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REVIEW PAPER

Use of probiotics in newborns: a neonatologist's perspective

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ABSTRACT

The neonatal period is crucial for microbiota development, especially in preterm infants, who face challenges, such as limited maternal contact and medical interventions. While breastfed full-term infants usually develop healthy gut flora naturally, preemies are at risk for conditions, including necrotizing enterocolitis (NEC), feeding intolerance, and sepsis. Probiotics may help reduce these risks. Guidelines from ESPGHAN and AGA cautiously support specific strains for NEC prevention, stressing the importance of strain selection, product quality, and safety. However, risks, such as probiotic-induced sepsis and lack of FDA-approved options, highlight the need for more research. Ethical concerns, informed consent, and close monitoring are essential. This article provided practical, evidence-based guidance for neonatologists, advocating responsible probiotic use to improve outcomes in preterm infants.

KEY WORDS:

probiotics, recommendations, newborns, preterm infants.

INTRODUCTION

Although bacterial DNA has been found in the placenta, amniotic fluid, and umbilical cord blood, the fetal gut is still considered sterile [1]. In healthy newborns, gut microbiota develops through contact with the mother, especially via breast milk [2]. Breast milk delivers a comprehensive array of essential nutrients, immunological agents, and a diverse consortium of commensal microbes vital for the healthy development of newborns. Recent progress in culture-independent analytical methods has uncovered the intricate nature of milk microbiome, and unveiled a broad spectrum of bioactive constituents. However, a cohesive understanding of these discoveries remains incomplete.

Even though research in this area is still emerging, multiple immunological functions of human milk have been well-documented, including the passive transfer of immunoglobulins, cytokines, and immunomodulatory peptides. Moreover, breast milk contains viable immune cells and stimulatory factors, which play a pivotal role in the development of both mucosal and systemic immune responses in the infant. Additionally, human milk is a natural source of prebiotics, particularly human milk oligosaccharides, which foster the selective growth of beneficial gut bacteria. This complex interplay between milk-derived components and the infant gut microbiome underscores the evolutionary significance of human milk in establishing early immune competence and promoting long-term health [3]. Therefore for breastfed, full-term infants, probiotics are not routinely recommended [4]. In contrast, preterm infants face disrupted microbiota development due to limited maternal contact, altered feeding, hospital exposure, and frequent use of antibiotics or steroids [5]. Preterm infants are at risk of serious illness and death, and the microbiome may be involved in these processes [5]. As a result, there is a growing interest in probiotic use in this group of patients. Given the common off-label drug use in neonatology [6], this narrative review was intended to assist neonatologists in the evidence-based selection and development of formal

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procedures related to the introduction of probiotics into clinical practice.

GENERAL CONSIDERATIONS

The selection and use of probiotics in preterm infants requires the consideration of several key issues.

SAFETY

Premature babies are born with immature immune system, making them more vulnerable to infections. Although probiotics are generally considered safe, there is a risk of bacterial translocation (transfer of bacteria from the gut into the bloodstream) and sepsis [7], especially in very small preterm infants. The presence of central venous catheters are also a risk factor [8]. Probiotics should not be administered to preterm infants experiencing severe, unstable general conditions, such as the development of sepsis or necrotizing enterocolitis (NEC), in congenital malformations of the gastrointestinal tract or after gastrointestinal surgery [7, 8]. Prophylactic administration of probiotics is intended to prevent NEC, but as soon as symptoms suggest this condition, probiotic supplementation should be discontinued [8].

Of the 1,569 published studies on newborns receiving probiotics, only 16 reports with 32 cases described probiotic sepsis (mostly in premature infants born before 32 weeks of gestation) [6]. In 25 cases, probiotic use was confirmed as the cause of sepsis based on full genomic analysis. The most frequently isolated bacteria were bifidobacteria (*Bifidobacterium infantis*) in 19 cases, lactic acid bacteria (*Lactobacillus rhamnosus* GG) in 10 cases, and *Saccharomyces boulardii* in 3 cases [7]. Two neonatal deaths from probiotic sepsis have been reported: one due to an unspecified cardiac issue [9] and second after administration of *Lactobacillus reuteri* [10].

However, it is essential to emphasize that side effects associated with probiotic administration in preterm infants are not common, and their risk should be compared with usual care and assessed using objective methods and tools. One such method is GRADEpro software, developed by the Grading of Recommendations, Assessment, Development, and Evaluations Working Group (GRADE). It evaluates the quality of evidence by assessing risk of bias, inconsistency, indirectness, and imprecision, each classified as not serious, serious, or very serious. Based on these assessments, the certainty of evidence is categorized as high, moderate, low, or very low [6].

STRAIN SELECTION AND DOSAGE

Different strains of probiotics may have different mechanisms of action and efficacy. For example, *Bifidobacterium lactis* BB-12/B94 could reduce NEC risk with a different size effect according to feeding type [11]. Also, many formulations include a combination of strains selected for specific indications. Choosing the right strain or combination that is well-studied and shows the desired effects in preterm infants is crucial. Dosage should be determined based on clinical studies, which consider gestational age, body weight, and the baby's health status. Excessive or insufficient dosage may be ineffective or even harmful.

DURATION OF THERAPY

Timing and length of probiotics administration can impact their overall effectiveness. For preterm infants, probiotic therapy is often started as early as possible (first or second day after birth) and continued until a certain post-conceptional age (34–36 weeks of gestation) or hospital discharge, lasting usually from 4 to 8 weeks [8].

ETHICAL AND PRACTICAL CONSIDERATIONS

Parents must be fully informed of the potential benefits and risks of probiotic therapy, and their informed consent is essential. It is important to be sure that the probiotics used are of high quality, clinically tested, and manufactured by reputable companies to ensure their efficacy and safety [12].

MONITORING AND EVALUATION

Continuous monitoring of the effects of probiotics is necessary to react quickly to any adverse events or lack of expected results. Monitoring should include short- and long-term health outcomes [12, 13]. Up till now, a few studies have shown that the use of probiotics in preterm infants had no long-term adverse events observed [13]. Seven randomized trials, which assessed the development of children aged 18–22 months, showed no impact on children's motor development, cognitive development, or the incidence of cerebral palsy, and visual and hearing impairment [14]. During monitoring probiotic strains invasiveness, it is recommended to report each event fully, analyze the cause-effect relationship, and assess its translocation potential. Antibiotic resistance monitoring requires phenotype and genotype assessment, and phenotypic resistance deviating from species norms needs special attention. According to WHO, risk/benefit for critical genes should be analyzed in detail, and the transmission potential and functionality of antibiotic resistance genes should be assessed. Probiotic-drug interactions (broad-spectrum antibiotics, for instance [15]) must also be monitored. Studies assessing the effects of probiotics on the microbiome are not required, but are necessary to conduct mechanistic analyses, which are essential for advancing the knowledge of probiotic use in preterm infants. In clinical studies among this age group, a follow-up period of at least 2 years is also recommended [13].

REGULATION AND PRODUCTION STANDARDS

Probiotics used in neonatology must be produced according to strict standards to ensure their high quality [12]. Products should be contaminant-free and thoroughly tested for strain specifications and quantities. The European Food Safety Authority (EFSA) has maintained and updated a list of species considered safe for human consumption since 2007 [16]. This qualification is based on taxonomic identification and comprehensive scientific data on the safety of the strain in question, which includes, among other things, its virulence, antibiotic resistance, and ability to transfer antibiotic resistance genes. It is important to emphasize that probiotics used in premature infants must be antibiotic-sensitive to treat probiotic-induced sepsis if it develops.

Probiotics used in preterm infants should meet strict quality requirements. Before introducing a particular probiotic (the bacterial strain chosen), the manufacturer and distributor should be contacted to ensure that the product offered meets highest standards of stringency and safety and, if in doubt, to assess the possibility of the manufacturer implementing additional requirements in the production and distribution process. Depends on legal requirements, a probiotic's manufacturer should have Good Manufacturing Practice (GMP) or Hazard Analysis Critical Control Points (HACCAP) certificates. The final specification of probiotic must include documentation of the manufacturing, packaging, and shipping process, and an analytical certificate. Dietary supplement label should include the strain identity for each micro-organism in the product, dosage in colony forming units (CFU), expiry date, storage instructions, and the manufacturer's contact details. Table 1 lists the information, which should be enclosed in the product label or insert, with any potential contaminants and pollutants and their acceptable limits [12].

TABLE 1. Information and limits required for using probiotics in preterm infants

Strain(s)	
1. Identity of the strain(s) for each micro-organism 2. Guaranteed number of CFU/AFU for each micro-organism in the product until expiry date	
Pollutants	**Limit**
Mycotoxins: Commission Regulation (EC) No 1881/2006, as amended (µg/kg), end product	
Aflatoxin B1	0.1
Aflatoxin M1	0.025
Ochratoxin A	0.5
Heavy metals: Commission Regulation (EC) No 1881/2006, as amended (µg/kg), end product	
Lead	0.01
Cadmium	1.0
Mercury	0.1
Polycyclic aromatic hydrocarbons (PAHs): Commission Regulation (EC) 2015/1993 and 835/2011 amending Commission Regulation (EC) 1181/2006 (µg/kg)	
Benzo(a)pyrene	1.0
Sum of: benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene	1.0
Microbiological requirements	
TAMC/ Number of aerobic bacteria (excluding lactic acid bacteria)	< 2,000 CFU/g; 5 samples of 10 g each
TYMC/ Number of moulds and yeasts	< 100 CFU/g; 10 samples of 10 g each
Enterobacteriaceae	Absent; 30 samples of 10 g
E. coli	Absent; 1 sample of 10 g
Cronobacter	Absent; 30 samples of 10 g
Staphylococcus aureus	Absent; 5 samples of 10 g
Salmonella spp.	Absent; 60 samples of 25 g
Listeria spp.	Absent; 10 samples of 25 g
Bacillus cereus	< 100 CFU/g; 5 samples of 10 g
Pesticides (mg/ kg): Commission Regulation (EC) 396/2005	
Pesticide residues	0.01
Other pesticide residues in accordance with Annex II and II of Commission Regulation (EU) 2016/128	Limit depending on type of pesticide (Annex II and III)

CFU – colony-forming unit; AFU – active fluorescent units

Despite the lack of clear recommendations globally on the use of probiotics in preterm infants, it is estimated that approximately 25% to 30% of neonatal intensive care units (NICUs) in Europe use probiotics in routine practice, with 12% in the UK and up to 68% in Germany [17, 18]. In the USA, where the approach is more conservative, 16.5% of NICUs report probiotic use [18], although following an FDA warning related to death of a premature infant with probiotic-induced sepsis, probiotic therapy has been virtually ceased in most NICUs [19]. The highest proportion, nearly 100% of probiotic therapy use in neonates is observed in New Zealand's NICUs [20].

EVIDENCE FOR USE IN MAJOR INDICATIONS

NECROTIZING ENTEROCOLITIS

NEC is a multi-factorial inflammatory disease of the intestine, leading to tissue necrosis and possible perforation. Its causes include intestinal and immune immaturity, ischemia, and dysbiosis, marked by low levels of *Lactobacillus* and *Bifidobacteria* as well as higher levels of pathogens, such as *Clostridium* and *Proteobacteria*. An excessive inflammatory response to normal gut flora may be another contributor. The risk increases with formula feeding and high osmolarity additives [21].

In terms of strain selection for NEC prevention, the latest 2022 ESPGHAN position paper recommended the use of *L. rhamnosus* GG ATCC 53103 (weak recommendation, low data reliability), or a combination of *B. infantis* BB-02, *B. lactis* BB-12, and *Streptococcus thermophilus* TH-4 (weak recommendation, low data reliability) [22]. The AGA in 2020 issued recommendations based on moderate to strong evidence that the most effective probiotics for NEC prophylaxis are a combination of *Lactobacillus* and *Bifidobacterium* species, such as *L. rhamnosus* ATCC 53103 and *B. infantis*; or *L. casei* and *B. breve*; or *L. rhamnosus*, *L. acidophilus*, *L. casei*, *B. infantis*, *B. bifidum*, and *B. longum*; or *L. acidophilus* and *B. infantis*; or *L. acidophilus* and *B. bifidum*; or *L. rhamnosus* ATCC 53103 and *B. longum* Reuter ATCC BAA-999; or *L. acidophilus*, *B. bifidum*, *B. lactis*, and *B. longum*; *B. lactis* (DSM 15954), or *L. reuteri* (DSM 17938 or ATCC 55730) or *L. rhamnosus* (ATCC 53103, ATC A07FA, or LCR 35) [23].

According to the recommendation of the East of England (EoE) Neonatal Units [8], probiotics for NEC prophylaxis should be used in neonates < 32 weeks of gestation or with a body weight < 1,500 g, optimally from the first day of life until 34 weeks of gestation. EoE recommended specific preparations: Labinic (*L. acidophilus* NCFM, *B. bifidum* BB-06, and *B. infantis* Bi-26) 1 × 5 drops; ProPrems (*B. infantis* BB-02 DSM 33361, *B. lactis* BB-12®, and *Streptococcus thermophilus* TH-4®) 1 × 1 sachet; Infloran (*B. bifidum* NCDO 2203 and *L. acidophilus* NCDO 1784) 1 × 1/2–1 capsules.

Since 2020, several meta-analyses have been published, confirming the strongest protective effect against NEC of multi-strain probiotics containing at least one strain of bifido and lactobacilli, and single-strain probiotics belonging to species, such as *B. animalis* subspecies *lactis*, *L. reuteri*, and *L. rhamnosus* [11]. Beneficial properties of L. *acidophilus* LB and *B. lactis* BB-12/B94 were also demonstrated, with the effect of the latter being particularly strong in children fed exclusively with breast milk [24].

Furthermore, the effectiveness of probiotic therapy in the prevention of NEC is confirmed by the experience of individual NICUs, e.g., the use of *L. reuteri* DSM 17938 at Sunnybrook Health Sciences Centre in Toronto reduced the incidence of NEC from 4.4% to 1.7% [25]. At the Oregon Health & Science University, there was a reduction in the incidence of NEC from 11% to 2.7% during a one-year administration of *B. infantis* EVC001 [26]. At the University of Utah Medical Center, the use of a multi-strain probiotic consisting of *B. breve* HA-129, *B. bifidum* HA-132, *B. infantis* HA-117, *B. longum* HA-135, and *L. rhamnosus* HA-111, resulted in a reduction in the incidence of NEC from 10% to 2% [27]. Since 2020, the Neonatal Intensive Care Unit (NICU) at Medical City Dallas has incorporated Visbiome® infant drops (containing 8 strains of bacteria: *L. paracasei*, *L. plantarum*, *L. acidophilus*, *L. helveticus*, *B. lactis*, *B. longum*, *B. breve*, and *S. thermophilus*) into nutritional management of preterm infants (< 1,500 grams and/or born before 34 weeks of gestation). Eligible neonates receive a daily dose of 2 billion colony-forming units (CFUs), equivalent to 0.2 ml, administered with feedings until either 36 weeks of post-menstrual age or hospital discharge, depending on which occurs first. Following the integration of Visbiome® into feeding protocol, the unit observed a significant reduction in the incidence of NEC. Before this intervention, NEC rates have reached up to 10%, but since the change, these rates consistently declined to approximately 0.8% in both 2020 and 2021. Notably, no instances of probiotic-associated bacteremia or fungemia have been reported during this period [28].

An analysis involving 307,905 very low birth weight (VLBW) neonates in 807 hospitals in the USA found that routine use of probiotics in at least 20% of the hospitalized neonates reduced the risk of NEC in those hospitals by 18%, without affecting the incidence of sepsis or mortality [18]. However, it is important to note that the results of the above studies are inconclusive. According to a systematic review of 60 studies involving a total of 11,156 VLBW and extremely low birth weight (ELBW) neonates, the use of probiotics reduced the risk of NEC only in VLBW neonates, but had little or no effect in ELBW cohort, with the results characterized by poor data quality [29].

The effects of probiotics should be interpreted with caution. In a PiPS trial, which did not demonstrate a bene-

ficial effect of *B. breve*, it was found that approximately 20% of patients in the control group were incidentally colonized by *B. breve* by 2 weeks of age. By 36 weeks of corrected age, this figure had increased to 49%. Such colonization may have reduced the differences between the treatment and control groups, weakening the evidence's strength for the efficacy or inefficacy of the probiotic strain tested [30]. According to a study by Samara *et al*. [31], strains of *B. bifidum* HA-132, *B. longum* HA-135, and *B. breve* HA-129 (but not *B. infantis* HA-116 and *L. rhamnosus* HA-111) not only persisted in the stools of children who received them, but also colonized other hospitalized newborns up to 6 months after their supplementation in the study group. The study's results suggest that the use of probiotic therapy, even in some hospitalized patients, may affect the non-treated neonates in the ward.

ENTERAL FEEDING INTOLERANCE AND TIME OF PARENTERAL NUTRITION

Preterm infants, especially those under 1,500 g (VLBW), often show enteral feeding intolerance and require parenteral nutrition early in life. Respiratory support can worsen gastrointestinal symptoms, including bloating and gastric retention. Recent studies suggest that probiotics may improve feeding tolerance and reduce reliance on parenteral nutrition [32–34].

In a randomized clinical trial described by Sowden *et al*. [32], *L. acidophilus* NCFM, *B. bifidum* BB-06, and *B. infantis* Bi-26 were used in 100 VLBW infants (plus 100 VLBW in the control group). The probiotic group reached full enteral feeding in a shorter amount of time. Fewer babies with feeding intolerance (14% vs. 35%), vomiting (49% vs. 69%), and abdominal bloating (24% vs. 48%) were reported in the probiotic group compared with the control group. Furthermore, statistically significantly fewer days with the above symptoms were found in children receiving the probiotic. Participants in another randomized clinical trial were 120 preterm ELBW infants treated with *L. acidophilus* NCDO 1748 and *B. bifidum* NCDO 2203. Better tolerance of enteral feeding was assessed by a shorter time duration of total parenteral nutrition (29 vs. 35.5 days), which was observed in the extremely preterm infants receiving the probiotic [33]. In another clinical trial, administration of a probiotic (FloraBABY) to ELBW resulted not only in a shorter time to achieve full enteral feeding (by 1.8 days), but also in better tolerance of cow's milk protein at 6 months of age [34].

Among the combinations of other probiotic strains shown to reduce the time to achieve full enteral feeding are *L. rhamnosus*, *B. longum* subsp. *infantis*, *L. acidophilus*, *L. casei*, *B. bifidum*, and *B. longum* subsp. *longum*; *L. casei* and *B. breve*; *L. acidophilus* and *B. bifidum*; *L. acidophilus*, *B. animalis* subsp. *lactis*, *B. longum* subsp. *longum*, and *B. bifidum* [11]. Better efficacy in preterm infants' food tolerance has also been demonstrated with the single-strain probiotics of *L. reuteri* DSM 17938 and ATCC55730 [11]. According to a meta-analysis and systematic review published in 2023 by Wang's team, both single- and multi-strain probiotics demonstrated high efficacy in this regard [35].

In a systematic review and meta-analysis, incorporating data from eleven randomized controlled trials (RCTs) and three non-randomized studies with concurrent control groups, a combined total of 14,888 extremely preterm infants was encompassed. The meta-analyses indicated a reduction in the average time to achieve full enteral feeding (mean difference of 1.1 days less; 95% confidence interval [CI]: 7.83 days shorter to 5.56 days longer) and a shorter duration of parenteral nutrition (mean difference of 2.4 days less; 95% CI: 7.44 days shorter to 2.58 days longer) in neonates receiving probiotics. However, these differences did not reach statistical significance. In contrast, a statistically significant decrease was observed in the incidence of NEC, with a relative risk (RR) of 0.80 (95% CI: 0.68–0.93) as well as in all-cause mortality, with a RR of 0.56 (95% CI: 0.33–0.93) among infants supplemented with probiotics. Despite these promising findings, the certainty of evidence for all measured outcomes was assessed as low or very low [36].

LATE-ONSET SEPSIS

Late sepsis in neonates is a generalized infection after 72 hours of life. In the afore-mentioned study by Samara [31], it was shown that supplementation with a mixture of probiotics (*B. bifidum* HA-132, *B. longum* HA-135, *B. breve* HA-129, *B. infantis* HA-116, and *L. rhamnosus* HA-111) accelerated the maturation of the intestinal microbiome of children born prematurely, and reduced the concentration of inflammatory markers in neonatal stools. Therefore, probiotics may improve the integrity of intestinal barrier, which reduces permeability to pathogens and toxins, and limiting their ability to trigger a systemic inflammatory response. Another previously described clinical study confirms this finding. In that prospective cohort study of 120 ELBW preterm infants, late sepsis was found in 47.1% of children receiving a probiotic (*L. acidophilus* NCDO 1748 and *B. bifidum* NCDO 2203) compared with 70% of children in the control group [33]. An additional observational research of 4,529 preterm infants in New Zealand showed that the administration of probiotic (*L. acidophilus* NCDO 1748 and *B. bifidum* NCDO 2203, or *L. rhamnosus* GG with lactoferrin) significantly reduced the incidence of late sepsis and NEC in VLBW children [20]. Similarly, in a meta-analysis based on 25 randomized clinical trials, Aceti *et al*. [37] showed that the application of multi-strain probiotics to preterm infants significantly reduced the incidence of late-onset sepsis (LOS) in preterm infants (13.6% vs. 17.25%), with effects even more prominent in babies fed exclusively with breast milk.

A meta-analysis of more than 20,000 newborns (81 randomized clinical trials) published in 2023 reported that multi-species probiotics combined with lactoferrin had the most beneficial effect in reducing the incidence of LOS (moderate to high data quality). Multi-strain probiotics showed intermediate efficacy. Unfortunately, in this study, no single or even a few of the most effective probiotic strains were identified as effective on their own [35]. A cohort study involving 307,905 neonates with VLBW in 807 neonatal intensive care units between 2012 and 2019 found that adopting probiotics was not associated with significant changes in the incidence of LOS and infant mortality [18]. Moreover, Sharif *et al.* [29] in a systematic review of research published in 2023, concluded that probiotics may have little or no protective effect on the occurrence of LOS in preterm infants (low data quality).

This is in line with Morgan, who made similar conclusions regarding the development of LOS [11]. A meta-analysis involving 48 randomized clinical trials (12,258 preterm infants) demonstrated that none of the probiotic products tested showed a statistically significant reduction in the risk of LOS compared with placebo. Three combinations of bacterial species, including *Lactobacillus* and *Bifidobacterium*; *Lactobacillus*, *Bifidobacterium*, and *S. boulardii*; and *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, showed statistically insignificant effects (very low data quality).

Nevertheless, based on the available results of randomized clinical trials, the CPS concluded that sufficient evidence support the use of multi-strain probiotic combinations to reduce the risk of mortality in preterm and young infants with sepsis [38].

MORTALITY

Based on an analysis of a hospital course of 10,000 extremely preterm infants (born < 28 weeks gestation) born in the USA between 2013 and 2018, the mortality rate was shown to be 21.7% [39].

Unsurprisingly, the search for ways to improve the survival of extremely small newborns is ongoing. One method of great interest is the prophylactic use of probiotics. A pooled study from 2020 of various reports on the use of probiotic therapy in preterm infants found that multi-strain probiotics containing at least one strain each of the *Lactobacillus* and *Bifidobacterium* species, were the most effective in reducing death rates. The following combinations were employed in studies: *L. rhamnosus* ATCC53103 and *B. longum* subsp. *infantis*; *L. casei* and *B. breve*; *L. acidophilus* and *B. longum* subsp. *infantis*; *L. acidophilus* and *B. bifidum*; L. rhamnosus ATCC 53103 and *B. longum* subsp. *longum* Reuter ATCC BAA-999; *L. acidophilus*, *B. animalis* subsp. *lactis*, *B. longum* subsp. *longum*, and *B. bifidum* [40]. The high efficacy of multi-species probiotics in reducing mortality (high to moderate data quality) was confirmed by a meta-analysis and systematic review of randomized clinical trials from 2023 in JAMA Pediatrics [35].

Also, the AGA recommendations published in 2020 confirm that the use of probiotic bacteria, such as *L. rhamnosus* ATCC 53103 and *B. infantis*; or *L. casei* and *B. breve*; or *L. rhamnosus*, *L. acidophilus*, *L. casei*, *B. infantis*, *B. bifidum*, and *B. longum*; or *L. acidophilus* and *B. infantis*; or *L. acidophilus* and *B. bifidum*; or *L. rhamnosus* ATCC 53103 and *B. longum* Reuter ATCC BAA-999; or *L. acidophilus*, *B. bifidum*, *B. lactis*, and *B. longum*; or *B. lactis* (including DSM 15954); or *L. reuteri* (DSM 17938 or ATCC 55730); or *L. rhamnosus* (ATCC 53103, or ATC A07FA or LCR 35), reduces mortality among children receiving them [23].

The beneficial effect of probiotic therapy in reducing mortality among VLBW infants was not confirmed in a cohort study based on the analysis of preterm infants hospitalized in ITN units in the USA. It should be noted that the children received a variety of probiotics, and the analysis examined the impact of all probiotics used together [18]. A less optimistic conclusion was also reached by Sharif *et al.* [29], who showed that probiotic therapy may reduce mortality to a small extent in VLBW children (measured data quality), or have no effect on mortality from any cause in ELBW (low data quality).

An analysis involving 30 high-quality non-randomized studies and including data of 77,018 preterm infants across 18 countries, demonstrated that the implementation of routine probiotic supplementation (RPS) was significantly associated with a reduced risk of NEC stage ≥ II (based on 30 studies, $n = 77{,}018$; odds ratio [OR]: 0.60; 95% confidence interval [CI]: 0.50–0.73; $p < 0.00001$; heterogeneity $I^2 = 65\%$; level of evidence: moderate), a reduced incidence of LOS (based on 21 studies, $n = 65{,}858$; OR: 0.85; 95% CI: 0.74–0.97; $p = 0.02$; $I^2 = 74\%$; level of evidence: low), and a decreased all-cause mortality (based on 27 studies, $n = 70{,}977$; OR: 0.77; 95% CI: 0.68–0.88; $p = 0.0001$; $I^2 = 49\%$; level of evidence: low). Sub-group analyses indicated that in ELBW infants (birth weight < 1,000 g), RPS was associated with a lower rate of NEC stage ≥ II (4.5% vs. 7.9%), although no significant differences were observed in LOS or mortality in this subgroup. Furthermore, multi-strain probiotic formulations appeared to be more effective than single-strain preparations. One study reported three non-fatal cases of probiotic-associated sepsis. In conclusion, this systematic review of non-RCTs suggested that routine probiotic use in preterm neonates is linked to significant reductions in NEC stage ≥ II, LOS, and overall mortality, with the greatest benefit observed in NEC prevention, particularly in the general preterm and ELBW populations. Nonetheless, the quality of evidence remains moderate to low, highlighting the importance of further rigorous research and ongoing clinical surveillance [41].

TABLE 2. Species and strains recommended by scientific societies and used in the cited studies

Indications	Recommendations [Ref.]	Clinical trials
NEC	AGA (2020) [23] Min. 1 each of *L.* and *B.* strains 1. *L. rhamnosus ATCC 53103* + *B. infantis* 2. *L. casei* + *B. breve* 3. *L. rhamnosus* + *L. acidophilus* + *L. casei* + *B. infantis* + *B. bifidum* + *B. longum* 4. *L. acidophilus* + *B. infantis* 5. *L. acidophilus* + *B. bifidum* 6. *L. rhamnosus ATCC 53103* + *B. longum* Reuter ATCC BAA-999 7. *L. acidophilus* + *B. bifidum* + *B. lactis* + *B. longum* • or single-stranded 1. *B. lactis* DSM 15954 2. *L. reuteri* (DSM 17938 or ATCC 55730) 3. *L. rhamnosus* (ATCC 53103 or ATC + A07FA or LCR 35) ESPGHAN (2022) [22] 1. *B. infantis* BB-02, B. lactis BB-12, *Streptococcus thermophilus* TH-4 2. *L. rhamnosus* GG ATCC 53103 National Health Service (NHS), East of England Neonatal Units (2022) [8] 1. *L. acidophilus* NCFM, *B. bifidum* BB-06, *B. infantis* Bi-26 (Labinic) 2. *B. infantis* BB-02 DSM 33361, *B. lactis* BB-12®, *Streptococcus thermophilus* TH-4® (ProPrems) 3. *B. bifidum* NCDO 2203, *L. acidophilus* NCDO 1784 (Infloran)	Min. 1 each of L. and *B. strains* 1. *L. acidophilus* LB + B. *lactis* BB-12/B94 [24] 2. *B. breve* HA-129 + *B. bifidum* HA-132 + *B. infantis* HA-117 + *B. longum* HA-135 + *L. rhamnosus* HA-111 (Ultimate FloraBABY) [27] 3. *L. acidophilus* DSM24735/SD5212, *L. plantarum* DSM24730/SD5209, *L. paracasei* DSM24733/SD5218, *L. helveticus* DSM24734/SD5210, *S. thermophilus* DSM24731/SD5207, *B. lactis* DSM24736/SD5219, *B. breve* DSM24732/SD5206, *B. lactis* DSM24737/SD5220 (de Simone formulation, Visbiome®) [28] • or single-stranded 1. *L. rhamnosus* GG [11] 2. *L. reuteri* DSM 17938 (BioGaja) [25] 3. *B. infantis* EVC001 [26] 4. *B. lactis* [11]
Food intolerance	None	1. *L. acidophilus* NCDO 1748 + B. bifidum NCDO 2203 (Infloran) [33] 2. *L. acidophilus* NCFM + B. bifidum BB-06 + B. infantis Bi-26 (Labinic) [32] 3. *L. rhamnosus* + *B. longum subsp. infantis* + *B. longum subsp. longum* + *L. acidophilus* + *L. casei* + *B. bifidum* (VSL#3) [11] 4. *L. casei* + *B. breve* + *L. acidophilus* + *B. bifidum, L. acidophilus* + *B. animalis subsp. lactis* + *B. longum subsp. longum* + *B. bifidum* (Primadophilus Optima) [11] 5. *L. reutri* DSM17938 or ATCC55730 [11]
Late-onset sepsis	None	1. *B. breve* HA-129 + *B. bifidum* HA-132 + *B. infantis* HA-117 + *B. longum* HA-135 + *L. rhamnosus* HA-111 (Ultimate FloraBABY) [31] 2. *L. acidophilus* NCDO 1748 + *B. bifidum* NCDO 2203 (Infloran) [20, 33] 3. *L. rhamnosus* + lactoferrin [20]
Mortality	None	1. *L. rhamnosus* ATCC53103 + *B. longum* subsp. *infantis* [11, 23] 2. *L. casei* + *B. breve* [11, 23] 3. *L. acidophilus* + *B. longum* subsp. *infantis* [11, 23] 4. *L. acidophilus* + *B. animalis* subsp. *lactis* [11,23] 5. *L. rhamnosus ATCC 53103* + *B. longum subsp. longum* Reuter ATCC BAA-999 [11, 23] 6. *L. acidophilus* + B. animalis subsp. lactis + B. longum subsp. longum + B. bifidum [11, 23] 7. *L. rhamnosus* + *L. acidophilus* + *L. casei* + *B. infantis* + *B. bifidum* + *B. longum* [23] 8. *B. lactis* DSM 15954, or *L. reuteri* (DSM 17938 or ATCC 55730) or *L. rhamnosus* (ATCC 53103, ATC + A07FA or LCR 35) [23]
Caesarean section	None	1. *L. fermentum* CECT 5716 [46] 2. *L. rhamnosus* LC705 + *B. breve* Bb99 + *Propionibacterium freudenreichii* spp. *shermanii* JS [47] 3. *B. animalis subspecies lactis* BB-12 [50]

CAESAREAN SECTION

Term newborns are generally healthy and quickly return to a low-risk home environment, often bypassing hospital stays entirely due to home births. Thus, probiotic use is mainly considered for infants born by caesarean section. C-section delivery alters gut colonization, and is linked to higher risks of allergies, asthma, obesity, and autoimmune diseases [42, 43]. Probiotics can help restoring gut microbiota, mainly boosting bifidobacteria levels, especially when started at birth and combined with breastfeeding [44, 45].

Based on three randomized clinical trials with a total of 173 children, it was shown that the use of *L. fermentum* CECT 5716 significantly (by 73%) reduced the incidence of gastrointestinal infections in children in the first year of life compared with the control group, but without affecting the incidence of respiratory infections [46].

In another study, three probiotic strains in combination were administered to children at risk of allergy from birth to six months: *L. rhamnosus* LC705, *B. breve* Bb99, and *Propionibacterium freudenreichii* spp. *shermanii* JS. Fewer IgE-related skin rashes were observed in children receiving probiotics, who were born via caesarean section during the first five years of life [47]. After 13 years of follow-up of the afore-mentioned group of children, Peldan *et al.* [48] showed that the prevalence of IgE allergy to any allergen was high in all children studied and increased with age. There was no significant difference in the prevalence of IgE allergy to any of the allergens tested between the groups. According to the S3 guidelines of the German Society of Allergy and Immunology and the German Pediatric Society, probiotics should not be used in newborns and infants for allergy prevention (strong recommendation) [49].

The effect of *B. animalis* subspecies *lactis* BB-12 utilization in the first months of life on the immune response after vaccination against influenza, tetanus, and polio, was also analyzed in children born via caesarean section. Higher levels of tetanus and polio antibodies, and lower levels of influenza antibodies were seen in children in the study group compared with the control group [49]. The administration of *B. lactis* in caesarean born children did not reduce the incidence of diarrhea in the first year of life. Also, there were no differences in anthropometric measurements of year-old children in the test and control groups [50].

CONCLUSIONS

The use of probiotics in preterm infants can offer significant benefits, but it must be carried out with caution and based on robust clinical trial data confirming efficacy and safety. To summarize, the species and strains recommended by scientific societies and used in the cited studies are shown in Table 2. Despite the limited recommendations from these scientific organizations regarding probiotic therapy in the neonatal period, the number of published studies of various kinds on the subject show how widely it is used and how varied its effects can be. It should be remembered that probiotic therapy can benefit the smallest patients who are only in the vicinity of the neonates receiving it (horizontal transmission), but on the other hand, it can be dangerous for them by leading to the development of probiotic sepsis (it was found in 2 out of 32 described cases of sepsis). The lack of studies, involving the most vulnerable infants, on NEC, sepsis, or mortality of those being < 28 weeks of gestation, must be also highlighted. It is very important to meet the formal requirements, which consider the safety and quality of probiotics used (Table 1). This is a task not only for neonatologists, but also for hospital pharmacists, who must assess the quality aspects of probiotics used among this age group.

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