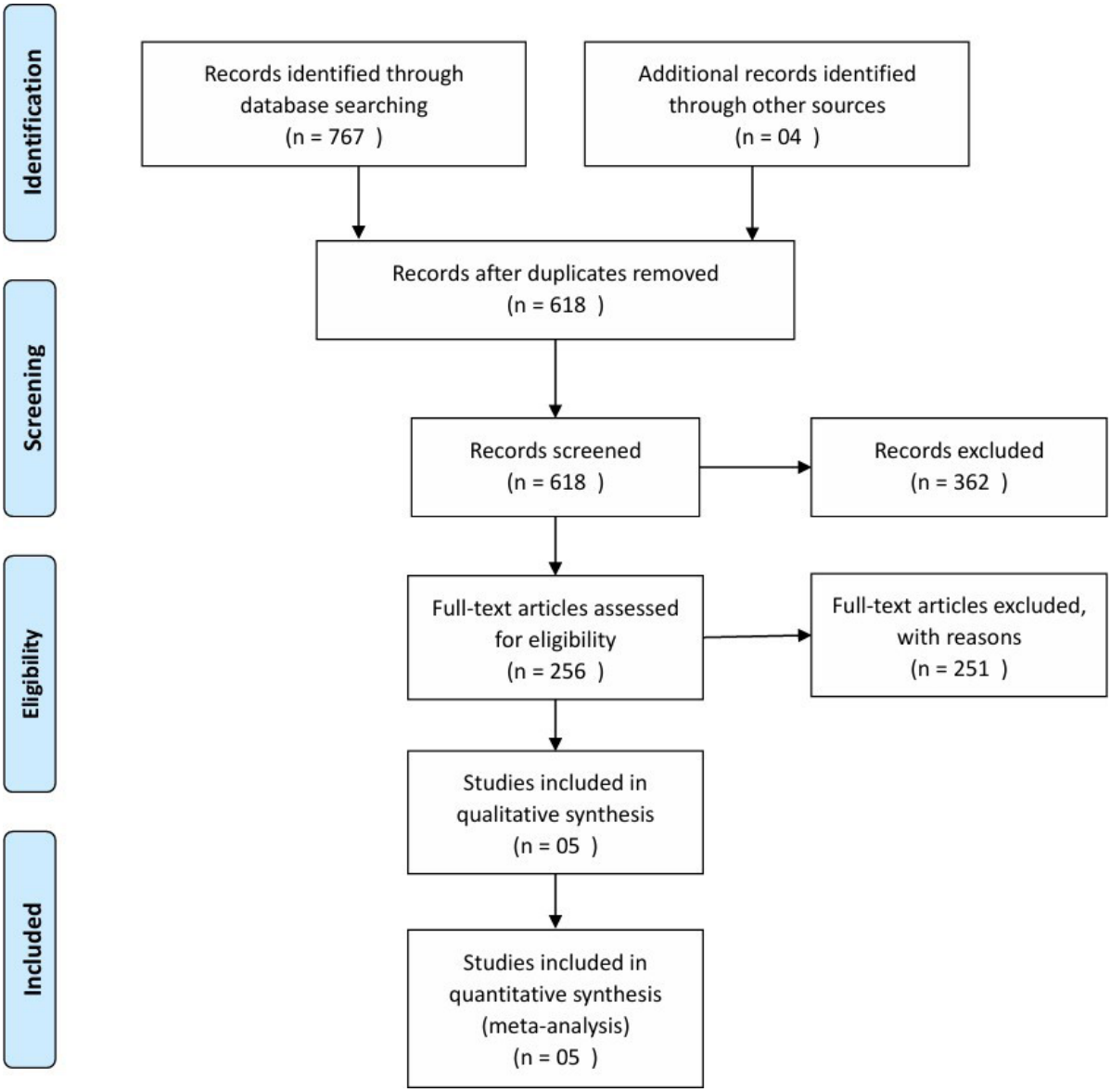

Figure 1: PRISMA flowchart for the results of the study selection process.

Figure 2: Graph showing the risk of bias of the included studies. **Note:** Low risk of bias ( ); Unclear risk of bias ( ); High risk of bias ( );

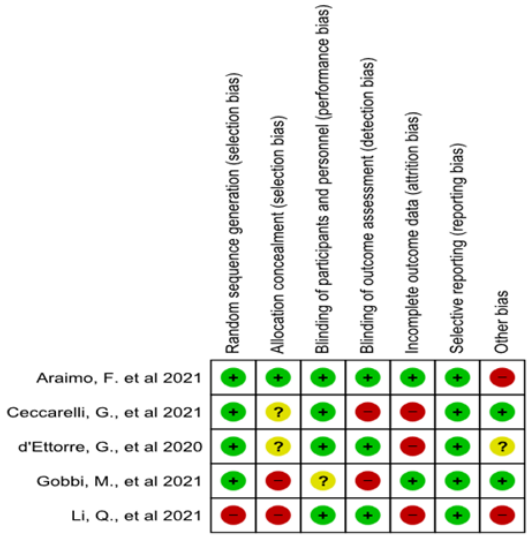

Figure 3: Graphical representation of the risk of bias in percentage (%).

Discussion

Discussion of the quality of the included studies

The random sequence generation index pertains to the randomization process of participants into intervention groups [39]. Among the five included studies, four of them [23-26] demonstrated a low risk percentage of 80%, while the remaining study was assessed as high risk, constituting 20% of the total. This discrepancy can be rationalized by contextual factors. The study categorized as high risk [22] was conducted relatively early in the context of the SARS-CoV-2 outbreak compared to the others. Initiated in February, 2020, at a time when knowledge about COVID-19 was limited, researchers might have faced challenges in ensuring a fully randomized participant allocation. The constraints imposed by the emergent nature of the crisis could have restricted opportunities for randomization and necessitated organizational adjustments in the study design. Despite these limitations, the study remains pertinent as it investigates the impact of probiotics, specifically in capsule form, aligning with the overarching objective of the meta-analysis.

Allocation concealment: The second criterion, allocation concealment, pertains to the detailed description of methodological procedures aimed at concealing the interventional allocation sequence [39].

Among the five studies, two were deemed to have a high risk, accounting for 40% of the total [22,24]. In the case of study [22], the inability to prognosticate the health status of patients and the high possibility of complications during the emergent phase of the COVID-19 outbreak rendered concealment impractical. The study [24] was also categorized as high risk because participants were admitted to rehabilitation services after exiting the acute phase of COVID-19, necessitating increased monitoring and targeted nutritional interventions.

Additionally, two studies representing 40% of the total, were characterized by unclear bias due to a lack of information to assess the risk level [23,26]. Conversely, the remaining study [25], constituting 20% of the total, was assessed as low risk. This classification was based on its minimal scale of participants (only 28), enabling independent and closely monitored therapeutic procedures.

Blinding of participants and personnel: This third criterion describes all the measures used, if any, to blind study participants to the nature of the interventions they received and staff to know which group

they were assigned to [39]. Four of the five studies evaluated as low risk cover a percentage of 80% because the patients [22-26], in these studies do not know which intervention group they belong to. Blinding of participants in human clinical studies is often recommended because it has a clear positive influence on the body's response and psychological state, which gives results and sometimes ensures perfect resistance against diseases. This leads the patient to believe that they are taking the effective treatment, and the same applies to the remaining patients, to avoid bias in the results. Thus, for a correct study of the real effect of each treatment against the other, blinding is essential. The remaining study five is scored as indefinite risk [24], as it is unclear and lacks sufficient information for this procedure.

Blinding of outcome assessment: The fourth criterion of this assessment refers to the process of concealing the identity of the treatment group from the outcome assessors after their assignment to the treatment by randomization to minimize the occurrence of biased assessments influencing the outcome of the research [39].

Three studies out of Five were rated as low risk, covering a percentage of 60% [22,25,26]. In the study, the epidemiological and clinical characteristics of each patient, and laboratory parameters [22], are monitored and collected from time to time by persons independent of the specialists, which facilitated the use of probiotics or hydroxychloroquine protocol. The study does not present serious health changes that require the intervention of previous clinicians to administer the treatment to the (28) participants [25]. In study, during admission, the participants underwent blood tests, and then, after continuous dosing of oxygen with probiotics and drugs for each group, the clinicians did not repeat the gas and blood analysis, considering the periodic monitoring unnecessary [26].

The remaining Two studies were considered as indefinite risk, which covers a percentage of 40% [23,24]. In the study, a set of measures was implemented to predict several changes (Blood urea, Blood pressure, Respiratory rate) to achieve the goal of reducing mortality as much as possible [23]. The study considers it necessary to carry out blood tests periodically to assess the effectiveness of the mentioned interventions on muscle strength and the restoration of physical conditions [24].

Incomplete outcome data: The fifth criterion provides a comprehensive description of the outcome data for each primary outcome, including attrition and exclusions from analysis (death, withdrawal), or due to the complication of certain health conditions in each intervention group [39].

Three out of five studies are assessed as high risk, comprising 60%, due to the exclusion of patients out of the total number, usually due to deaths in all three studies [22,23,26]. The study reports a total of 44 deaths in females and males [23]. In this study, it is stated that participants died, but the exact number is not specified, following complications due to elevated serum IL-6 levels, which indicate deteriorating health [26]. There were many exclusions due to deaths; 30 people died in the probiotic group, which is far fewer than in the other group [22].

The remaining Two studies out of five are considered low risk, covering 40%, meaning no participants were excluded for these reasons [24,25]. In study, the 28 participants completed the study successfully without any deaths [25]. In study, the 48 participants completed the study without withdrawal or death [24].

Selective reporting: In the sixth criterion, it is revealed how the possibility of selective outcome reporting was examined by the review authors, and what was found raises the question: Are the study reports free from suggestions of selective outcome reporting? [39].

All five studies are rated as low risk, comprising a percentage of 100%, as all protocols used in the studies are available and advised by associations and organizations, and all specified outcomes (primary and secondary) have a prespecified impact [22-26].

Other biases: The final criterion of other biases describes the status of significant concerns about bias not addressed in the previous six areas, generally by limitations of each included study [39].

Two studies out of the five show a low risk of bias, comprising a percentage of 40%, as they lack serious biases and major limitations that may hinder or limit the course taken by these studies [23,24].

Then two studies out of five were noted as high risk, covering a percentage of 40% [22,25]. In the study, many limitations are reported, including the small sample size, the short follow-up, and the impossibility to discriminate the specific effects and the exact influence of the different drugs used [25]. In the study, an important limitation is present: the design of this retrospective study is monocentric, meaning from a single source [22]. In the future, we need large-scale research to conclude high-quality evidence and thus exclude cases of death from COVID-19.

Finally, the remaining study is assessed as being of unclear risk, as there are no reports of bias regarding the limitations of this study [26]

Discussion of the parameters analyzed

In the discussion and interpretation, the parameters analyzed necessarily depend on the location on the vertical axis, either on the left or on the right, depending on the intervention effect of each included study.

- The effect is displayed on the left position (considered in favor of the control) when the value is SMD<0.00.
- The effect is located in the right-hand position (considered in favor of the intervention groups, generally experimental) when the value is SMD>0.00.
- The average position in the middle indicates no effect when the value is SMD=0.00.

C-Reactive Protein (CRP): In this first analyzed parameter of CRP, which indicates the degree of inflammation in the total number of 587 patients, we noted (Figure 4) that there are four studies out of five after the intervention of probiotics from different sources (drug, nutritional food supplement) that belong to different strains (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*) [22-25].

Out of the two studies out of four [24,25], the probiotic agents gave SMD values in the following order: -0.08 MG/L, including an increase in CI (-0.66 to 0.50), and -0.49 MG/L, also with an increase of (-1.24 to 0.26). These two were placed in the left position of the vertical axis, meaning that this position can be translated as a favorable effect in the control group regarding these two studies.

The remaining studies showed other SMD values in the range of 0.21 MG/L with an increase (CI: -0.02 to 0.44) and 0.54 MG/L (CI: 0.26 to 0.83) [22,23], placed in the right position of the vertical axis, which means that there is an effect in favor of the intervention and an increase compared to the previous values; this difference translates into COVID-19 replication in lung cells.

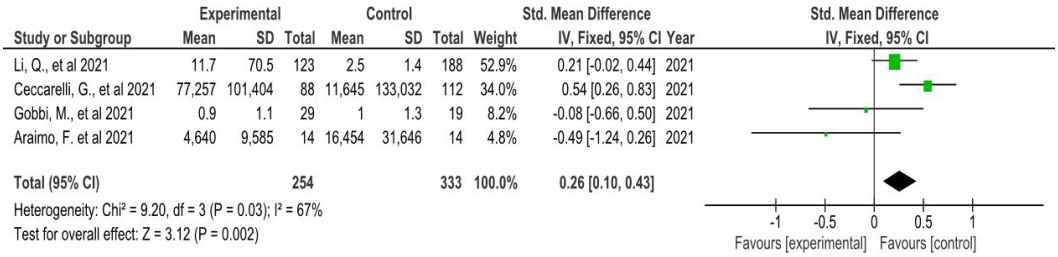

Figure 4: Forest plot showing the association between experimental probiotic use and other control interventions on CRP levels.

The black diamond at the bottom of the table summarizes the overall effect of all previous interventions in each study included in the analysis of this endpoint. Its positioning corresponds to the statistical calculations of the mean between the confidence interval and the SMD. This effect translates into an overall value of SMD center, which is 0.26 MG/L, A statistically insignificant increase of a bad effect after a set of probiotic administration and intervention procedures. It is important to note that study [23], with the highest SMD effect of 0.54 MG/L (high inflammation), shows that clinicians involved the therapeutic protocol in both groups (experimental and control). Only those with respiratory complications due to inflammation received high doses of probiotics. This explains the negative influence of probiotics on the inflammation index (CRP) in the experimental group due to this imbalance. However, overall, we find that the survival of patients is in favor of those treated with probiotics.

What makes the CRP levels high in the experimental groups is the presence of inflammation caused by COVID-19, which mainly targets the lungs, much more than in the control group. In contrast to this result, several scientific publications based on human or animal trials show the positive effect of probiotics and suggest them as a better alternative to other drugs used in the control group to reduce inflammation levels (CRP) caused by respiratory viruses, which have a similar mechanism of action to COVID-19, such as Influenza H7N9. The results show a non-significant effect for the probiotic groups [40,41].

However, this is not sufficient, and in the future, we need more randomized studies to better understand the impact of probiotics on the inflammation index in patients with COVID-19. Better results can be obtained with the proliferation of studies on the subject. The insufficiency of journals that deal with these pathologies and the research, still at the experimental level, does not allow for an exhaustive and definite opinion on the issue.

Body Mass Index (BMI): The second parameter, the Body Mass Index (BMI), which summarizes a quick assessment of corpulence with weight and height, is included in the quantitative analysis in (Figure 5). This includes four out of five studies, covering a total of 346 participants. Only one out of four studies is on the left side of the vertical axis after treatment with probiotics and administration of an oxygen/ozone (O_2/O_3) mixture [23-26]. This resulted in an SMD value of -0.17 KG/m² with subsequent increases (CI: -0.91 to 0.57), indicating an effect in favor of the control group.

We observe that one study took the mean middle of the vertical axis with an SMD value of 0.00 KG/m² after the use of probiotics contained in milk powder [24]. This means that there is no change in this index before and after therapeutic nutritional treatment in both groups, whether they received probiotics or not.

Then, two studies [23,26] took the right position of the vertical axis after the intervention of probiotic strains from the same source (SivoMixx) but with different high concentration doses (16×10 and 24×10^{11} CFU/per day). These showed SMD values of 0.37 KG/m² and 0.38 KG/m², indicating an effect in favor of the probiotic intervention experimental groups.

Finally, the marker consisting of the black diamond on the graph took a value of SMD=0.28 KG/m², a significant increase indicating a beneficial effect in the experimental group, which generally took probiotics. To prove the viability of my result, there is a recent study that shows the beneficial impact on the reduction of URTI symptoms by using probiotic strains (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*), the same ones used in our clinical experiments [42]. This study shows the impact on the body mass index in COVID-19 positive adults and children, resulting in weight loss and improvement of some metabolic parameters, which positively affect BMI.

T cells: The third parameter analyzed, T cells, are the basis of adaptive immunity, as they manufacture and aggregate several immune cells (CD4, CD8, NK), and help motivate B cells to manufacture antibodies against microbial agents [32].

For the evaluation of total T cells, four studies out of five include this parameter in the meta-analysis (Figure 6) [22-25]. The four studies occupy the left side in variable proportions on the SMD values in the following order: 0.01, 0.10, 0.19, 0.13 after treatment with the original probiotics (supplements, nutritional material sub-form, capsule), showing a significant effect in favor of the experimental group.

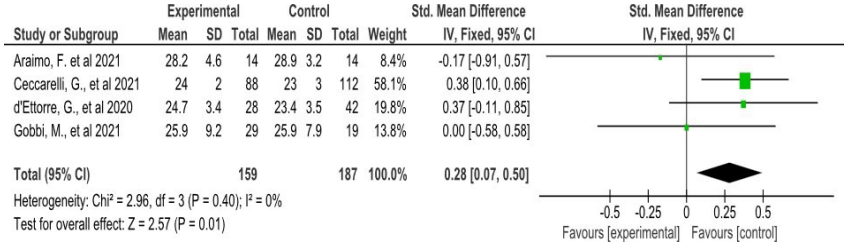

Figure 5: Forest plot showing the association between experimental probiotic use and other control interventions on BMI levels.

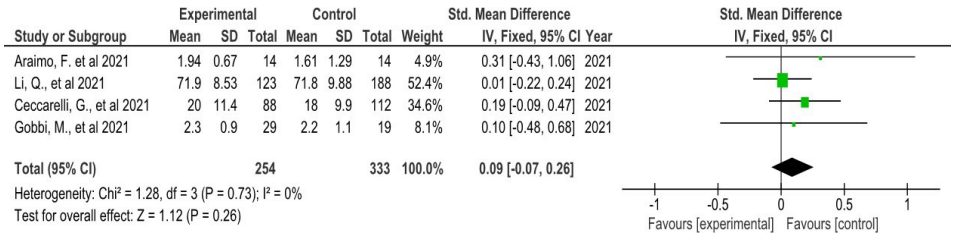

Figure 6: Forest plot showing the association between experimental probiotic use and other control interventions on T-cells levels.

In these four included studies, there is a diversity in effect and levels of the immune response in each study, as shown by comparison of SMD values. Although in two studies out of four the probiotics that are orally administered are from the same source (SivoMixx), they have values of (0.19 G/L), according to the study by [23] and (0.31 G/L) for the study by [25]. This leads to the important observation that this difference is a direct result of the doses administered: (24×10^{11} CFU/Per day>16×10 CFU/Per day), which results in these clearly distinct effects, i.e., when the amount of probiotics is increased, a more positive beneficial effect in the T-cell count can be observed; but the doses must, of course, respect the limits imposed by FAO and WHO in 2002 [43].

The overall effect size of the included SMD studies is estimated to be 0.09 G/L, and the increase is within the confidence interval of (-0.07 to 0.26), which translates to a significant increase in a small effect but is still the best possible index.

In fact, according to WHO (World Health Organization), COVID-19, when replicating in the cells of the organism, often uses subterfuge capacities against it, preventing the resistance of the immune cells, generally the T lymphocytes. When it penetrates the cells, it only displays its long external coronary receptors, which are of a total protein composition. In this sense, it allows the cells to grow as a protein support (energy), which makes them relax their defense, thus avoiding the involvement of T cells. Instead, the viral load continues to increase until the body collapses. The results show the role of probiotics in making a difference in increasing T cell levels, which are involved in resistance against all undesirable agents (as shown in our results indicating an immune response against COVID-19). Several reviews highlight one of the main characteristics of probiotics in modulating the immune system [44].

Albumin: For the determination of albumin levels, three out of five studies were included in the analysis (Figure 7), involving the intervention of probiotics and other therapeutic protocols such as hydroxychloroquine, tocilizumab, and O_2/O_3 administration, with the SMD values of each study amounting in order: -0.03, 0.00, 0.39 [23-25].

The study with the value of -0.03 indicates an effect in favor of the control group. The value of 0.00 shows no change in albumin levels in the two treated groups, and finally, the SMD value of 0.39 indicates an effect in favor of the experimental group that generally received probiotics.

The black diamond, summarizing the overall effect size in these experiments, shows an SMD value of 0.28 G/DL, indicating a significant increase in albumin levels. Moreover, the positive effect of this blood index in favor of the participants in the experimental groups who received probiotics is supported by the study [45]. The increase in albumin levels for those consuming probiotic intakes compared to the control group showed an effect of 0.2 G/DL up to 3.4 G/DL. This increase in albumin levels translates to good transport of enzymes, hormones, and many substances that provide the necessary elements for the body as they circulate in the blood [46].

IL-6: To determine the fifth parameter, interleukin 6, Two SMD values are provided after the analysis of two out of five studies in (Figure 8) [22,25]. The SMD values are as follows: A large effect in favor of the control group with 0.06, with a confidence interval between (-1.75 to -0.18), and another small effect compared to the experimental group.

The overall effect of the two SMD studies is 0.67, reflecting a significant increase in IL-6 levels. This indicates a statistically significant calculation in favor of the experimental groups, which received probiotics, in contrast to the control groups of these 2 studies included in this parameter, which did not receive this oral bacteriotherapy. This increase in IL-6 suggests good anti-inflammatory activities against COVID-19 in the probiotic experimental group.

LDH: Regarding lactate dehydrogenase, a total of two studies out of five were analyzed in (Figure 9), showing different positions on the left and right vertical axes after treatment with probiotics (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*) and Hydroxychloroquine, Lopinavir/Ritonavir, Tocilizumab, etc [22,23]. Analysis of the statistical data of these two studies after blood sampling, at the end of the treatment duration, yielded the following SMD values: -0.04 mmol/L (favoring the control group) and 0.23 mmol/L (favoring the experimental group).

The overall effect of this parameter analysis shows that the consumption of probiotics increased the LDH level to 0.12 mmol/L, representing almost a minimal effect in favor of the experimental group. This increase is statistically non-significant. The study by demonstrated a non-significant effect of probiotics [47], with results differing (showing a decrease in LDH levels treated by probiotics) from our study due to the reduced number of articles included in the meta-analysis of this index.

Ferritin: Finally, for ferritin, after the previously mentioned two-group treatment procedures, only two studies were included in the analysis out of five in (Figure 10) [24,25]. This illustrates that there are 2 vertical axes, each taking different positions, including SMD values rising from -0.17 mmol/L, according to the study by [24], in favor of the control group, and 0.86 mmol/L in favor of the test group, for patients with COVID-19 [25].

The overall effect size of the studies included in the analysis shows a significant increase effect in favor of the probiotic-treated groups, with the value of SMD 0.19 mmol/L. This indicates an increase in ferritin levels in favor of those consuming the probiotics. For example, in this included study, they consumed more (24×1011 CFU).

The increase in ferritin levels explains good availability of iron in the bloodstream to compete against respiratory infections, which are the main symptoms of COVID-19 (Figure 11).

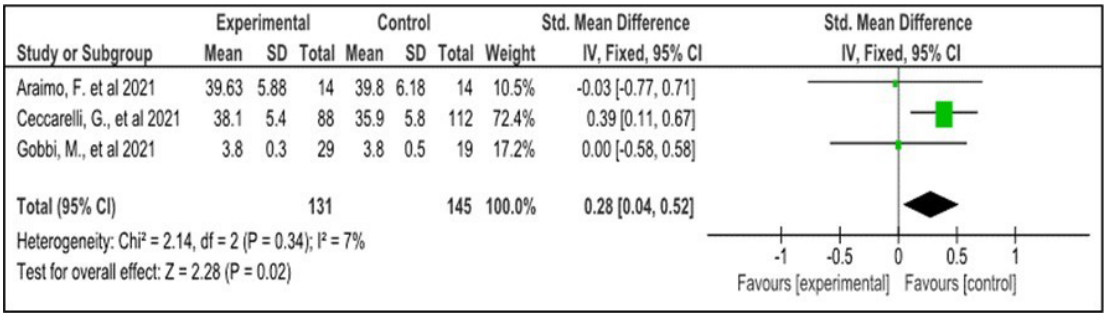

Figure 7: Forest plot showing the association between experimental probiotic use and other control interventions on albumin levels.

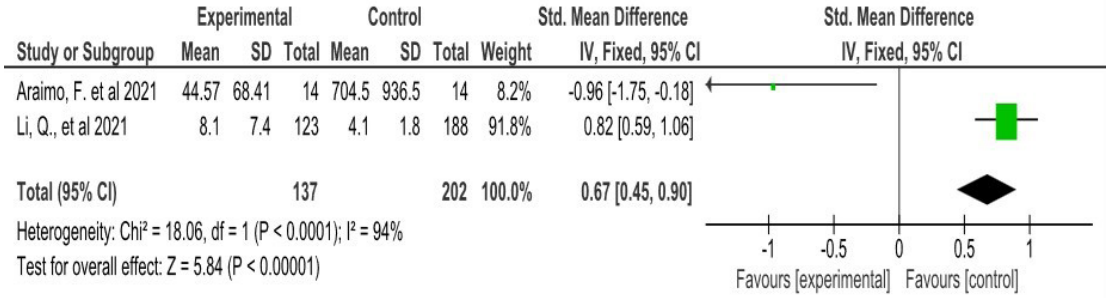

Figure 8: Forest plot showing the association between experimental probiotic use and other control interventions on IL-6 levels.

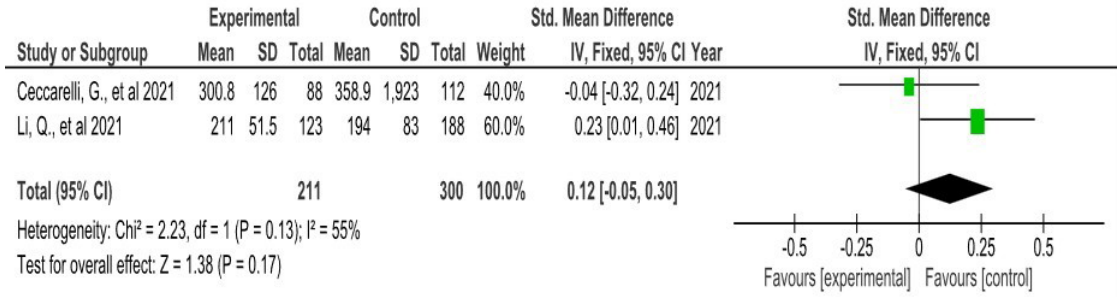

Figure 9: Forest plot showing the association between experimental probiotic use and other control interventions on LDH levels.

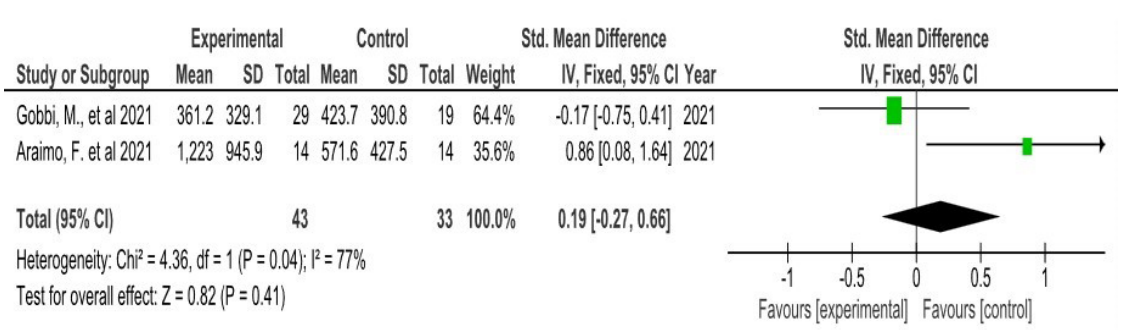

Figure 10: Forest plot showing the association between experimental probiotic use and other control interventions on ferritin levels.

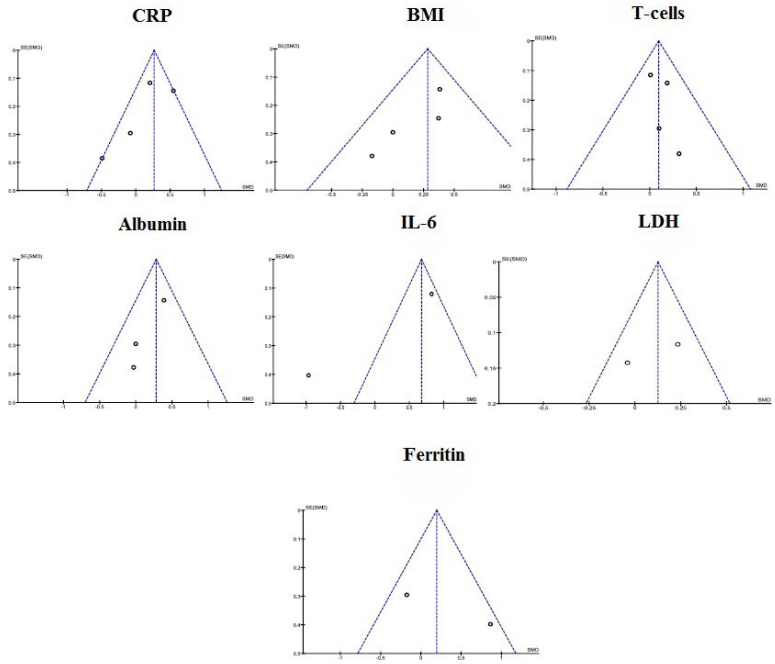

Figure 11: Forest plot showing the association between illustrates minimal indications of publication bias across the five selected studies.

Conclusion

In conclusion, probiotics, which have demonstrated effective roles in modulating the immune system and gut microbiota, are essential agents in the prophylaxis and treatment of SARS-CoV-2. Through the meta-analysis presented herein, involving the selection and filtration of 776 articles from seven databases, along with data extraction and statistical analysis of five included studies structured in seven parameters, we were able to assess the level of respiratory infections related to COVID-19, which is a main symptom. Quantitative analysis of data from the five included studies indicates a statistically non-significant increase in LDH levels and the inflammatory index, including CRP levels, with a lack of significance by the degree of heterogeneity (I^2=0%), and a statistically significant increase in each of the following parameters: BMI, T Cells, Albumin, IL-6, and Ferritin, with varying levels of heterogeneity ranging from low to high.

This meta-analysis, based on human randomized clinical trials, highlights the need for additional clinical data in the near future to fully identify the specific role of probiotics in SARS-CoV-2-related respiratory infections. Further investigation of RCTs needs to be conducted through additional research. During our search for eligible studies to be included in this analysis, we encountered significant difficulties in gathering sufficient information to provide a comprehensive overview of the issue. This problem can be addressed by conducting more specific and confidential experiments, with considerations for blinding of participants, investigators, and researchers, along with larger sample sizes and multicenter studies. Such efforts will contribute to improving our understanding of the efficacy of probiotics in combating COVID-19-related respiratory infections.

Declarations

Competing interests: All authors declare that they have no competing interests. All authors have no conflicts of interest to disclose.

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