STUDY PROTOCOL

Probiotic effect in preterm neonates with sepsis - A systematic review protocol [version 3; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background: The microbiota in the intestine is made up of trillions of living bacteria that coexist with the host. Administration of antibiotics during neonatal infection causes depletion of gut flora resulting in gut dysbiosis. Over the last few decades, probiotics have been created and promoted as microbiota management agents to enrich gut flora. Probiotics decrease the overgrowth of pathogenic bacteria in the gut of preterm neonates, reducing the frequency of nosocomial infections in the Neonatal Intensive Care Unit (NICUs).

Methods: The systematic review will include randomized control trials (RCTs) of preterm neonates with sepsis. Studies will be retrieved from global databases like Cochrane CENTRAL, CINAHL Plus via EBSCO host, MEDLINE via PubMed, EMBASE, SCOPUS, Ovid, Web of Science, ProQuest Medical Library, Microsoft academic, and DOAJ by utilizing database-specific keywords. Screening, data extraction, and critical appraisal of included research will be carried out separately by two review writers. Findings will be reported in accordance with the PRISMS-P 2020 guidelines.

Conclusions: The findings of this systematic review will help to...
translate the evidence-based information needed to encourage the implementation of potential research output in the field of neonatal intensive care, guide best clinical practise, assist policy making and implementation to prevent gut dysbiosis in neonates with sepsis by summarising and communicating the evidence on the topic.

**PROSPERO registration number:** This systematic review protocol has been registered in PROSPERO (Prospective Register of Systematic Reviews) on 10th March 2022. The registration number is CRD42022315980.

**Keywords**
Probiotics, Preterm, Neonatal Sepsis, Gut health, Mortality, Morbidity, Growth

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Introduction
According to the Global Burden of Disease (GBD), there are 1.3 million yearly incident cases of neonates sepsis and associated infections (about 937 instances per 100,000 live births) and 203,000 sepsis-related neonatal deaths. A preterm infants’ immature intestine predisposes it to infection and inflammation, as it has immature immunity, barrier function, and peristalsis resulting in gut dysbiosis. Co-morbidities like c-section deliveries, antenatal and post-natal antibiotic exposure, parenteral nutrition and prolonged stays in a neonatal intensive care unit (NICU), lead to a rapid decrease in taxonomic richness and diversity of good commensal gut bacteria contributing to the loss of colonization resistance. The gut is the centre of microbial activity; it is essential to address neonatal microbiota dysbiosis as many of the events like period of gestation, method of delivery, dietary patterns, weaning and antibiotic administration play an important role in framing gut microbiota and may have an impact on long term health outcomes. The host and gut microbiota have unique and cryptic interlinkage; the formation of an individual’s gut microbiota starts right from birth and is shaped by various factors. Starting from birth, the gut microbiota plays three critical roles: protective, metabolic, and trophic. Gut microbiota carries out their effects by modulating immunologic, endocrine, and neurological pathways.

The gut also serves as a significant source of noninflammatory immune stimulators in healthy people throughout their lives. However, these beneficial health-promoting factors of the gut microbiota are not infallible and can be altered due to dysbiosis or neonatal infection. Numerous studies have shown that antibiotic consumption causes dysbiosis and disturbance of the gut microbiome in infants, children and adults. Probiotics have been shown to improve the gut health of preterm infants. Probiotics are by definition: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. This definition is a consensus statement by the International Scientific Association for Probiotics and Prebiotics and aligns with the definition accepted by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) published in 2001 (FAO/WHO, 2001) and the guidelines to assist with this definition published in 2002 (FAO/WHO, 2002). Probiotics can enhance your immune system while preventing the growth pathogenic gut bacteria. A shift in the composite microbiota system has increased disease risk. The use of probiotics facilitates the restoration of the gut microbial profile. It may protect high-risk neonates by preventing the movement of microbes and microbial metabolites across the mucosa, elimination of pathogenic organisms, modifying host reactions to microbial supplements and enhancing nutrition by upregulation of immune response. A relative reduction in the Bifidobacteriaceae counts and an increase in Enterobacteriaceae and Clostridiaceae may be a good criterion to define dysbiosis in the initial months of life. It can also explain gut colonization between birth, which can serve as the basis for introducing nutritional strategies targeted at the microbiota.

These nutritional interventions, based mainly on probiotics, may be used for favourably altering the gut microbiome and preventing neonatal sepsis. Dysbiosis of the gut microbiome invites the expansion of the pathobiont population, leading to sepsis in neonates. Probiotic supplementation aims to rebuild the gut bacteria, preventing necrotizing enterocolitis (NEC), infection and other problems. The initial days of life are crucial for an adequate microbiome that facilitates gut maturation and neonatal health. Therefore, strategies focused primarily on establishing microbiota and consuming probiotic supplements that can help balance the gut microbiota composition, improve well-being, and lower disease risk in neonates. Thomas et al. (2023) conducted a Bayesian network meta-analysis suggesting that probiotics may significantly reduce sepsis and mortality rates in VLBW neonates, emphasizing their importance in resource-limited settings. This review included 29 RCTs with 4,906 neonates and 24 probiotics, but only 11 studies had a low risk of bias. Probiotics were compared with placebos, not with each other. The combination of B. longum, B. bifidum, B. infantis, and L. acidophilus may reduce mortality (RR 0.26; 95% CrI 0.07 to 0.72), sepsis (RR 0.47; 95% CrI 0.25 to 0.83), and NEC (RR 0.31; 95% CrI 0.10 to 0.78), though evidence is uncertain. B. lactis alone may also reduce mortality (RR 0.21; 95% CrI 0.05 to 0.66) and NEC (RR 0.09; 95% CrI 0.01 to 0.32), but evidence is low-certainty.
systematic review, highlighted both the benefits and potential risks of probiotic use, stressing the need for rigorous trials to confirm safety and efficacy.\textsuperscript{20} Despite these comprehensive reviews, gaps remain in the evidence regarding the optimal strains, dosages, and treatment durations of probiotics, as well as their long-term effects on neonatal health. This systematic review protocol aims to address these gaps by synthesizing current evidence on the probiotic effect in preterm neonates with sepsis.

**Description of the intervention?**

Probiotics supplements help revive the disrupted gut microbiota and prevent gut inflammation and other intestinal diseases.\textsuperscript{21} Probiotics decrease the overgrowth of pathogenic bacteria in the gut of preterm neonates, reducing the frequency of nosocomial infections in the Neonatal Intensive Care Unit (NICU).\textsuperscript{22} According to systematic evaluations of randomized controlled trials (RCT), probiotics have substantial potential to lower mortality and morbidity in premature neonates. A systematic assessment of RCT narrated the benefits of probiotics in low- and middle-income countries.\textsuperscript{11} The supplements may restore the composition of the gut microbiome and introduce beneficial functions to gut microbial communities. According to recent randomized controlled research, early probiotic use may have the capacity to alter the gut microbiota during antibiotic treatment recovery.\textsuperscript{23}

Probiotics are becoming more well acknowledged as a valuable tool for promoting health and preventing adverse health outcomes in premature neonates.\textsuperscript{24,25} They may protect high-risk neonates by increasing the barrier for migration of bacteria and their products across the mucosa, by elimination of potential pathogens, by modifying host response to microbial products, and enhancing nutrition by upregulation of the immune response.\textsuperscript{26} This study aims to evaluate the effect of probiotics on the gut health and growth of preterm infants in neonates with sepsis. We hypothesized that the administration of specific probiotics could improve the gut microbiota of preterm neonates by lowering the risk of morbidity and mortality with sepsis.

**How might the intervention work?**

Over the last few decades, probiotics have been created and promoted as microbiota management agents to enrich gut microbiota. Early life is a crucial time for the gut of neonates to be gradually colonised with different species of bacteria that collectively promote initial gut maturation. Bacterial diversity in preterm neonates is low and comparatively different from term neonates.\textsuperscript{21} Probiotic supplements may help to restore the gut microbiome composition and introduce beneficial functions to gut microbial communities, resulting in the improvement or prevention of inflammation of the gut and other phenotypes of systemic or intestinal diseases.\textsuperscript{27}

Probiotic supplement in preterm has been proven to decrease pathogen overgrowth and promote the maturation of the intestinal mucosa by facilitating the growth and proliferation of probiotic bacteria in the intestinal tract.\textsuperscript{28} Antibiotic exposure was observed to significantly alter gut microbiota, with a considerable reduction in *Bifidobacterium* and *Lactobacillus*, according to Zhong et al., 2021.\textsuperscript{21} The administration of probiotics concurrently with antibiotics was more advantageous to the gut microbiota than waiting until after antibiotic therapy to use probiotics, especially in terms of increasing *Bifidobacterium* abundance.\textsuperscript{23} Pneumonia and sepsis accounted for 46% of all admission diagnoses and were attributed to 29% of the overall death rate, compared to 26% globally.\textsuperscript{29}

The mode of delivery influences the gut microbial pattern. Several high-quality studies suggest that bacterial diversity differs among babies based on the mode of delivery. Neonates born by vaginal delivery swallow the vaginal bacteria on their way down the birth canal, which results in the primary source for initial seeding of microbiota to the neonatal gut. Over the initial year of life, there is an abundance of *Bifidobacterium* spp. and a decrease of *Enterococcus* spp. and *Klebsiella* spp.\textsuperscript{31} Microbiota of caesarean section neonates show distinctly different gut microbiota than vaginally delivered infants and are more likely to have skin, breastmilk, oral and environmental bacteria.\textsuperscript{31}

**Why it is important to do this review**

Infections spread rapidly in preterm infants, resulting in severe disease and death; therefore, infection prevention directly reduces neonatal morbidity and mortality. In a systematic review conducted by Balasubramanian et al. among preterm infants in India, metaanalysis showed a significantly lower risk in blood culture positive Late-Onset Sepsis (LOS) after 48 hours of birth in the probiotic group (p < 0.001).\textsuperscript{32} A double-blinded, placebo-randomized controlled trial was conducted using *Bacillus clausii* to prevent LOS in 244 preterm infants. Of that, 120 were extremely preterm neonates, of which 59 received a placebo and 61 received probiotics. On other hand, 124 babies were stratified as preterm, 61 neonates received a placebo and 61 received probiotics. The study concluded that prophylactic administration of *B. clausii* to preterm neonates did not result in a significant difference in LOS incidence compared with placebo.\textsuperscript{32}

The gut microbiome is a complex and dynamic population of hundreds of bacteria responsible for transporting nutrition, controlling intestinal epithelial maturation, and building an innate immune defence in neonates. An RCT
was conducted by Panigrahi et al. to prevent sepsis among rural Indian neonates. Researchers used *Limosilactobacillus plantarum* plus fructooligosaccharide as a probiotic on 4,556 neonates of birthweight < 2000 grams, gestational age ≥ 35 weeks with no sign of sepsis and morbidity and were recruited and monitored for 60 days; a significant decrease in neonatal sepsis (culture-positive sepsis, culture-negative sepsis) and lower respiratory tract infections were observed. The study’s findings suggested that neonatal sepsis in developing countries could be effectively prevented using a symbiotic containing *L. plantarum* ATCC-202195. The findings of this systematic review would definitively answer whether early administration of specific probiotics could improve the gut microbiota of preterm neonates by lowering the risk of infant morbidity and mortality in neonates with sepsis, and assist healthcare providers and policymakers in developing a probiotic supplement guideline for sepsis neonates.

**Methods**

**Ethical considerations**
As this is a systematic review protocol, ethical approval is not required as we will not be directly involving human participants.

**Review questions**
How effective is probiotics in lowering the risk of morbidity and mortality among preterm neonates with sepsis?

**Specific objectives**
To determine the impact of probiotics on lowering the risk of morbidity and mortality among preterm neonates with sepsis.

**Design**
We will systematically review existing randomized and non-randomized control trial studies and execute a meta-analysis when acceptable data is available. This protocol follows the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analyses of Protocols (PRISMA-P) 2015. The Participants, Interventions, Comparators and Outcomes elements used for the systematic review are listed in Table 1.

**Search strategy**
A comprehensive search would be conducted in global databases like Cochrane CENTRAL, CINAHL Plus via EBSCO host, MEDLINE via PubMed, EMBASE, SCOPUS, Ovid, Web of Science and ProQuest Medical Library by utilizing the stated search keywords: “Premature neonates”, “Preterm birth”, “Very low birth weight”, “Low birth weight”, Preterm, “Preterm infants”, “extremely low birth weight”, “Neonates”, “Neonatal infection”, Infection*, “Late-onset sepsis”, “Early-onset sepsis”, Sepsis, “Neonatal period”, “intestinal infection”, “Nosocomial infection”, septicaemia, Bacteraemia, “septic shock”, probiotic*, “Probiotic supplement”, Lactobacilli, Lactobacillus, Bifidobacterium, “Lactic acid

| Table 1. Eligibility criteria. NICU: Neonatal Intensive Care Unit; NRCT's: Non-randomized controlled trials. |
|-------------------------------------------------|-------------------------------------------------|
| **Inclusion and exclusion criteria**             |                                                  |
| **PICO component**                               | **Inclusion criteria**                          | **Exclusion criteria**                          |
| Population (P)                                   | Preterm neonates (Gestational age <36 weeks) with sepsis. | Term neonates and preterm neonates without sepsis. |
| Intervention (I)                                 | All types of probiotic supplements administered to preterm with sepsis. | Administered prebiotics and synbiotic supplements to preterm with sepsis. |
| Comparison (C)                                   | Placebo or standardized NICU treatment.         | Prebiotics and synbiotic supplements.           |
| Outcome (O)                                      | **Primary outcome:** Morbidity, and mortality |                                                  |
|                                                  | **Secondary outcome:** Length of NICU Stay      |                                                  |
| Time (T)                                         | Articles published from January 2010 to December 2022 with full text in English language. |                                                  |
| Study design (S)                                 | RCTs, NRCT’s.                                   | Observational studies, Qualitative studies, reviews, editorials, conference abstracts, study protocols, reports, and letters. |
Search for other resources

Additional references will be found in the reference lists of all primary research and review papers. To get necessary supplementary information, we will reach out to relevant field experts and researchers from the included papers. For data management, records will be exported to EndNote X7.

Selection of studies

Two review authors (FI, SN) shall execute independent searches of the study titles and abstracts of the indicated study sources. We shall exclude the articles which do not meet the inclusion criteria. If there is a disagreement among those reviewers, it will be resolved through conversation, and the complete text will be examined. Two reviewers will independently assess the included abstracts as “include” or “exclude” after obtaining the whole manuscript. If required, we will work with a supervisory review author (LEL) to address any disagreements. We will keep track of why the articles were refused. We will look for and eliminate duplicates and compile numerous reports from the same research.

Data extraction and management

Data will be extracted by examining general features such as gestational age, research participants, setting, identifiers, type of pathogen isolated, LOS, early-onset sepsis (EOS), type of probiotic, study selection criteria, and results. To organize the list of intervention parameters to assist intervention replication and research comparability, we will use the TIDier checklist (Template for Intervention Description and Replication). Additionally, we will extract data of specific characteristics such as type of probiotic used, dosage, antibiotic exposure, gestational age and type of delivery. Two review authors will independently use the data extraction templates to abstract the data from included articles in the review. The included studies will have the following data collected: research title and authors, sample size, study setting, intervention features, assessment procedures, outcomes, findings, conclusions, and year of publication. If there is a disagreement, we will discuss it until we reach an agreement, or we will handle it with the assistance of a third reviewer (MKR).

Assessment of quality of the studies

We will assess the quality of included randomized controlled trials using the Revised Cochrane Risk of Bias Tool (RoB 2.0). The RoB 2.0 tool considers the following factors: a) The randomization method, b) deviation from the planned intervention, c) outcome measurement, d) missing outcomes, and e) selective reporting. Two authors will independently evaluate each paper. RCT risk will be assessed and classified into three categories: low risk, high risk, and some concerns. To assess the quality of the included non-randomized controlled trials, we will use the Joanna Briggs Institute (JBI) Critical Appraisal Checklist. If there is any disagreement, we will solve it by contacting a methodology expert (BSN).

Dealing with missing data

We will contact the primary authors whenever possible to obtain the missing data. The most important empirical data, including screening, randomization, intention-to-treat, as-treated, and per-protocol groups, will be investigated thoroughly. The article will be excluded from the review if the authors do not respond within two weeks after communicating through email. If this is not possible, we will consider the missing data a major bias, and the article will be removed from consideration.

Data synthesis

Two independent reviewers (FI and SN) will extract the data from each study and enter it into a Microsoft Excel file Version 16.61. Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) standards will be used to provide evidence regarding the effect of probiotics on the gut health of preterm neonates with sepsis (Extended data).

If there is sufficient homogeneity in study design and study subjects among the selected studies, meta-analyses will be conducted. As a result, continuous and dichotomous outcomes will be integrated for meta-analysis purposes. Statistical analyses will be conducted with the help of the Cochrane Review Manager (RevMan 5).

Sensitivity analysis

Sensitivity analysis will be performed to investigate the robustness of the results by examining the effects of including and excluding studies with a high risk of bias and studies with missing data. The results’ robustness will be evaluated using a variety of impact size measures, such as risk ratio and odds ratio, and various statistical models, such as fixed effects and random-effects models.
**Discussion**

The microbiota in the intestine is made up of trillions of living bacteria that coexist with the host. Depleting gut microbiota in neonates, especially in vulnerable preterm neonates, may elevate the risk of neonatal infections resulting in administration of antibiotics. Antibiotics are pharmacological agents that destroy the bacteria cells, but not viruses that are also possible gut pathogens. Probiotic supplementation has become increasingly popular in the fight against gut microbiota depletion.

The impact of probiotics on neonatal health has been extensively studied, and several randomized controlled trials have demonstrated significant benefits. A randomized control trial was conducted by Zhong et al. to assess the impact of probiotic supplements on 90 neonates with gestational age ≥ 37 weeks. They concluded that antibiotics cause a decrease in the microbial richness and variety of the gut microbiota in neonates and the attenuation of several bacteria, especially *Bifidobacterium* and *Lactobacillus*. A significant reduction in newborn sepsis has been reported in randomized controlled trials (RCTs) of various probiotic strains and combinations given to preterm infants of different gestational ages and birth weights. Panigrahi et al., in their randomized control trial, examined the effect of probiotics in neonatal sepsis and showed a significant reduction in neonatal sepsis cases when supplemented with *Limosilactobacillus plantarum*. Costelo et al. evaluated the effect of *Bifidobacterium breve* strain BBG-001 on the development of sepsis or NEC in very low birth weight neonates. At two weeks postnatal age, he observed *B. breve* colonisation in 1186 (94%) survivors. There were 85% in the probiotic group and 37% in the placebo group.

**Strengths and limitations of the study**

This systematic review will include randomized controlled trials and all types of non-randomized control trials. Meta-analysis will be carried out with the results of RCTs, and narrative description will be used to analyse non-randomized control trials regarding the effect of probiotics among preterm neonates with sepsis. This review includes only the use of probiotics in preterm infants, excluding the studies that assessed the effect of prebiotics and synbiotics among preterm neonates. Only studies published in English will be included; thus, eligible studies published in other languages will be excluded.

**Data availability**

**Underlying data**

No underlying data are associated with this article

**Extended data**

Figshare: supplementary_file1.docx. https://doi.org/10.6084/m9.figshare.19839604

This project contains the following extended data:

- Supplementary_File1.docx (Appendix 1 - Search Strategy; Appendix 2 – Search Terms)

Figshare: Supplementary file 2. https://doi.org/10.6084/m9.figshare.19839604

This project contains the following extended data:

- Supplementary_file2.pdf (PRISMA flow chart of Systematic review)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Reporting guidelines**

Figshare: PRISMA-P checklist for ‘Probiotic effect in preterm neonates with sepsis - A systematic review protocol’, https://doi.org/10.6084/m9.figshare.19839241

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Open Peer Review

Current Peer Review Status:  

Yao Wang  
The Chinese University of Hong Kong, Hong Kong, Hong Kong

Sepsis is a severe condition that threatens fetal health. Applying probiotics is a feasible and potential approach to prevent this disease. Therefore, this study is meaningful with significant clinical implications. The methods and study design are clear and reasonable. Importantly, the authors will extract specific data such as the type of probiotic used, dosage, antibiotic exposure, gestational age, and type of delivery.

One suggestion is that the details of probiotics should be clearly documented at the species or strain level if the data allows. Moreover, the prescribed model, including the starting point of administration during pregnancy and the duration of probiotic use, should also be included. All these contents are crucial to help clinicians to understand and make decisions. An example study is Bekalu et al., AJOG FMF 2023. 10.1016/j.ajogmf.2023.101148.

I am overall content if the authors could further improve above comments.

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Is the rationale for, and objectives of, the study clearly described?  
Yes

Is the study design appropriate for the research question?  
Yes

Are sufficient details of the methods provided to allow replication by others?  
Yes
Are the datasets clearly presented in a useable and accessible format?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiome, Maternal-fetal health, Exercise Intervention, RCT,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Reviewer Report 13 June 2024**

https://doi.org/10.5256/f1000research.157165.r278782

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**Sabina Fijan**
Faculty of Health Sciences, University of Maribor, Maribor, Slovenia

**Probiotic effect in preterm neonates with sepsis - A systematic review protocol: Review report**

This manuscript, submitted to the journal f1000Research, is a crucial topic and describes the protocol to conduct a systematic review of the probiotic effect in preterm neonates with sepsis.

**General comments**

Recent systematic reviews on this subject have been published and should be referenced with the rationale for conducting similar research (Kulkarni et al., 2022; Thomas et al., 2023).

Probiotics are by definition: **“live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”** (Hill et al., 2014). This definition is published as a consensus statement by the International Scientific Association for Probiotics and Prebiotics and should be referenced when citing the correct definition. This definition is in line with the definition accepted by the Food and Agriculture Organization of the United Nations and the World Health Organisation (FAO/WHO) published in 2001 (FAO/WHO, 2001) and the guidelines to assist with this definition published in 2002 (FAO/WHO, 2002).

The human microbiota comprises of bacteria, archaea, viruses, fungi and other eukaryotes that coexist in and on various sites of the human body (Hou et al., 2022), while the microbiome is the collection of all genetic information of this microbiota (Grice & Segre, 2012; Ogunrinola et al., 2020). The term ‘flora’ is outdated and should be replaced by the taxonomically correct term...
When comparing the modulation of gut microbiota, the abundance or ratio of certain phyla is usually compared (Walsh et al., 2014). However, it is necessary to use up-to-date nomenclature (Oren & Garrity, 2021). Firmicutes are now Bacillota, Proteobacteria are now Pseudomonadota, Actinobacteria are now Actinomycetota, Bacteroidetes are now Bacteroidota etc.

Up-to-date terminology should be used for lactobacilli (Zheng et al., 2020). This genus *Lactobacillus* has been divided and now consists of 25 genera. It is necessary to include the new nomenclature of many species that contain probiotic strains, for example: *Limosilactobacillus reuteri* (previously *Lactobacillus reuteri*), *Limosilactobacillus reuteri* (previously *Lactobacillus reuteri*), *Lactiplantibacillus plantarum* (previously *Lactobacillus plantarum*), *Levilactobacillus brevis* (previously *Lactobacillus brevis*), *Lactcaseibacillus rhamnosus* (previously *Lactobacillus rhamnosus*), *Ligilactobacillus salivarius* (previously *Lactobacillus salivarius*). The new nomenclature can be found at the website: http://lactotax.embl.de/wuyts/lactotax/. Other genera that also include probiotic strains such as *Bacillus*, *Clostridium*, *Propionibacterium* have also undergone taxonomic reclassification (Gupta et al., 2020; Lawson et al., 2016; Scholz & Kilian, 2016).

Another important factor is the meaning of probiotics. Only certain strains are probiotic microbes. The strains that are labeled as a probiotic must have at least one quality double blind randomised controlled clinical study that exhibits a statistically significant difference in the group that was supplemented with probiotics compared to the placebo group (Binda et al., 2020). And in many instances the health benefits are strain-specific, therefore strain information must be included. For example, *Lactcaseibacillus rhamnosus* GG (previously known as *Lactobacillus rhamnosus* GG) is a well-known probiotic with several proven health benefits (McFarland et al., 2021; Tan et al., 2021). However, this does not mean that all strains of the species *Lactcaseibacillus rhamnosus* are probiotics, let alone all lactobacilli. The same applies for *Limosilactobacillus reuteri* strains, *Lactiplantibacillus* strains, and many others.

**Minor corrections**
- Introduction, paragraph 3: please correct the sentence to ‘probiotics can enhance your immune system while preventing the growth pathogenic gut bacteria’.
- Assessment of quality of the studies: please add the foreseen Joanna Briggs Institute Critical appraisal tool checklist for randomised controlled trials.
- Discussion: paragraph 1: please use accurate phrases. antibiotics are pharmacological agents that destroy the bacteria cells (Patel P et al., 2023), but not viruses that are also possible gut pathogens.
- Discussion: paragraph 1: some connective sentences should be included, not just the presentation of results of randomised controlled trials. When referring to the conductors of clinical trials please use the term ‘they’.

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**Is the rationale for, and objectives of, the study clearly described?**
Yes

**Is the study design appropriate for the research question?**
Yes
Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: probiotics, beneficial microbes, fermented foods, health benefits

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Jun 2024

Faiza Iqbal

Thank you for your detailed review and constructive feedback. We have addressed each of your comments as follows:

Comment #1: Recent systematic reviews on this subject have been published and should be referenced with the rationale for conducting similar research (Kulkarni et al., 2022; Thomas et al., 2023).
Authors Reply: Recent systematic reviews by Kulkarni et al. (2022) and Thomas et al. (2023) has referenced in the introduction to provide context and rationale for conducting similar research.

Comment #2: Probiotics are by definition: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host“ (Hill et al., 2014). This definition is published as a consensus statement by the International Scientific Association for Probiotics and Prebiotics and should be referenced when citing the correct definition. This definition is in line with the definition accepted by the Food and Agriculture Organization of the United Nations and the World Health Organisation (FAO/WHO) published in 2001 (FAO/WHO, 2001) and the guidelines to assist with this definition published in 2002 (FAO/WHO, 2002).
Authors Reply: The definition of probiotics has been updated to include the consensus statement by Hill et al. (2014) and references to the FAO/WHO definitions from 2001 and 2002.

Comment #3: The human microbiota comprises of bacteria, archaea, viruses, fungi and other eukaryotes that coexist in and on various sites of the human body (Hou et al., 2022), while the microbiome is the collection of all genetic information of this microbiota (Grice & Segre, 2012; Ogunrinola et al., 2020). The term 'flora' is outdated and should be replaced by the taxonomically correct term 'microbiota.'
Authors Reply: The term ‘flora’ has been replaced with ‘microbiota' throughout the manuscript.
Comment #4: When comparing the modulation of gut microbiota, the abundance or ratio of certain phyla is usually compared (Walsh et al., 2014). However, it is necessary to use up-to-date nomenclature (Oren & Garrity, 2021). Firmicutes are now Bacillota, Proteobacteria are now Pseudomonadota, Actinobacteria are now Actinomycetota, Bacteroidetes are now Bacteroidota etc.

Authors Reply:
Up-to-date nomenclature for bacterial phyla and genera has been adopted, following the guidelines by Oren & Garrity (2021) and Zheng et al. (2020).

Comment #5: Up-to-date terminology should be used for lactobacilli (Zheng et al., 2020). This genus Lactobacillus has been divided and now consists of 25 genera. It is necessary to include the new nomenclature of many species that contain probiotic strains, for example: Limosilactobacillus reuteri (previously Lactobacillus reuteri), Limosilactobacillus reuteri (previously Lactobacillus reuteri), Lactiplantibacillus plantarum (previously Lactobacillus plantarum), Levilactobacillus brevis (previously Lactobacillus brevis), Lacticaseibacillus rhamnosus (previously Lactobacillus rhamnosus), Ligilactobacillus salivarius (previously Lactobacillus salivarius). The new nomenclature can be found at the website: http://lactotax.embl.de/wuyts/lactotax/. Other genera that also include probiotic strains such as Bacillus, Clostridium, Propionibacterium have also undergone taxonomic reclassification (Gupta et al., 2020; Lawson et al., 2016; Scholz & Kilian, 2016).

Authors Reply:
Modified accordingly

Comment #6: Another important factor is the meaning of probiotics. Only certain strains are probiotic microbes. The strains that are labeled as a probiotic must have at least on quality double blind randomised controlled clinical study that exhibits a statistically significant difference in the group that was supplemented with probiotics compared to the placebo group (Binda et al., 2020). And in many instances the health benefits are strain-specific, therefore strain information must be included. For example, Lacticaseibacillus rhamnosus GG (previously known as Lactobacillus rhamnosus GG) is a well-known probiotic with several proven health benefits (McFarland et al., 2021; Tan et al., 2021). However, this does not mean that all strains of the species Lacticaseibacillus rhamnosus are probiotics, let alone all lactobacilli. The same applies for Limosilactobacillus reuteri strains, Lactiplantibacillus strains, and many others.

Authors Reply:
Modified accordingly in manuscript

Minor corrections

Comment #7: Introduction, paragraph 3: please correct the sentence to 'probiotics can enhance your immune system while preventing the growth pathogenic gut bacteria'.

Authors Reply:
The sentence has been corrected to "Probiotics can enhance your immune system while preventing the growth of pathogenic gut bacteria."

Comment #8: Assessment of quality of the studies: please add the foreseen Joanna Briggs Institute Critical appraisal tool checklist for randomised controlled trials.
**Authors Reply:**

For the assessment of the quality of randomized controlled trials (RCTs) used in our study, we have utilized the Revised Cochrane Risk of Bias Tool (RoB 2.0), not the Joanna Briggs Institute Critical Appraisal Tool. The methodology section has been updated to reflect this, ensuring clarity on the quality assessment process used in our research.

**Comment #9:** Discussion: paragraph 1: please use accurate phrases. Antibiotics are pharmacological agents that destroy the bacteria cells (Patel P et al., 2023), but not viruses that are also possible gut pathogens.

**Authors Reply:**
The sentence has been corrected.

**Comment #10:** Discussion: paragraph 1: some connective sentences should be included, not just the presentation of results of randomised controlled trials. When referring to the conductors of clinical trials please use the term ‘they’.

**Authors Reply:**
Connective sentences has been added for clarity, and when referring to the conductors of clinical trials, the term ‘they’ has been used.

We hope these revisions will adequately address your concerns and improve the clarity and accuracy of our manuscript. Please let us know if any further modifications are required.

Thank you for your valuable feedback and guidance.

**Competing Interests:** No Competing Interests
Not applicable

Is the study design appropriate for the research question?
Not applicable

Are sufficient details of the methods provided to allow replication by others?
Not applicable

Are the datasets clearly presented in a usable and accessible format?
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** My area of expertise is on clinical nutrition specifically chemical and microbiological composition of human milk.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Author Response 30 Oct 2023

**Faiza Iqbal**

Dear Dr. Cecile Leah T Bayaga,

Thank you for your feedback and for taking the time to review our systematic review protocol. We appreciate your comments and suggestions.

We have carefully considered your previous feedback and have made efforts to improve the clarity and focus of the protocol.

Regarding your suggestion to compare the protocol with a checklist on STROBE, we appreciate the recommendation for a structured evaluation. However, it's important to note that STROBE is primarily designed for reporting observational studies, and our protocol is focused on the systematic review protocol. Hence, we have utilized the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) checklist, which is tailored to assess the quality and completeness of systematic review protocols.

We have written the protocol based on the PRISMA-P checklist to ensure that it adheres to recognized standards for systematic review protocol, which is provided in Extended data under reporting guidelines. You can find the checklist at this link: https://doi.org/10.6084/m9.figshare.19839241.

Once again, we appreciate your valuable feedback, and if you have any further comments or suggestions, please feel free to share them.
Sincerely,
Faiza Iqbal

**Competing Interests:** No competing interest

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**Version 1**

**Reviewer Report 14 September 2023**

https://doi.org/10.5256/f1000research.134189.r198892

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**Cecile Leah T Bayaga**

Department of Food Science and Nutrition, College of Home Economics, University of the Philippines, Quezon City, Philippines

**General Observation**

From my perspective, the paper is unfinished. There are no results of the systematic review. The tense used is in the future; hence it will still be implemented.

**Specific Observation**

**Abstract**

Following the PRISMA guidelines, the abstract lacks the following details:

1. Explicitly stated objective
2. Inclusion/Exclusion criteria
3. Methods on how bias was lessened
4. Methods on how data were synthesized
5. No major results and discussion
6. No mention of funding agency

**Introduction**

First, majority of the systematic reviews published do not have subsections under this portion. Having sub sections probably is beneficial when the author writes the Introduction. However, reading is made smooth if there were no sub sections. Second, the specific objective which was supposed to be in this section was found in the Methods section. Last, the stated objective of the study does not jive with the stated hypothesis. The premise “growth of preterm infants” was included in the objective but absent in the hypothesis.
Methods
There is a need to refine the methods to indicate it has been implemented, make the connection seamless, and revise the statements to make them clearer.

Discussion
There was no discussion as there were no results presented.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Partly

Are sufficient details of the methods and analysis provided to allow replication by others?
No

Is the statistical analysis and its interpretation appropriate?
No

Are the conclusions drawn adequately supported by the results presented in the review?
No

If this is a Living Systematic Review, is the ‘living’ method appropriate and is the search schedule clearly defined and justified? (‘Living Systematic Review’ or a variation of this term should be included in the title.)
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My area of expertise is on clinical nutrition specifically chemical and microbiological composition of human milk.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 14 Sep 2023
Faiza Iqbal

Dear Reviewer,

Thank you for taking the time to review our systematic review protocol. We appreciate your feedback and would like to address your observation that the paper appears unfinished due to the absence of results and the use of future tense.

It’s essential to clarify that a systematic review protocol is a prelude to the actual systematic review. Protocols are written before the systematic review and data extraction phases commence, and they serve as a comprehensive plan that outlines the steps and procedures
that will be followed during the review process.

The use of future tense in the protocol is intentional and necessary because, protocols are typically written before starting the search and data extraction process. At this stage, the review team has not yet gathered and analyzed the data, so it would be premature to present any results. Instead, the protocol serves as a blueprint for the systematic review, detailing the research questions, eligibility criteria, search strategy, data extraction methods, and analysis plan.

Rest assured that once the systematic review is completed, we will publish the results in a separate paper or report, where we will present and discuss our findings based on the data collected and analyzed during the review process. This follow-up publication will provide a comprehensive overview of the results and their implications.

We hope this clarifies the purpose and scope of our systematic review protocol. We value your input and appreciate your understanding of the process. If you have any further questions or suggestions, please feel free to share them.

Sincerely,
Faiza Iqbal

Competing Interests: No competing interests