Effect of probiotics on the intestinal microbiota of preterm and low birth weight infants: systematic review

Efeito dos probióticos no microbiota intestinal de bebês prematuros e com baixo peso ao nascer: revisão sistemática

Efecto de los probióticos en la microbiota intestinal de los recién nacidos prematuros y de bajo peso al nacer: revisión sistemática

DOI:10.34119/bjhrv7n2-184

Originals received: 02/19/2024
Acceptance for publication: 03/08/2024

Laressa Rios Cardoso
Master in Pharmaceutical Sciences
Institution: Universidade Estadual de Feira de Santana
Address: Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana - Bahia, CEP: 44036-900
E-mail: laressa_rios@hotmail.com

Letícia Cerqueira Pereira
Master's student in Pharmaceutical Sciences
Institution: Universidade Estadual de Feira de Santana
Address: Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana - Bahia, CEP: 44036-900
E-mail: leticia.cerqueira@hotmail.com

Andressa Rios Cardoso
Master in Pharmaceutical Sciences
Institution: Universidade Estadual de Feira de Santana
Address: Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana - Bahia, CEP: 44036-900
E-mail: andressariosc@gmail.com

Kaio Vinicius Freitas de Andrade
PhD in Public Health
Institution: Universidade Estadual de Feira de Santana
Address: Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana - Bahia, CEP: 44036-900
E-mail: kaiovinnicius@yahoo.com.br

Manoelito Coelho dos Santos Junior
PhD in Biotechnology
Institution: Universidade Estadual de Feira de Santana
Address: Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana - Bahia, CEP: 44036-900
E-mail: manoelito@uefs.br

ABSTRACT
Premature infants have an immature intestinal microbiota when compared to a full-term baby, colonized with a reduced number of beneficial bacterial species and, therefore, are more likely to have their microbiota populated by pathogenic species. The administration of probiotics can positively influence the intestinal colonization of premature babies. Thus, this study aimed to systematically review evidences of the effect of administering probiotics on the microbiota of premature newborns and their safety. It was designed as follows: population (premature newborns), intervention (probiotics), comparison (placebo or no treatment), outcome (changes in the microbiota of premature newborns), study type (randomized clinical trials). The studies were searched in the Cochrane Library, Medline via PubMed and Embase databases and, in a complementary way, through manual searches on Google Scholar and the Brazilian CAPES journal portal (www.periodicos.capes.gov.br). We included 23 studies involving 3,670 preterm infants, of which 65.2% (n=15) were classified as having a low risk of bias, 17.4% (n=4) with some risk of bias and 17.4% (n= 4) with high risk of bias. Probiotics have been used in order to colonize the intestinal microbiota. Finally, some *Lactobacillus* and *Bifidobacterium* strains tested seem to have benefits and safety for the microbiota and health of premature newborns.

Keywords: effect, probiotics, premature, microbiota, *Bifidobacterium*, *Lactobacillus.*

RESUMO
Bebês prematuros possuem microbiota intestinal imatura quando comparados a bebês a termo, colonizados por um número reduzido de espécies bacterianas benéficas e, portanto, são mais propensos a ter sua microbiota povoada por espécies patogênicas. A administração de probióticos pode influenciar positivamente na colonização intestinal de bebês prematuros. Este estudo objetivou revisar sistematicamente as evidências sobre efeitos da administração e segurança na utilização de probióticos na microbiota de recém-nascidos prematuros. Foi desenhado da seguinte forma: população (recém-nascidos prematuros), intervenção (probióticos), comparação (placebo ou nenhum tratamento), desfecho (alterações na microbiota de recém-nascidos prematuros), tipo de estudo (ensaios clínicos randomizados). A busca dos estudos foi realizada na Biblioteca Cochrane, Medline via bases de dados PubMed e Embase e, de forma complementar, por meio de buscas manuais no Google Acadêmico e no portal de periódicos da CAPES. Foram incluídos 23 estudos envolvendo 3.670 prematuros, dos quais 65,2% (n=15) foram classificados com baixo risco de viés, 17,4% (n=4) com algum risco de viés e 17,4% (n=4) com alto risco de viés. Probióticos têm sido utilizados para colonizar a microbiota intestinal. Por fim, algumas cepas de *Lactobacillus* e *Bifidobacterium* testadas parecem trazer benefícios e serem seguras para a microbiota e saúde de recém-nascidos prematuros.

Palavras-chave: efeito, probióticos, prematuro, microbiota, *Bifidobactéria, Lactobacilos*.

RESUMEN
Los recién nacidos prematuros tienen una microbiota intestinal inmadura en comparación con los recién nacidos a término, colonizada por un número reducido de especies bacterianas beneficiosas, por lo que es más probable que su microbiota esté poblada por especies patógenas. La administración de probióticos puede influir positivamente en la colonización intestinal de los bebés prematuros. Este estudio tenía como objetivo revisar sistemáticamente las pruebas sobre los efectos de la administración y la seguridad de los probióticos en la microbiota de los recién nacidos prematuros. Se diseñó de la siguiente manera: población (recién nacidos prematuros), intervención (probióticos), comparación (placebo o ningún tratamiento), resultado (cambios en la microbiota de los recién nacidos prematuros), tipo de estudio (ensayos clínicos

aleatorizados). La búsqueda de estudios se realizó en la Cochrane Library, Medline a través de las bases de datos PubMed y Embase y, además, mediante búsquedas manuales en Google Scholar y en el portal de revistas CAPES. Se incluyeron 23 estudios con 3.670 prematuros, de los cuales el 65,2% (n=15) se clasificaron como de bajo riesgo de sesgo, el 17,4% (n=4) como de cierto riesgo de sesgo y el 17,4% (n=4) como de alto riesgo de sesgo. Los probióticos se han utilizado para colonizar la microbiota intestinal. Por último, algunas cepas de Lactobacillus y Bifidobacterium probadas parecen ser beneficiosas y seguras para la microbiota y la salud de los recién nacidos prematuros.

Palabras clave: efecto, probióticos, prematuros, microbiota, *Bifidobacterias*, *Lactobacilos*.

1 INTRODUCTION

The gut microbiota harbors numerous living microorganisms such as beneficial and pathogenic bacteria, fungi, archaea and bacteriophages. The balance of these microbiomes is important to perform functions such as barrier formation for protection against pathogens, preservation of the mucosa and maturation of immunity, which are important for the homeostasis of the human organism [1, 2].

Premature newborns with gestational age of less than 37 weeks have a fragile and vulnerable intestinal microbiota, which undergoes changes in its composition and functions, due to the influence of gastrointestinal immaturity, type of delivery, prolonged use of antibiotics and reduced breastfeeding. These factors can influence intestinal imbalance. Intestinal immaturity is characterized by impaired mucosal barrier function, resulting in increased intestinal permeability, which may affect the protection of the sterile fetal environment. Thus, babies are exposed to the hostile outside world and pathogenic bacteria before their barrier function is fully developed, which can result in morbidity and mortality [3, 4, 5, 6, 7].

Probiotics are living microorganisms, with different strains, which in adequate doses have positive effects on the intestine. Among the various types of probiotic strains, the most studied are Bifidobacterium and Lactobacillus, each one playing a role, such as modulation of the imbalance in the composition of the intestinal microbiota, prevention of uncontrolled growth of pathogens and alteration in intestinal permeability. Thus, the administration of probiotics can influence the promotion of intestinal microbiota colonization and healthy development in premature newborns [8, 9]. This study aimed to analyze the evidence of the effect and safety of probiotic supplementation on the microbiota of newborns with prematurity. For that, a systematic review was carried out.

2 MATERIALS AND METHODS

This review protocol was registered in PROSPERO (Prospective Systematic Reviews Register) (ID: CRD42022308753). This report was performed in accordance with the Preferred Reporting Items for Extended Systematic Reviews and Meta-analyses [10]. Ethical approval was not required.

To prepare the research question, the PICOT method was used: population (premature newborns), interventions (probiotics), comparison (placebo or no treatment), outcome (changes in the microbiota of premature newborns), type of study (randomized clinical trials). The study-oriented question was "Do probiotics have an effect on the microbiota of premature newborns?"

Participants of reviewed studies were premature newborns with a gestational age of less than 37 weeks, with extremely low birth weight (less than 1,000 g), very low birth weight (less than 1,500 g) or low birth weight (less than 2,500 g), born through cesarean section or vaginal delivery, any gender and ethnicity. Studies carried out in pregnant women, full-term babies (between 37 to 41 weeks and 6 days of gestation), and post-term babies (above 42 weeks of gestation) were excluded.

Intervention of interest was supplementation of probiotics with single strains or combination of strains, regardless of strain or dose. Studies carried out with the administration of prebiotics and other types of supplements or with a focus on drug treatments were excluded.

Comparators were placebo or no treatment. Primary outcome was improvement of the intestinal microbiota, measured by: increased numbers of beneficial bacteria and their intestinal activities; reduction of pathogenic species; improvement in intestinal permeability. Secondary outcomes were assessment of eventual adverse events and mortality/death. We reviewed only randomized clinical trials. Observational studies, systematic and narrative reviews were excluded.

Studies were searched in *The Cochrane Central Register of Controlled Trials (CENTRAL)* databases in the *Cochrane Library*, *Medline* via *PubMed* and *Embase*. Publications from the years 2000 to 2022 were selected, without language restrictions. In addition, manual searches were performed on Google Scholar, the Capes journal portal and on the reference lists of systematic reviews on the subject. Searches were updated in December 2022. Manual searches were performed in CAPES (www.periodicos.capes.gov.br), Google Scholar and in the reference lists of systematic reviews on the subject. Search strategies are reported in Table 1.

Table 1: Database search strategy and manual searches.

Database	Keywords according to DeCS and MeSH	Total without filter	Total with the filter on the side applied	Publication date	Publication type	Species	Language	Selected
PubMed	"Effect" AND "Microbiota, Gastrointestinal" OR "Intestinal Flora" AND "Premature Infant" OR "Preterm Infant" AND "Probiótics" NOT "Prebiotics"	23,627 results	1,156 results	2000-2022	Clinical trial and randomized controlled trial	Humans	Without restriction	15
Cochrane Library	"Effect" AND "Microbiota, Gastrointestinal" OR "Intestinal Flora" AND "Premature Infant" OR "Preterm Infant" AND "Probiótics" NOT "Prebiotics"	2,001 results	1,978 results	2000-2022	Essay	-	Without restriction	2
Embase	"Effect" AND "probiotics" AND "microbiota" OR "gastrointestinal" AND "premature" OR "preterm"	188 results	45 results	2000-2022	Article	Fetus, newborn or infant	Without restriction	3
Manual searches	-	-	-	-	-	-	-	-
Google acadêmico	"Effect" AND "Microbiota, Gastrointestinal" AND "Preterm Infant" AND "Probiótics" AND"Randomized Controlled Trial"	13,300 results	1,450 results	2000-2022	"Summaries" and "all"	-	Without restriction	2
Portal CAPES	"Microbiota" AND "Preterm Infant" AND "Effect" AND "Randomized Controlled Trial" AND "Probiótics" NOT "systematic review"	44 results	44 results	2000-2022	Articles	-	Without restriction	1

Source: Author, 2023.

3 SELECTION PROCESS

The selection of studies was carried out independently and paired by two reviewers (CL and CA). A search was carried out in the selected databases and manual searches, in which the search results were analyzed through the title of the articles and, if available, the abstracts. In addition, duplicates and incompatible studies were excluded. Reviewers CL and CA compared selected data, and discrepancies were resolved by a third reviewer.

Selected articles were read in full to confirm pre-specified eligibility criteria, and information from included articles was collected and recorded on Microsoft Excel spreadsheets (including author/year, title, study site, study design, population, intervention and dosage, comparison, evaluated outcomes, treatment time) and in the Zotero software (reference manager) (author/year, title, base journal, URL and DOI).

The risk of bias of the included studies was analyzed according to the Cochrane risk of bias tool for randomized trials (RoB 2) using five domains: randomization process, deviations from intended interventions, outcome data, outcome measurement and selection of reported outcomes. All review steps were performed by two independent reviewers (CL and CA).

A qualitative synthesis (systematic review) was performed without a quantitative synthesis (meta-analysis). A table with study characteristics (author/year, participant profile, results and risk of bias) was presented, comparing the study variables with critical interpretation and validation of the applicability of the results.

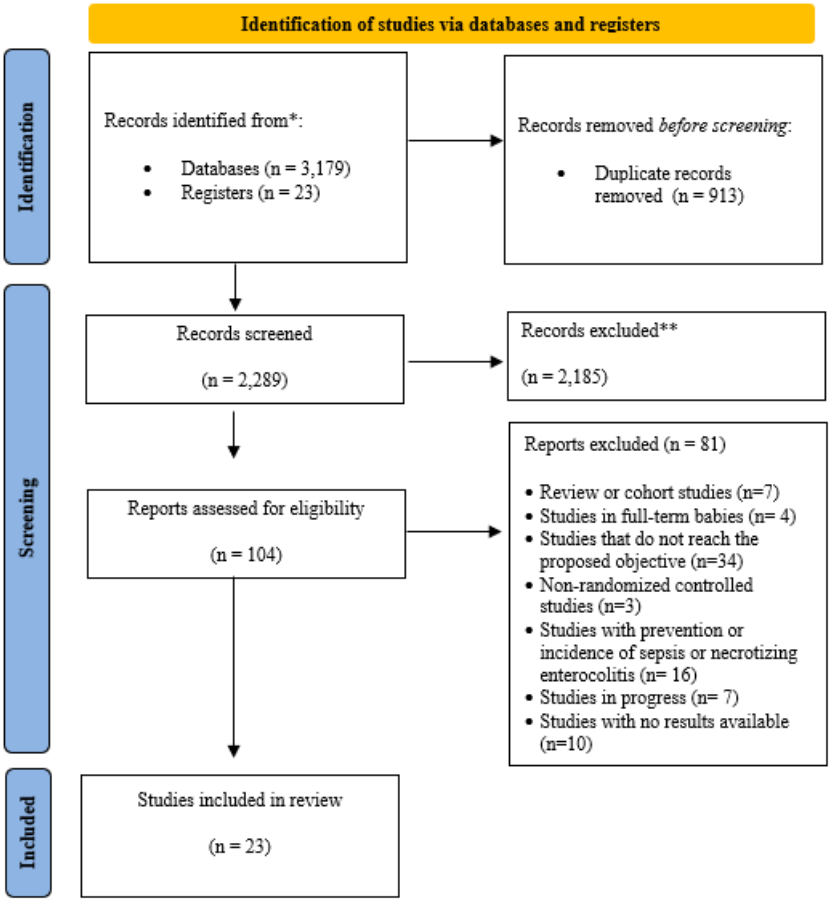
4 RESULTS

4.1 STUDY SELECTION

We found 3,179 registers. A manual search identified a further 23 references. After reading titles, abstracts and removing duplicates, 2,289 potentially relevant publications were selected. However, 2,185 publications were excluded because they contained review, cohort or observational studies; studies with other supplements, foods, drugs or formula; studies with probiotic supplementation in term infants, children, adults or pregnant women; animal studies; studies related to pathologies in children or adults; microbiota transplantation studies.

The 104 full-text articles were retrieved and evaluated for eligibility. We excluded 81 articles that were not included because they were studies in full-term babies; studies that do not reach the proposed objective; non-randomized controlled studies; studies with prevention or incidence of sepsis or necrotizing enterocolitis; ongoing studies; studies with no results available. Finally, 23 randomized controlled trials (RCTs) were included in the systematic review. The flowchart of the study selection process is shown in Figure 1.

Figure 1: Study search and selection strategy flowchart.



Source: Author, 2023.

4.2 CHARACTERISTICS OF THE STUDIES

Characteristics of the included studies are given in Frame 1. The RCTs included a total of 3,670 patients and were conducted in 13 countries. Of the included studies, they used single-strain probiotics (n=10, 43.5%), combinations of probiotic strains (n=11, 47.8%) and analyzed both single-strain and combinations of strains (n=2, 8.7%). The comparison found in the studies was performed with placebo (n=21, 91.3%) and without treatment (n=2, 8.7%). Outcomes inclued changes in microbiota, composition, colonization and intestinal permeability. Treatment duration was less than 1 year in 3 studies, 1 year in 4 studies, greater than 1 year in 13 studies; 3 studies did not report that. The most recent studies were conducted in 2018 (n=2, 8.7%), 2019 (n=2, 8.7%), 2020 (n=1, 4.3%), 2021 (n=3, 13.1%) and 2022 (n=4, 17.4%).

4.3 RISK OF BIAS ASSESSMENT

Of the included studies, 65.2% (n=15) were considered as low risk of bias, 17.4% (n=4) considered as some risk of bias, and 17.4% (n=4) considered as high risk of bias. The studies were considered as low risk of bias for the randomization process domain (D1) (n=19, 82.6%),

deviations from intended interventions (D2) (n=19, 82.6%), data of missing results (D3) (n=23, 100%), measuring results (D4) (n=23, 100%) and selecting the reported result (D5) (n=19, 82.6%). The methods used for random sequence generation were clearly described in all trials: stratified randomization (n=14, 60.9%); en bloc (n=4, 17.4%); computer-generated random lists (n=4, 17.4%); or draw of cards (n=1, 4.3%).

In 21 (91.3%) trials, treatment allocation was adequately concealed, most studies used sealed, opaque numbered envelopes. In the study [11], treatment allocation was conducted by a third party who was not involved in the study (nutritional service). In 8.7% (n=2) of the studies, allocation concealment was not clearly demonstrated or described [12, 13].

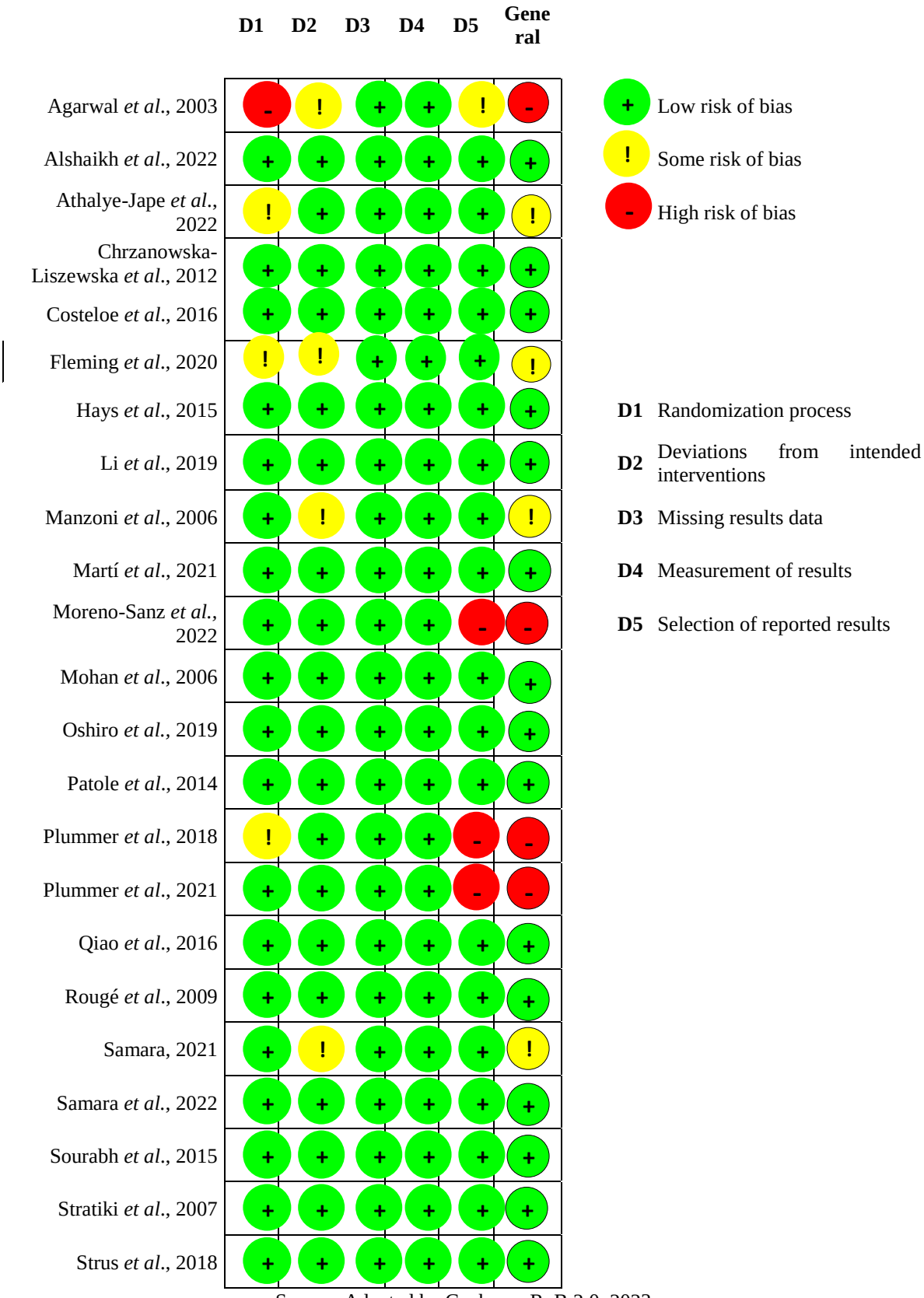
Only 8.7% of the studies had a baseline imbalance, which was a potential source of bias. In the first study, 24 babies (less than 1,500g) and 23 babies (1,500-1,999g) were enrolled in the treatment group, whereas in the control group there were 15 (less than 1,500g) and 9 (1,500-1,999g) [12]. The two groups compared in the study (TS and SS) had similar characteristics, TS group (n=87 preterms) and SS group (n=86 preterms), but when compared to the placebo group (REF) (n=29) preterms, there was a disproportion in the number of preterm infants [14]. And finally, in the last trial, 38 premature infants were registered in the probiotic-treated group and 28 premature infants in the placebo group [15].

Blinding of study participants, caregivers and evaluators was clearly made in 60.9% (n=14) of the trials. In 13.0% (n=3) of the trials, there was not enough information about the method of blinding participants or staff to make a judgment [13, 16, 17]. In the studies [15, 18], the participants and the team were blinded, but there was no information regarding the blinding of the evaluators. And in the trials [19, 20] participants and raters were blinded, but a research assistant (nurse) opened the envelope to reveal the study group.

The reported outcome data were satisfactory for 73.9% (n=17) of the included studies. Missing outcome data were missing in 26.1% (n=6) of the studies, but were balanced by sensitivity analysis [13, 15, 21, 22, 23, 24]. Selective reporting (reporting bias): In all studies, results pre-specified in the methods section were reported in the results section. In 17.4% (n=4) of the studies, there were multiple eligible outcome measures [13, 15, 18, 25].

The summary of the individual results is given in Table 2 (Appendix). Increase in beneficial bacteria was reported in 39.1% (n=9) of the studies, the alteration of pathogenic species was reported in only 4.3% (n=1) of the studies, the increase in beneficial bacteria and a reduction in pathogenic species was reported in 47.8% (n=11) of the studies, and an increase in beneficial bacteria and intestinal permeability were reported in only 8.7% (n=2) of the studies.

Figure 2: Risk of bias in the reviewed studies.



Source: Adapted by Cochrane RoB 2.0, 2023.

4.4 PRIMARY OUTCOME

The increase in beneficial bacteria was reported in 39.1% (n=9) of the studies, of which five of the trials address the increase in *Bifidobacterium*, one on the increase in *Lactobacillus*, and three of the trials with an increase in both bacteria (*Bifidobacterium* and *Lactobacillus*).

The probiotics that took part were *Bifidobacterium (B. breve M-16, B. longum, B. longum subsp. Infants M-63, B. longum subsp. Longo BB536, BBG-001, B. lactis, B. bifidum, B. animalis subp. Lactis)* present in 21 studies; *Lactobacillus* (*L. rhamnosus GG- L. casei, L. plantarum, L. reuteri DSM, L. salivarius, L. acidophilus)* present in 15 studies; *Saccharomyces boulardii* present in one study; and *Streptococcus Trermophillus* in two studies.

4.5 SECONDARY OUTCOMES

4.5.1 Eventual Adverse Events

In 52.2% (n=12) articles, there were no reports of adverse effects caused during the process. In 43.5% (n=10) articles, it was reported that no adverse events were associated with probiotic supplementation, including probiotic sepsis, abdominal distension, vomiting and diarrhea, in which supplementation would be interrupted. In only 4.3% (n=1) of the articles, there was the presence of abdominal distention and vomiting, but these were not caused by the administration of the probiotic, but by intercurrences.

4.5.2 Mortality/ Death

There was no information about mortality/death in 69.6% (n=16) of the articles during the process. In 26.1% (n=6) articles, mortality occurred during the experiment in patients receiving probiotics or placebo/no treatment, but in those that received supplementation, mortality was not attributed to probiotics, but to other causes, such as sepsis or necrotizing enterocolitis, candida, injuries, septic shock, hemorrhages, respiratory failure, among others. In only 4.3% (n=1) of the article there was no death.

5 DISCUSSION

This systematic review was undertaken to provide further data to support the use of probiotics in preterm infants, specifically *Lactobacillus* and *Bifidobacterium* both in single strains and in combinations of strains. For the bacteria to live and colonize the intestinal microbiota, the dosage of probiotics must reach the adequate amount to provide a health benefit.

Our results showed that premature babies supplemented with probiotics either in a single strain (*Bifidobacterium* or *Lactobacillus*) or in a combination of strains, provided beneficial

changes in the microbiota influencing positively for the abundance of *Lactobacillus* or *Bifidobacterium* or both strains, with or without reduction of pathogenic species in the intestinal microbiota.

In an observational study developed [34], samples from two cohorts were compared, in which preterm infants were supplemented with Infloran® (combination of *Bifidobacterium* and *Lactobacillus*), administered twice a day, compared to preterm infants not supplemented with probiotics (control group). The group supplemented with probiotics had an abundance of *Bifidobacterium* and a small amount of *Lactobacillus* was detected in the microbiota of preterm infants, in addition to a reduction in pathogenic species such as *Klebsiella*, *Escherichia*, *Enterobacter* and *Clostridium*. Thus, it is indicated that the combination of supplemented strains may persist in the microbiota of premature infants and/or stimulate the colonization of other *Bifidobacterium* and *Lactobacillus*.

Another observational study [35], with 76 babies enrolled, found that 31 premature newborns with a gestational age of less than 28 weeks were supplemented with a probiotic (Infloran: *Lactobacillus acidophilus* and *Bifidobacterium longum subspecies infantile*), compared with 35 preterm infants aged 28 to 31 weeks who were not supplemented and 10 healthy full-term infants without supplementation. In their results, supplemented preterm infants had a greater abundance of *Bifidobacterium* in the intestinal microbiota a few days after administration of probiotics compared to non-supplemented preterm infants and full-term infants. Also, *Lactobacillus* colonizatin increased by up to 4 months in all 3 groups.

As for the results of the non-randomized clinical trial [36], the administration of the probiotic *Bifidobacterium breve M-16V* led to the colonization in the intestinal microbiota of preterm infants with low birth weight for at least a few weeks, but the evidence of this colonization in longer time is limited. The intestinal microbiota of the group of infants supplemented showed a greater abundance of *Bifidobacterium* and lower *Proteobacteria* in the microbiota compared to the control group of non-supplemented preterm infants, where *Bifidobacterium* was significantly lower and *Proteobacteria* was higher.

Strengths of the systematic review include the robust methodology, comprehensive nature, and unique focus on RCT of probiotics in preterm infants. Limitations include lack of meta-analysis for a more reliable study; and few studies were found for the applied methodology, with some having unavailable results.

For rational decision-making and consideration of therapies in the future, information about the development of the microbiota in preterm infants is essential. Although studies on the

microbiota in preterm infants have been carried out, many are more specific for the analysis of pathologies and fail in prevention.

In a final consideration, it was observed that some tested strains of Lactobacillus and mainly, of Bifidobacterium have benefits for the intestinal microbiota of premature newborns, contributing to their health. The studies did not indicate risks associated with probiotics, since the safety data in most of the RCTs included in this analysis did not report adverse events during the process, in addition to the fact that the mortalities observed in some studies were due to other pathological causes, guaranteeing the safety of the probiotics.

REFERENCES

1 MOHAMMADKHAH, AI; SIMPSON, EB; PATTERSON, SG; FERGUSON JF. Development of the gut microbiome in children, and lifetime implications for obesity and cardiometabolic disease. **Children**, v. 5, n. 12, p. 160, 2018. DOI: 10.3390/children5120160

2 ROYO, SM; TARRAZÓ, M; GARCÍA-MANTRANA, I; GÓMEZ-GALLEGO, C; SALMINEM, S; COLLADO, MC. Shaping microbiota during the first 1000 days of life. **Probiotics and Child Gastrointestinal Health**, p. 3-24, 2019. DOI: 10.1007/5584_2018_312

3 PAULA, MB; CAMPBELL, CSG; ALVES, AFC; RIBEIRO FILHO, ES; FERREIRA, MIM; MARÇAL, FA. Microbiota intestinal infantil: do nascimento aos 5 anos de idade. **Brazilian Journal of Health Review**, v. 4, n. 6, p. 26235-26252, 2021. DOI:10.34119/bjhrv4n6-210.

4 TIRONE, C; PEZZA, L; PALADINI, Â; TANA, M; AURÍLIA, C; LIO, A; et al. Gut and lung microbiota in preterm infants: immunological modulation and implication in neonatal outcomes. **Frontiers in immunology**, v. 10, p. 2910, 2019. DOI: 10.3389/fimmu.2019.0291.

5 CASTELLANO, MGSR; TOFFOLO, MCF. O uso de probióticos na gestação – quando indicar? uma revisão integrativa. **Brazilian Journal of Health Review**, v. 5, n. 4, p. 12448-12463, 2022. DOI:10.34119/bjhrv5n4-041

6 CUNA, A; MOROWITZ, MJ; AHMED, I; UMAR, S; SAMPATH, V. Dynamics of the preterm gut microbiome in health and disease. **American Journal of Physiology-Gastrointestinal and Liver Physiology**, v. 320, n. 4, p. G411-G419, 2021. DOI: 10.1152/ajpgi.00399.2020

7 SANDERS, ME; AKKERMANS, LMA; HALLER, D; HAMMERMAN, C; HEIMBACH, J; HÖRMANNSPERGER, G; et al. Safety assessment of probiotics for human use. **Gut microbes**, v. 1, n. 3, p. 164-185, 2010. DOI:10.4161/gmic.1.3.12127

8 AZAD, M; SARKER, M; LI, TJ; YIN, J. Probiotic species in the modulation of gut microbiota: an overview. **BioMed research international**, v. 2018, 2018. DOI: 10.1155/2018/9478630

9 ALVES, J. L. B.; DE OLIVEIRA, Y; CARVALHO, NNC; CAVALCANTE, RGS; LIRA, MMP; DO NASCIMENTO, LCP; et al. Gut microbiota and probiotic intervention as a promising therapeutic for pregnant women with cardiometabolic disorders: Present and future directions. **Pharmacological Research**, v. 145, p. 104252, 2019. DOI:10.1016/j.phrs.2019.104252

10 MOHER, D.; LIBERATI A; TETZLAFF J; ALTMAN DG; THE PRISMA GROUP. Itens de relatório preferidos para revisões sistemáticas e meta-análises: a declaração PRISMA. PloS Med. 2009. DOI: 10.5123/S1679-49742015000200017

11 STRATIKI, Z; COSTALOS, C; SEVASTIADOU, S; KASTANIDOU, Ó; SKOUROLIAKOU, M; GIAKOUMATOU, A; et al. The effect of a *bifidobacter* supplemented bovine milk on intestinal permeability of preterm infants. **Earrly human development**. V.83, n.9, p. 575-579, 2007. DOI:10.1016/j.earlhumdev.2006.12.002

12 AGARWAL, R.; SHARMA, N.; CHAUDHRY, R.; DEORARI, A.; PAULO, V.K.; GEWOLB, I.H. et al. Effects of Oral *Lactobacillus GG* on Enteric Microflora in Low-Birth-Weight Neonates. **Revista de gastroenterologia e nutrição pediátrica**, v. 36, n. 3, pág. 397-402, 2003. DOI: 10.1097/00005176-200303000-00019

13 FLEMING, P; WILKS, M; EATON, S; PANTON, N; HUTCHINSON, R; AKYEMPON, A; et al. *Bifidobacterium breve BBG-001* e função da barreira intestinal em bebês prematuros: estudos exploratórios do estudo PiPS. **Pediatric Research** , v. 89, n. 7, pág. 1818-1824, 2021. DOI:10.1038/s41390-020-01135-5

14 ATHALYE-JAPE, G; ESVARAN, M; PATOLE, S; SIMMER, K; NATHAN, E; DOHERTY, D; et al. Effect of single versus multistrain probiotic in extremely preterm infants: a randomised trial. **BMJ open gastroenterology**, v. 9, n. 1, p. e000811, 2022. DOI:10.1136/bmjgast-2021-000811

15 PLUMMER, EL; BULACH, DM; MURRAY, GL; JACOBS, SE; TABRIZI, SN; GARLAND, SM; et al. Gut microbiota of preterm infants supplemented with probiotics: sub-study of the ProPrems trial. **BMC microbiology**, v. 18, n. 1, p. 1-8, 2018. DOI:10.1186/s12866-018-1326-1

16 MANZONI, P; MOSTERT, M; LEONESSA, ML; PRIOLO, C; FARINA, D; MONETTI, C; et al. Oral supplementation with *Lactobacillus casei* subspecies *rhamnosus* prevents enteric colonization by *Candida* species in preterm neonates: a randomized study. **Clinical infectious diseases**, v. 42, n. 12, p. 1735-1742, 2006. DOI:10.1086/504324

17 SAMARA, J; MOOSAVI, S; ALSHAIKH, B; ORTEGA, VA; PETTERSEN, VK; FERDOUS, T; et al. Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. **Cell Host & Microbe**, v. 30, n. 5, p. 696-711. e5, 2022. DOI:10.1016/j.chom.2022.04.005

18 PLUMMER, EL; DANIELEWSKI, J; GARLAND, SM; SU, J; JACOBS, SE; MURRAY, GL. The effect of probiotic supplementation on the gut microbiota of preterm infants. **Journal of medical microbiology**, v. 70, n. 8, 2021. DOI:10.1099/jmm.0.001403

19 ALSHAIKH, B; SAMARA, J; MOOSAVI, S; FERDOUS, T; SORAISHAM, A; DERSCH-MILLS, D; et al. Multi-strain probiotics for extremely preterm infants: a randomized controlled trial. **Pediatric Research**, p. 1-8, 2022. DOI:10.1038/s41390-022-02004-z

20 SAMARA, J. **The Gut Microbiome of Premature Infants: An Ecological Analysis of a Probiotic Intervention Study**. 2021. Dissertação de Mestrado. Cumming School of Medicine.

21 COSTELOE, K; HARDY, P; JUSZCZAK, E; WILKS, M; MILLAR, MR. *Bifidobacterium breve BBG-001* in very preterm infants: a randomised controlled phase 3 trial. **The Lancet**, v. 387, n. 10019, p. 649-660, 2016. DOI:10.1016/S0140-6736(15)01027-2

22 MARTÍ, M; SPRECKELS, JE; RANASINGHE, PD; WEJRYD, E; MARCHINI, G; SVERREMARK-EKSTRÖM, E; et al. Effects of *Lactobacillus reuteri* supplementation on the gut microbiota in extremely preterm infants in a randomized placebo-controlled trial. **Cell reports medicine**, v. 2, n. 3, p. 100206, 2021. DOI: 10.1016/j.xcrm.2021.100206

23 PATOLE, S; KEIL, AD; CHANG, A; NATHAN, E; DOHERTY, D; SIMMER, K; et al. Effect of Bifidobacterium breve M-16V supplementation on fecal *Bifidobacteria* in preterm neonates--a randomised double blind placebo controlled trial . **Plos one**, v. 9, n. 3, pág. e89511, 2014. DOI:10.1371/jornal.pone.0089511

24 STRUS, M; HELWICH, E; LAUTERBACH, R; RZEPECKA-WEGLARZ, B; NOWICKA, K; WILINSKA, M; et al. Effects of oral probiotic supplementation on gut *Lactobacillus* and *Bifidobacterium* populations and the clinical status of low-birth-weight preterm neonates: a multicenter randomized, double-blind, placebo-controlled trial. **Infection and Drug Resistance**, v. 11, p. 1557, 2018. DOI:10.2147/IDR.S166348

25 MORENO-SANZ, B; MONTES, MT; MANZANO, S; ESPINOSA-MARTOS, I; CÁRDENAS, N; ESTEBAN, S; et al. Randomized, Double-Blind, Placebo-Controlled Study to Assess the Effect of Two Probiotics on the Preterms' Gut Microbiota. **Journal of Pediatric Gastroenterology and Nutrition**, v. 74, n. 6, p. e153-e159, 2022. DOI:10.1097/MPG.0000000000003427

26 OSHIRO, T; NAGATA, S; WANG, C; TAKAHASHI, T; TSUJI, H; ASAHARA, T; et al. *Bifidobacterium* Supplementation of Colostrum and Breast Milk Enhances Weight Gain and Metabolic Responses Associated with Microbiota Establishment in Very-Preterm Infants. **Polo de Biomedicina** , v. 4, n. 3, pág. 1-10, 2019. DOI:10.1159/000502935

27 HAYS, S; JACQUOT, A; GAUTHIER, H; KEMPF, C; BEISSEL, A; PIDOUX, O; et al. Probiotics and growth in preterm infants: a randomized controlled trial, PREMAPRO study. **Clinical nutrition**, v. 35, n. 4, p. 802-811, 2016. DOI:10.1016/j.clnu.2015.06.006

28 SOURABH, D.; PALLAB, R.; ANIL, N. Comparison of Stool Colonization in Premature Infants by Three Dose Regimes of a Probiotic Combination: A Randomized Controlled Trial. **Am J Perinatol**, , 2015. DOI: 10.1055/s-0034-1395473

29 QIAO, LX; ZHU, WY; ZHANG, HY; WANG, H. Effect of early administration of probiotics on gut microflora and feeding in pre-term infants: a randomized controlled trial. **The Journal of Maternal-Fetal & Neonatal Medicine**, v. 30, n. 1, p. 13-16, 2017. DOI: 10.3109/14767058.2016.1163674

30 MOHAN, R; KOEBNICK, C; SCHILDT, J; SCHMIDT, S; MUELLER, M; POSSNER, M; et al. Effects of *Bifidobacterium lactis Bb12* supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. **Journal of Clinical Microbiology**, v. 44, n. 11, p. 4025-4031, 2006. DOI:10.1128/JCM.00767-06

31 LI, YF; ZHU, CR; GONG, XL; LI, HL; XIONG, LK; WANG, KJ; et al. Beneficial Effects of Probiotic Treatment on Gut Microbiota in Very Low Birth Weight Infants. **Pesquisa e prática em gastroenterologia**, 2019. DOI:10.1155/2019/3682836

32 CHRZANOWSKA-LISZEWSKA, D; SELIGA-SIWECKA, J; KORNACKA, MK. The effect of *Lactobacillus rhamnosus GG* supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial. **Early human development**, v. 88, n. 1, p. 57-60, 2012. DOI:10.1016/j.earlhumdev.2011.07.002

33 ROUGÉ, C; PILOQUETE, H; BUTEL, MJ; BERGER, B; ROCHAT, F; FERRARIS, L; et al. Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. **The American journal of clinical nutrition**, v. 89, n. 6, p. 1828-1835, 2009. DOI:10.3945/ajcn.2008.26919

34 ALCON-GINER, C; DALBY, MJ; CAIM, S; KETSKEMETY, J; SHAW, A; SIM, K; et al. A suplementação de microbiota com *Bifidobacterium* e *Lactobacillus* modifica a microbiota intestinal e o metaboloma do bebê prematuro: um estudo observacional. ***Cell Rep***. *Med.* 2020. DOI:10.1016/j.xcrm.2020.100077

35 ESAIASSEN, E; HJERDE, E; CAVANAGH, JP; PEDERSEN, T; ANDRESEN, JH; RETTEDAL, SI; et al. Effects of probiotic supplementation on the gut microbiota and antibiotic resistome development in preterm infants. **Front. Pediatr.** 2018. DOI:10.3389/fped.2018.00347

36 HORIGOME, A; HISATA, K; ODAMAKI, T; IWABUCHI, N; XIAO, JZ; SHIMIZU, T. Colonization of supplemented *Bifidobacterium breve M-16V* in low birth weight infants and its effects on their gut microbiota weeks post-administration. Front Microbiol, 2021. DOI:10.3389/fmicb.2021.610080

APPENDIX

Frame 1: Characteristics of the included studies

Article	Author/year	Título	Study location	Study design	Population	Intervention and dosage	Comparison	Evaluated outcomes	Treatment time
3	Athalye-Jape et al., 2022	*Effect of single versus multistrain probiotic in extremely preterm infants: A randomised trial*	Australia	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=173	SS probiotic group: *B. breve M-16V* (3×10^9 UFC/day); TS probiotic group: Probiotic group TS: Mixture of *B. breve M-16V*, *B. longum subsp. infantis M-63 and B. longum subsp. longo BB536* (3×10^9 UFC/day).	Placebo REF Group	Analysis of the fecal microbiota	21 months
4	Chrzanowska-Liszewska et al., 2012	*The effect of Lactobacillus rhamnosus GG supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial*	Poland	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=47	*Lactobacillus rhamnosus GG* 6×10^9 cells per unit	Placebo	Increase in the amount of bifidogenic flora in the stool; Decreased pathogenic colonization of the intestine.	12 months
5	Costeloe et al., 2016	*Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial*	England	Phase 3 multicenter, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=1,310	*B breve* BBG-001 a daily dose of 8.2 to 9.2 log10 UFC	Placebo	Bacterial colonization	37 months

Article	Author/year	Título	Study location	Study design	Population	Intervention and dosage	Comparison	Evaluated outcomes	Treatment time

6	Fleming et al., 2020	*Bifidobacterium breve BBG-001* and intestinal barrier function in preterm babies: Exploratory Studies from the PiPS Trial	England	Randomized controlled, placebo-controlled trial	Premature newborns with gestational age less than 37 weeks n=94	*Bifidobacterium breve BBG-001* probiotic No dosage	Placebo	Intestinal permeability; Composition of the intestinal microbiota.	39 months
7	Hays et al., 2015	*Probiotics and growth in preterm infants: a randomized controlled trial*	France	Prospective , randomized, double-blind controlled study	Premature newborns with gestational age less than 37 weeks n=197	Probiotics (group P). Group P comprised three subgroups: P1 received *Bifidobacterium lactis*, P2 received*Bifidobacterium longum* and P3 received *B. lactis* and *B. longum*. No dosage	Placebo	Evaluation of the administration of probiotics.	Not declared
8	Li et al., 2019	*Beneficial Effects of Probiotic Treatment on Gut Microbiota in Very Low Birth Weight Infants*	China	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=16	*L. plantarum LK006* 20%, *B. longum LK014* 40% *and B. bifidum LK012* 40%; each probiotic capsule contains 500mg of 510 colony-forming units	Placebo	Evaluation of bowel changes microbiota; Analysis of the intestinal microbial composition correlation.	16 months

Article	**Author/year**	**Título**	**Study location**	**Study design**	**Population**	**Intervention and dosage**	**Comparison**	**Evaluated outcomes**	**Treatment time**
9	Manzoni et al., 2006	*Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm*	Italy	Prospective , randomized, double-blind study	Premature newborns with gestational age less than 37 weeks n=80	*Lactobacillus casei* subespecies *rhamnosus*; 6 10^9 ufc/day added to breast milk	Placebo	Reduction in the incidence of fungal colonization by *Candida sp*	12 months

		neonates: a randomized study							
10	Martí et al., 2021	*Effects of Lactobacillus reuteri supplementation on the gut microbiota in extremely preterm infants in a randomized placebo-controlled trial*	Sweden	Part of a prospective, multicenter, double-blind, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=134	Probiotic *Lactobacillus reuteri DSM 17938* (1,25 × 10^8 bactéria/day)	Placebo	Analysis of the effect of the probiotic on the composition of the intestinal microbiota	24 months
11	Moreno-Sanz et al., 2022	*Randomized, Double-blind, Placebo-controlled Study to Assess the Effect of Two Probiotics on the Preterms' Gut Microbiota.*	Spain	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=30	(1 10^9 UFC de *L.salivarius PS11603* and 1 10^8 UFC ofe *B.longum subsp. infantis* PS10402).	Placebo	Colonization of the gastrointestinal tract	13 months

Article	**Author/year**	**Título**	**Study location**	**Study design**	**Population**	**Intervention and dosage**	**Comparison**	**Evaluated outcomes**	**Treatment time**
12	Mohan et al., 2006	*Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study*	Germany	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=69	2x10^9 of *Bifidobacterium lactis Bb12* per gram of formula powder (Nestle' FM 2000A) 1st to 3rd day: 1,6X10^9 cells from 4th day: 4.8X10^9 cells	Placebo	Modification of the intestinal microbiota; Reduced colonization of antibiotic-resistant organisms; Increased beneficial and pathogenic cell counts.	21 months
13	Oshiro et al., 2019	*Bifidobacterium Supplementation of Colostrum and Breast*	Japan	Blinded, randomized controlled trial	Premature newborns	*B. breve (BBG-01):* 10^9 viable cells	Placebo	Microbiota composition.	27 months

		Milk Enhances Weight Gain and Metabolic Responses Associated with Microbiota Establishment in Very-Preterm Infants.			with gestational age less than 37 weeks n=35				
14	Patole et al., 2014	*Effect of Bifidobacterium breve M-16V supplementation on fecal bifidobacteria in preterm neonates--a randomised double blind placebo controlled trial*	Australia	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=153	*Bifidobacterium breve M-16V* probiotic ($36x10^9$ cfu/day)	Placebo	Evaluation of the effect of *B.breve M 16V* on *B.breve* levels in feces; Evidence of a bifidogenic effect (increase in *Bifidobacterium* in the stool).	19 months

Article	Author/year	Study location	Study location	Study design	Population	Intervention and dosage	Comparison	Evaluated outcomes	Treatment time
15	Plummer et al., 2018	*Gut microbiota of preterm infants supplemented with probiotics: sub-study of the ProPrems trial*	Australia	Multicentre, double-blind, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=66	Combination of probiotic supplementation (*Bifidobacterium longum subsp. infantis* (BB-02, 300×10^6), *Streptococcus thermophilus* (TH-4, 350×10^6) and *Bifidobacterium animalis subsp. lactis* (BB-12, 350×10^6) with 1×10^9 total organisms per 1.5 g, in a maltodextrin-based powder)	Placebo	Development of the intestinal microbiota; Analysis of the intestinal bacterial microbiota.	2 months
16	Plummer et al., 2021	*The effect of probiotic supplementation on the gut microbiota of preterm infants*	Australia	This study was part of an open-label, randomized	Premature newborns with gestational age less than 37 weeks	A combination of probiotics at 1.5 g per day [1×10^9 organisms totais of *B. longum subsp. infantis BB-02*	Placebo	Microbiota composition.	23 months

				controlled trial	n=459	(300 x 10^6), *S. thermophilus* TH-4 (350 x 10^6) and *B. animalis subsp. lactis BB-12* (350×10^6; baby ABC dophilus probiotic powder; solgar) maltodextrin-based powder			

Article	Author/year	Title	Study location	Study design	Population	Intervention and dosage	Comparison	Evaluated outcomes	Treatment time
17	Qiao et al., 2016	*Effect of early administration of probiotics on gut microflora and feeding in pre-term infants: a randomized controlled trial*	China	Randomized, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=60	Combined probiotics of *Bifidobacterium*, *Lactobacillus acidophilus* was >0.5*10^7 UFC-2x/day	Placebo	Composition: Measurement of *Lactobacillus* and *Bifidobacterium* in the intestine	2 weeks
18	Rougé et al., 2009	*Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial*	France	Randomized, biocentric, double-blind controlled trial	Premature newborns with gestational age less than 37 weeks n=94	10^8 lyophilized cells per unit of probiotic *L. rhamnosus GG* and *B. longum BB536* and maltodextrin	Placebo	Composition of the intestinal microbiota.	22 months
19	Samara, 2021	*The Gut Microbiome of Premature Infants: An Ecological Analysis of a Probiotic Intervention Study*	Canadá	Randomized, open-label controlled trial	Prematures newborns with gestational age less than 37 weeks n=62	One 0.5-g FloraBABY sachet contains 4 billion total CFU of 4 species of *Bifidobacterium* (1.2X10^9 CFU *B. breve*, 8X10^8 CFU *B. bifidum*, 6X10^8 CFU *B. longum infantis* and 6X10^8 *B. longum*) together with 10^9 CFU of	Placebo	Colonization and composition of the microbiota.	Not declared

						Lacticaseibacillus rhamnosus, mixed with dextran.			
Article	**Author/year**	**Title**	**Study location**	**Study design**	**Population**	**Intervention and dosage**	**Comparison**	**Evaluated outcomes**	**Treatment time**
20	Samara et al., 2022	*Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants*	Canada	This study was part of an open-label, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=57	Florababy probiotic (0.5g/day): 4 strains of *Bifidobacterium* from species that are common and dominant in the infant intestine *B. breve* 1:2 3 10^9 UFC, *B. bifidum* 8 3 10^8 UFC, *B. infantis* 63 10^8 UFC and *B. longum* 63 10^8) together with *Lacticaseibacillus rhamnosus* 13 10^9 UFC	No treatment	Composition and functioning of the microbiome.	37 months
21	Sourabh et al., 2015	*Comparison of Stool Colonization in Premature Infants by Three Dose Regimes of a Probiotic Combination: A Randomized Controlled Trial*	India	Blinded, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=149	A and B: high dose (10 billion UFC: *L. acidophilus* , *L. rhamnosus* , *B. longum* , *S. boulardii*) C: low dose (1 billion UFC : *L. acidophilus* , *L. rhamnosus* , *B. longum* , *S. boulardii*)	D-Placebo	Colonization of feces.	Not declared
Article	**Author/year**	**Title**	**Study location**	**Study design**	**Population**	**Intervention and dosage**	**Comparison**	**Evaluated outcomes**	**Treatment time**
22	Stratiki et al., 2007	*The effect of a bifidobacter supplemented*	Greece	Prospective randomized,	Premature newborns with		Placebo	Decreased intestinal permeability.	24 months

		bovine milk on intestinal permeability of preterm infants		double-blind study	gestational age less than 37 weeks n=75	Supplemented with *Bifidobacter lactis* (2×10^7 ufc/g of powdered milk)			
23	Strus et al., 2018	*Effects of oral probiotic supplementation on gut Lactobacillus and Bifidobacterium populations and the clinical status of low-birth-weight preterm neonates: A multicenter randomized, double-blind, placebo-controlled trial*	Poland	Multicenter, double-blind, randomized controlled trial	Premature newborns with gestational age less than 37 weeks n=177	Ffbaby ® probiotic in powder form (One dose contained 10^6 UFC of a bacterial mixture including freeze-dried *Lactobacillus rhamnosus KL53A* and *Bifidobacterium brevePB04* and auxiliary substances: maltodextrin and ascorbic acid 2x/day	Placebo	Change in intestinal microbiota	12 months

Source: Author, 2023.

Table 2: Summary of results

Article	Author/year	Profile of participants		Results (Intervention+placebo or treatment)	Risk of bias
1	Agarwal et al., 2003	**Treatment** **Birth weight less than 1,500g** Mean gestational age: 31s Mean weight: 1,176g Female: 11 Male: 13 **Birth weight 1,500-1,999g** Mean gestational age: 34.14s Mean weight: 1745g Female:12 Male: 11	**Control** **Birth weight less than 1,500g** Mean gestational age: 30.7s Mean weight: 1190g Female: 8 Male:7 **Birth weight 1,500-1,999g** Mean gestational age: 33.42s Mean weight:1717g Female: 3 Male:6	Moderate colonization of *Lactobacillus GG (LGG)* in preterm infants weighing 1,500 to 1,999g; Poor colonization in preterm infants weighing less than 1,500g.	High risk of bias
2	Alshaikh et al., 2022	**Treatment** Mean gestational age: 25.8s Mean weight: 763g Female: 15 Male:16	**Control** Mean gestational age: 25.6s Mean weight 751g Female 10 Male:21	Elevation of *Bifidobacterium* and *Lactobacillus*; Marked reduction of species of *Candida sp* .	Low risk of bias

Article	Author/year	Profile of participants		Results (Intervention+placebo or treatment)	Risk of bias
3	Athalye-Jape et al., 2022	**Treatment** **Group TS** Mean gestational age: 26.3s Mean weight: 870g Female::41 Male:46 **Group SS** Mean gestational age: :26.2s Mean weight::828g Female::38 Male:48	**Control** **Group REF** Mean gestational age: 26.1s Mean weight: 810g Female: 13 Male:16	TS and SS groups: Effective in reducing dysbiosis (reducing gammaproteobacteria, clostridia, streptococcus, proteobacteria); Elevating *Bifidobacterium.*	Some risk of bias
4	Chrzanowska-Liszewska et al., 2012	**Treatment**	**Control**	7th and 21st: increase in the number of *LGG*	Low risk of bias

		Mean gestational age: 29.62s Mean weight: 1,227.3g Female::8 Male:13	Mean gestational age: 29.46s Mean weight: 1,282.5g Female::16 Male:10	Day 42: low elevation of *LGG* with minimal difference to placebo. In addition to the increase of *Enterobacteriaceae* and *Enterococcus sp* without causing harm. May not change the picture of pathogenic colonization	
5	Costeloe et al., 2016	**Treatment** Mean gestational age: 28s Mean weight: 1,039g Female: 276 Male:374	**Control** Mean gestational age: 28s Mean weight: 1,043g Female: 290 Male:370	2 weeks: colonization of Bifidobacterium breve BBG (B.breve BBG-001) greater than 36 weeks: colonization remained high, and in the placebo group there was an increase in the microbiota	Low risk of bias

Article	**Author/year**	**Profile of participants**		**Results (Intervention+placebo or treatment)**	**Risk of bias**
6	Fleming et al., 2020	**Treatment** **Intestinal permeability (Sugar absorption test/Bacterial translocation/ Stool alpha-1-antitrypsin)** Mean gestational age: 27.5s/ 26.93s/ 26.71s Mean weight: 900g/ 869g/ 970g Female: No information Male:No information **Intestinal microbiota** Mean gestational age: 26.71s Mean weight: 890g Female:: No information Male:No information	**Control** **Intestinal permeability (Sugar absorption test/Bacterial translocation/ Stool alpha-1-antitrypsin)** Mean gestational age: 27.5s/ 26.93s/ 26.71s Mean weight: 900g/ 869g/ 970g Female: No information Male:No information **Intestinal microbiota** Mean gestational age: 26.71s Mean weight::890g Female: No information Male:s No information	Colonization of *Bifidobacterium*, with elevation of *B.breve BBG-001*; Increased loads of *enterococci;* *no* change in intestinal permeability	Some risk of bias
7	Hays et al., 2015	**Treatment** **P1/P2/P3** Mean gestational age: 29s Mean weight: 1,170g Female:: No information Male:No information	**Control** Mean gestational age: 29.4s Mean weight::1,170g Female:: No information Male:No information	Elevation of *Bifidobacterium*	Low risk of bias

Article	**Author/year**	**Profile of partticipants**	**Results (Intervention+placebo or treatment)**	**Risk of bias**

8	Li et al., 2019	**Treatment** Mean gestational age: 29.3s Mean weight:1,176g Female: 2 Male:6	**Control** Mean gestational age: 30.4s Mean weight: 1,326g Female: 5 Male:3	*Lactobacillus* elevation; Reduction of *proteobacteria* and *enterobacteria*.	Low risk of bias
9	Manzoni et al., 2006	**Treatment** Mean gestational age: 30s Mean weight: 1,200g Female: 19 Male:20	**Control** Mean gestational age: 29s Mean weight: 1,170g Female: 20 Male: 21	Significant reduction of gastrointestinal colonization of *Candida sp*	Some risk of bias
10	Martí et al., 2021	**Treatment** Mean gestational age: 25.5s Mean weight: 727.5g Female:31 Male:23	**Control** Mean gestational age: 25.5s Mean weight: 763g Female: 19 Male:35	Increase in bacterial diversity and composition, elevation of *Lactobacillus:* Reductions of *Proteobacteria, Enterobacteria* and *Staphylococcus.*	Low risk of bias
11	Moreno-Sanz et al., 2022	**Treatment** Mean gestational age: 29s Mean weight: 1,270g Female:8 Male:6	**Control** Mean gestational age: 29s Mean weight: 1,150g Female:7 Male:6	*Lactobacillus* and *Bifidobacterium* colonization; Significant reduction of *Enterococcus* and *Coprococcus.*	High risk of bias
12	Mohan et al., 2006	**Treatment** Mean gestational age: Less than 37s Mean weight: No information Female:23 Male:14	**Control** Mean gestational age: Less than 37s Mean weight: No information Female:20 Male:12	Increase in the number of *Bifidobacterium;* Reduction of *Enterobacteria* and *Clostridium spp* in the intestinal microbiota of preterm infants with the use of probiotics.	Low risk of bias

Article	**Author/year**	**Profile of partticipants**		**Results (Intervention+placebo or treatment)**	**Risk of bias**
13	Oshiro et al., 2019	**Treatment** Mean gestational age: 28.1s Mean weight: 1.049g Female:15 Male: 6	**Control** Mean gestational age: 28.2s Mean weight: 1,002g Female:10 Male: 7	Elevation *Bifidobacterium*	Low risk of bias
14	Patole et al., 2014	**Treatment** Mean gestational age: Less than 32.6s Mean weight: 1,090g Female:32 Male: 45	**Control** Mean gestational age: Less than 32.6s Mean weight: 1,025g Female:35 Male: 41	Increased levels of *B.breve* in the stool of preterm infants supplemented with probiotics	Low risk of bias

15	Plummer et al., 2018	**Treatment** Mean gestational age: 28.6s Mean weight: 1,040g Female:19 Masculino: 19	**Control** Mean gestational age: 27.5s Mean weight: 1,000g Female:14 Male: 14	*Bifidobacterium rates found in greater abundance in premature infants who received probiotics, in addition to the reduction of Enterococcus.*	High risk of bias
16	Plummer et al., 2021	**Treatment** Mean gestational age: 28.6s Mean weight: 1,058g Female:114 Male: 115	**Control** Mean gestational age: 28.1s Mean weight: 1,080g Female:117 Male: 113	*Increased Bifidobacterium (B.longum subsp.infants and B.animalis subsp lactis) in the probiotic group, and there were no differences in detection of Streptococcus.thermophilus between groups.*	High risk of bias

Article	**Author/year**	**Profile of partticipants**		**Results (Intervention+placebo or treatment)**	**Risk of bias**
17	Qiao et al., 2016	**Treatment** Mean gestational age: 32.4s Mean weight: 1,653 g Female: 13 Male: 17	**Control** Mean gestational age: 32.1s Mean weight: 1,532 g Female: 14 Male: 16	1st and 2nd weekend: Higher amounts of *Bifidobacterium* and *Lactobacillus*	Low risk of bias
18	Rougé et al., 2009	**Treatment** Mean gestational age: 28.1s Mean weight: 1,115g Female: 17 Male: 28	**Control** Mean gestational age: 28.1s Mean weight: 1,057g Female: 23 Male: 26	*Bifidobacterium* and *Lactobacillus* colonization was higher in the experimental group. In addition to the reductions in *Clostridia* in the 1st week and *Enterococcus* in the 2nd week.	Low risk of bias
19	Samara, 2021	**Treatment** Mean gestational age: 25.8s Mean weight: 763g Female: 15 Male: 16	**Control** Mean gestational age: 25.6s Mean weight: 751g Female: 11 Male: 20	Significantly higher number of *Bifidobacterium* cells (*B.breve, B.bifidum and B. longum subsp longum*); Reduction of *Candida sp.* in the probiotic group.	Some risk of bias
20	Samara et al., 2022	**Treatment** Mean gestational age: 23s-29s Mean weight: Less than	**Control** Mean gestational age: 23s-29s Mean weight: Less than 1,000g Female: No information Male: No information	High colonization of *Bifidobacterium* less than *B.infantis HA-116* in probiotic group for 6 months; Reduction of *Staphylococcus* and *Enterococcus.*	Low risk of bias

		1,000g Female: No information Male: No information			

Article	Author/year	Profile of partticipants		Results (Intervention+placebo or treatment)	Risk of bias
21	Sourabh *et al*., 2015	**Treatment** **Group A** Mean gestational age: 30.64s Mean weight: 1,286.08g Female: 13 Male: 25 **Group B** Mean gestational age: 31.08s Mean weight: 1,335.97 g Female::15 Male: 23 **Group C** Mean gestational age: 30.89s Mean weight: 1,413.32 g Female: 18 Male: 20	**Control** **Group D** Mean gestational age: 30.82s Mean weight: 1,252.27 g Female: 12 Male: 23	High numbers in *Lactobacillus spp.* and *Bifdobacterium spp.* in the 3 probiotic groups compared to placebo. There was no difference in colonization between the probiotic groups, only Group A had a higher change.	Low risk of bias
22	Stratiki et al., 2007	**Treatment** Mean gestational age: 31s Mean weight: 1,500 g Female: 41 Male: 23	**Control** Mean gestational age: 30.5s Mean weight: 1,500g Female: 31 Male: 17	Increase in *Bifidobacterium*; Significant change in intestinal permeability.	Low risk of bias
23	Strus et al., 2018	**Treatment** Mean gestational age: 29.73s Mean weight: 1,281.24 g Female: 47 Male: 42	**Control** Mean gestational age: 29.67s Mean weight: 1,350.11 g Female: 38 Male: 50	Weeks 2-3: Increased *Bifidobacterium*; Week 2-7; Increased *Lactobacillus* count.	Low risk of bias

Source: Author, 2023.