Svāsthya: Trends in General Medicine and Public Health
https://doi.org/10.70347/svsthya.v3i5.163

Review Article

Probiotics, Gut Microbiome, Antibiotic Resistance, and Necrotizing Enterocolitis in Preterm Infants: A Narrative Review

Thalia Anggrea Noor [1*], Conny Riana Tjampakasari[2]

[1] Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia; [2] Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, RW 5, Kenari, Kec. Senen, Kota Jakarta Pusat, DKI Jakarta 10430, Indonesia

ABSTRACT

Background: Premature infants are susceptible to infections owing to immature immunity and underdeveloped gut microbiomes. Prolonged antibiotic use increases resistance and dysbiosis, and prematurity remains the leading cause of neonatal death worldwide. **Objective:** This study aimed to evaluate the role of probiotic supplementation in modulating the gut microbiome, antibiotic resistome, and risk of necrotizing enterocolitis (NEC) in premature infants. **Methods**: This study used a narrative review informed by a structured search of PubMed/MEDLINE, Scopus, Web of Science, and Cochrane Library publications from January 2018 to March 2025, supplemented by landmark studies and current guidelines. Eligible sources were used to examine the effects of probiotics in premature infants (<37 weeks gestation) on NEC, the gut microbiota, antibiotic resistance outcomes, and underlying biological mechanisms. **Results**: Probiotic supplementation with Bifidobacterium and Lactobacillus restored gut microbial communities disrupted by antibiotic exposure. Probiotics act through competition with pathogens, bacteriocin, and organic acid production, which inhibits bacteria and increases secretory IgA. In very preterm or very-low-birth-weight infants, probiotics may reduce NEC (low-certainty evidence) and slightly reduce all-cause mortality (moderate-certainty evidence), but the benefit in extremely preterm or extremely low-birth-weight infants remains uncertain. The effects on the antibiotic resistome are inconsistent, and the benefits appear to increase with exclusive milk feeding. **Conclusion**: Probiotic supplementation is a strain-specific strategy for preventing NEC in premature infants, but its role in limiting antibiotic resistance remains unestablished. Its use in neonatal protocols requires quality-assured products, infant selection, and surveillance for probiotic-associated sepsis.

KEYWORDS

Infan, probiotics, drug resistance, microbial, gastrointestinal microbiome, Lactobacillus

ARTICLE INFO

Received Feb 17, 2026
Revised Nov 20, 2026
Accepted Nov 20, 2026
Online Nov 21, 2026

EDITOR

dr. Ronald Pratama Adiwinoto, M.Ked.Trop

CORRESPONDENCE

Thalia Anggrea Noor, Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, email: thalianoor633@gmail.com

CITE THIS ARTICLE

Noor, T. A., & Tjampakasari, C. R. (2026). Probiotics, gut microbiome, antibiotic resistance, and necrotizing enterocolitis in preterm infants: a narrative review. *Svāsthya: Trends in General Medicine and Public Health*, 3(5), e163. https://doi.org/10.70347/svsthya.v3i5.163

Supplemental data for this article can be accessed online at https://doi.org/10.70347/svsthya.v3i5.163
©2026 The Author(s). Published with license by PT. Mega Science Indonesia
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0). This license allows reusers to copy and distribute the material in any medium or format in unadapted form only, for noncommercial purposes only, and only so long as attribution is given to the creator. The terms on which this articlehas been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

INTRODUCTION

Premature birth, defined as birth before 37 weeks of gestation, is a global health problem that contributes to neonatal morbidity and mortality. An estimated 13.4 million infants were born preterm in 2020, representing 9.9% of all live births, and preterm birth is the leading cause of neonatal mortality [1,2]. In Indonesia, the 2023 Indonesian Health Survey reported a low birth-weight proportion of 6.1%, and low birth weight and prematurity remain among the main causes of neonatal death [3]. Premature infants experience immaturity in multiple organ systems, including the immunological and gastrointestinal systems. The immaturity of the gut microbiome in premature infants results in high susceptibility to pathogenic bacterial colonization and nosocomial infections. This condition triggers prolonged empirical antibiotic use as a therapeutic intervention to prevent or treat neonatal sepsis and gastrointestinal infection. The gut microbiome of premature infants differs substantially from that of full-term infants, with a dominance of Enterobacteriaceae and a relative deficiency of protective *Bifidobacterium* and *Lactobacillus* species [4,5].

The use of broad-spectrum antibiotics during the neonatal period, although life-saving in certain contexts, has negative consequences for the ecology of the intestinal microbiome. Prolonged antibiotic exposure eliminates not only target pathogens but also beneficial commensals, inducing dysbiosis and creating selective pressure that facilitates the proliferation of resistant strains of bacteria. Antibiotic resistance mechanisms involve genetic mutations, horizontal transfer of resistance genes via plasmids and transposons, and the modification of antibiotic targets that reduce drug affinity [6]. Prolonged empirical antibiotic therapy in extremely low-birth-weight infants is associated with an increased incidence of necrotizing enterocolitis and mortality [7]. Necrotizing enterocolitis (NEC) is a life-threatening gastrointestinal emergency in premature infants and is characterized by acute intestinal inflammation and mucosal necrosis. The pathophysiology of NEC is multifactorial and involves intestinal barrier immaturity, dysregulation of the mucosal immune response, and microbiome dysbiosis. In the premature intestine, increased epithelial expression of Toll-like receptor 4 (TLR4) amplifies the response to lipopolysaccharide from gram-negative colonizers [8]. An imbalance in the microbiota, exacerbated by antibiotic use, can trigger excessive inflammatory responses, bacterial translocation, and progressive intestinal tissue damage. NEC mortality ranges from 15% to 30%, with long-term morbidity, including short bowel syndrome and neurodevelopmental disorders [9,10].

Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, offer an approach to modulating the gut microbiome of premature infants. The genera *Lactobacillus* and *Bifidobacterium* are the most extensively studied probiotics in the neonatal population, although the former genus was reclassified in 2020 into 25 genera; for example, *Lactobacillus rhamnosus* is now *Lacticaseibacillus rhamnosus,* and *Lactobacillus reuteri* is now *Limosilactobacillus reuteri* [11]. The protective mechanisms of probiotics include the competitive exclusion of pathogens through competition for nutrients and adhesion sites, the production of antimicrobial metabolites such as lactic acid and bacteriocins, the strengthening of the intestinal epithelial barrier, and the modulation of adaptive and innate immune responses [12,13].

Breast milk is an optimal source of endogenous bacteria for transfer to premature infants. Culture-dependent and culture-independent studies have shown that human milk supplies staphylococci, streptococci, lactic acid bacteria, and bifidobacteria to the infant gut [14], and an Indonesian review identified *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Lactobacillus* as the dominant genera [15]. In addition to bacteria, breast milk is rich in human milk oligosaccharides, which function as selective prebiotics, immunoglobulins (especially secretory IgA), lactoferrin, lysozyme, and growth factors that support intestinal maturation (Figure 1). The bioactive components of breast milk work synergistically with probiotics to optimize the colonization of beneficial microbiota and strengthen mucosal defenses [5,13]. Feeding preterm infants formula rather than donor breast milk increases the risk of NEC (risk ratio [RR] 1.87, 95% confidence interval [CI] 1.23–2.85) [16].

Although scientific evidence supports the effectiveness of probiotics in preventing NEC and modulating the microbiome, their clinical implementation still faces challenges. The heterogeneity of probiotic strains used in various studies, variability in dosage and duration of supplementation, and limited availability of breast milk among mothers with premature infants are factors that influence the applicability of probiotic interventions in clinical practice. In addition, the safety of probiotics in infants with severe immunocompromise and the risk of bacteremia or fungemia due to probiotic strain translocation remain concerns [17,18]. Professional bodies have reached different conclusions regarding this issue. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) issued a conditional, strain-specific recommendation [19], whereas the American Academy of Pediatrics does not support routine universal administration, particularly in infants weighing <1000 g [20].

This study aimed to critically evaluate the role of probiotic supplementation in modulating the gut microbiome and antibiotic resistance and reducing the risk of NEC in premature infants. A deep understanding of the probiotic–microbiome–host interaction in the context of premature birth can inform the development of evidence-based probiotic supplementation protocols to improve neonatal health outcomes. The results of this study are expected to contribute to the formulation of practical recommendations for the implementation of probiotics in the routine care of premature infants in neonatal intensive care units, with attention to settings where product regulation is limited.

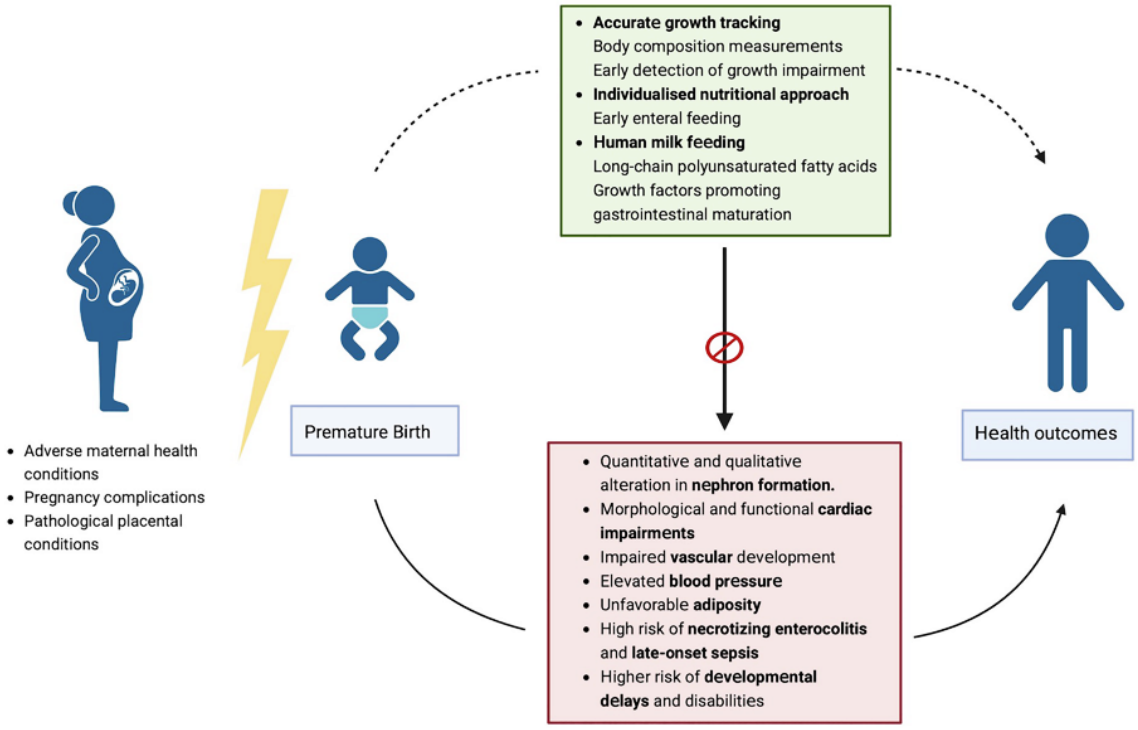


Figure 1. Contribution of human milk to the health and development of preterm infants. Reproduced from Morniroli *et al.* [5], published under the Creative Commons Attribution (CC BY) license.

MATERIALS AND METHODS

Study design and setting

This study used a narrative review design informed by a structured literature search to analyze scientific publications examining the use of probiotics in premature infants in the context of preventing antibiotic resistance and NEC. The review was prepared according to the Scale for the Assessment of Narrative Review Articles (SANRA) [21]. Literature searches were conducted in the PubMed/MEDLINE, Scopus, Web of Science, and Cochrane Library electronic databases for publications from January 2018 to March 2025. Earlier seminal publications on gut microbiota development, antibiotic resistance mechanisms, and NEC pathophysiology were added through

backward citation searching, together with current position statements and regulatory communications. The search strategy used a combination of Medical Subject Headings (MeSH) and keywords: ("Infant, Premature"[MeSH] OR "preterm infant" OR "very low birth weight") AND ("Probiotics"[MeSH] OR probiotic OR *Bifidobacterium* OR *Lactobacillus*) AND ("Enterocolitis, Necrotizing"[MeSH] OR "Drug Resistance, Microbial"[MeSH] OR "antibiotic resistance gene" OR resistome OR "Gastrointestinal Microbiome"[MeSH] OR "gut microbiota"). The syntax was adapted to each database, and the last search was performed in March 2025.

Population and selection criteria

The target population of the study was premature infants with a gestational age of < 37 weeks who received probiotic intervention for the prevention of conditions related to dysbiosis and antibiotic resistance. The inclusion criteria were as follows: (1) randomized controlled trials, cohort studies, or systematic reviews with or without meta-analyses evaluating the effectiveness or safety of probiotics in premature infants, together with position statements and regulatory safety communications; (2) studies measuring primary outcomes such as NEC incidence, intestinal microbiota colonization, or antibiotic resistance markers; (3) publications in English with full-text accessibility; and (4) studies using *Lactobacillus* and/or *Bifidobacterium* probiotic strains alone or combined with other genera such as *Streptococcus thermophilus*. The exclusion criteria were as follows: (1) editorials and case reports, except case reports of probiotic-associated invasive infections used for safety synthesis; (2) studies in term or nonneonatal infants; (3) studies with outcomes not relevant to the microbiome or antibiotic resistance; and (4) duplicate publications or data that could not be extracted. When a primary trial had already been pooled in an included meta-analysis, its results were not counted again as independent evidence.

Research procedure

The study selection process was independently conducted by two reviewers. The first stage involved screening the titles and abstracts to identify potentially relevant studies. The second stage involved evaluating full-text articles against the inclusion and exclusion criteria. Disagreements between the reviewers were resolved through consensus discussions or consultations with a third reviewer. Because this was a narrative review, a new risk of bias assessment was not performed. Instead, the certainty ratings and risk-of-bias judgments reported by the source systematic reviews were extracted and reported.

Data extraction

Data were extracted using a standardized form covering study characteristics (author, year of publication, country, study design), population characteristics (sample size, gestational age, birth weight), intervention characteristics (probiotic strain, dose, duration of administration, route of administration), and outcomes (incidence of NEC, changes in microbiota composition, antibiotic resistance markers, and adverse events). Quantitative data were extracted in the form of the mean±standard deviation, median with interquartile range, risk ratio with 95% confidence interval, or odds ratio with 95% credible interval according to the reporting format of each study.

Data analysis

Analysis was performed descriptively and qualitatively, with a narrative synthesis of the findings of the included studies. No new quantitative pooling was performed, and pooled estimates are reported as published by the source meta-analyses. Thematic analysis was used to identify patterns of probiotic mechanisms involved in modulating the microbiome and influencing antibiotic resistance. Categorization was based on (1) the biological mechanisms of probiotics (nutritional competition, bacteriocin production, and immune modulation), (2) the probiotic strains used (single-strain versus multistrain), (3) the type of feeding (exclusive human milk versus formula alone or mixed feeding), and (4) clinical outcomes (NEC prevention, reduction in antibiotic resistance, and changes in the microbiome). Where available, the certainty of evidence is reported as rated by the source reviews using the grade of recommendations, assessment, development, and evaluations (GRADE) approach.

RESULTS

Characteristics of preterm infants and susceptibility to infection

Premature infants are a neonatal population whose physiological characteristics differ substantially from those of full-term infants. Birth before 37 weeks of gestation results in the immaturity of multiple organ systems, particularly the immunological and gastrointestinal systems. The neonatal immune system in premature infants is characterized by quantitative and functional neutrophil deficiency, limited production of proinflammatory cytokines, and intestinal mucosal barrier immaturity. The gut microbiome of premature infants shows low diversity, with a predominance of facultative anaerobic bacteria from the Enterobacteriaceae family, in contrast to that of breast-fed full-term infants, who are dominated by *Bifidobacterium*, an obligate anaerobe, with a smaller contribution of aerotolerant lactobacilli [4,13]. Preterm infants almost universally receive early and often extended antibiotic therapy [22]. In extremely low-birth-weight infants with sterile initial cultures, a longer initial course of empirical antibiotics was associated with higher rates of NEC or death [7]. Prematurity has consequences that extend beyond the neonatal period, including later growth and neurodevelopmental and cardiometabolic risks [2,5].

Effects of antibiotics on the gut microbiome and resistance

Antibiotic exposure during the neonatal period induces extensive and persistent disruption of the intestinal microbiome. A longitudinal metagenomic study of 401 stool samples from 84 preterm infants revealed that meropenem, cefotaxime, and ticarcillin-clavulanate were associated with reduced species richness, whereas the effects of vancomycin and gentamicin varied among infants. Multidrug-resistant *Escherichia*, *Klebsiella*, and *Enterobacter* dominated the preterm gut, and every antibiotic regimen enriched resistance genes with no known activity against the administered drugs [22]. The mechanism of antibiotic resistance in the neonatal microbiome involves the horizontal transfer of resistance genes through mobile genetic elements (MGEs), such as plasmids, transposons, and integrons. Functional metagenomic selection in preterm infant stools revealed 794 antibiotic resistance genes (ARGs), 79% of which had not been previously classified as resistance genes [22]. Clinically relevant ARG families include extended-spectrum beta-lactamases (*blaTEM*, *blaSHV*, *blaCTX-M*), aminoglycoside-modifying enzymes (*aac*, *aph*, *ant*), and fluoroquinolone resistance determinants (*qnr*, *aac(6′)-Ib-cr*). The persistence of ARGs in the intestinal microbiome creates a reservoir of resistance that can be transferred to opportunistic pathogens, increasing the risk of therapy-resistant infections [6,22].

Probiotics in the prevention of antibiotic resistance

Probiotic supplementation has been demonstrated to mitigate the negative effects of antibiotics on the intestinal microbiome through multiple biological mechanisms (Figure 2). Primary mechanisms include competitive exclusion through competition for nutrients and adhesion sites on the intestinal epithelium, which limits the proliferation of pathogenic bacteria. *Lactobacillus* and *Bifidobacterium* produce organic acids (lactate and acetate) that lower the luminal pH, thereby creating an unfavorable environment for the growth of Enterobacteriaceae and *Clostridioides difficile*. The production of bacteriocins by certain strains (nisin by *L.lactis* and plantaricin by *L. plantarum*) provides antimicrobial activity against pathogens, and some bacteriocins have a narrow activity spectrum that may limit collateral damage to commensals [12,23]. However, clinical evidence on resistance outcomes remains limited.

A systematic review that screened 1,970 records included only six randomized or cohort studies involving newborns. Two studies suggested a favorable effect of probiotics on antibiotic resistance, one suggested an unfavorable effect, and three did not report a significant effect; thus, no firm conclusions could be drawn [24]. In a Norwegian multicenter cohort, extremely preterm infants given *Lactobacillus acidophilus* and *Bifidobacterium longum* subsp. *infantis* had a median relative abundance of *Bifidobacterium* of 64.7% at 7 days compared with 0.0% in non-supplemented preterm infants. Despite much higher antibiotic exposure, their microbial diversity and resistome did not differ from those of more mature infants at 4 weeks and 4 months of age

[25]. Probiotic strains can themselves carry ARGs, and ESPGHAN therefore requires that strains given to preterm infants be devoid of transferable resistance genes [19].

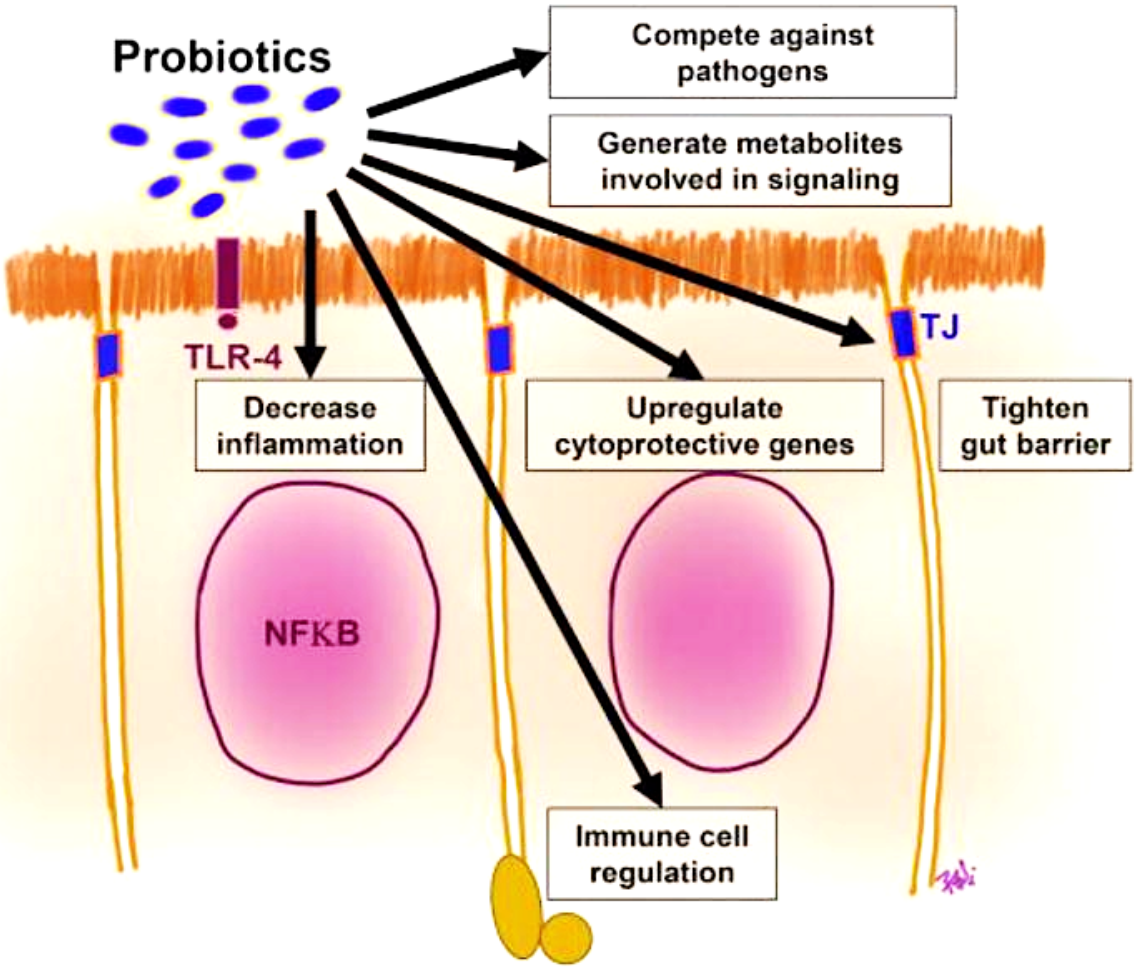


Figure 2. Mechanisms through which probiotics protect immature intestines. Adapted from Patel and Underwood [10].

Intestinal immune modulation by probiotics

Probiotics modulate the intestinal mucosal immune system by interacting with pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), on epithelial and dendritic cells. The cell wall components of gram-positive probiotics, such as lipoteichoic acid and peptidoglycan, are sensed mainly through TLR2, and this signaling favors the production of anti-inflammatory cytokines (IL-10 and TGF-β) and the differentiation of regulatory T cells. This response suppresses excessive proinflammatory cytokines (TNF-α and IL-1β) [12]. In contrast, TLR4 is the receptor for gram-negative lipopolysaccharide, and its exaggerated signaling in the premature gut is a central driver of NEC, which experimental probiotic strategies aim to attenuate [8,26]. This mechanism plays a role in maintaining intestinal immune homeostasis and preventing pathological inflammation underlying NEC [10].

Probiotics stimulate the maturation of the neonatal humoral immune system by increasing the production of secretory IgA (sIgA) by plasma cells in the lamina propria. Increased sIgA contributes to the neutralization of bacterial toxins, pathogen aggregation, and prevention of microbial adhesion to the intestinal mucosa. In addition, probiotics increase the population and function of dendritic cells and intestinal macrophages, which play a role in immune surveillance and elimination of invasive pathogens (Figure 3) [12,27]. Restoration of the perturbed infant microbiota has been reported after single-strain and, more consistently, multi-strain supplementation with *Bifidobacterium* and lactobacilli [28].

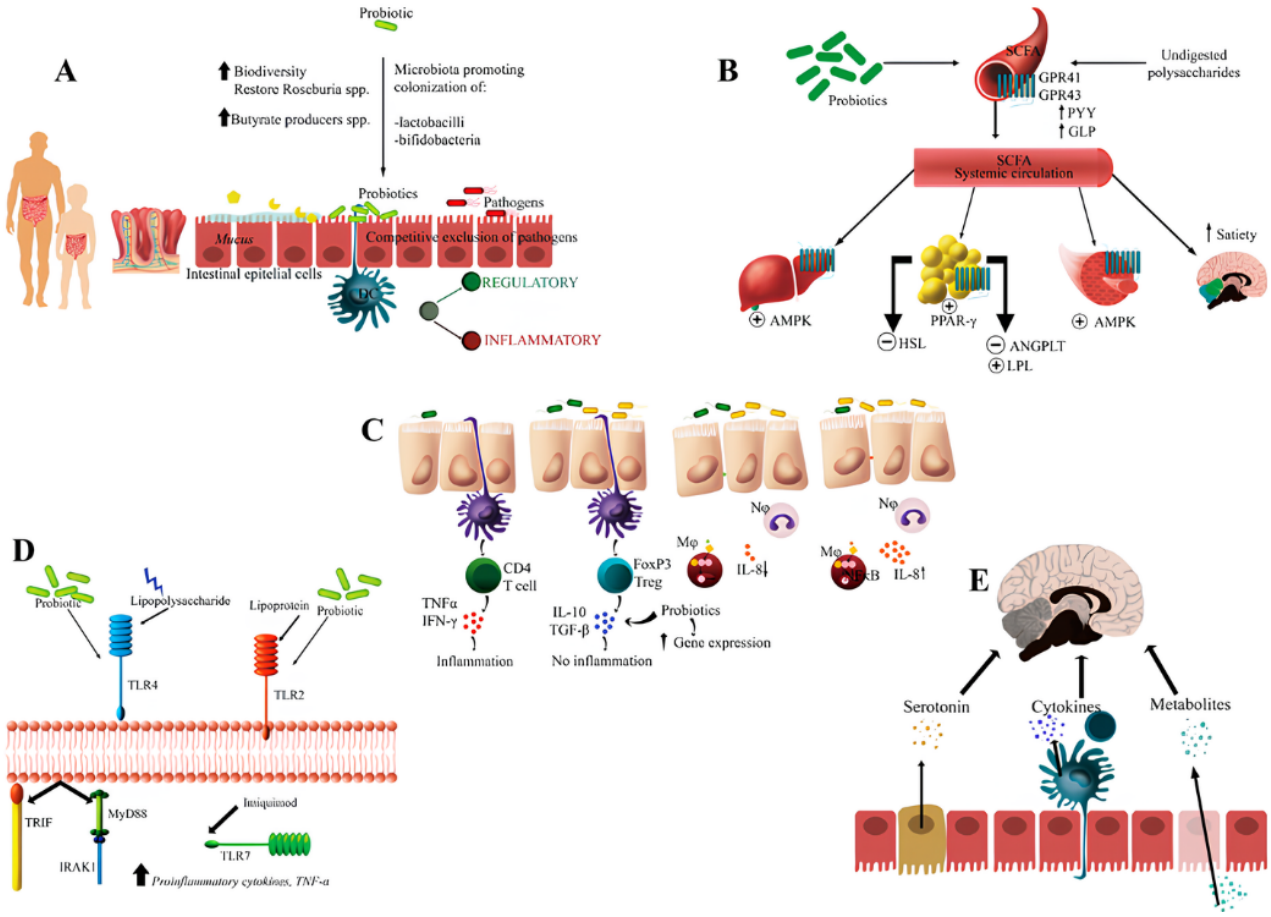


Figure 3. Mechanisms of probiotic action. Adapted from Plaza-Díaz *et al.* [12].

Effectiveness of probiotics in preventing necrotizing enterocolitis

The most recent Cochrane review included 60 trials with 11,156 very preterm (<32 weeks) or very low birth weight (VLBW, <1500 g) infants. Probiotics may reduce the risk of NEC (RR 0.54, 95% CI 0.46–0.65; 57 trials; 10,918 infants, low certainty) and probably reduce all-cause mortality (RR 0.77, 95% CI 0.66–0.90, moderate certainty). However, in extremely preterm or extremely low birth weight (ELBW, <1000 g) infants, probiotics may have little or no effect on NEC (RR 0.92, 95% CI 0.69–1.22; low certainty) [29]. A network meta-analysis of 51 trials (10,664 infants and 29 probiotic interventions) revealed that most strains were evaluated in a few small trials. The combination of *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Bifidobacterium longum* B94 reduced the NEC stage to ≥2 in both feeding groups [30]. On this basis, the ESPGHAN issued a conditional recommendation, with low certainty of evidence, for either *Lacticaseibacillus rhamnosus* GG ATCC 53103 or a combination of *Bifidobacterium infantis* Bb-02, *Bifidobacterium lactis* Bb-12, and *Streptococcus thermophilus* TH-4 [19].

The protective mechanism of probiotics against NEC involves multiple pathways: (1) competition with potentially pathogenic bacteria, such as *Clostridium perfringens* and *Escherichia coli*, which have been implicated in the pathogenesis of NEC; (2) production of short-chain fatty acids (acetate, propionate, and butyrate), which provide nutrients for enterocytes and strengthen intestinal tight junctions; (3) modulation of the inflammatory response by reducing the production of TNF-α and IL-8, which mediate tissue damage; and (4) stimulation of mucin production, which enhances the protective mucous barrier (Figure 4). Histopathological studies in animal models have demonstrated that probiotics reduce the degree of intestinal inflammation, enterocyte apoptosis, and bacterial translocation in groups at risk of NEC [8–10].

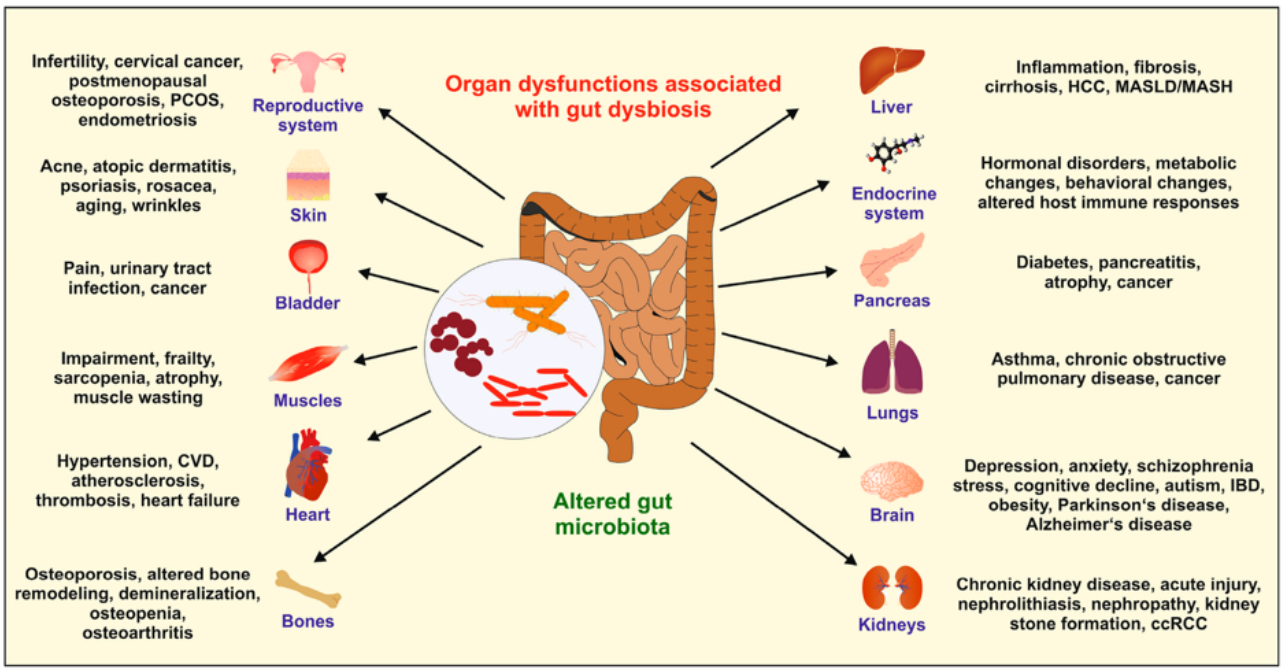


Figure 4. Organ dysfunction is associated with gut dysbiosis. Reproduced from Chandrasekaran *et al.* [27], published under the Creative Commons Attribution (CC BY) license.

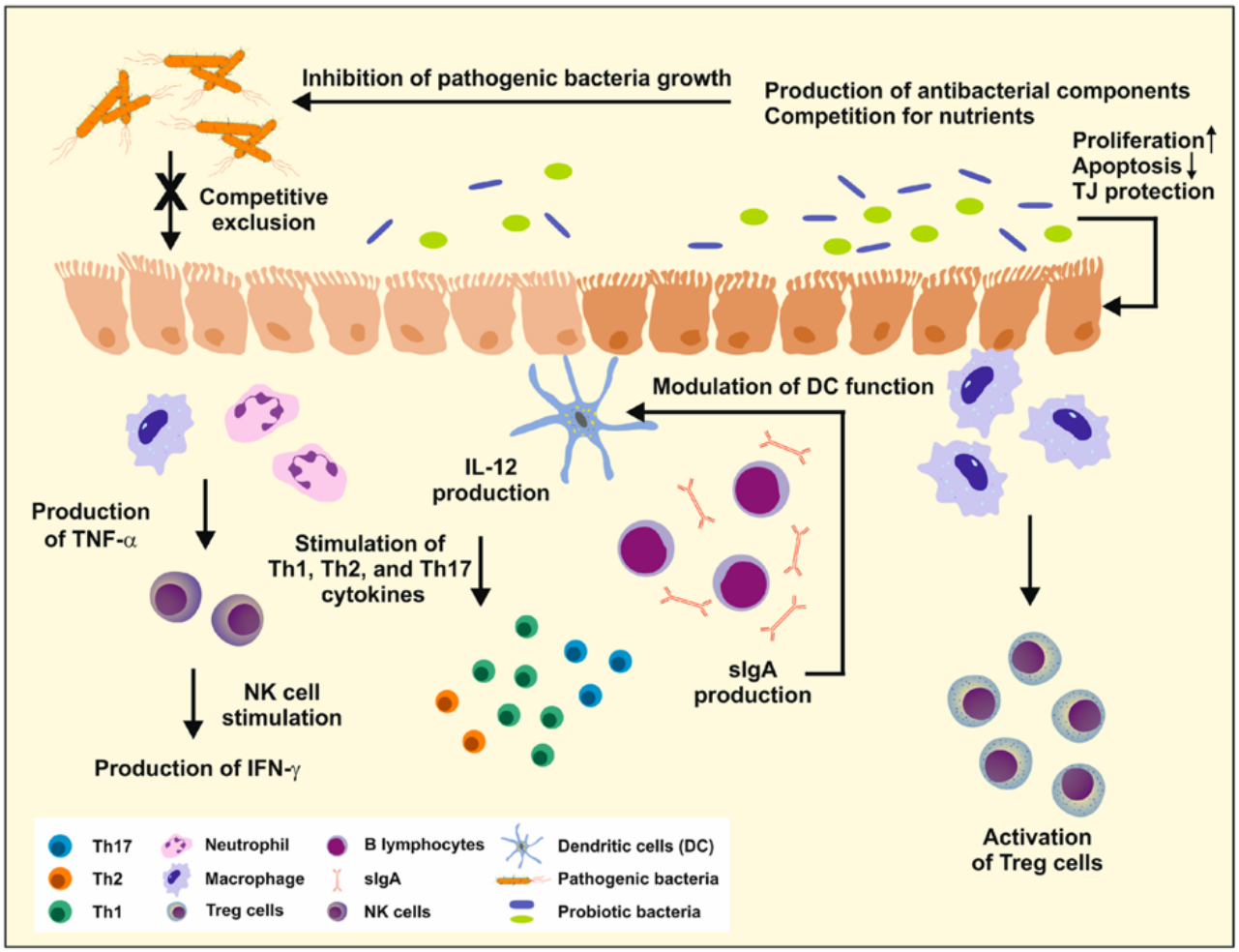


Figure 5. Role of probiotics in strengthening the host immune system. Reproduced from Chandrasekaran *et al.* [27], published under the Creative Commons Attribution (CC BY) license.

Safety and clinical considerations of probiotics

The safety profile of probiotics in preterm infants has been extensively evaluated through systematic reviews and meta-analyses. In the Cochrane review, probiotics likely had little or no effect on late-onset invasive infection (RR 0.89, 95% CI 0.82–0.97; 49 trials; 9,876 infants; moderate certainty) [29]. Trials are not powered to detect rare events, and probiotic-associated bacteremia has been documented. In a United Kingdom unit, the first case of *B. longum* subsp. *infantis* bacteremia in 10 years of routine use was confirmed by whole-genome comparison to originate from a probiotic product [18]. In 2023, the United States Food and Drug Administration reported that preterm infants given probiotics are at risk of invasive, potentially fatal disease after the death of an infant with a birth weight <1000 g whose blood isolate genetically matched the administered *B. longum* subsp. *infantis* product from sepsis [32]. Case series of patients with septicemia have also kept routine administration under debate [17,28].

Considerations for selecting probiotic strains for premature infants include metabolic profiles, particularly D-lactate production. Several *Lactobacillus* strains (e.g., *L. acidophilus* and *L. delbrueckii*) produce D-lactate as a fermentation metabolite. The accumulation of D-lactate in premature infants with immature kidney function can induce D-lactic acidosis, which manifests as metabolic acidosis, encephalopathy, and neurological disorders. Therefore, the recommended strains for premature infants are those whose lactate isomer profiles have been documented. *Bifidobacterium* species are not lactate free because they ferment hexoses through the fructose-6-phosphate phosphoketolase pathway (bifid shunt) and release acetate and L-lactate [13,31]. Quality control of probiotic products includes verification of strain identity, CFU viability, absence of pathogenic contamination, absence of transferable ARGs, and stability during storage [17,19].

DISCUSSIONS

The evidence presented here supports two conclusions concerning unequal strength. The effect of probiotics on NEC in preterm and VLBW infants is based on a large body of randomized evidence, although its certainty is low and its benefit is not demonstrated in ELBW infants [29]. The claim that probiotics limit antibiotic resistance rests on mechanistic plausibility and a small number of heterogeneous observational and randomized studies, which do not yet permit a firm conclusion [24,25]. The mechanism of competitive exclusion between probiotics and pathogens is a plausible pathway for limiting antibiotic resistance.

Lactobacillus and *Bifidobacterium* have strain-dependent adhesion to the intestinal mucosa through interactions with mucin, fibronectin, and glycoprotein receptors on enterocytes. The occupation of ecological niches by probiotics may limit the colonization of Enterobacteriaceae and *C. difficile*, which often develop antibiotic resistance. The clinical data are less consistent than this mechanism implies. Only two of the six studies in the systematic review by Fernandes *et al.* reported a favorable effect on resistance outcomes [24]. This heterogeneity probably reflects differences in strains, antibiotic exposure among the comparison groups, sampling time points, and the use of targeted culture rather than metagenomic resistome profiling. This competitive mechanism operates not only at the spatial level (adhesion sites) but also at the nutritional level, where probiotics consume the substrates required by pathogens for proliferation [12].

The production of antimicrobial metabolites by probiotics is an additional mechanism contributing to the inhibition of pathogen growth. Organic acids produced through fermentation (lactate, acetate, and propionate) lower the luminal pH, which is bacteriostatic against gram-negative pathogens such as *E. coli*, *Salmonella*, and *Klebsiella*. The antimicrobial activity spectrum of bacteriocins produced by certain strains is more specific. Nisin produced by *Lactococcus lactis* is active against gram-positive bacteria, such as *S. aureus* and *C. difficile*. The advantage of narrow-spectrum bacteriocins over conventional antibiotics is that they may limit disruption to beneficial commensal microbiota, although resistance to bacteriocins can also emerge [12,23]. Modulation of the intestinal immune response by probiotics is an aspect that distinguishes this intervention from conventional antimicrobial agents. The immunological effects of probiotics reported in experimental and clinical studies are mainly regulatory, namely, the induction of regulatory T cells and IL-10 and TGF-β [12]. This profile is relevant to NEC

because the premature intestine is prone to an exaggerated TLR4-dependent inflammatory response to gram-negative colonizers [8,26]. Most of these mechanistic data are derived from animal or *in vitro* models and adult or term infant studies, and their transfer to extremely preterm infants requires caution. Increased sIgA contributes to pathogen immune exclusion, toxin neutralization, and microbial aggregation for elimination via intestinal peristalsis.

The effectiveness of probiotics in preventing NEC, as summarized in this review, aligns with evidence from multiple systematic reviews and meta-analyses [29,30]. The protective mechanism of probiotics against NEC is multifactorial and involves not only modulation of the microbiome and immune system but also improvement of intestinal barrier integrity. Short-chain fatty acids produced by probiotics and the microbiota they support, especially butyrate, are the main energy source for colonocytes and stimulators of tight junction proteins (occludin, claudin, and ZO-1) [12]. Strengthening tight junctions reduces paracellular permeability, preventing the translocation of bacteria and endotoxins that trigger the inflammatory cascade in NEC pathogenesis. Animal models of NEC have shown reduced bacterial translocation and preserved barrier function after probiotic exposure [8,10].

Comparisons of the effectiveness of various probiotic regimens show marked heterogeneity. Large meta-analyses have demonstrated the efficacy of multi-strain preparations in reducing NEC and all-cause mortality, whereas the efficacy of single-strain preparations is less certain [20]. However, network meta-analyses have shown that most individual strains or combinations have been tested in few small trials; thus, strain-level rankings remain imprecise [30]. The mechanism of strain combination synergism has not been fully elucidated, but the hypotheses include the following: (1) metabolic complementarity, where different strains utilize different substrates, optimizing nutrient utilization; (2) a broader spectrum of antagonism against diverse pathogens; and (3) more comprehensive immune modulation through the activation of multiple pathways. However, the implementation of multistrain combinations faces practical challenges related to formulation, stability, and inter-strain compatibility that require further investigation.

The role of breast milk as an optimal delivery system for probiotics in preterm infants is underappreciated in the literature. Breast milk provides not only endogenous bacteria but also prebiotic substrates (HMOs) that selectively promote *Bifidobacterium* growth and immunological components that work synergistically. The greater effect of Bb-12/B94 in exclusively human-milk-fed infants [30], together with the higher NEC risk of formula than of donor milk [16], suggests that probiotic efficacy depends on the feeding substrate. The mechanisms of synergism include the following: (1) HMOs as cosubstrates that increase probiotic viability and metabolic activity; (2) lactoferrin, which chelates iron, limiting its availability to pathogens; and (3) lysozyme and lactoperoxidase, which amplify the antimicrobial activity of probiotics. The limited availability of breast milk in mothers with premature infants is a barrier to implementing this strategy, which requires donor human milk banks or formula fortification with synthetic HMOs.

The safety of probiotics in premature infants is a legitimate concern given that immunocompromised and invasive procedures increase the risk of translocation. However, evidence from large-scale meta-analyses has shown no increase in late-onset invasive infection [29], although trials are underpowered for rare events. Isolated cases of probiotic-associated sepsis reported in the literature are generally associated with (1) product contamination with pathogens; (2) use in extremely preterm infants <26 weeks with severe immunodeficiency; and (3) administration in infants with established necrotizing enterocolitis or intestinal perforation [17,18]. ESPGHAN states that probiotic supplementation is acceptable only when product quality is assured, strains are free of transferable ARGs, and local microbiology laboratories can routinely detect probiotic sepsis [19]. The fatal case reported by the United States Food and Drug Administration in an infant weighing <1000 g [32] shows that these conditions apply most strongly to ELBW infants, the group in which efficacy is also least certain [20,29].

Consideration of probiotic strain selection on the basis of the lactate metabolic profile is practical. D-lactic acidosis is a potential complication of probiotics that produce D-lactate in infants with immature renal function. Reported cases show manifestations of metabolic acidosis with anion gap elevation, neurological symptoms (lethargy, hypotonia), and increased serum D-lactate levels. Preference for strains with a documented L-lactate profile is a prudent way to

minimize this risk, given that *Bifidobacterium* species also produce lactate [31]. Quality assurance of probiotic products includes whole-genome sequencing to confirm strain identity, testing for antibiotic resistance genes to ensure the absence of transmissible resistance, and shelf-life stability testing to verify CFU viability until the expiration date [19].

The implementation of probiotics in clinical practice faces several challenges that need to be addressed. First, the heterogeneity of probiotic formulations in various studies (strain, dose, and duration) makes it difficult to standardize protocols. The optimal probiotic dosage for premature infants has not been definitively established, with the range used in studies ranging from 10^8–10^{10} CFU/day [19]. Second, the timing of probiotic initiation varies from birth to day 14 of life. Earlier initiation is theoretically more advantageous for establishing the microbiome before pathogen colonization; however, comparative head-to-head data are limited. Third, the optimal duration of supplementation is not clearly defined and ranged from 4 to 8 weeks in the majority of the studies. Prolonged supplementation until discharge or term-equivalent age may be beneficial for extremely preterm infants who are hospitalized for prolonged periods; however, this assumption has not been tested in adequately powered trials [29].

Future research perspectives in the area of probiotics for preterm infants include several directions. First, personalized probiotic therapy based on baseline microbiome profiling could optimize the selection of strains most beneficial for individual infants. Metagenomic sequencing can identify specific dysbiosis patterns requiring targeted intervention, and resistome profiling should become a standard outcome in probiotic trials [22,24]. Second, studies on the safety and efficacy of engineered or next-generation probiotics, such as *A. muciniphila* and *E. prausnitzii*, which are beneficial for adults, are needed. Third, synbiotics (a combination of probiotics and prebiotics) may amplify benefits through the provision of selective substrates for probiotics. The combination of *Bifidobacterium* with galacto-oligosaccharides or HMOs has shown promising results in preliminary studies. Fourth, the postbiotic approach using metabolites or probiotic cell components (short-chain fatty acids, exopolysaccharides, and surface proteins) can provide benefits without the risk of viable organism translocation [12].

The implications of antibiotic stewardship programs in neonatal intensive care units are important. Probiotics can be integrated as a component of a multimodal strategy to minimize antibiotic exposure and sequelae. The strategy includes the following: (1) consideration of a quality-assured, guideline-supported probiotic for very preterm or VLBW infants, with particular caution in ELBW infants [19,20]; (2) shortened duration of empirical antibiotic therapy with early discontinuation if cultures are negative at 48–72 hours [7]; (3) preference for narrow-spectrum antibiotics when the pathogen is identified; and (4) avoidance of prolonged antibiotic courses unless clinically indicated. Implementation requires multidisciplinary collaboration among neonatologists, clinical pharmacists, infection control practitioners, and microbiologists to develop evidence-based protocols and monitor compliance.

Clinical and public health implications

The findings of this study have implications for neonatal clinical practice and for public health policy. The implementation of probiotic supplementation in very premature infants in neonatal intensive care units can reduce NEC-related morbidity and mortality [29]. The prevention of NEC through probiotics can reduce the burden on the healthcare system and improve the long-term quality of life of survivors. In Indonesia and other settings where probiotics are marketed as food supplements, the absence of pharmaceutical-grade, quality-assured products is a practical barrier that must be addressed before probiotics can be used more widely [20,32]. From a public health perspective, probiotics may support global antimicrobial resistance mitigation efforts, although direct evidence remains limited [24]. Reducing antibiotic use and preventing the proliferation of resistant bacteria in the neonatal population can reduce the abundance of antibiotic resistance genes in the community. Educational programs for healthcare providers on appropriate probiotic use, safe strain selection, and monitoring of adverse events are essential for successful implementation. Involving parents in education about the importance of exclusive breastfeeding and the benefits and risks of probiotic supplementation can increase acceptance and adherence to interventions.

Limitations

This study has several limitations that need to be considered when interpreting the results. First, the methodological heterogeneity of the studies analyzed, including variability in probiotic strains, doses, duration, and route of administration, makes it difficult to generalize the findings. Second, most studies were conducted in developed countries with optimal neonatal intensive care resources; therefore, the applicability of the findings in resource-limited settings requires further validation. Third, long-term follow-up to evaluate the impact of probiotics on neurodevelopmental outcomes and metabolic health was limited in the analyzed literature. Fourth, this study did not perform a quantitative meta-analysis or a new risk-of-bias and GRADE assessment; it relied on the certainty ratings of the source reviews. Fifth, the restriction to English-language publications and the narrative design may have introduced selection bias. Sixth, evidence on antibiotic resistance outcomes is drawn from few studies with heterogeneous methods.

CONCLUSIONS

Probiotic supplementation containing *Lactobacillus* and *Bifidobacterium* is a promising preventive strategy against necrotizing enterocolitis in preterm and VLBW infants, with low-certainty evidence for NEC reduction and moderate-certainty evidence for a small reduction in mortality, whereas the benefit in ELBW infants is uncertain. Its effect on antibiotic resistance is biologically plausible but not yet established. Protective mechanisms include restoration of the intestinal microbiome, competition with pathogens, production of antimicrobial metabolites, and modulation of the mucosal immune response. Multi-strain preparations and exclusive human milk feeding appear to enhance efficacy. Future research recommendations include standardization of supplementation protocols, resistome profiling as a trial outcome, evaluation of personalized probiotic therapy on the basis of microbiome profiling, and assessment of long-term outcomes. The implementation of probiotics in routine neonatal care protocols requires evidence-based guidelines, quality assurance of probiotic products, and surveillance monitoring to ensure optimal safety and efficacy.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision: T.A.N, and C.R.T

FUNDING

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

None

DATA AVAILABILITY STATEMENT

No new data were generated in this study. All the data analyzed are available in the published sources cited in the reference list of this article.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

This study used artificial intelligence (AI) tools with the following capacity: manuscript writing support, in which AI-based language models were employed for language refinement, and technical writing assistance in structuring the revised text. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were made solely by the authors.

REFERENCES

[1] Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. Lancet 2023;402:1261-71. https://doi.org/10.1016/S0140-6736(23)00878-4.

[2] Khandre V, Potdar J, Keerti A. Preterm birth: an overview. Cureus 2022;14:e33006. https://doi.org/10.7759/cureus.33006.

[3] Health Development Policy Agency. Thematic report on the Indonesian Health Survey (Survei Kesehatan Indonesia) 2023. Jakarta: Ministry of Health of the Republic of Indonesia; 2024. https://www.badankebijakan.kemkes.go.id/laporan-tematik-ski/.

[4] Schwiertz A, Gruhl B, Löbnitz M, Michel P, Radke M, Blaut M. Development of the intestinal bacterial composition in hospitalized preterm infants in comparison with breast-fed, full-term infants. Pediatr Res 2003;54:393-9. https://doi.org/10.1203/01.PDR.0000078274.74607.7A.

[5] Morniroli D, Tiraferri V, Maiocco G, De Rose DU, Cresi F, Coscia A, et al. Beyond survival: the lasting effects of premature birth. Front Pediatr 2023;11:1213243. https://doi.org/10.3389/fped.2023.1213243.

[6] Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr 2016;4:VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.

[7] Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics 2009;123:58-66. https://doi.org/10.1542/peds.2007-3423.

[8] Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016;13:590-600. https://doi.org/10.1038/nrgastro.2016.119.

[9] Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364:255-64. https://doi.org/10.1056/NEJMra1005408.

[10] Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin Pediatr Surg 2018;27:39-46. https://doi.org/10.1053/j.sempedsurg.2017.11.008.

[11] Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, et al. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol 2020;70:2782-858. https://doi.org/10.1099/ijsem.0.004107.

[12] Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. Adv Nutr 2019;10:S49-66. https://doi.org/10.1093/advances/nmy063.

[13] O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol 2016;7:925. https://doi.org/10.3389/fmicb.2016.00925.

[14] Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, et al. The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 2013;69:1-10. https://doi.org/10.1016/j.phrs.2012.09.001.

[15] Syahniar R, Suri AA. Profil mikrobiota ASI dan perannya terhadap saluran cerna bayi [Profile of breast milk microbiota and its role in the infant gastrointestinal tract]. Muhammadiyah J Nutr Food Sci 2020;1:8-17. https://doi.org/10.24853/mjnf.1.1.8-17.

[16] Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 2018;6:CD002971. https://doi.org/10.1002/14651858.CD002971.pub4.
[17] Seghesio E, De Geyter C, Vandenplas Y. Probiotics in the prevention and treatment of necrotizing enterocolitis. Pediatr Gastroenterol Hepatol Nutr 2021;24:245-55. https://doi.org/10.5223/pghn.2021.24.3.245.
[18] Acuna-Gonzalez A, Kujawska M, Youssif M, Atkinson T, Grundy S, Hutchison A, et al. Bifidobacterium bacteraemia is rare with routine probiotics use in preterm infants: a further case report with literature review. Anaerobe 2023;80:102713. https://doi.org/10.1016/j.anaerobe.2023.102713.
[19] van den Akker CHP, van Goudoever JB, Shamir R, Domellöf M, Embleton ND, Hojsak I, et al. Probiotics and preterm infants: a position paper by the European Society for Pediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Pediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 2020;70:664-80. https://doi.org/10.1097/MPG.0000000000002655.
[20] Poindexter B, Committee on Fetus and Newborn. Use of probiotics in preterm infants. Pediatrics 2021;147:e2021051485. https://doi.org/10.1542/peds.2021-051485.
[21] Baethge C, Goldbeck-Wood S, Mertens S. SANRA-a scale for the quality assessment of narrative review articles. Res Integr Peer Rev 2019;4:5. https://doi.org/10.1186/s41073-019-0064-8.
[22] Gibson MK, Wang B, Ahmadi S, Burnham CAD, Tarr PI, Warner BB, et al. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nat Microbiol 2016;1:16024. https://doi.org/10.1038/nmicrobiol.2016.24.
[23] Cotter PD, Ross RP, Hill C. Bacteriocins - a viable alternative to antibiotics? Nat Rev Microbiol 2013;11:95-105. https://doi.org/10.1038/nrmicro2937.
[24] Fernandes MA, Delgado L, Almeida AC. The impact of probiotics on antibiotic resistance in newborns: a systematic review. J Neonatal Perinatal Med 2024;17:509-18. https://doi.org/10.3233/NPM-240008.
[25] 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;6:347. https://doi.org/10.3389/fped.2018.00347.
[26] Duess JW, Sampah ME, Lopez CM, Tsuboi K, Scheese DJ, Sodhi CP, et al. Necrotizing enterocolitis, gut microbes, and sepsis. Gut Microbes 2023;15:2221470. https://doi.org/10.1080/19490976.2023.2221470.
[27] Chandrasekaran P, Weiskirchen S, Weiskirchen R. Effects of probiotics on gut microbiota: an overview. Int J Mol Sci 2024;25:6022. https://doi.org/10.3390/ijms25116022.
[28] Navarro-Tapia E, Sebastiani G, Sailer S, Toledano LA, Serra-Delgado M, García-Algar Ó, et al. Probiotic supplementation during the perinatal and infant period: effects on gut dysbiosis and disease. Nutrients 2020;12:2243. https://doi.org/10.3390/nu12082243.
[29] Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants. Cochrane Database Syst Rev 2023;7:CD005496. https://doi.org/10.1002/14651858.CD005496.pub6.
[30] Beghetti I, Panizza D, Lenzi J, Gori D, Martini S, Corvaglia L, et al. Probiotics for preventing necrotizing enterocolitis in preterm infants: a network meta-analysis. Nutrients 2021;13:192. https://doi.org/10.3390/nu13010192.
[31] Pokusaeva K, Fitzgerald GF, van Sinderen D. Carbohydrate metabolism in Bifidobacteria. Genes Nutr 2011;6:285-306. https://doi.org/10.1007/s12263-010-0206-6.
[32] U.S. Food and Drug Administration. Warning regarding use of probiotics in preterm infants: Dear Healthcare Provider Letter, 29 September 2023. Silver Spring (MD): FDA; 2023. https://www.fda.gov/media/172606/download.