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Neonatal Evidence Based Reviews

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Reducing Risk Factors for Necrotizing Enterocolitis

What Is the Recent Evidence and Biologic Plausibility Supporting Probiotics?

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ABSTRACT

Background: Development of necrotizing enterocolitis is multifactorial, with contributing factors that are unique to the preterm infant in the neonatal intensive care unit (NICU). The use of probiotics may reduce these risk factors.

Purpose: To evaluate evidence of biologic plausibility for probiotic supplementation to mitigate key risk factors implicated in the development of disease and show recent evidence of safety and effectiveness.

Data Sources: A literature survey of electronic databases, including PubMed, Cochrane Database of Systematic Reviews, and CINAHL, was conducted.

Study Selection: Selection terms included "necrotizing enterocolitis," "probiotics," and "prematurity." Reviews that were included were full text, in English, and published in the last 5 years. Ten systematic reviews of randomized controlled trials were extracted from 749 records. Excluded were studies that used adjuncts to probiotics, such as lactoferrin or prebiotics, and studies of probiotics given antenatally.

Data Extraction: Two independent reviewers extracted data to AMSTAR 2, a critical appraisal tool for systematic reviews of randomized or nonrandomized studies of healthcare interventions.

Results: All the reviews found statistically significant reductions in necrotizing enterocolitis rates after supplementation with probiotics. None of the reviews reported adverse effects.

Implication for Practice and Research: Probiotic supplementation with specific strains reduces risk for necrotizing enterocolitis. To advance probiotic use in the NICU, additional high-quality trials are needed to focus on specific strains or combinations of strains and to evaluate dosing and duration of treatment.

Video Abstract available at https://journals.lww.com/advancesinneonatalcare/Pages/videogallery.aspx.

Key Words: biologic, necrotizing enterocolitis, NICU, prematurity, prevention, probiotics

Despite advancements in care of the preterm infant (<37 weeks), necrotizing enterocolitis (NEC) remains one of the most damaging gastrointestinal (GI) complications.[1] NEC rates are inversely proportional to weight and gestational age, with 90% of the cases seen in preterm infants.[1] With more preterm infants surviving, the at-risk population has increased. Outbreaks of NEC may display common infectious agents, but the cause is unknown.[2] Development of NEC is multifactorial, with morbidities that include short-bowel disease, liver disease associated with parenteral feedings, growth restriction, and neurodevelopmental disabilities.[3] The mortality rate is 15% to 30%.[2] Many contributing factors are unique to preterm infants, including GI compromise, bacterial dysbiosis, type and timing of trophic feeding, mode of delivery, and nosocomial infections.[1,4]

The interaction of genes and environment shapes the neonatal immune system,[5] and the neonatal intensive care unit (NICU) environment influences GI tract colonization and immunomodulation.[6] Risk factors associated with NEC in the preterm infant, such as GI compromise and loss of enteric tight junctions, abnormal colonization with pathogenic species, feeding type and timing, exposure at delivery, and sepsis,[1,7] may be positively impacted by probiotics. Proposed biologic mechanisms of select probiotic strains include competitive colonization,[8] strengthening immunoglobulin A (IgA) mucosal response,[9,10] maintaining tight junctions in the GI tract,[11-13] antimicrobial peptides,[5] and upregulation of the immune response.[14]

Probiotics are defined by a working group of the Food and Agricultural Organization/World Health Organization (FAO/WHO) as "Live micro-organisms which when administered in adequate amounts confer a health benefit on the host."[15] Supplemental probiotics must be of human origin, be able to resist gastric and bile acid, adhere to mucous, and competitively

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displace pathogens.[16] *Lactobacillus* and *Bifidobacterium* are normal commensals in the infant GI tract and have been established as safe in foods and supplements worldwide.[15] Probiotics are routinely used in the NICU in Canada, Australia, Colombia, Japan, and Europe without significant adverse effects.[17] Probiotics, while commonly in use over the counter in the United States, have been slow to be adopted in the NICU setting. This is, in part, due to the US Food and Drug Association (FDA) restricting approval for use of probiotics as a live biotherapeutic product for prevention, mitigation, or treatment of disease.[18]

The data on probiotic efficacy and safety are extensive compared with other interventions in the NICU.[19] There have been concerns, however, regarding variable quality and quantity of organisms in commercially available products.[20] Probiotics are implicated in side effects, including unwanted metabolic activity such as production of D-lactate or bile salt deconjugation, excessive stimulation of the immune system, gene transfer, and systemic infections.[15] Large-scale studies have not found a significant increase in sepsis. However, there have been case reports of catheter-related infection and fungemia,[21,22] and in 2014, an infant died from mucormycosis associated with a contaminated product.[20] Numerous observational cohort studies and randomized controlled trials (RCTs) have reported minimal negative outcomes. At the same time, safety data in RCT studies are not held to the same standard as are pharmacologic products.[23] For this reason, the FAO/WHO published science-based criteria for evaluating function and safety of probiotic supplements. Their probiotic guidelines specify importance of knowing genus/species/strain; many effects of probiotics are strain specific, and strain identity will allow accurate epidemiologic follow-up. Nomenclature must be scientifically recognized using specific approved lists, and products must have an effective, viable concentration at the end of shelf life. In vivo validation of testing for safety and effectiveness is necessary, and good manufacturing practices regarding production and quality assurance must be applied[15] (Table 1).

The purpose of this evidence-based practice brief is to compile recent research on probiotic use for reduction of NEC. A second aim is to examine risk factors for the development of NEC and mechanisms of action of probiotic strains that mitigate them.

SEARCH STRATEGY

Methods

A review of the literature according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA)[24] was conducted utilizing 3 databases: Cumulative Index to Nursing and Allied Health Literature (CINAHL), PubMed, and Cochrane Database of Systematic Reviews. Data were limited to using only systematic reviews of RCTs or observational studies published in the last 5 years, in English or translated to English. No unpublished literature was included. The most recent Cochrane Database of Systematic Reviews was selected.

Two independent reviewers performed a literature review. Search terms included Medicine's Medical Subject Headings (MeSH) terms ((“Enterocolitis, Necrotizing/prevention and control” [Mesh]) AND “Probiotics/therapeutic use” [Mesh]) AND “Infant,

TABLE 1. Probiotic Safety Joint FAO/WHO Working Group[a]

	Science-Based Criteria for Evaluation of Function and Safety of Probiotics in Food
Genus, species, strain	Classification into taxonomic groups recognized by the International Code of Nomenclature for probiotics. Strain identification by internationally recognized methods, eg, genetic typing.
Minimum number of each strain	Minimum daily amount shown in vitro studies to confer benefit. Viable concentration at the end of shelf life. Serving size delivery of effective dose.
Clinical evaluations	Randomized, double-blind, placebo-controlled human studies of probiotic strains that measure both beneficial and adverse effects, with adequate sample size for statistically significant results.
Proper storage requirements	Proof that viability of strains is maintained throughout processing and storage.
Consumer information	Epidemiologic follow-up of adverse outcomes available to consumers. Corporate contact details on label. Evidence of Quality Assurance and Good Manufacturing Practice.
Antibiotic resistance	Testing for antibiotic resistance and virulence factors. Must be absent or nontransferable.
Metabolic activities	Testing for the potential for D-lactate production or bile salt deconjugation.
Side effects	Testing for interactions with other food/drugs.
Mammalian toxins/cytokines	Testing for toxin production.
Hemolytic activity	Determination of any potential for hemolytic activity.
Plasmids	Plasmids in *Lactobacilli* and *Bifidobacterium* must be sequenced and safe from genes encoding antibiotic resistance.

Abbreviation: FAO/WHO, Food and Agriculture Organization/World Health Organization.
[a]*Data adapted from the FAO/WHO.*[15]

Premature" [Mesh]). In total, 749 sources were found in the 3 databases, including 84 from CINAHL, 663 from PubMed, and 2 from Cochrane Database of Systematic Reviews. Duplicate reviews, those without full text, and those that did not substantively meet the criteria were removed, leaving 162 articles. After 135 sources that were not systematic reviews or meta-analyses of the data were excluded, 27 sources remained. Both reviewers independently reexamined the 27 articles, followed by discussion for inclusion in the review.

Systematic reviews were included with target populations preterm (<37 weeks), very preterm (<32 weeks), less than 2500 g (low birth weight [LBW]), and less than 1500 g (very low birth weight [VLBW]). Primary outcome was incidence of NEC stage II or above using Bell's staging[25] after administration of probiotics.

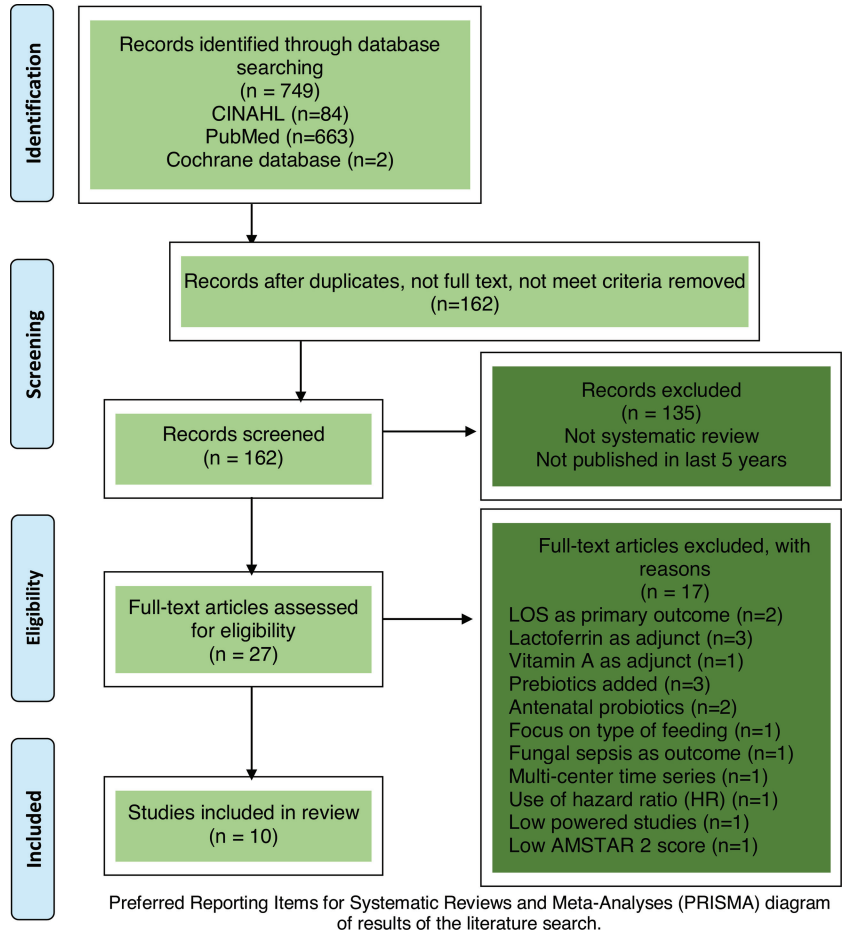
Independently, the authors extracted data into AMSTAR 2, a critical appraisal tool for systematic reviews of randomized or nonrandomized studies of healthcare interventions. AMSTAR 2 uses 16 specific criteria to judge quality of research, including risk of bias (ROB) and heterogeneity of reviews.[26] Reviews with 14 to 16 of the criteria were graded as high quality, 10 to 14 as medium quality, and fewer than 10 as low quality. Studies with the highest AMSTAR 2 scores were selected. Risk ratio (RR) and odds ratio (OR) to determine whether there is an association between intervention and risk were included when statistical outcomes (RR or OR) met a 95% confidence interval (CI). Reported *P* values of less than .05% were considered significant. Reviews were selected when a consensus was made regarding low ROB, including publication bias, and adequate statistical methodology. Seventeen additional articles were excluded for reasons listed on the PRISMA diagram (Figure 1).

RESULTS

Overview

Ten recent large-scale reviews were included after a search of the literature. All 10 were systematic review and meta-analysis of RCTs or network meta-analysis (NMA), with the following inclusion criteria: preterm infants (<37 weeks) with LBW (<2500 g), use of probiotics as the intervention in comparison with placebo or no probiotics, or to each other,

FIGURE 1



Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram of results of the literature search.

PRISMA diagram. LOS indicates late-onset sepsis.

and at least one primary outcome of NEC stage II or greater. One review included RCT and observational studies; only the RCT subgroup analysis was used here.[27] All reviews included effect estimates (RR or OR) with 95% CI (Table 2). Five reviews addressed certainty of evidence for each individual study using the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) approach.[23,27,28,30,34] Three assigned JADAD scores to assess methodological quality of the individual studies.[29,31,32] The remaining 2 reviews used Cochrane ROB tools.[33,35] Four reviews considered all combinations of any probiotic.[27,28,34,35] Four based their results on a probiotic mixture.[23,30,31,33] One study looked at only *Lactobacillus*,[29] and one considered only *Bifidobacterium*.[32]

Sharif et al[28] searched all RCTs and quasi-RCTs from 1946. Included in the Cochrane database were results pertaining to very preterm or VLBW infants. Trials included all probiotic combinations. Although the meta-analysis found probiotics may reduce risk of NEC, evidence was downgraded to low certainty due to trial design limitations and funnel plot asymmetry suggesting possible publication bias. Morgan et al[23] compared single- to multiple-strain probiotics in an NMA of RCTs. They determined moderate to high degree of certainty using GRADE for combinations of 1 or more *Lactobacillus* species plus 1 or more *Bifidobacterium* species over alternative single- and multiple-strain probiotics. Limitations include relative lack of studies comparing strains to each other rather than placebo. A review of *Lactobacillus* (subgroup analysis) by Liu et al[29] observed significant positive results with the absence of publication bias. Limitations included inconsistency of dosage and duration of treatment, as well as variable gestational age and birth weight. Bi et al[30] in an NMA of probiotic strategies to reduce NEC found significant reduction in NEC after probiotic mixture was administered. Evidence of publication bias lowered certainty of evidence. In addition, preselection of probiotic strategy may bias results. Jiang et al[31] found significant effect for mixed probiotics to reduce NEC. Publication bias was accounted for by funnel plot and Egger's regression test. They did not find significant heterogeneity overall but noted discrepancies in dose and duration and noted poor quality of some studies. A review of *Bifidobacterium* by Zhu et al[32] detected significant results for efficacy and safety of probiotics, with 80% of included studies scoring high on the JADAD scale. Unclear ROB was seen in allocation concealment and attribution, and inconsistency in dose and duration were noted as limitations. A subgroup and meta-analysis of multiple-strain probiotics by Chang et al[33] found reduced development of NEC compared with placebo. No

TABLE 2. Recent Systematic Reviews/Meta-analyses[a]

Year	Author	Number of Trials and Infants	Design	Statistically Significant Reduction in NEC
2020	Sharif et al[28]	53 trials 10,812 infants	Cochrane Database of Systematic Reviews	RR = 0.54; 95% CI, 0.54-0.65
2020	Morgan et al[23]	63 trials 15,712 infants	NMA of RCTs	OR = 0.35; 95% CI, 0.20-0.59 (1 or more *Lactobacillus*)
2020	Liu et al[29]	23 trials 4686 infants	Systematic review RCTs	RR = 0.34; 95% CI, 0.25-0.46; $P < .00001$ (*Lactobacillus*)
2019	Bi et al[30]	34 trials 9161 infants	Systematic review and NMA of RCTs	OR = 0.38; 95% CI, 0.27-0.54 (probiotic mixture)
2019	Jiang et al[31]	27 trials 9522 infants	Systematic review RCTs	RR = 0.39; 95% CI, 0.26-0.57 (probiotic mixture)
2019	Zhu et al[32]	24 trials 6155 infants	Systematic review RCTs	RR = 0.38; 95% CI, 0.25-0.58; $P < .00001$ (*Bifidobacterium*)
2017	Chang et al[33]	25 trials 7345 infants	Meta-analysis RCTs	OR = 0.36; 95% CI, 0.24-0.53; $P < .00001$ (multiple strain)
2017	Deshpande et al[34]	23 trials 4783 infants	Systematic review RCTs	RR = 0.46; 95% CI, 0.34-0.61; $P < .00001$
2017	Dermyshi et al[27]	29 trials 8535 infants	Systematic review RCTs	RR = 0.57; 95% CI, 0.47-0.70; $P < .00001$
2016	Sawh et al[35]	38 trials 10,520 infants	Systematic review RCTs	RR = 0.53; 95% CI, 0.42-0.66

Abbreviations: CI, confidence interval; NEC, necrotizing enterocolitis; NMA, network meta-analysis; OR, odds ratio; RCT, randomized controlled trial; RR, risk ratio.

[a]*RR and OR with a value less than 1 indicates protective effect of probiotic(s).*

publication bias was measured with funnel plot and Egger's regression test. A JADAD score was assigned to each study, with 20 of 25 trials scoring high with a 4 or 5. Limitations included heterogeneity of multiple-strain products and inadequate power from small sample size in some studies. In a review of 4 continents of low-income and medium-income countries, Deshpande et al[34] observed risk of NEC was significantly lower in the probiotic cohort. Quality of evidence was deemed high using GRADE guidelines. High score was based on a low risk of random sequence generation and allocation concealment, as well as large sample size, low *P* value, and mild heterogeneity. Limitations reflect variations in probiotic protocols and high ROB in many domains. Dermyshi et al[27] conducted a systematic review and meta-analysis of RCTs and observational studies using a GRADE approach. Only the RCT results were considered for this review. No publication bias was measured using the funnel plot and the Egger and Beggs tests. Administration of probiotics significantly reduced the incidence of NEC in preterm infants. Subgroup analysis of RCTs found significance of mixed probiotics over single species. Listed limitations were inconsistency in dosages and preparations and evidence of publication bias. A review of preterm infants by Sawh et al[35] observed NEC was significantly reduced after receiving probiotics compared with placebo. Evidence was considered high quality using Cochrane handbook tools. Limitations included uncertainty around randomization, blinding, allocation concealment, and a degree of selective reporting in some of the studies.

All the included reviews found significant improvement in rates of NEC after probiotic supplementation. None of the included systematic reviews reported any adverse effects from supplemented probiotics including late-onset sepsis (LOS) or negative neurodevelopmental outcomes, although long-term follow-up was not described. Critical appraisal of the reviews using the AMSTAR 2 tool is detailed in Table 3.[26]

Biological Mechanisms of Probiotics That Reduce the Risk of NEC

GI Compromise

The skin and mucosa of the preterm infant are fragile, and compromises in both structure and function

TABLE 3. AMSTAR 2 Critical Appraisal Tool

AMSTAR 2[a]	Sharif et al[28]	Morgan et al[23]	Liu et al[29]	Bi et al[30]	Jiang et al[31]	Zhu et al[32]	Chang et al[33]	Sawh et al[35]	Dermyshi et al[27]	Deshpande et al[34]
1. Components of PICO	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
2. Methods established prior	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3. Study designs for inclusion	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4. Comprehensive literature search	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5. At least 2 reviewers	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
6. At least 2 extractors	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
7. List of exclusion factors	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
8. Details of included studies	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
9. ROB of each study	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10. Source of funding for each study	N	N	N	N	N	N	N	N	N	N
11. Statistical methods	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
12. Impact of individual ROB	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
13. Individual ROB	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
14. Discuss heterogeneity	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
15. Publication bias impact	Y	Y	Y	Y	Y	U	Y	Y	Y	Y
16. Conflict of interest/ funding	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Abbreviations: ROB, risk of bias; N, no; U, unable to determine; Y, yes.

[a]AMSTAR 2: A critical appraisal tool for systematic reviews of randomized or nonrandomized studies of healthcare intervention.[26]

leave them vulnerable to bacterial and fungal sepsis.[36] Intestinal epithelia have tight junctional complexes that allow transcellular or paracellular permeability for nutrients and water. Decreased numbers of tight junctions in preterm infants result in opportunistic infection and translocation of intestinal bacteria into the bloodstream.[11] Premature infants have fewer goblet cells that secrete mucin for protection; intestinal secretion and absorption do not begin to develop until 26 weeks of gestation.[2] Antimicrobial peptides, which protect against gram-positive and gram-negative bacteria, some fungi, and viruses, are fewer in the premature infant intestine than in term infants, and this increases the risk of pathogenic invasion.[5] In addition, higher levels of innate immune receptors lead to overreactivity, exaggerated inflammatory response, and prooxidant stress and result in mucosal breakdown, bacterial translocation, and development of NEC.[5] Preterm infants display a lack of intestinal innate and adaptive immune defense mechanisms, with reduced maternal antibody transfer further adding to the risk factors.[5]

Inflammatory cascades may be activated in the gut by hypoxia or sepsis, stimulating an ischemic–reperfusion injury in utero. Release of inflammatory mediators, including tumor necrosis factor alpha (TNF- α), activate neutrophils and release reactive oxygen species. These cause vasoconstriction and increased permeability.[1] Immature regulation of intestinal circulation with decreased blood flow to the endothelium contributes to hypoxic ischemia[2]

Select probiotics counteract the compromise of tight junctions. A randomized, crossover, human study showed the positive effect of *Lactiplantibacillus plantarum* WCSF1 on recruitment of zonulin and occludin, two transmembrane proteins that increase tight junction structure and function.[11] This helps prevent pathogenic translocation from the GI tract to lymph nodes, liver, and bloodstream.[11]

The stress of hypoxia or sepsis adversely affects the neuroendocrine and GI systems, inducing intestinal permeability and impaired functionality that may be regulated by probiotics.[12] A retrospective clinical study from 2018 administered *Bifidobacterium* triple live capsules to neonates with initial Apgar scores of less than 7 points. Levels of serum corticotropin-releasing factor (CRF), a neuroendocrine peptide regulating the stress response, D-lactate, diamine oxidase (DAO), procalcitonin (PCT), and high-sensitivity C reactive protein (Hs-CRP), were made to determine stress response and tight junction permeability. The stress response levels (cortisol, CRF, and CRP) were significantly lower in the probiotic group ($P < .001$). Measures of intestinal permeability and damage (D-lactate, PCT, and DAO) were also significantly lower in the probiotic cohort ($P < .001$).[12]

A subset of patients with LOS may be related to translocation of pathogens from impaired gut barrier function in preterm infants that may be mitigated with colonization by probiotic bacteria.[13] A multicenter, randomized, double-blind (DB), placebo-controlled study dosed preterm infants with *Lactobacillus rhamnosus* KL53A and *Bifidobacterium breve* PB04, both stimulators of enteric tight junction proteins. Follow-up of infants who did not develop coagulase-negative *Staphylococcus* sepsis found significantly higher numbers of the administered *Lactobacillus* and *Bifidobacterium* ($H = 8.4150$; $df = 3$; $P = .0382$) than controls.[13]

Other probiotic mechanisms to enhance the integrity of the gut mucosa include stimulating epithelial growth factor receptors, activating pattern recognition receptors to seal tight junctions, protection against oxidative stress and apoptosis, and production of antimicrobial peptides by select *Lactobacillus* strains to competitively exclude pathobionts and modulate immunity.[16]

Bacterial Dysbiosis

Dysbiosis represents an imbalance in enteric ratio of beneficial (Firmicutes, Bacteroidetes) to pathogenic (Proteobacteria) and gram-negative organisms.[37] Perturbations in the gut microbiome, in addition to genetic predisposition and prematurity, are implicated in the development of NEC.[7] Dysbiosis antedates NEC frequently in preterm infants, whereas infants who do not develop NEC display higher numbers of Firmicutes and lower numbers of Proteobacteria.[37] Bacterial overgrowth with gram-negative bacteria is a key finding in NEC. Pneumatosis represents gaseous waste products of bacterial fermentation trapped in the bowel wall. *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Escherichia coli* are suspect and have been found in some outbreaks of NEC.[37] Modified Bradford Hill criteria to assess causality or strong association found strong support for temporality and plausibility for the relationship of gram-negative bacteria and the onset of NEC.[37]

Previously thought to be sterile in utero, the fetus may be exposed to microbes from the maternal vagina, GI tract, placenta, and mouth before birth.[5,7] Before rupture of membranes, the fetus may also be exposed to microbes in amniotic fluid, which begin the formation of a neonatal microbiome.[38] The GI tract is further colonized at birth.[39] Protective commensal bacteria affect cytokine expression on immune pathways by producing beneficial short-chain fatty acids such as acetate and butyrate crucial for decreasing levels of the pro-inflammatory cytokines TNF-α and interleukin 6 (IL-6).[40] Intestinal microbes, including *Lactobacillus*, are associated with lower levels of IL-6 and increased levels of anti-inflammatory transforming growth factor beta (TGF-β), whereas pathogenic *E coli* and inflammatory TNF-α compromise the intestinal barrier and function.[8,11] Normal microflora resists pathogenic colonization from exogenic

pathogenic species and overgrowth of endogenic opportunistic organisms. This is achieved by competition for nutrients and attachment sites and by production of inhibiting substances. Intestinal flora interfaces with undigested food for metabolism and secretion of conjugates.[41] A study of fecal samples from preterm hospitalized infants demonstrated a significant increase in microbial similarity of these infants as compared with full-term breastfed infants ($P < .05$), indicating an acquisition of hospital-based bacterial communities, with the potential for dysbiosis.[6]

A meta-analysis of RCTs in 2018 reviewed the effects of probiotics on inflammatory biomarkers. In 42 controlled trials with 2258 subjects, levels of inflammatory cytokines were lower in the probiotic group than in the placebo group, including Hs-CRP (standard mean difference [SMD] = −0.39; 95% CI [−0.50 to −0.28], I^2 = 83.8%), serum IL-6 (SMD = −0.37; 95% CI [−0.51 to −0.24], I^2 = 69.7%), TNF-α (SMD = −0.21; 95% CI [−0.34 to −0.08], I^2 = 85.5%), IL-12 (SMD = −0.47; 95% CI [−0.67 to −0.27], I^2 = 85.2%), and IL-4 concentrations (SMD = −0.48; 95% CI [−0.76 to −0.20], I^2 = 0.0%). An increase was seen in anti-inflammatory IL-10 (SMD = 0.21; 95% CI [0.04 to 0.38], I^2 = 48.5%).[14] This suggests probiotics reduce inflammation from presumed dysbiosis in some instances.

A randomized, DB, controlled study of infants less than 34 weeks and less than 1500 g administered *Lactiplantibacillus plantarum* found an association between the probiotic cohort and an increase in Firmicutes and decrease in Proteobacteria. Researchers propose the decrease in harmful bacteria was replaced by probiotic species.[8] The probiotic group displayed a decrease in serum IL-6 and an increase in TGF-β, a protective cytokine that suppresses inflammation.[8]

Enteral Feeding

The type and timing of human milk feeding affect humoral immune system maturation through colonization and development of IgA and IgM-secreting cells, providing improved mucosal protection for intestinal epithelium.[9] Degradation of mucin by the microbiota of the neonate begins later in breastfed infants.[41] Colonization of the intestine with human milk bacteria modulates indigenous microflora toward stability and is associated with lower morbidity and mortality.[42] Formula-fed infants display *Bacteroides* dominance and higher numbers of *Enterobacter*, *Enterococcus*, and *Clostridium* than human milk–fed infants who display predominance of *Bifidobacterium* over potentially pathogenic bacteria.[41] Low availability of mother's milk and delayed enteral feedings are probable risk factors for NEC.[39] Type and timing of enteral feedings are 2 of the few modifiable risk factors for developing NEC.[1]

An RCT of infants less than 35 weeks contrasted infants given a fermented, heat-inactivated formula that contained *B breve* C50 and *Streptococcus thermophilus* 065 versus a control formula. Fecal flora was examined for TNF-α and fecal calprotectin, 2 inflammatory markers, and secretory IgA, an immunological marker and participant in maintenance of mucosal barriers. Decreases were seen in abdominal distention after 2 weeks ($P = .016$) and fecal calprotectin ($P = .01$), suggesting less inflammation. Secretory IgA was increased with both mother's milk and fermented formula.[10]

Administration of *Bifidobacterium* triple live capsules to neonates 37 to 41 weeks was linked to higher daily milk intake than controls (16.57 ± 2.58 mL vs 13.26 ± 1.87 mL).[12]

Mode of Delivery

Vaginally delivered infants have bacterial compositions that are like those of their mother's vagina, including *Bacteroides*, *Lactobacillus*, *Prevotella*, and *Sneathia*. Infants delivered by cesarean birth (C-section) are dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* commonly found on the skin and in the hospital environment. Delivery mode may be a factor in susceptibility to pathogens and disease predisposition.[43]

Cesarean delivery may be a medical necessity with potential to compromise bacterial colonization that may be ameliorated by probiotic supplementation. In a blinded, randomized trial of 428 C-sectioned infants, study participants were given *B breve* Bb99, *Propionibacterium* freudenreichii subsp *shermanii* JS, *Lactobacillus rhamnosus* LC705, and *Lactobacillus rhamnosus* GG added to galacto-oligosaccharides (GOS) for the first 6 months after birth compared with controls. Fecal samples from the supplemented group did not demonstrate a decline in *Bifidobacterium* ($P = .01$) and *Bacteroides* ($P < .001$) compared with the control C-sectioned group. In addition, increases in *Enterococcaceae*, *Clostridiaceae*, and *Veillonellaceae* seen in the control group were not present in the probiotic cohort. Fecal metagenomes of the C-sectioned control group demonstrated decreases in amino acid and folic acid synthesis pathways unchanged in the supplemented group.[44]

Nosocomial Infections

Inappropriate colonization of the infant gut related to the hospital environment may be a risk factor for NEC.[6] Bacterial fecal samples of hospitalized infants initially display limited diversity and acquire a more diverse community over time. Profiles of these infants become more like each other, demonstrating cross-transmission of bacteria in the hospital setting. This similarity is seen regardless of birth weight, feeding type, or antibiotic therapy.[6] In contrast, patterns of human milk–fed, full-term infants show little similarity with each other or with hospitalized

infants. Instrumentation, procedures, antibiotics, and antacids alter neonatal microflora. In a multivariate analysis of extremely low birth-weight (ELBW) infants weighing less than 1000 g, prolonged antibiotic therapy was associated with NEC risk. Each treatment day is associated with increasing odds of developing disease.[45] Antibiotic and antacid therapy that favors Proteobacteria over Firmicutes is believed to increase the risk of developing NEC.[7,33] Changes to the gut microbiome provide conditions that allow pathogens to colonize, dysregulate the immune response, and decrease production of beneficial short-chain fatty acids, thus promoting sepsis that further disrupts homeostasis.[40]

A randomized, DB, placebo-controlled study of probiotic supplementation with *Lactobacillus rhamnosus* KL53A and *B breve* PB04 found high levels of *B breve* colonization of the gut were associated with lower rates of sepsis.[13]

In a prospective study of LOS and other sepsis in preterm infants, randomized infants received a probiotic supplement of *Lactobacillus acidophilus* subsp *gasseri*, *Bifidobacterium infantis*, and *Enterococcus faecium* versus control. Subsequent monitoring of the infants found less evidence of sepsis in the probiotic cohort (40.0% vs 72.5%; $P = .006$). No probiotic side effects were found clinically.[46] It should be noted that not all studies support a decrease in LOS associated with supplemented probiotics.[4,47]

DISCUSSION

In this evidence-based review, data are presented that support the use of probiotics for statistically significant reduction in NEC.[23,27-35] A high certainty of evidence is demonstrated for multiple-strain products, especially one or more *Lactobacillus* plus one or more *Bifidobacterium*.[17,23,33] Dosages vary across clinical trials, with an optimum dose suggested as 3×10^9 colony-forming units beginning with enteral feeding and continued until 36 to 37 weeks.[17] There is little research on ELBW infants[21] who may not have adequately developed receptors for probiotic colonization.[48] Positive findings from one dual-strain product given to 4,683 ELBW infants found less NEC and lower mortality with no reported adverse effects.[49] The negative sequelae of NEC with potential for long-term morbidities including severe neurodevelopmental disability and increased mortality[3] must be balanced with concern for safety regarding probiotics.

There is research to support strains currently available commercially. A retrospective cohort study of 311 neonates weighing less than 1000 g found prophylaxis of *Lactobacillus reuteri* DSM 17938 significantly lowered rates of NEC Bell stage II or greater, preventing one case of NEC for every 8 neonates treated (15.1% vs 2.5%; $P = .0475$).[4] A retrospective observational study measured rates of NEC Bell stage II or greater for infants less than 32 weeks and less than 1500 g over 10 years. Initially, infants given dual-strain *L acidophilus* and *Bifidobacterium bifidum* and then changed to a multiple-strain *Lactobacillus acidophilus*, *B bifidum*, and *Bifidobacterium longum* subsp *infantis* showed rates of NEC decreased by half from 7.5% to 3.1% (adjusted sub-hazard ratio = 0.44; 95% CI, 0.23 to 0.85; $P = .014$).[50] An RCT of probiotic combination *B infantis*, *S thermophilus*, and *Bifidobacterium lactis* administered to infants less than 32 weeks and less than 1500 g reduced NEC Bell stage II or more (2.0% vs 4.4%; RR = 0.46; 95% CI [0.23 to 0.93], $P = .03$). They did not find significant reduction in LOS or mortality.[47]

Biologic plausibility both for dysbiosis as a risk factor for development of NEC and for select strains of probiotic to reduce dysbiosis via various mechanisms of action described here has been established.[8,13,51] Probiotic research is continuing to advance. Large-scale well-designed trials have begun to emerge that are more specific for choice of strains. Further testing of specific combinations of strains will add to the pool of data.

The Cochrane Database of Systematic Reviews surveyed RCTs covering the last 20 years and a wide geography, including Europe, Asia, Africa, and the United States. A limitation to these kinds of review is the heterogeneity among studies regarding strains, dosing, and duration of treatment. Complementary therapies to reduce NEC in research studies may also serve as confounders to probiotic effects. The use of only English language studies in this review may leave out other important contributions.

IMPLICATIONS FOR PRACTICE

Probiotic supplementation is common in other countries to reduce the incidence of NEC.[52] Recent large-scale systematic reviews discussed here confirm earlier findings that probiotics given orally to preterm infants decrease NEC and mortality and may lessen LOS.[40,42] Additional benefits include decreased feeding intolerance and decreased length of hospital stay.[53] Parents can be recruited to help in reducing sepsis and NEC. Informational handouts covering benefits of breastfeeding or expressing milk, handwashing, and sterilization of equipment are very useful.[36] Additional literature for parents on probiotics is found on the NEC Society Web site[54] and may be provided in units where probiotics are given.

IMPLICATIONS FOR RESEARCH

High-quality clinical trials are needed to further narrow the field of safe and effective probiotics. Recent systematic reviews have focused on specific strains and number of strains. More information regarding

Summary of Recommendations for Practice and Research	
What we know:	• Probiotic supplementation is common in other countries. • An extensive number of RCTs of 10,000 infants and observational cohort trials of 30,000 infants have been published. • Biologic plausibility of probiotic mechanisms to reduce factors related to NEC has been researched. • Sepsis and death related to probiotics are uncommon and not statistically significant across all RCTs. • There is consensus that human milk feeding decreases NEC.
What needs to be studied:	• High-quality clinical trials comparing genus and species, doses, timing, and length of therapy. • Studies of long-term outcomes after probiotics. • Clinical trials evaluating probiotics for ELBW infants weighing <1000 g. • Evaluation of probiotics on subsets of NEC after transfusion, allergy or intolerance, viral/bacterial, hypoxic/ischemic, antibiotic exposure. • Influence of mother–infant genetic polymorphisms on probiotic response. • Study of varied human milk oligosaccharides (HMOs) and influence as prebiotic or symbiotic.
What can we do today:	• Increase human milk feedings. • Follow standardized feeding protocols. • Establish standardized transfusion guidelines. • Observe antibiotic stewardship. • Consider handouts like the one written by the NEC Society (https://necsociety.org/wp-content/uploads/2018/01/probiotic-information-for-parents-2018.pdf).

dosing and duration of supplementation will contribute to our understanding. Long-term follow-up of neurodevelopmental outcomes and development of allergies will provide important data on safety. Information is particularly needed on the effectiveness and safety in the ELBW population, the most vulnerable to developing NEC. Advances in mapping the human genome will provide insight into personalized prebiotic–probiotic combinations to address specific mother–infant microbiomes.

CONCLUSION

Despite advances in care of the preterm infant, NEC continues to be a common and damaging GI complication. No established etiology is agreed upon, and development is multifactorial with many subsets of definition for NEC. Some risk factors unique to the preterm NICU patient include GI compromise, bacterial dysbiosis, enteral feedings, mode of delivery, and nosocomial infections. Probiotic mechanisms are effective at decreasing intestinal permeability, resisting colonization by pathobionts, and benefitting the developing infant immune system. Recent systematic reviews and meta-analyses confirm previous findings that probiotic administration in the preterm infant is an efficacious and safe means to reduce risk factors for development of NEC.

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