The Use of Probiotics for Prevention of Late-Onset Sepsis in Very Preterm or Very Low Birth Weight Infants: A Scoping Review

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Abstract
Background
Neonatal late-onset sepsis (LOS) is a major cause of morbidity and mortality in very preterm and/or very low birth weight (VLBW) infants. Immaturity of the gut and immune system, together with microbial dysbiosis, increases susceptibility to bacterial translocation. Probiotics have been proposed as a preventive strategy, but evidence for their efficacy remains uncertain.

Objective
This scoping review aimed to collect and analyse existing information regarding the prophylactic effect of probiotics on the incidence of LOS in very preterm (gestational age (GA) <32 weeks) and/or VLBW infants (birth weight <1500 grams).

Design
A search was conducted on 06/08/2023 in the PubMed (Medline) database, yielding 1073 articles after deduplication. Randomised, double-blinded, and placebo-controlled, with a population consisting of very preterm and/or VLBW infants, and with incidence of LOS as primary or secondary outcome, were included. In total, twelve articles met the criteria.

Results
Three studies reported a significant reduction in LOS with probiotic supplementation. In one of these studies, this difference was observed only in the subgroup with GA ≥ 28 weeks. However, eight studies, including the one with the largest study population (N=1310), did not yield significant results, and one study did not provide results from statistical analysis.

Conclusion
Due to clinical and statistical heterogeneity, it is difficult to draw a conclusion about the efficacy of probiotics in preventing LOS in very preterm and/or VLBW infants. Additionally, no clear answer could be provided regarding the optimal probiotic strain, dosage, and duration of treatment. Further, more homogeneous research is needed.

Introduction

Late-onset neonatal sepsis (LOS), defined as sepsis occurring at least 72 hours after birth until discharge, is a common and serious complication (1). Definitions vary widely across studies, with studies employing two major criteria interchangeably. One criterion relies on a positive blood culture, often termed as 'culture-proven sepsis'. The other criterion relates to clinical signs of sepsis without a positive blood culture, termed as 'culture-negative sepsis' or 'clinical sepsis'. This variation makes it challenging to compare clinical trials (1).

Despite advances in neonatal care, LOS rates remain high, affecting 12–50% of very preterm and/or very low birth weight (VLBW) infants, whereas the incidence in term infants is only 1.6% (1, 2). Consequently, LOS remains a major cause of morbidity and mortality in the Neonatal Intensive Care Units (NICU) and a significant complication of prematurity (3).

Late-onset sepsis is primarily caused by Gram-positive bacteria, most commonly coagulase-negative *Staphylococcus* (CNS) and *Staphylococcus aureus*, which may be introduced via invasive procedures. However, in very preterm and VLBW infants, Gram-negative bacteria and fungal infections have been shown to account for a larger proportion of infections (4). These pathogens are predominantly introduced through translocation across the intestinal barrier in the gastrointestinal tract. Preterm infants are more prone to bacterial intestinal translocation due to numerous factors such as immaturity of the barrier function

of the intestinal mucosa, immaturity of the intestinal immune response and the impact of broad-spectrum antimicrobial drugs and invasive procedures on the gut microbiome in an intensive care hospital environment (3). The acquisition of the gut microbiome in preterm infants is mainly driven by the NICU environment, where early-life clinical practices may interrupt the normal colonisation of the infant gut microbiome with a reduced microbial diversity (3, 5). There is a decreased colonisation by beneficial bacteria such as Lactobacilli and Bifidobacteria, which are typically predominant in healthy full-term infants, and increased abundance of potentially pathogenic, such as Gram-negative Enterobacteriaceae (3, 5, 6). While in full-term infants, the gut microbiome primarily arises from maternal genital tract, skin and breastmilk (3, 5). Interestingly, infants receiving mothers' own milk (MOM) harbour more beneficial *Clostridiales*, *Lactobacillales*, and *Bacillales* compared to those fed human donor milk (HDM) or formula, who have more pathogenic *Enterobacteriaceae* (7).

Management of LOS relies on antibiotics and supportive measures like hemodynamic stabilization (2). Early therapy is crucial but the diagnosis is difficult due to the nonspecific symptoms (1). Given its severity and diagnostic challenges, prevention of LOS is a major focus (1). For fungal infections, prophylactic systemic antifungal therapy is already in use and has been shown to significantly reduce the incidence of invasive fungal infections (8).

Probiotics represent another promising preventive strategy, currently under investigation.

These live organisms, which closely resemble the beneficial gut flora of the human gastrointestinal tract, may, when administered in adequate amounts, confer health benefits to the host by interacting with the gut microbiota and supporting immune function. Probiotics could strengthen the intestinal barrier function, prevent the gastrointestinal overgrowth of pathogenic bacteria, and inhibit the translocation of pathogens across the intestinal wall. Through these combined effects, probiotics could contribute to a reduction in the risk of life-threatening infections, including LOS (2, 3).

However, probiotic supplementation in neonatal medicine remains controversial. A major challenge is the considerable variability in probiotic strains used across studies, which makes it difficult to compare outcomes and to develop standardised guidelines for prevention of LOS. In addition, potential risks and major concerns have been identified. Probiotics may increase bacterial adherence to the intestinal mucosa, potentially promoting bacterial translocation and leading to probiotic-associated sepsis. Although rare, such cases have been reported and have led to guidelines recommending cautious use of probiotics, particularly in (preterm) neonates and in infants with immune deficiencies (3, 9). In addition, there is a risk of promotion of antimicrobial resistance, fungal or bacterial contamination of commercial products, and cross-colonisation on the NICU which was prevalent in at least one large randomised controlled trial (10).

Despite current concerns about the safety of probiotics, multiple reports suggest that their use can be considered safe in preterm infants, with few reported side effects (11, 12). For instance, probiotics are already used in the prevention of necrotising enterocolitis (NEC), which is another major cause of death and comorbidities in infancy, linked to antibiotic exposure and gut dysbiosis (13-15). Like LOS, it occurs more frequently in infants with low gestational age (GA) and/or birth weight (BW) (16). However, the role of probiotics in NEC lies beyond the scope of our review.

This scoping review explores the potential role of probiotics to prevent LOS in very preterm (<32 weeks GA) and/or VLBW (<1500 g) infants in the NICU, aiming to identify key insights, controversies, and research gaps in this area.

Methods

The study entailed a scoping review for which the search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist (PRISMA) (17) .

The study objective was to evaluate the effect of the use of probiotics on the incidence of LOS in very preterm infants (<32 weeks GA) and/or VLBW infants (<1500 grams), when probiotics are administered prophylactically. This was investigated by comparing the currently available data on this topic, which has already been acquired through RCTs.

This scoping review included all prospective, mono- or multicentre, double-blind, randomised, placebo-controlled trials looking at preterm infants with a <32 weeks GA and/or with a VLBW <1500g with no other serious medical or surgical conditions or severe malformations, with probiotics supplementation as intervention and written in the English language, with as primary or secondary outcome the incidence of both clinical and culture-proven LOS.

We excluded all non-randomised, retrospective, case-control studies, systematic reviews, and meta-analyses. Additionally, articles published in any language other than English or involving a population with incorrect criteria (>32 weeks GA, >1500g BW) or with severe medical or surgical conditions or severe malformations were excluded. Studies without a placebo group for control were also excluded.

An overview of these inclusion and exclusion criteria can be found in table 1.

A search was conducted in the PubMed (Medline) database on the 6th of August 2023 with the following three concepts: (A) probiotics, (B) premature, (C) low birth weight. The complete search can be found in the appendix.

After the search, all duplicates were first removed using the Endnote 21 software (18). Subsequently, the inclusion process commenced, conducted by one reviewer, and comprised two consecutive phases. In the initial phase, articles were screened based on their title and abstract. The inclusion and exclusion of articles was carried out according to the preceding criteria (table 1). Next, the remaining articles were screened based on the full text, again following the same criteria. This inclusion process was conducted using the Rayyan software program (19).

Afterwards, 'snowballing' was performed within the relevant systematic reviews published between 2021 and 2023, to additionally include any potentially missed eligible RCTs. This was also conducted by one reviewer.

Information about the study design, key characteristics of the study, the patient population, the used probiotic strain and dose and the reported incidence of LOS were collected from these studies. Additionally, results investigating the occurrence of probiotic-induced sepsis, the safety of probiotics and cross-contamination were also included.

As this concerns a purely literature-based study, no approval was required from the authorized medical ethics committee.

Results

The search was conducted on the 6th of August in 2023 in the PubMed (Medline) database and yielded 1075 articles. After deduplication, 1073 articles were screened by title and abstract, of which 267 articles underwent full-text review. Eleven articles met the inclusion criteria, with one additional article identified through snowballing, resulting in a total of twelve included articles. This is schematically presented in the PRISMA flowchart in the appendix as figure 1.

TABLE 1: Inclusion and exclusion criteria of the review.

Category	Inclusion criteria	Exclusion criteria
Study objective	Incidence of LOS as primary or secondary outcome Intervention: probiotics	No outcomes regarding sepsis
Population	Very preterm <32 weeks GA Very low birth weight <1500 g No other severe medical or surgical conditions or severe malformations	>32 weeks GA >1500 g BW With other severe medical or surgical conditions or severe malformations
Language	English	All other languages
Type of literature	Prospective, mono- or multicenter, double blind, randomized, placebo-controlled trials	Non-randomized, retrospective, case-control studies, systematic reviews, and meta-analyses
Publication date	No restrictions	/

Abbreviations: BW = birth weight; GA = gestational age; LOS = late-onset sepsis

In total, 4314 participants were involved in the studies, with no overlap between the different studies. The sample size of the studies ranged from 35 to 1310 participants (3, 20). The most relevant demographic data of the included patients are listed below. The mean GA varied between 25 weeks and 31 weeks and the mean BW varied between 736 grams and 1648 grams (21-23).

In most of the included studies, different types of feeding were administered, being MOM, HDM and preterm formula (3, 5, 6, 16, 24, 25). Only one study involved infants exclusively fed MOM (9). In another study, pasteurised human milk (MOM or HDM) was provided to 70% of VLBW infants, with the remaining infants receiving preterm formula (26). Another investigation reported that all infants received exclusive breastfeeding until reaching a weight of 2000 grams, and the milk was fortified with bovine protein fortifier once enteral feeds reached 100mL/kg/day (23). In contrast, Costalos et al. included only infants receiving preterm formula, as breastfeeding was an exclusion criterion (21). In one study all infants additionally received their mother's colostrum, and the majority were exclusively fed MOM (20). Finally, one study did not specify the type of feeding administered to the infants (3).

Probiotic interventions varied regarding strain, dose, timing, frequency and the duration of treatment across the different studies. Two studies both used the same *Bifidobacterium* breve BBG-001 strain as a probiotic, although in different doses and administration schedules. For instance, Costeloe et al. administered it, as soon as possible after birth, at a dose ranging from $10^{8.3}$ to $10^{8.8}$ colony-forming units (CFU) once daily until a postmenstrual age (PMA) of 36 weeks or discharge (3). On the other hand, Oshiro et al. initiated the probiotic supplementation within a few hours after birth, administering it once daily at a dose of $2.5x10^8$ CFU until discharge (20). Two other studies utilised *Lactobacillus rhamnosus* GG, although one of these studies combined it with another probiotic. Moreover, the dosage and administration schedule also differed between these two studies. Dani et al. administered *Lactobacillus rhamnosus* GG at a dose of $6x10^9$ CFU once daily starting from the first feeding until discharge (25). Whereas Rougé et al. combined *Lactobacillus rhamnosus* GG with *Bifidobacterium longum* BB536, administering them at a dose of 10^8 CFU four times daily from the initiation of enteral feeding until discharge (6). Three other studies utilised *Lactobacillus reuteri* DSM 17938. In Marti et al., it was administered once daily from birth until a PMA of 36 weeks, with no specified dose (22). In the study by Oncel et al., *Lactobacillus reuteri* DSM 17938 was administered at a dose of $1x10^8$ CFU once daily from birth until discharge (16). Wejryd et al. administered the same probiotic within three days after birth until a PMA of 36 weeks but at a dose of $1.25 x10^8$ CFU once daily (23).

The study by Patole et al. used a different strain, *Bifidobacterium breve* M16, with a different dose and administration schedule. In this study, probiotic supplementation began when the infant could tolerate enteral feeding until a PMA of 37 weeks, with a dose of $3x10^9$ CFU once daily (5, 24). *Lacticaseibacillus paracasei* was only used in the study by Matin et al., and was administered once daily at a dose of $1.5x10^9$ CFU from 48 to 72 hours after birth for a total of 28 days (9). Only one study used *Saccharomyces boulardii*, a probiotic yeast strain, administered every 12 hours at a dose of 10^9 CFU from the initiation of enteral feeding for a total of 30 days (21).

Different types of administered probiotics were also investigated either head-to-head as single-strain or in combination as multi-strain products and compared with placebo. One study compared the effect of two different species of *Bifidobacterium*. One group received *Bifidobacterium lactis* once daily at a dose of 10^9 CFU, another group received *Bifidobacterium longum* once daily at 10^9 CFU, and the third group received a combination of *Bifidobacterium lactis* and *Bifidobacterium longum* at a dose of 10^9 CFU. All three groups received this supplementation for 4 to 6 weeks (26). In the ProPrems study, the probiotics group received a combination of bacteria, including *Bifidobacterium infantis* BB-02, *Streptococcus thermophilus* TH-4, and *Bifidobacterium lactis*, at doses of 300 $x10^6$, 350x 10^6, and 350 $x10^6$ CFU respectively, once daily if tolerating enteral feeds of at least 1 mL every 4 hours until a PMA of 40 weeks or discharge.

Three studies showed a significant difference in the incidence of LOS between the probiotic and placebo group (5, 9, 16). However, these benefits were largely confined to specific subgroups. In Jacobs et al. the effect was significant only among infants with GA ≥ 28 weeks; no significant difference was observed when the complete study population was analysed (5). Oncel et al. demonstrated a significant benefit of probiotic supplementation, particularly in infants weighing less than 1000 grams (16). Eight other included studies showed no significant effect, where one of these studies was terminated prematurely due to a lack of effect (6). One study only mentioned the number of cases of sepsis in the probiotic and placebo group, but not the effect accompanied by the statistical analysis (22). Detailed results of the included studies are summarised in table 2.

Dani et al. reported that, in the placebo group, 75% of the sepsis cases were caused by CNS and 25% by *ß-haemolytic Streptococci*, whereas in the probiotic group 64% were due to coagulase negative *Staphylococcus* (CNS) and 36% to *Enterobacteriaceae* (25). Matin's study reported three sepsis cases, all occurring in the placebo group, caused by *Staphylococcus aureus*, CNS, and *Escherichia coli* (9). In the study by Costeloe et al., Gram-negative bacteria (*Enterobacteriaceae* and *Enterococcus* species) were the main causative pathogens, followed by *Staphylococcus aureus* and fungal organisms (3). Another study reported a greater proportion of Gram-positive than Gram-negative pathogens, followed by fungal organisms (16). Hays et al. identified CNS as the most frequent pathogens, followed by *Staphylococcus aureus* and *Candida* species (26). None of these studies demonstrated a difference in

TABLE 2: Descriptive of the included studies.

Authors, Reference number, Year	Study Design	Participants	Description of the Study	Most Relevant Outcomes	Results: Number of Cases (%) and p-value or Confidence Interval	Conclusion
Costalos, et al. **Reference number: 21** **2003**	Prospective, monocentric double-blind, randomised, placebo-controlled trial	Preterm infants with GA 28-32w. N= 87 - Placebo: n= 36 - Probiotics: n= 51	Intervention: *S. boulardii* 1x/12h vs placebo 1x/12h. Duration: - Start: when enteral feeding started - Stop: after 30 days	Primary outcomes: - Toleration of *S. boulardii* supplementation - Does *S. boulardii* supplementation result in a reduction of the size of the bowel reservoir of nosocomial pathogens - Role of *S. boulardii* on gastrointestinal function Secondary outcomes: - Duration of supplementation - Incidence of NEC - Sepsis	Culture-proven sepsis: Placebo: 3 (8,3%) Probiotics: 3 (5,8%) → p= 0,7	No significant difference in incidence of culture-proven sepsis in probiotic group. No results on causative pathogens.
Costeloe, et al. **Reference number: 2** **2016**	Prospective, multicentre, double-blind, randomised placebo-controlled phase 3 trial.	Preterm Infants with GA 23 0/7-30 6/7w. N= 1310 - Placebo: n= 660 - Probiotics: n= 650	Intervention: *B. breve* BBG-001 1x/d vs placebo 1x/d. Duration: - Start: as soon as possible - Stop: PMA 36w or discharge	Primary outcome: - NEC bell stage 2-3 - Blood culture positive sepsis (>72h after birth and before 46 weeks PMA or discharge or death) - Death before discharge from hospital Secondary outcome: - Composite of the three primary outcomes - Number of infants with any positive blood culture with an organism recognized as a skin commensal - Number of infants with blood stream infections with pathogens categorised by organism	Culture-proven sepsis: - Placebo: 77 (12%) - Probiotics: 73 (11%) → CI: 0,97 (0,73–1,29) Causative pathogens: - *Enterobacteriaceae* • Placebo: 29 (4%) • Probiotics: 23 (4%) → CI: 0,80 (0,41-1,59) - *Enterococcus species* • Placebo: 14 (2%) • Probiotics: 13 (2%) → CI: 0,92 (0,35-2,43) - *Staphylococcus species* • Placebo: 17 (3%) • Probiotics: 21 (3%) → CI: 1,26 (0,56-2,82) - Fungi • Placebo: 5 (1%) • Probiotics: 5 (1%) → CI: 1,00 (0,20-5,06) - Othor non-skin commensals • Placebo: 22 (3%) • Probiotics: 22 (3%) → CI: 0,93 (0,44-1,96)	No significant difference in incidence of culture-proven sepsis in probiotic group. No significant differences in pathogen distribution.
Dani, et al. **Reference number: 23** **2002**	Prospective, multicentre, double-blind, randomised, placebo- controlled trial.	Infants with GA <33w or BW <1500g. N= 585 - Placebo: n= 290 - Probiotics: n= 295	Intervention: *L. rhamnosus* GG 1x/d vs placebo 1x/d. Duration: - Start: with first feed - Stop: discharge	Primary outcome: - NEC (7 days after start supplementation) - Sepsis, confirmed by positive blood cultures. (7 days after start supplementation)	Sepsis: - Placebo: 12 (4,1%) - Probiotics: 14 (4,7%) → p> 0,05 Causative pathogens: - Placebo: • CNS: n= 9 • β-haemolytic *Streptoccoci*: n= 3 - Probiotics: • CNS: n=9 • *Enterobacteriacea*: n= 5 → no CI was available	No difference in incidence of sepsis in the probiotic group. The study did not report statistical data on significance in differences in pathogen distribution.
Hays, et al. **Reference number: 24** **2015**	Prospective, multicentre, randomised, double-blind, placebo-controlled trial	Preterm infants with GA between 25-31w and BW between 700-1600g. N= 199 - Placebo: n= 52 - Probiotics: n= 147 • P1: n= 50 • P2: n=49 • P3: n=47	Intervention: P1: *B. lactis* OR P2 *B. longum* OR P3 *B. lactis* and *B. longum* 1x/d vs placebo 1x/d. Duration: 4-6 weeks	Primary Outcomes: - LOS	Culture-proven sepsis: - Placebo: 19% - Probiotics: 17% • P1: 18% • P2: 16,7% • P3: 17%) → p=0,912 Causative pathogens: - CNS • Placebo: 80% (55-100) • Probiotics: 56% (37-76) • P1: 67% (36-98) • P2: 38% (4-72) • P3: 63% (30-97) - *Staphylococcus aureus* • Placebo: 0% (0-0) • Probiotics: 28% (10-46) • P1: 11% (0-31) • P2: 50% (15-85) • P3: 25% (0-55)	No significant difference in incidence of culture-proven sepsis in probiotic group. No significant differences in pathogen distribution.

					- *Candida* • Placebo: 10% (0-29) • Probiotics: 0% (0-0) • P1: 0% (0-0) • P2: 0% (0-0) • P3: 0% (0-0) - Others • Placebo: 10% (0-28) • Probiotics: 16% (2,30) • P1: 22% (0-49) • P2: 13% (0-36) • P3: 13% (0-36)	
Jacobs, et al. **Reference number: 4** **2013**	Prospective multicentre, double-blind, randomised, placebo-controlled trial	Preterm infants with GA <32w and BW <1500g. N= 1099 - Placebo: n= 551 - Probiotics: n= 548	Intervention: *B. infantis* BB-02 and *S. thermophilus* TH-4 and *B. lactis* BB-12 (3)1x/d vs placebo 1x/d Duration: - Start: intake PO of 1ml every 4h - Stop: discharge or PMA 40w	Primary outcome: - At least one episode of culture-proven LOS <40 weeks PMA or discharge home • Culture-proven sepsis • Clinical sepsis Secondary outcomes: - Incidence culture proven/clinical sepsis - Composite outcome of culture proven/clinical sepsis - Number and duration of antibiotic treatment - Incidence of culture-proven sepsis with probiotic species - Mortality - Incidence of NEC and NEC bell stage 2≤	Culture-proven LOS: - Placebo: 89 (16,2%) - Probiotics: 72 (13,1%) → p=0,16 Subgroup analyses (culture-proven LOS): - GA: • <28w: ◦ Placebo: 55 (23,4%) ◦ Probiotics: 54 (24,7%) → p= 0,75 • ≥28w: ◦ Placebo: 34 (10,8%) ◦ Probiotics: 18 (5,5%) → p= 0,01 - BW: • <1000g: ◦ Placebo: 58 (24,3%) ◦ Probiotics: 53 (22,6%) • ≥1000g: ◦ Placebo: 31 (9,9%) ◦ Probiotics: 19 (6,1%) Clinical LOS - Placebo: 83 (15,1%) - Probiotics: 75 (13,7%) → p= 0,52 Clinical or Culture-proven LOS - Placebo: 146 (26,5%) - Probiotics: 129 (23,5%) → p= 0,26 Causative pathogens: - CNS ◦ Placebo: 43 (7,8%) ◦ Probiotics: 40 (7,3%) → CI: 0,94 (0,62-1,42)	No significant difference in incidence of LOS with probiotic combination. Significant reduction in culture-proven LOS in probiotic subgroup ≥28w GA (p=0,01), not for <28w (p=0,75). No differential effect on LOS in subgroup analyses for BW. No difference in number of infants with 1 episode of clinical LOS (p=0,52) or with the composite outcome of culture proven or clinical sepsis (p=0,26). No difference was observed in episodes of LOS caused by conventional pathogens or CNS between the placebo and probiotics groups, and the study did not report numerical data on the number of cases.
Martı, et al. **Reference number: 19** **2021**	Prospective, multicentre, double-blind, randomised, placebo-controlled trial	Infants with BW ≤1000g. N= 134 - Placebo: n= 66 - Probiotics: n= 68	Intervention: *L. reuteri* DSM 17938 1x/d vs placebo 1x/d Duration: - Start: from birth - Stop: PMA 36 weeks	Secondary outcomes: Analyse microbiota composition in relation to NEC, sepsis	Culture-proven sepsis: - Placebo: 23 - Probiotics: 25	Significance level not mentioned. Cases of culture-proven sepsis: placebo: n= 23, probiotics: n= 2. No results on causative pathogens.
Matin, et al. **Reference number: 12** **2022**	Prospective, monocentre, double-blind, randomised, placebo-controlled trial	Breastfeeding mothers and infants with BW ≤1000g. N= 78 infants and 75 mothers Placebo to both mother and infant: - Infants: n= 26 - Mothers: n= 25 Placebo to infant, probiotic to mother: - Infants: n= 26 - Mothers: n= 25 Probiotic to infant, placebo to mother: - Infants: n=26 - Mothers: n= 25	Intervention: *L. paracasei* 1x/d vs placebo 1x/d Duration: for 28 days.	Secondary infant outcomes: - Occurrence of serious neonatal problems until 40 days of infancy • Death • NEC • Positive blood culture	Serious problems: → p= 0,035 Causative pathogens: - 3 cases of LOS all in the placebo group: • One with CNS • One with *Staphylococcus aureus* • One with *Escherichia coli*	Sepsis was classified under broader term of "serious problems". Statistical analysis for serious problems showed significantly lower incidence of serious problems in probiotics group. The study did not report statistical data on significance in differences in pathogen distribution.

Authors, Reference number, Year	Study Design	Participants	Description of the Study	Most Relevant Outcomes	Results: Number of Cases (%) and p-value or Confidence Interval	Conclusion
Matin, et al. **Reference number: 12** **2022**	Prospective, monocentre, double-blind, randomised, placebo-controlled trial	Breastfeeding mothers and infants with BW ≤1000g. N= 78 infants and 75 mothers Placebo to both mother and infant: - Infants: n= 26 - Mothers: n= 25 Placebo to infant, probiotic to mother: - Infants: n= 26 - Mothers: n= 25 Probiotic to infant, placebo to mother: - Infants: n=26 - Mothers: n= 25	Intervention: *L. paracasei* 1x/d vs placebo 1x/d Duration: for 28 days.	Secondary infant outcomes: - Occurrence of serious neonatal problems until 40 days of infancy • Death • NEC • Positive blood culture	Serious problems: → p= 0,035 Causative pathogens: - 3 cases of LOS all in the placebo group: • One with CNS • One with *Staphylococcus aureus* • One with *Escherichia coli*	Sepsis was classified under broader term of "serious problems". Statistical analysis for serious problems showed significantly lower incidence of serious problems in probiotics group. The study did not report statistical data on significance in differences in pathogen distribution.
Oncel, et al. **Reference number: 8** **2013**	Prospective, monocentre, double-blind, randomised, placebo-controlled trial	Infants with GA ≤32w and BW ≤1500 g. N= 400 - Placebo: n= 200 - Probiotics: n= 200	Intervention: *L. reuteri* DSM 17938 1x/d vs placebo 1x/d Duration: - Start: with first feed - Stop: until discharge	Primary outcomes: - Death beyond the 7th day of life - NEC ≥2 Secondary outcomes: - Culture-proven sepsis Adverse events were also recorded, included: - Culture- proven sepsis attributable to *L. reuteri*	Total: culture-proven sepsis - Placebo: 25 (12,5%) - Probiotics: 13 (6,5%) → p= 0,041 1000-1500g: culture-proven sepsis: - Placebo: 6 (6,2%) - Probiotics: 7 (6,5%) → p= 0,57 <1000g: culture-proven sepsis - Placebo: 19 (18,4%) - Probiotics: 6 (6,5%) → p= 0,01	Frequency of culture-proven sepsis was significantly lower in probiotic group. Subgroup analysis in BW 1000-1500g group showed no significant difference in incidence of culture-proven sepsis. Subgroup analysis in BW <1000g group showed significant difference in incidence of sepsis. No significant differences in pathogen distribution.
Oshiro, et al. **Reference number: 18** **2019**	Prospective, monocentre, double-blind, randomised, placebo-controlled trial	Infants with GA 24-31w and BW <1500g. N= 35 - Placebo: n= 18 - Probiotics: n= 17	Intervention: *B. breve* BBG-01 1x/d vs placebo 1x/d Duration: - Start: several hours PP. - Stop: until discharge	Secondary outcomes: - Incidences of NEC - Incidences of sepsis	Sepsis: - Placebo: 0 - Probiotics: 3 (16,7%) → p>0,05	No significant difference in incidence of LOS in probiotic group. No results on causative pathogens.
Patole, et al. **Reference number: 22** **2014**	Prospective, monocentric, double-blind, randomised, placebo-controlled trail	Infants with GA ≤32w + 6d and BW <1500g. N= 159 - Placebo: n= 80 - Probiotics: n= 79	Intervention: *B. breve* M-16V 1x/d vs placebo 1x/d Duration: - Start: when ready for enteral feeds - Stop: PMA 37w	Secondary outcomes: - Incidence of NEC (≥stage II) - All cause death - Blood culture positive LOS (≥72h of life)	Suspected episodes LOS: none: - Placebo: 43 (57%) - Probiotics: 48 (62%) → p= 0,744 Proven episodes LOS: none: - Placebo: 64 (84%) - Probiotics: 48 (62%) → p= 0, 465	No significant difference in incidence of LOS in probiotic group. No results on causative pathogens.
Rougé, et al. **Reference number: 5** **2009**	Prospective, bicentric, double-blind, randomised, placebo-controlled trial	Infants with GA <32w and BW <1500g and a postnatal age ≤ 2w. N= 94 - Placebo: n= 49 - Probiotics: n= 45	Intervention: *L. rhamnosus* GG and B. longum BB536 4x/d vs placebo 4x/d Duration: - Start: when enteral feeding started - Stop: until discharge.	Secondary outcomes: - Nosocomial infections - Sepsis with positive blood culture - Duration of antibiotic use - NEC - Death	Culture-proven sepsis - Placebo: 13 (26,5%) - Probiotics: 15 (33,3%) → p= 0,47	Trial was discontinued after fourth sequential analysis concluded a lack of effect. No significant difference in incidence of LOS in probiotic group.
Wejryd, et al. **Reference number: 20** **2018**	Prospective, multicentre, double-blind, randomised, placebo-controlled trial	Infants between GA 23w + 0d and 27w +6d and BW <1000g. N= 134 - Placebo: n= 66 - Probiotics: n= 68	Intervention: *L. reuteri* DSM 17938 1x/d vs placebo 1x/d Duration: - Start: within 3d after birth - Stop: PMA 36w + 0d	Secondary outcomes: - All-cause mortality - NEC (≥stage 2) Culture-proven sepsis	Culture-proven sepsis: - Placebo: 23 (35%) - Probiotics: 25 (37%) → p= 0,82	No significant difference in incidence of LOS in probiotic group. (p= 0,82). No results on causative pathogens.

pathogen distribution between the probiotic and placebo groups (3, 9, 16, 25, 26). The ProPrems study reported sepsis due to CNS only (7,3% in the probiotic and 7,8% in the placebo group), with no data on other pathogens. The supplemented probiotic was not isolated in any case (5). For further details, see Table 2.

The remaining six studies did not mention the causative bacteria of the reported sepsis cases (6, 20-24).

The anticipated adverse events of probiotic supplementation were vomiting, abdominal distension, and diarrhoea. Five studies reported no adverse events which indicated that probiotic supplementation was, overall, well tolerated (5, 6, 9, 16, 21, 24). One study reported no increase in severe adverse events in the probiotics group compared to the placebo, but the specific adverse events that occurred are not mentioned (23). In the study by Hays et al., multiple adverse events were documented, but none of these adverse events were linked to probiotic supplementation. It was not specified what these adverse events were (26). The study conducted by Costeloe et al. documented two cases of serious adverse events: toxic epidermal necrolysis and pulmonary haemorrhage. It was deemed unlikely that these severe side effects were caused by the probiotic supplementation. Furthermore, the study concluded that the participants tolerated the treatment well (3). Two studies did not mention the safety of the probiotic supplementation (20, 22).

Another potential adverse event is the risk of bacteraemia caused by the supplemented probiotic strain. Ten studies concluded that the supplemented probiotic was not the causative agent of the sepsis cases (3, 5, 6, 9, 16, 21, 23-26). It was not mentioned in two studies (20, 22).

Cross-contamination likely occurs during the preparation of the probiotic and placebo supplements, leading to unintended probiotic supplementation in the placebo group. This was observed in two studies, where probiotic levels in stool increased over time in both groups (20, 24).

Discussion

Late-onset sepsis is a serious condition that primarily affects preterm and VLBW infants. Although supportive therapy exists, the mortality rate remains high, underscoring the need for new preventive strategies. Gut microbiome dysbiosis has been observed to precede both clinical and culture-proven LOS, typically involving *Staphylococcus epidermidis* and other *Staphylococci* and *Bacillales* (27). These disrupted microbial patterns suggest a potential mode of action through which probiotics may exert their effects. As a non-invasive intervention, probiotics aim to restore this dysbiosis and promote a healthy gut microbiome, resembling the gut microbiome of exclusively breastfed healthy term infants.

Overall, the studies included in this review were well-randomised regarding the GA and BW across different studies (3, 5, 6, 9, 16, 20-26). However, significant clinical heterogeneity in other patients' characteristics limited comparability. The type of feeding is likely to influence clinical outcomes in this population, as breastfeeding will reduce the incidence of LOS. Infants receiving MOM exhibit a more beneficial gut microbiome than infants exclusively receiving formula feeding (7). In the three included studies that reported a significant difference, the majority received exclusive breastfeeding (5, 9, 16). In contrast, the study by Costalos et al., in which exclusively formula feeding was provided, showed no reduction in the incidence of LOS (21). This suggests that for the prevention of LOS, exclusive breastfeeding with probiotic supplementation may potentially offer added value compared to formula feeding. This could be because most common probiotics studied, such as *Bifidobacterium infantis*, grow most effectively in the presence of human milk oligosaccharides which are lacking in the studied cow-milk formula (28).

The included studies used a wide variety of probiotic strains or combination of strains, with varying concentrations or treatment durations, making comparison between studies challenging. Current literature provides no clear evidence that one single probiotic strain is superior for infant supplementation, and each strain appears to exert its own specific effects (29). Overall, *Lactobacillus* and *Bifidobacterium* species are the most used, as they are predominantly found in the gut microbiome of healthy, exclusively breastfed infants (26, 30). Notably, the three studies demonstrating a statistically significant reduction in LOS used these probiotics: *Lactobacillus reuteri* and *Lactobacillus paracasei* as a single strain intervention and in the ProPrems study, a combination of *Bifidobacterium infantis* BB-02, *Streptococcus thermophilus* TH-4, and *Bifidobacterium lactis* BB-12 was administered (5, 9, 16). Based on these findings, no conclusion can be drawn regarding the superiority of combination therapy over single-strain therapy, nor about which specific strains are most effective. One study included the probiotic yeast *Saccharomyces boulardii*, however, cases of bloodstream infections with this organism have been reported, leading to its discontinuation in neonatal care (31).

Additionally, there was a significant diversity in the sepsis definitions applied. Some studies focused on culture-proven sepsis, while others addressed clinical sepsis. Furthermore, several studies did not report which definition they adhered to. Moreover, the timing of sepsis onset was often unspecified, making it challenging to differentiate between early onset and LOS. These inconsistencies further complicated the comparison of results.

Most included studies, including the study with the largest sample size, showed no effect of probiotic supplementation on the incidence of LOS (3, 6, 20-26). However, the true effect of probiotics may be underestimated due to cross-contamination, in which the placebo preparation becomes unintentionally contaminated with the probiotic strain, given to the intervention group, resulting in inadvertent probiotic exposure in the placebo group (3, 20, 24). In addition, many studies had relatively small sample sizes, limiting their power to detect difference in LOS. No RCT was adequately powered to individually demonstrate a reduction in LOS, which may have further obscured potential benefits. Furthermore, the extensive use of antibiotics in this population, also alters the gut microbiome and may reduce the ability of supplemented probiotics to colonise the gastrointestinal tract, diminishing their efficacy (3).

Three studies did report a beneficial effect of probiotic supplementation. Remarkably, the ProPrems study showed a significant reduction in LOS only in subgroup analyses, specifically among infants with GA ≥ 28 weeks (5). This finding aligns with observations by Costeloe et al., who reported that successful colonisation with the administered probiotic increases with GA (3). As the efficacy of probiotics depends on successful colonisation of the gastrointestinal tract, reduced colonisation in infants born at <28 weeks' gestational age may potentially limit probiotic effectiveness. Interestingly, in the study by Oncel et al., a total significant overall effect was observed, yet subgroup analyses showed a significant benefit only among infants with BW <1000 gram (16). This conflicting outcome may be explained by the higher baseline risk of LOS in the <1000g subgroup, in whom a greater impact of microbiome modulation by probiotics may be observed.

In 2023, the Food and Drug Administration (FDA) released a warning regarding the use of probiotics in preterm infants, claiming that "preterm infants receiving probiotics risk invasive, potentially fatal infections from probiotic organisms" (32). Moreover, the American Academy of Pediatrics states that "due to the lack of FDA-regulated pharmaceutical-grade products, conflicting safety and efficacy data, and potential harm in this vulnerable population, routine probiotic use in preterm infants, particularly those <1000 g, is not currently recommended" (32). However, the collected data suggests a relatively safe use of probiotics. No mild to severe adverse events were reported that

could be linked to the probiotic use. Additionally, no sepsis cases were observed with the same supplemented probiotic strain. However, it is possible that these side effects are very rare and that the sample sizes were too small to capture them. Moreover, we are specifically focusing on very preterm and/or VLBW infants who are otherwise healthy. It is possible that infants with additional medical conditions may be even more sensitive to the potential side effects of probiotics.

This review identified major research gaps including a lack of well-powered RCTs, substantial heterogeneity in definitions, outcomes, and probiotic use (including dosage, strain, and administration regimen), and limited data in the most vulnerable populations. In addition, strain-specific evidence remains scarce, and not all studies report causative pathogens. The role of gastrointestinal pathogen–related sepsis is also unclear due to a lack of studies examining gastrointestinal colonisation and microbiota modulation by probiotics. Finally, safety data on probiotic use in this population remain limited.

Encouragingly, two large trials are currently ongoing: the WHO PROPS trial (NCT03978000), evaluating *Lactobacillus rhamnosus* GG and *Bifidobacterium longum subsp. infantis* in preterm and small-for-gestational-age infants, and the CONNECTION trial (NCT03978000), assessing *Lactobacillus reuteri* for the prevention of necrotising enterocolitis in preterm infants (GA 23-32 weeks and BW 500–1500 g).

The strength of this review lies in our target population (very preterm infants (<32 weeks GA) and/or VLBW (<1500 grams)) being the most vulnerable to developing LOS. This approach minimises distortion in assessing the effect of probiotics on the incidence of LOS. All included studies were required to be randomised, double-blinded, and placebo-controlled RCT, ensuring that all reported data are objective and unlikely to be subject to allocation bias. Additionally, the sample size is increased by pooling data from various RCTs. However, the review has several limitations. First, the search was conducted in a single database, namely Medline, and both the search and subsequent inclusion were performed by a single reviewer, raising the possibility of selection bias. Second, studies in which the control group received only milk feeding without an added placebo substance were classified "non-placebo controlled" during the study selection process. This classification led to a reduced number of eligible articles. In addition, inclusion was not restricted to well-powered studies, which may have resulted in both under- and overestimation of the probiotic effect. Lastly, the extensive clinical and statistical heterogeneity makes it difficult to draw conclusions regarding the efficacy of probiotics in very preterm and/or VLBW infants including the ideal probiotic strain, dosage, and duration of treatment.

Conclusion

This review indicates that further research is required, as the inconsistency of the available evidence is insufficient to either confirm or refute the efficacy of probiotics as a prophylactic measure for LOS. Moreover, the findings highlight the need for greater methodological uniformity, emphasising the importance of studies using consistent definitions (such as sepsis occurring >72 hours after birth, and clinical vs culture-proven sepsis) to facilitate comparison. In addition, further investigation for each subtype of probiotics is warranted, given the difficulty in comparing different types with each other. Specifically, this scoping review demonstrates the need for well-powered RCTs focusing on the preventive use of probiotics in gastrointestinal pathogen–related sepsis among the most vulnerable NICU populations, particularly infants with a BW <1000 g. Finally, additional studies are required to further assess the safety profile of probiotics in this popular

Statements

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APPENDIX:
Search Strategy

((("Intensive Care, Neonatal"[Mesh] OR "neonatal intensive care"[tiab] OR "NICU"[tiab])OR ("Infant, Low Birth Weight"[Mesh] OR "low birth weight*"[tiab] OR "Birth weight Low"[tiab] OR "Birth weights low"[tiab]) OR "VLBW"[tiab] OR "LBW"[tiab] OR "ELBW"[tiab]) OR ("Infant, Premature"[Mesh] OR "Premature infant*"[tiab] OR "Preterm infants"[tiab] OR "Preterm infant"[tiab] OR "premature infants"[tiab] OR "premature infant*"[tiab] OR "Neonatal prematurity"[tiab] OR "Preterm newborn*"[tiab] OR "Premature newborn*"[tiab] OR "preterm newly born"[tiab] OR "premature newly born"[tiab] OR "preterm bab*"[tiab] OR "premature bab*"[tiab])) AND ("Probiotics"[Mesh] OR "Probiotic*"[tiab] OR "synbiotic*"[tiab] OR "Lactobacillus"[Mesh] OR "lactobacillus"[tiab] OR "Lactobacillus acidophilus"[Mesh] OR "Saccharomyces"[Mesh] OR "Saccharomyces"[tiab] OR "Bifidobacterium"[Mesh] OR "bifidobacterium"[tiab])