Probiotics and Necrotizing Enterocolitis: Finding the Missing Pieces of the Probiotic Puzzle

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Necrotizing enterocolitis (NEC) is one of the leading causes of death in the neonatal intensive care unit. Morbidity and mortality rates significantly increase with decreases in gestational age and birth weight. Strong evidence suggests probiotic prophylaxis may significantly decrease the incidence of NEC and should therefore be incorporated into the standard of care for preterm infants. However, debate still remains because of limitations of completed studies. The purpose of this review was to provide an overview of the controversies regarding probiotic use in preterm infants and to shed light on the practical considerations for implementation of probiotic supplementation.

INDEX TERMS necrotizing enterocolitis, premature infant, probiotics

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INTRODUCTION

Despite remarkable advances over the past 2 decades in the field of neonatology, answers to the prevention and management of necrotizing

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enterocolitis (NEC) remain elusive. Although increased rates have been observed in some centers along with increased survival of extremely low-birth-weight infants, the overall prevalence rates of NEC have remained relatively unchanged (7%-14%) in the United States. Mortality rates secondary to NEC are as high as 20% to 30%, making it one of the leading causes of death in premature infants.¹ In addition to acute morbidity and mortality associated with NEC, significant long-term adverse effects impact survivors. NEC is the leading cause of short bowel syndrome in the United States, which results in the prolonged need for parenteral nutrition and its associated complications. In addition, neurodevelopmental delays occur in up to 25% of infants who suffer from NEC.² Survivors of NEC are at high risk for growth failure, cerebral palsy, vision and hearing impairments, and poor neurodevelopment. Altogether, NEC results in annual healthcare costs of

up to \$1 billion in the United States.³

Current research has focused on enhancing our understanding of the risk factors and pathophysiology of NEC. Great interest surrounds the use of probiotic supplementation for the prevention of NEC. This review describes the theories regarding the benefits of probiotic use in preterm infants, the evidence supporting the use of probiotics for the prevention of NEC, the safety of probiotic supplementation in preterm infants, and practical considerations for the selection and administration of probiotics in the neonatal intensive care unit (NICU).

BACKGROUND

Our understanding of the pathophysiology of NEC remains elementary, potentially because of its multifactorial nature. NEC is thought to be the result of an initial intestinal injury that may involve alterations in mucosal defenses, gastrointestinal (GI) microbiota, and/or imbalances of GI inflammatory responses. Numerous factors have been associated with an increased risk of NEC including prematurity, alterations in GI microbial composition, steroid use, broad spectrum antibiotic use, hemodynamic compromise (e.g.,

patent ductus arteriosus [PDA]and/or sepsis), and enteral administration of hyperosmolar substances.^{4,5} To date, the only strategies associated with a reduced risk of NEC are conservative feeding practices and the use of maternal breast milk. The use of breast milk alone has been shown to significantly reduce the risk of NEC compared to formula feeding.^{6,7} The reader is referred to several detailed reviews of the pathophysiology, presentation, and management of NEC.^{2,4,5,8} It is important to differentiate NEC from spontaneous intestinal perforation when evaluating literature regarding NEC, as they are believed to represent distinct entities.

MECHANISMS FOR PREVENTION

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.”⁸ In order for a microorganism to be considered a probiotic, it must be of human origin and be nonpathogenic in nature.⁹ Probiotic microorganisms are also often referred to as commensal bacteria, or protective microorganisms which are part of the normal flora. In contrast, prebiotics such as fructo-oligosacchrides, galacto-oligosaccharides, and lactulose are supplements that enhance the growth of potentially beneficial intestinal microbes such as *Bifidobacterium* species.¹⁰ Unlike probiotics, prebiotics require an initial appropriate colonization of the gut to be beneficial.

Despite the lack of identification of a specific cause, numerous factors are believed to contribute to the risk for development of NEC in premature infants. The GI tract of the premature infant has abnormal bacterial colonization and immature barrier function, mesenteric circulation, and immune defenses.^{11–14} Probiotics are theorized to convey benefits in the prevention of NEC via multiple mechanisms which influence these GI deficiencies.

Bacterial Colonization

The most basic theory by which probiotics influence NEC rates is through “normalization” of GI colonization. By approximately 10 days of life, a term breastfed infant’s GI tract becomes colonized with *Bifidobacterium* and *Lactobacillus* species. However, in a formula-fed infant, the colonization is less diverse and is composed of only ~50% of the *Bifidobacterium* organisms ob-

served in breastfed infants.^{15,16} The colonization of preterm infants is not only delayed but is more likely to be dominated by pathogenic bacteria such as members of the *Enterobacteriaceae* family and *Clostridium* species. There is a significant association between pathogenic bacterial colonization and frequent use of antibiotics.¹⁷

These pathogenic bacteria are more likely to stimulate an immune response, which may lead to the initial injury, resulting in the cascade of events leading to NEC. Probiotic supplementation may have the ability to inhibit pathogenic colonization and stimulate anti-inflammatory effects.⁴ In addition to competing with pathogenic bacteria within the environment of the neonatal GI tract, commensal bacteria are also known to have antimicrobial effects on pathogenic bacteria. *Lactobacilli* have been shown to secrete lactic acid to lower local pH, which inhibits the growth of pathogenic bacteria.¹⁸ *Lactobacillus* organisms are also known to communicate directly with pathogenic bacteria to reduce the pathogenic bacteria’s gene expression for binding to host cells, while *Lactobacillus* itself is able to bind to epithelial cells, further inhibiting pathogen attachment.^{19,20} Thus, colonization with commensal bacteria may reduce the virulence of pathogenic bacteria.

Colonization with commensal bacteria can be achieved with probiotic administration in preterm infants. Several investigations have evaluated the impact of probiotic supplementation on intestinal colonization rates.^{21–26} Supplementation of premature infants with *Lactobacillus* species increases intestinal colonization with *Lactobacillus*; however colonization is variable. Reduced colonization rates were reported in low-birth-weight infants, infants with antibiotic exposure, and those receiving formula feedings.^{21,23,24} Despite effective colonization with *Lactobacillus*, pathogenic organisms were not eliminated from the colon.^{23,24} Similar findings were observed in studies evaluating supplementation with *Bifidobacterium*.^{25,27} Interestingly, supplementation with *Lactobacillus* has also been shown to reduce intestinal colonization with *Candida* species.^{26,28} Not all strains of bacteria, even within a species, exert the same effect on bacterial colonization, and we have yet to determine which strains are most beneficial.

Finally, although probiotic supplementation may improve colonization with commensal bacteria, numerous factors influence colonization, and complete elimination of pathogenic

bacteria seems unlikely. Oligosaccharide molecules within human milk have been shown to influence microbiota colonization, selecting for *Bifidobacterium infantis* over other species of *Bifidobacterium*.^{29,30} In addition, acid-suppressive therapy has been shown to alter microbial colonization rates and has been associated with increased rates of NEC.^{31,32}

Immature Intestinal Function and Development

Colonization by commensal bacteria is believed to be critical for the normal development and growth of the GI tract, particularly for epithelial barrier function and vascularization.^{5,33} The intestinal tract of the premature infant is known to have structural and biochemical deficiencies which predispose infants to NEC. Commensal bacteria have been shown to regulate the expression of genes, which enhances barrier function and stimulates normal growth.⁵

Two barrier defenses of the intestinal mucosa are tight junctions between epithelial cells and a thick layer of mucus which covers the epithelial lining.^{5,33} Each defense is immature in the preterm infant, allowing penetration and adherence of bacteria and increasing intestinal permeability to toxic substances. *Lactobacillus* bacteria have been shown to stimulate production and secretion of mucins that enhance the mucous layer to prevent bacterial adhesion as well as to increase their clearance. Probiotics also help to maintain the tight junctions between epithelial cells to preserve a barrier against bacterial translocation.³⁴ Finally, breaks in the mucosal barrier itself can be prevented by reducing epithelial cell apoptosis.³⁵ Epithelial cells in the premature infant are highly susceptible to exaggerated apoptosis. Probiotics have been shown to stimulate epithelial cell barrier function and decrease epithelial apoptosis; however, it is unclear which strains have the most beneficial effects. Recent molecular data also suggest commensal bacteria stimulate gene expression to enhance GI motility and neurotransmission.³⁶ Enhancement of GI motility is believed to decrease GI transit time to reduce contact with potential toxins or pathogens.³⁶

A recent concern has arisen regarding an association between red blood cell transfusions and NEC. Many investigators have theorized that this is the result of altered mesenteric blood flow.³⁷ Although most evidence points to abnormal bacterial colonization and inflammation as

the major influences on development of NEC in preterm infants, hypoxia resulting from immature intestinal circulatory regulation may play a secondary role. Maintenance of mucosal blood flow is indeed essential for mucosal integrity. It is known that premature infants have an altered balance of prostaglandins, nitric oxide, and epidermal growth factor, all of which influence mucosal blood flow, oxygenation, and as a result, mucosal integrity.³⁸⁻⁴² Probiotics may play a role in intestinal perfusion as well. Havranek and colleagues⁴³ recently reported that probiotic supplementation enhanced intestinal blood flow in extremely low-birth-weight infants. The effects on intestinal blood flow of probiotics are theorized to be the result of production of vasoactive amines.

Inflammatory versus Anti-inflammatory Imbalance

A close balance between anti-inflammatory and proinflammatory responses is critical to ensure the immune system functions as an effective defense system. Of equal importance, it is imperative that there is not an overreaction to normal antigens in the environment (e.g., self- or food antigens). Intestinal epithelial cells must be able to detect and discriminate self-antigens, food antigens, and normal bacteria from pathogenic species.^{44,45} The immature immune system of the premature GI mucosa predisposes the infant to "hyper-inflammatory" responses to microbial stimuli. Excessive recruitment of inflammatory leukocytes and proteases can further damage intestinal mucosa, allowing pathogenic bacterial invasion and stimulating a cycle of further inflammation and injury.⁵

Normally, after initial microbial colonization in the intestine, the term infant attenuates its response to microbial stimulation. However, the preterm infant is less able to modulate the inflammatory response triggered by colonization with gram-negative organisms. Toll-like receptors on the surface and within the cytoplasm of enterocytes sense bacteria that are able to penetrate the mucous layer. Toll-like receptor 4 (TLR4) is a receptor for lipopolysaccharide, a bacterial outer membrane component which, upon stimulation, results in activation of the transcription factor nuclear factor kappa B (NF- κ B). NF- κ B then triggers the synthesis of proinflammatory mediators such as interleukin-8 (IL-8).⁴⁶ Thus, excessive TLR4 expression in preterm infants is believed to result

in their “hyper-reactivity” to microbial stimulation. Interestingly, animal studies have shown increased expression of TLR4 with formula feeding, which may partly explain the increased risk of NEC observed in formula-fed infants.⁴⁷ Polymorphisms in TLR4 expression also may explain the observed genetic predisposition to NEC. Thus, the inability of the premature infant to down-regulate TLR4 expression may result in exaggerated immune responses to pathogens and a predisposition to NEC.⁴⁶

Colonization with commensal bacteria is believed to modulate these proinflammatory processes while priming the immune system against pathogenic bacteria.⁴⁴ Studies have shown that probiotics, after stimulation of TLR4 receptors, inhibit the transcription and production of NF- κ B, thereby attenuating the proinflammatory responses.^{4,10} Commensal bacteria appear to be required to prime and control TLR4 responses to maintain mucosal integrity.⁴⁴ However, not all probiotic strains have the same effects regarding inflammatory processes. Some commensal bacteria are able to inhibit activation of NF- κ B, while others have been shown to activate NF- κ B and increase production of IL-6, such that varying the probiotic strain results in different effects on the inflammatory system. This may explain the strain-specific benefits observed in clinical trials (e.g., adults with irritable bowel syndrome respond to *Bifidobacterium* but not *Lactobacillus*).¹⁰ Finally, some probiotic strains stimulate the production of immunoglobulin A, which protectively binds pathogens,^{33,45,54} while others have effects on T-cell immunity in the GI tract.^{33,45,55,56} Clearly, probiotics have a variety of anti-inflammatory effects, many of which may be strain-specific. Further research is needed to determine strain-specific influences on the immune system.

PROBIOTICS: EVIDENCE

The potential benefits of probiotics in preterm infants which have been evaluated include time to full feeds (TFF),^{57,58} feeding tolerance,^{6,59} NEC rates,^{57,58,61–68} and sepsis rates.^{28,57,58,61–66} The level of evidence supporting the use of probiotics for the prevention of NEC is the greater, while limited evidence exists to support use for the other endpoints. The following discussion focuses on the evidence for probiotic prevention of NEC.

Level of Evidence for Efficacy

In 1999, Hoyos and colleagues⁶⁷ sparked interest in the use of probiotic supplementation for the prevention of NEC with a partially retrospective study of 2519 infants, completed in Bogota, Columbia. Since then, there has been a growing amount of evidence including several recent meta-analyses. In 2010, Deshpande and colleagues⁶⁹ performed a meta-analysis of 11 randomized controlled trials (RCTs), which detected a statistically significant benefit of probiotic prophylaxis by reducing NEC rates from 6.56% to 2.37% (placebo versus probiotic, respectively) as well as reducing all-cause mortality (relative risk [RR] 0.42). Despite the lack of an effect on length of stay, the authors concluded that additional trials were “unnecessary if a suitable probiotic product is available.”⁶⁹ More recently, Mihatsch and colleagues⁷⁰ performed an updated meta-analysis using more stringent criteria in 15 RCTs.⁷⁰ The analysis by Mihatsch et al. included studies with NEC reduction as either a primary or secondary outcome. That analysis included 10 of the trials evaluated by Deshpande and colleagues and 5 additional studies in which 2 of the largest observed a very low baseline rate of NEC (<5%). In their analysis, the authors did not find a statistically significant benefit of probiotics for any outcome (NEC, death, sepsis, TFF); however, the collective data for risk reduction was not provided for each analysis performed. The authors performed additional analyses stratifying the data by probiotic strain, which found no effect on NEC, death, sepsis, or TFF. Unfortunately, the stratification reduced the patient numbers, diminishing the strength of the conclusions.⁷⁰ The opinions from those two analyses varied dramatically because of the various qualities of the studies and outcomes included, along with different statistical analyses that were used. Despite differences in the conclusions of the meta-analyses, the authors agreed that the baseline NEC rate was a major factor that influences the potential benefit of probiotic supplementation in a population.

Efficacy by Baseline NEC Rates

In the United States, the average rates of NEC range from 5% to 7% at most institutions, while NEC rates greater than 10% may be observed.^{4,8} The benefits of probiotic supplementation appear to be of greater significance in the setting

of high NEC rates. Table 1 summarizes the RCTs that have been performed evaluating the use of probiotics for the prevention of NEC. Trials performed at institutions with high NEC rates have observed significant benefit from probiotics, while those institutions with low NEC rates have shown limited effects. Individual studies are examined below.

The study by Bin-Nun and colleagues⁵⁷ included 145 very-low-birth weight (VLBW) neonates (≤ 1500 g) in Israel at a NICU with a prestudy NEC incidence rate of 15%. With first feed, neonates were randomized to receive either a once-daily supplement of a combination probiotic consisting of *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* (ABC Dophilus, Solgar, Leonia, NJ) or unsupplemented milk. Approximately 60% of the infants in both groups received only mother's expressed breast milk (EBM), while infant formula (Similac Special Care, Abbott Laboratories, Abbott Park, IL) was given to the remainder of neonates because of insufficient EBM or lack of availability. As seen in Table 1, the average birth weight (BW) and gestational age (GA) between the probiotic and control group were similar. The rate of NEC was lower in the probiotic-supplemented group than in the control group (4% vs. 16.4%, respectively, $p=0.03$). The overall incidence of NEC was reduced by 12.4%, with 8 as the number needed to treat (NNT) to prevent one case of NEC. NEC-associated mortality was lower in the probiotic group; however this was not statistically significant. The study was funded by the manufacturer of ABC Dophilus.⁵⁷

In 2010, Sari and colleagues⁶⁵ published an RCT investigating the efficacy of *Lactobacillus sporogenes* for NEC prevention in a total of 221 infants from a NICU in Turkey. That study was conducted in VLBW infants, but inclusion criteria also incorporated preterm infants with a GA < 33 weeks. Infants in the study group received EBM or formula supplemented with *L sporogenes* once daily, initiated at first feed and continued until discharge, while the control group received only the feeds alone. The incidence of NEC in the study group versus the control group was not different (5.8% vs. 9%, respectively, $p=0.515$). None of the infants from the study group died of NEC, while 1 infant from the control group (1%) died from NEC. It is also important to note that the authors overestimated the baseline NEC rate

at 32% (based upon recent NEC rates at the institution) to calculate the sample size, which may have contributed to the study's inability to show a statistically significant effect of probiotics.⁶⁵

In a similar trial, Samanta and colleagues⁵⁸ enrolled 186 eligible preterm (< 32 weeks) and VLBW infants in a NICU in India over a span of 6 months. The study group received a probiotic mixture of *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Lactobacillus acidophilus* twice daily upon initiation of feeds until discharge, while the control group received only breast milk. The incidence of NEC was significantly lower in the study group than in the control group (5.5% vs. 15.8%, respectively, $p=0.032$). Results demonstrated a 10.3% reduction in NEC with the use of probiotics, resulting in a NNT to prevent one case of NEC of 10. Aspects of this trial such as baseline incidence of NEC as well as breastfeeding rates require clarification. It is unclear whether the NEC rate of 15.8% represents the usual NEC rates for the institution.⁵⁸

A recent study by Awad and colleagues⁶⁴ reported dramatic effects of probiotics in a unit with a high baseline NEC rate of 31% in Egypt. That investigation compared killed *L acidophilus* versus living *L acidophilus* versus placebo in the reduction of stage 2 or higher NEC. A total of 150 neonates were enrolled in that single-center prospective trial, 89 of whom were preterm. The overall rate of NEC was significantly reduced (0%, 2.7%, 31.3%, respectively) in the preterm infants, but there were no significant differences in rates of sepsis. The NNT to prevent one case of NEC in the preterm infants with live probiotic was 3. Unfortunately, the delay of intake along with the type of feeds provided for the infants were not addressed in detail. The method of stratification in the preterm population was unclear; the process appears as a posthoc stratification as there was no information provided under the methods sections.⁶⁴

In determining the potential impact of probiotic supplement in the United States, it is important to examine studies with relatively low NEC rates. Two studies conducted in Taiwan by Lin and colleagues^{62,63} provide the best representation of US NEC incidence rates. Both of the studies completed by Lin and colleagues were partially blinded, with the breast milk team knowing the group assignments.^{62,63} The 2005 single-center

study evaluated VLBW infants in an NICU who received breast milk with the addition of *L acidophilus* and *B infantis* (Infloran, Swiss Serum and Vaccine Institute, Berne, Switzerland), while the control group received only breast milk feeds. The incidence of NEC was significantly lower in the probiotics group than in the control group (1.1% vs. 5.3%, respectively, $p=0.03$) with an NNT of 24.⁶² Lin and colleagues followed up this study with a multicenter trial of similar design published in 2008. That study included a larger patient sample (434 infants) at seven NICUs in Taiwan, with the same brand of probiotic.⁶³ Of note, the company that distributed Infloran had been bought out by another company after the completion of the first study, and the formulation was subsequently changed to include *B bifidum* instead of *B infantis* (Infloran, Laboratorio Farmaceutico, Italy).⁶³ Infants in that study were supplemented with additional formula feedings when EBM was inadequate in both groups; approximately 60% of neonates received only EMB. Again, the results of the study demonstrated a lower incidence of NEC in the study group than in the control (1.8% vs. 6.5%, respectively, $p=0.02$) with the NNT to prevent one case of NEC of 21.³⁸ Both of these studies had lower than expected NEC rates (23% in the 2005 study and 25% in the 2008 study) by approximately 4-fold, but the NEC rates observed (~5%-7%) in these studies closely resembles the NEC incidence in the United States.^{62,63} Results of the studies by Lin et al^{62,63} provided strong evidence suggesting potential benefit of probiotics for the prevention of NEC in the United States.

In contrast, the efficacy of probiotics tends to decrease in studies reporting very low NEC rates. The trial completed by Dani and colleagues⁶¹ is one of the studies most frequently mentioned for NEC prevention.⁶¹ It is the largest prospective study currently completed, with 585 newborn infants, with GA of <33 weeks or BW <1500 g in 12 Italian NICUs. Infants in the study by Dani et al.⁶¹ received breast milk or formula feeds supplemented or not with *Lactobacillus rhamnosus* GG (Dicoflor, Dicofarm, Rome, Italy) once daily starting at first feed. Sixty-three percent of the infants received EBM alone, while the remaining infants received either formula or mixed feeding. The incidence of NEC was reduced in the study group versus that of the control (1.4% vs. 2.8%, respectively), but the difference was not

statistically significant. This lack of significance is possibly because of the fact that the incidence of NEC in that study was drastically lower than the expected rate of 10%. There was no death resulting from NEC in the probiotic group; however, there was an NEC-associated mortality rate of 25% in the control group. Dani and colleagues⁶¹ concluded that future studies should focus on areas where NEC is more prevalent.

Braga and colleagues⁶⁶ enrolled 231 preterm infants ranging from 750 to 1499 g at birth in a NICU located in northeast Brazil. Similar to the study by Dani et al.,⁶¹ the sample size was based on an institutional baseline NEC rate of 10% at the time of the study. Infants were randomized to receive human milk with or without probiotic supplementation, *Lactobacillus casei*, and *Bifidobacterium breve* (Yakult LB, São Paulo, Brazil). The results of the study showed a reduction in the occurrence of NEC in the study group versus that in control (0% vs. 3.6%, respectively); however, the statistical significance of this result was not mentioned in the study. The low rate of NEC may be related to the exclusive use of breast milk in study infants. Even though the overall effect of probiotics was deemed statistically insignificant in these two studies, there was a trend toward reduced incidence of NEC.

NEC rate should be one of the first determinants when considering the use of probiotics for the prevention of NEC in the NICU. According to the evidence currently available, probiotics can significantly lower the rate of NEC in units with a high incidence. Unfortunately, the effect decreases dramatically with lower occurrences of NEC. Further studies will be required prior to establishing a benefit in areas with lower NEC rates. Limitations to future trials may be large sample sizes required to demonstrate probiotic benefit. For example, Mihatsch et al.⁷⁰ calculated that with an NEC incidence of 5%, at least 714 infants per group would be required to demonstrate a 50% reduction rate ($\alpha = 0.05$, $\beta = 0.2$).⁷⁰ Despite larger clinical trials currently underway, there is no ongoing trial targeting a sample size that large.

Efficacy by Probiotic Strain

A major factor confounding assessment of the efficacy of probiotics in neonates is the wide variation in strains used in clinical trials. The potential benefit of supplementation with mul-

multiple probiotic strains is supported by emerging evidence suggesting that not all probiotic strains have the same mechanisms of action.⁶ As mentioned, beneficial effects of a probiotic may be highly strain-specific and may not even be generalizable to strains of the same species.³³ Although many trials of probiotic use for the prevention of NEC have used single-strain probiotics, some investigators argue that the use of multiple species is necessary to create an environment similar to that of a healthy infant. Of available RCTs, five studies used multistrain formulations, while three studies used single-strain species. It is interesting to note that prospective RCTs using single-strain formulations resulted in data that were not statistically significant, with the exception of the studies by Awad et al.^{28,61,64,65,72-74} Guthmann and colleagues⁷⁵ performed a meta-analysis evaluating probiotic use for the prevention of NEC, stratifying the analysis by multiple versus single-strain probiotic evaluations. Four of the 11 trials used a single-strain probiotic, 4 studies used a multiple-strain probiotic product, and 3 studies used a specific combination of *L acidophilus* plus *Bifidobacterium* spp. Trials using more than one strain as probiotic prophylaxis tend to produce greater reductions in the incidence of NEC, with *Bifidobacterium* spp. and *L acidophilus* being the most commonly used combination. However, head-to-head comparisons of single- versus multiple strains have not been performed.⁷⁰

Efficacy by Birth Weight

Low BW is the most significant risk factor for developing NEC; therefore, VLBW infants and extremely low-birth-weight infants may potentially benefit the most from probiotic supplementation. Mortality rates secondary to NEC in infants weighing 501 to 750 g are 42%, but only 15.9% in infants weighing 1251 to 1500 g.¹ Unfortunately, GI colonization with probiotic organisms has been shown to be more difficult in preterm infants. This may be related to increased antibiotic exposure, greater delays to initiation of feeds, differences in lactose concentrations in feedings (premature infant formulas contain less lactose required for lactobacillus growth), and longer duration of hospitalization and/or exposure to pathogenic bacteria.^{21,23-25}

Studies published to date that support the use of probiotics have included infants with an average BW of >1000 g (Tables 1 and 2). Evaluations

of the efficacy in extremely low-birth-weight infants are limited. Lin and colleagues^{62,63} attempted to evaluate the effect of BW on probiotic efficacy. A post hoc analysis of the 2008 probiotic trial stratified their results by weight (<750 g, 751-1000 g, and >1000 g). For infants <750 g, probiotic supplementation resulted in a lower combined incidence of death and NEC in the probiotic group than in the control group (3.03% vs. 27.77%, respectively, $p=0.02$).⁶³ The incidence of NEC greater than stage 2 was significantly lowered only in infants weighing 1001 to 1500 g. At this point, there is not enough evidence to support the efficacy of probiotics in extremely low-birth-weight infants, and future well-designed studies are needed.

Limitations

It is not surprising to note that each trial has its own limitations. The available trials do not look at one specific product, dosing regimen, or protocol. Methods of randomization, blinding, and feeding regimens are vague or unpublished. Some authors have stated that the studies published to date are underpowered to establish any appropriate conclusions.⁷⁰ Future studies should not be focused on questioning the benefits of probiotics, rather they should further delineate the ideal probiotic, target group, and duration of therapy. Despite the lack of consensus regarding the benefit of probiotics, many NICUs are routinely giving probiotic supplements to preterm infants. From the current data, it appears that NICUs with high incidence rates of NEC are more likely to benefit from probiotic supplementation. Although multistrain products may be more effective than single-strain products, evidence is still lacking in this area, along with their efficacy in extremely low-birth-weight infants.

SAFETY CONCERNS WITH PROBIOTICS

A major concern regarding the use of probiotics in preterm infants is probiotic-associated sepsis. Although probiotics are believed to have many protective effects on mucosal integrity, the potential for disruption of mucosal integrity is unknown. In the presence of an altered mucosal barrier, bacteria can translocate and enter the systemic circulation causing sepsis. Safety monitoring for sepsis, whether from probiotic strains or from pathogenic bacteria, is necessary until

these questions can be answered.

Lactobacillus and *Bifidobacterium* colonizations are infrequent causes of sepsis in infants. To date, there have been only six cases of probiotic-associated sepsis reported in infants, of which only two occurred in the newborn period (Table 3). Four of those cases were associated with *Lactobacillus* organisms following probiotic administration during a period when intestinal mucosal integrity may have been acutely compromised.⁷⁶⁻⁷⁸ Two reports of *Bifidobacterium* bacteremia have been reported to date.^{79,80} The first occurred in a term infant (status-post omphalocele repair) after 10 days of probiotic administration, while the second case occurred in a premature infant after 9 days of probiotic supplementation. Although reports of *Bifidobacterium* sepsis are limited, under-reporting may be a problem because *Bifidobacterium* is an anaerobic bacteria which does not grow well under aerobic conditions.⁷⁹ The only other report of probiotic associated sepsis in a preterm infant was caused by *Escherichia coli* NISSLE 1917 in a 28-week premature infant with gastroenteritis.⁸¹ Each case of probiotic-associated bacteremia in infants is believed to have resulted from translocation of bacteria across an impaired intestinal mucosa. However, the potential for contamination of central venous catheters after opening and manipulating capsules could not be ruled out.⁷⁶ In RCTs, up to 35% of infants randomized to receive placebo have been reported to be colonized with a probiotic strain of bacteria, suggesting cross-contamination is prevalent.⁸² All infants in the case reports listed in Table 3 were subsequently effectively treated with antibiotics, with resolution of the respective infections.

It is reassuring that no case of probiotic-associated sepsis have been reported in any of the published trials evaluating probiotic use in preterm infants, which include collectively over 1200 patients.^{59,70} Additionally, surveillance data from countries where probiotic use is widespread have not found evidence of increased probiotic-associated sepsis.⁸³

In addition to the risk of sepsis from strains within a probiotic supplement, there is also the concern that the probiotic could induce changes locally in the GI tract or systemically by altering immune responses to increase the rate of sepsis by other pathogens. Again, although one of the proposed benefits of probiotics in preterm infants is to reduce neonatal sepsis, the opposite

effect is not beyond the realm of possibility.⁸⁴ For this reason, trials evaluating probiotics for the prevention of NEC have closely evaluated sepsis rates. Collectively, there has not been an increased risk of sepsis observed with probiotic use in meta-analyses of available RCTs.^{69,70,75} Only the trial by Lin and colleagues⁶³ reported an increased rate of sepsis in the probiotic group. Sepsis, as a secondary outcome, was more common in the probiotic group in that trial; however, it was not statistically significant when controlled for confounding variables such as BW, center, umbilical artery catheter use, ventilation, and duration of hospitalization.⁶³ The pathogens causing sepsis were typical catheter-related organisms, and overall sepsis was more common in infants <1000 g.

The safety concern regarding the potential consequences of administering only a sole probiotic strain must also be considered. The normal microbiota of the GI tract are responsible for many roles, including regulation of the production of vitamins B and K.⁸⁵ In a study examining the administration of a single-strain probiotic (*B. breve*), a 24-week gestation infant developed a prolonged partial thromboplastin time at 30 days of life. The patient's stool cultures showed *Bifidobacterium* flora alone. The authors speculated the patient may have had a vitamin K deficiency as *Bifidobacterium* cannot produce vitamin K and stressed the importance of monitoring for vitamin K deficiency if such supplementation is used.²⁵

The long-term effects of probiotic supplementation in preterm infants remain to be elucidated. There are numerous chronic disorders for which molecular research indicates a link with alterations in gut flora. The role of probiotic supplementation in the development of such disorders, including inflammatory bowel disease, systemic autoimmune diseases (e.g., multiple sclerosis), and obesity, is unknown.⁸⁶ The most critical outcome of concern for preterm infants is long-term neurodevelopmental outcome. There are numerous risk factors for poor neurodevelopmental outcomes in the preterm infant, including NEC, intraventricular hemorrhage (IVH), chronic lung disease (CLD), and PDA. In an effort to determine long-term effects of probiotic supplementation in preterm infants, Chou and colleagues⁸⁷ performed a follow-up neurodevelopmental evaluation of preterm infants

Table 1. Randomized Controlled Trials: Probiotics for NEC Prevention as Primary Outcome

Study (sample size)	Study Group				Probiotic Regimen (product: organism, count; Rx)		
	GA (wks)	P (wks)	C (wks)	BW (g)	P (g)	IP (g)	C (g)
Dani et al. ⁶¹ (n=585)	<33	30.8 ± 2.4	30.7 ± 2.3	<1500	1325 ± 361		1345 ± 384
Dicoflor: <i>L. rhamnosus</i> GG, 6 × 10 ⁹ CFU; once daily from first feed until discharge							
Bin-Nun et al. ⁵⁷ (n=145)				<1500	1152 ± 262		1111 ± 278
ABC Dophilus: <i>Streptococcus thermophilus</i> , 0.35 × 10 ⁹ CFU; <i>B. infantis</i> , 0.35 × 10 ⁹ CFU; and <i>B. bifidum</i> , 0.35 × 10 ⁹ CFU; once daily from first feed to 36 wks corrected age							
Lin et al. ⁶² (n=367)				<1500	1104 ± 242		1071 ± 243
Infloran: <i>L. acidophilus</i> , 1.0 × 10 ⁶ organisms; and <i>B. infantis</i> 1 × 10 ⁶ organisms; BID from day 7 until discharge							
Lin et al. ⁶³ (n=434)	<34 [‡]			<1500	1028.9 ± 246		1077.3 ± 214.4
Infloran: <i>B. bifidum</i> , 1 × 10 ⁹ CFU; <i>L. acidophilus</i> , 1 × 10 ⁹ CFU for 6 wks ⁷²							
Samanta et al. ⁵⁸ (n=186)	<32	30.12 ± 1.63	30.14 ± 1.59	<1500	1172 ± 143		1210 ± 143
Brand not stated [§] ; <i>B. infantis</i> , <i>B. bifidum</i> , <i>B. longum</i> and <i>L. acidophilus</i> , 2.5 billion CFU;							
Awad et al. ⁶⁴ (n=150; 89 preterm)**				~1100-4300 (all infants)	1830 ± 520	1860 ± 750	2100 ± 750
Brand not stated; <i>L. acidophilus</i> 6 × 10 ⁹ CFU; BID until discharged							
Sari et al. ⁶⁵ (n=221)	<33	29.5 ± 2.4	29.7 ± 2.4	<1500	1231 ± 262		1278 ± 282
Brand not stated; <i>L. sporogenes</i> 3.5 × 10 ⁸ CFU; once daily starting at first feed until discharge							
Braga et al. ⁶⁶ (n=231)				150-1499	1194.7 ± 206.3		1151.4 ± 224.9
Yakult LB [§] : <i>B. breve</i> and <i>L. casei</i> , 3.5 × 10 ⁷ to 3.5 × 10 ⁹ CFU; once daily starting on day 2 for 30 days							

BID, twice daily; BW, birth weight (grams); C, control group; CFU, colony-forming units; GA, gestational age (weeks); FM, formula milk; IP, inactivated probiotic group (contained only killed probiotics); MX, mixed feeding (human and formula); NEC, necrotizing enterocolitis; NNT, number needed to treat; NS, not statistically significant; P, probiotic group; TPN, total parenteral nutrition; TTF, time to full feeds (days); UTI, urinary tract infection

* NS
‡ Secondary outcome
‡ Specifics not provided
§ Breakdown by individual species not available
** Study looked at all infants, but was also stratified for preterm newborns
†† Premature infants only

Table 1. Randomized Controlled Trials: Probiotics for NEC Prevention as Primary Outcome (*cont.*)

Study (sample size)	Type of Feed (no. of patients/n) (% of total)						Outcomes (no. of patients/n) (% of total)						Other Primary Outcomes	NEC Rate from Study			
	HM			Mx			FM			NEC					Sepsis		
	P	C	P	C	P	C	P	C	P	C	P	C			P	C	%
Dani et al. ⁶¹ (n=585)	63	64	1	2	36	35	4/295 (1.4)*	8/290 (2.8)*	14/295 (4.7)*	12/290 (4.1)*	UTI				2.8		
Bin-Nun et al. ⁵⁷ (n=145)	42/72	47/73	12/72	9/73	18/72	17/73	3/72 (4.2)	12/73 (16.4)	31/72 (43)	24/73 (32.9)	Death; TFF; Days until TPN was stopped				16.4	8	
Lin et al. ⁶² (n=367)	56/180	61/187					2/180 (1.1)	10/187 (5.3)	31/180 (17.2) [†]	60/187 (32.1) [†]	Death				5.3	24	
Lin et al. ⁶³ (n=434)	69	62					4/217 (1.8)	14/217 (6.5)	40/217 (18.4)**	24/217 (11.1)**	Death				6.5	21	
Samanta et al. ⁵⁸ (n=186)	— [‡]						5/91 (5.5)	15/95 (15.8)	13/91 (14.3)	28/95 (29.5)	TFF Death Hospital stay				15.8	10	
Awad et al. ⁶⁴ (n=150; 89 preterm)**	undclear on feeds for all data						0/36 (0) ^{††}	1/37 (2.7) ^{††}	5/16 (31.3) ^{††}	18/36 (50.0) ^{††}	25/37 (67.6) ^{††}	12/16 (75.0) ^{††}			31.3	3	
Sari et al. ⁶⁵ (n=221)	24	33	76	67			6/110 (5.5)*	10/111 (9.0)*	29/110 (26.4)**	26/111 (23.4)**	Death				9.0		
Braga et al. ⁶⁶ (n=231)	HM						0/119 (0)	4/112 (3.6)	40/119 (33.6)*	42/112 (37.5)*	Death				3.6	28	

BID, twice daily; BW, birth weight (grams); C, control group; CFU, colony-forming units; GA, gestational age (weeks); FM, formula milk; HM, human milk; IP, inactivated probiotic group (contained only killed probiotics); Mx, mixed feeding (human and formula); NEC, necrotizing enterocolitis; NNT, number needed to treat; NS, not statistically significant; P, probiotic group; TPN, total parenteral nutrition; TFF, time to full feeds (days); UTI, urinary tract infection

* NS

[†] Secondary outcome

[‡] Specifics not provided

[§] Breakdown by individual species not available

** Study looked at all infants, but was also stratified for preterm newborns

^{††} Premature infants only

Table 2. Probiotic Trials in Preterm Infants Without NEC as Primary Outcome

Study (sample size)	Study Group				Probiotic Regimen (brand; organism, count; Rx)	
	GA (wks)	P (wks)	C (wks)	BW (g)	P (g)	C (g)
Reuman et al. ²⁴ (n=45)				<2000	1366 ± 302	1377 ± 344
Millar et al. ²³ (n=20)	<33	26-33	24-33		800-2560	830-2150
Kitajima et al. ²⁵ (n=91)				<1500	1026 ± 241	1026 ± 205
Hoyos et al. ⁶⁷ (n=2519)					(inpatient) 2600* (transfer) 2746**	(inpatient) 2594** (transfer) 2630**
Agarwal et al. ²¹ (n=71)**				<1500 1500-1999	1176 ± 220 1745 ± 143	1190 ± 207 1717 ± 181
Costalos et al. ⁷² (n=87)	28-32	31.1	31.8		1651	1644
Li et al. ⁹⁵ (n=30)**				<1500	Group 1: 1523 ± 490 Group 2: 1354 ± 280	1480 ± 237
Manzoni et al. ²⁸ (n=80)	<37	29.6 ± 5	29.3 ± 4		1212 ± 290	1174 ± 340
Mohan et al. ⁹⁶ (n=69)**	<37					
Stratiki et al. ⁷³ (n=77)	~27-37	31 (27-37)	30.5 (26-37)		1500 (900-1780)	1500 (700-1900)
Chou et al. ⁸⁷ (n=300)					1103.6 ± 232.4**	1097.2 ± 231.4**
Rouge et al. ⁶⁰ (n=94)	<32	28.1 ± 1.9	28.1 ± 1.8	<1500	1115 ± 251	1057 ± 260
Manzoni et al. ²⁸ (n=472)				<1500	(with BL) 1138 ± 244	1109 ± 269
Luoto et al. ⁶⁸ (n=3342)††	<30***			≤1500***		
Mihatsch et al. ⁷⁰ (n=180)	<30	26.6 ± 1.8	26.7 ± 1.7	<1500	856 ± 251	871 ± 287
Al-Hosni et al. ⁵⁹ (n=101)	<34	25.7 ± 1.4	25.7 ± 1.4	501-100	778 ± 138	779 ± 126
Indrio et al. ²² (n=42)					(with FM) 4990 ± 631***	5100 ± 739††
Romeo et al. ²⁶ (n=249)	<37	(with <i>L. reuteri</i>) 33.8 ± 1.8 (with <i>L. rhamno-</i> <i>sus</i>) 33.3 ± 1.6	33.3 ± 2.1	<2500	(with <i>L. reuteri</i>) 1998.7 ± 439 (with <i>L. rhamnosus</i>) 1940.7 ± 590	1945.7 ± 465

Table 2. Probiotic Trials in Preterm Infants Without NEC as Primary Outcome (cont.)

Study (sample size)	Outcome					Sepsis			Primary Outcome
	P	BL	NEC	PGI	C	P	BL	C	NNT
Reuman et al. ²⁴ (n=45)									Gut colonization
Millar et al. ²³ (n=20)									Gut colonization
Kitajima et al. ²⁵ (n=91)									Gut colonization
Hoyos et al. ⁶⁷ (n=2519)	37/1237 (3.0%) ^{‡§}				85/1282 (6.6%) ^{‡§}	(inpatient) 36/918 ^{‡§} (transfer) 38/319 ^{‡§}		(inpatient) 39/935 ^{‡§} (transfer) 31/347 ^{‡§}	28
Agarwal et al. ²¹ (n=71)**									Gut colonization
Costalos et al. ⁷² (n=87)	5/51 (9.8%) ^{‡§}				6/36 (16.7%) ^{‡§}	3/51 (5.9%) ^{‡§}		3/36 (8.3%) ^{‡§}	Gut function; Stool colonization
Li et al. ⁹⁵ (n=30)**									Intestinal flora
Manzoni et al. ²⁶ (n=80)	2.5% of 39 ^{‡§}				5% of 41 ^{‡§}				Gut colonization by <i>Candida</i> species
Mohan et al. ⁹⁶ (n=69) ^{††}									Gut colonization by <i>B. lactis</i> and enteric pathogens
Stratiki et al. ⁷³ (n=77)	0/41 (0%) ^{‡§}				3/36 (8.3%) ^{‡§}	0/41 (0%) ^{‡§}		3/36 (8.3%) ^{‡§}	Intestinal permeability
Chou et al. ⁸⁷ (n=300)	0/152 (0%) ^{‡§}				2/148 (1.4%) ^{‡§}	21/153 (13.7%) ^{‡§}		30/148 (20.3%) ^{‡§}	Death; growth; neurodevelopment
Rouge et al. ⁶⁰ (n=94)	2/45 (4.4%) ^{‡§}				1/49 (2.0%) ^{‡§}	15/45 (33.3%) ^{‡§}		13/49 (26.5%) ^{‡§}	Enteral feed intake at day 14
Manzoni et al. ²⁸ (n=472)	(+BL) 0/151 (0%) [‡]	3/153 (2.0%) [‡]			10/168 (6.0%) [‡]	(+BL) 7/151 (4.6%)		9/153 (5.9%)	
Luoto et al. ⁶⁸ (n=3342) ^{††}	19/418 (4.5%)				18/1024 (1.8%)			29/168 (17.3%)	
Mihatsch et al. ⁷⁰ (n=180)	2/91 (2.2%) ^{‡§}				4/89 (4.5%) ^{‡§}				Nosocomial infections
Al-Hosni et al. ⁵⁹ (n=101)	3/50 (6.0%) ^{‡§}				4/51 (7.8%) ^{‡§}	13/50 (26.0%) ^{‡§}		16/51 (31.4%) ^{‡§}	Weight; feeding tolerance; antimicrobial treatment days
Indrio et al. ²² (n=42)									Frequency of regurgitation; gastric emptying
Romeo et al. ²⁶ (n=249)									Gut colonization; Bacterial and/or fungal infections; neurological effects

BID, twice daily; BL, bovine lactoferrin (mammalian milk glycoprotein); BW, birth weight (grams); C, control group; CFU, colony-forming units; FM, formula milk; GA, gestational age (weeks); GER, gastroesophageal reflux; IP, inactivated probiotic group (contained only killed probiotics); NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; NNT, number needed to treat; NS, statistically not significant; P, probiotic group; PGI, probiotic "on demand" group (infants with gastrointestinal problems who received probiotics); PMA, postmenstrual age (weeks); TTF, time to full feeds (days); UTI, urinary tract infection

Studies included in shaded rows did not assess for NEC or sepsis

* ICU

† Partially retrospective

‡ Secondary outcome

§ NS

** Had 2 probiotic groups

†† Specifics not provided

‡‡ Weight below the 10th percentile for GA

§§ Breakdown by individual species not available

*** Retrospective

††† Infants with GER

Table 3. Infant Probiotic-Associated Bacteremia Case Reports

Study	PNA	Underlying Risk Factor(s)	Probiotic	Outcome
Kunze et al. ⁷⁷	3 mo	36 wks GA, short gut syndrome (secondary to intestinal atresia); TPN; cholestasis; friable/inflamed intestines on endoscopy	<i>Lactobacillus</i> GG (Culturelle) 1 capsule GT daily	<i>Lactobacillus</i> bacteremia (day 23 of probiotic)
	6 mo	34 wks GA; short gut syndrome (secondary to gastroschisis); TPN; cholestasis; mildly inflamed intestines	<i>Lactobacillus</i> , 1 capsule JT daily	<i>Lactobacillus</i> bacteremia (day 169 of probiotic); confirmation of probiotic strain
DeGroote et al. ⁷⁶	11 mo	26 wks GA twin; short gut syndrome (secondary to NEC); CVC; TPN; cholestasis; rotavirus diarrhea	<i>L. rhamnosus</i> GG, 1/8 capsule GT twice daily	<i>Lactobacillus</i> and <i>Candida</i> bacteremia (week 5 of probiotic); confirmation of probiotic strain
Land et al. ⁷⁸	6 wks	Term, status -post cardiac defect repair; CVC	<i>Lactobacillus</i> GG (Culturelle), 1 capsule GT daily	<i>Lactobacillus</i> bacteremia (day 20 of probiotic); presumed endocarditis; confirmation of probiotic strain
Ohishi et al. ⁷⁹	2 wks	37 wks GA; status-post omphalocele repair; PCVC	<i>B. breve</i> BBG-01 (Yakult)	<i>Bifidobacterium</i> bacteremia (day 10 of probiotic); confirmation of probiotic strain
Guenther et al. ⁸¹	2 wks	28 wks GA (935 g); rotavirus and adenovirus diarrhea; CVC	<i>E. coli</i> NISSLE 1917, 1 mL daily	<i>E. coli</i> bacteremia (day 10 of probiotic); confirmation of probiotic strain
Jenke et al. ⁸⁰	18 days	27 5/7 wks GA; status-post PDA closure with indomethacin	<i>B. bifidum</i> , <i>L. acidophilus</i> (Infloran)	<i>B. longum</i> and <i>infantis</i> bacteremia (day 9 of probiotic); confirmation of probiotic strain

CVC, central venous catheter; GA, gestational age; GT, gastric tube; JT, jejunostomy tube; PCVC, percutaneous central venous catheter; PNA, postnatal age; TPN, total parenteral nutrition

who received probiotic supplementation in the trial of Lin and colleagues.^{63,87} In the 301 infants evaluated at 3 years corrected age, no statistically significant differences were observed in growth or neurodevelopmental outcomes including cerebral palsy, visual or hearing impairments, or mental development.⁸⁷ The potential effect of probiotic supplementation on other conditions common in preterm infants such as CLD, IVH, and periventricular leukomalacia has yet to be determined.⁶³

PRACTICAL CONSIDERATIONS

Despite the growing body of evidence supporting the routine use of probiotic supplementation for the prevention of NEC in preterm neonates, many practitioners in the neonatal community have urged continued caution regarding routine use.^{4,88} Before considering the addition of routine probiotic supplementation, it is prudent to minimize other potential risk factors associated with the development of NEC discussed previously.

Additionally, it is imperative to emphasize the use of human milk, as this alone has been shown to significantly reduce the risk of NEC compared to formula feeding.⁷

One of the predominant concerns associated with routine probiotic supplementation remains which probiotic product, or combination of products, is most efficacious. As shown in this review, wide variability exists in the probiotics evaluated (Tables 1 and 2). While the probiotic strains most frequently studied in neonatal clinical trials have been species of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, the same combinations of probiotic strains have not been repetitively studied.⁶³ Few studies have evaluated commercially available products (Table 4), and most of the products evaluated in premature infants are not manufactured or marketed in the United States.⁸⁹ Due to the extensive variability in the current body of literature, the ideal probiotic strain(s) and regimen is difficult to discern. This ambiguity has important implications for both proposed routine probiotic use as well as for future research. Ques-

Table 4. Commercially Available Probiotic Products Evaluated in Premature Infants*

Product (manufacturer)	Strain(s)	Available in the United States (form)	Storage	Allergen Considerations
ABC Dophilus (Solgar Inc., Leonia, NJ) ⁹⁸	<i>B infantis</i> (BB-02), 0.30×10^9 CFU; <i>S thermophilus</i> (TH-4), 0.35×10^9 CFU; <i>B bifidum</i> (BB-12), 0.35×10^9 CFU	Yes (powder)	Refrigerate after opening	Contains no major allergens: 100% dairy-, lactose-, and milk-free
Align (Proctor & Gamble, Cincinnati, OH) ⁹⁹	<i>B infantis</i> , 1×10^9 CFU	Yes (capsule)	Room temperature	Does not contain lactose, soy, or gluten
Culturelle for Kids (Amerifit, Inc., Cromwell, CT) ¹⁰⁰	<i>L rhamnosus</i> GG LGG, 1.0×10^9 CFU	Yes (packets)	Refrigeration recommended but not required; store in a cool, dry place away from direct sunlight; do not exceed 75°F	No dairy, artificial dyes, colors, preservatives, flavors, yeast, wheat, gluten, or lactose
Culturelle Natural Health and Wellness (Amerifit, Inc., Cromwell, CT) ¹⁰¹	<i>L rhamnosus</i> GG LGG, 1.0×10^{10} CFU	Yes (capsule)	Refrigeration recommended but not required; store in a cool, dry place away from direct sunlight; do not exceed 75°F	Gluten-free; contains milk proteins
Dicoflor 60 (S.I.I.T. s.r.l., Italy) ¹⁰²	<i>L rhamnosus</i> GG 6.0×10^9 CFU	No (powder)	Store below 20°C (68°F)	Casein derivate is used during production, which is analytically nondetectable in the final product
Infloran (Laboratorio Farmaceutico SIT srl, Mede, Pavia, Italy) ⁹⁷	<i>L acidophilus</i> 1.0×10^9 CFU <i>B bifidum</i> 1.0×10^9 CFU	No (capsule)	Store at a temperature range of +2°C to +8°C, away from light	Contains lactose

*Complete product and manufacturing information is not currently available for Prenan Nestle or Yakult LB

tions regarding practical aspects of strain selection, formulation, and lack of regulation remain at the forefront of the probiotic debate.^{4,85,88}

Formulation

Considering the multifactorial nature of NEC pathophysiology and the multitude of proposed mechanisms for how probiotic supplementation may prevent NEC, it stands to reason that multiple strains with individual, specific mechanisms of action may provide additional benefit compared to a single-probiotic strain. Ideally, all microorganisms used in a probiotic preparation should be clearly identified and should consist of strains with known safety and efficacy profiles. The culture medium used during the probiotic manufacturing process must also be identified

and taken into account.³³ For example, certain formulations of *Lactobacillus* GG are grown on whey and may contain trace levels of casein and whey, which are proteins found in milk.⁸⁸ This is an especially important consideration in patients with food intolerances or allergies. Last, the type of feedings must also be a consideration. Specific strains of *Bifidobacterium*, such as *B infantis*, specifically use oligosaccharides abundant in human milk and have been shown to grow selectively in breast-fed infants.⁹⁰

The importance of dosage form must not be overlooked. Probiotic products are currently available in various dosage forms including but not limited to capsules, granules, powders, and liquids. Despite growing interest in the administration of probiotic supplements to preterm

infants, most probiotic products are not manufactured with the neonatal population in mind. Thus, dosage and administration of probiotics to preterm infants can be associated with a unique set of challenges. Many preterm infants are initially started on small feeding volumes through oral or nasogastric tubes, which has implications when selecting dosage forms. For example, many powder formulations require a minimum amount of fluid volume to properly dissolve, granules may not be easily dissolvable, and liquid formulations can be associated with relatively large volumes with respect to feeding volumes. Anecdotal reports have noted that the administration of probiotic granules may result in clogged oral or nasogastric tubes.

Once the desired combination of probiotic strains and a suitable probiotic product is decided upon, several other practical considerations must be taken into account in the development of a NICU policy regarding probiotic supplementation for preterm infants. First and foremost, an institution must determine which GA and BW to include for routine supplementation. Contraindications, such as GI anomalies and short gut syndrome, should also be determined along with the time of initiation and duration of therapy. Institution-specific factors regarding storage, distribution, and documentation should be established. Many probiotic products require or recommend refrigeration to ensure product viability. Therefore, storage specifications of specific products as well as storage capabilities of an institution must be considered. Because probiotics are presently classified as dietary supplements in the United States, some institutions distribute them through dietary services rather than the pharmacy. Variability in distribution practices may lead to inconsistencies or lack of proper nursing documentation. Including the probiotic as part of the medication administration record has been suggested as an effective means to verify administration. Once distributed, every attempt should be made to minimize bedside manipulations of probiotic powders and capsules because of the potential for inadvertent contamination of intravenous lines.⁷⁶ Given the controversy surrounding probiotics in preterm infants, the development of such a policy taking into account all aforementioned issues is critical to promote patient safety and consistent practice.

Regulatory Concerns

Another key aspect of the probiotic controversy is the general lack of regulatory mechanisms in place to ensure the safety and quality of probiotic products. In the United States, probiotics can be marketed as ingredients in conventional foods, dietary supplements, foods for special dietary uses, drugs, and veterinary products. Each of these respective areas represents a different regulatory category. A product's regulatory category is dependent upon the product's intended use, formulation, route of administration, consumer target, and safety. The assigned regulatory category will directly impact the clinical research, development, manufacture, and marketing of the product. Most probiotics in the United States are currently sold as food ingredients, mostly in dairy products, and as dietary supplements.⁸ Labeling of probiotic products sold as dietary supplements will reflect the fact that product statements have not been evaluated by the Food and Drug Administration (FDA) and that the product is not intended to diagnose, treat, cure, or prevent any disease. Furthermore, concern has arisen that the contents of different commercial probiotic products may not always correspond to the claims stated on the label.⁹¹

The lack of an FDA-approved probiotic product for administration in preterm infants has been frequently cited as a deterring factor for many critics of routine probiotic use. In the United States, drugs are defined as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease," and as "articles (other than food) intended to affect the structure or function of the body of humans or other animals."⁹² While probiotic supplements may "affect the structure or function of the body," probiotics do not meet the legal definition of a drug unless they are also intended to diagnose, cure, mitigate, treat, or prevent disease. For a probiotic to be investigated as a drug for the prevention of NEC, an Investigational New Drug application would have to be filed with the FDA to conduct a clinical trial in the United States. Because the FDA requires detailed product information and stringent manufacturing processes to ensure quality, filing an Investigational New Drug application would be both a costly and time-intensive endeavor for a probiotic manufacturer. As opposed to a drug product, the FDA does not currently evaluate dietary supplements, such as probiot-

ics, for safety and effectiveness. The probiotic manufacturer is solely responsible for ensuring the safety of the product before it is marketed.

In October 1994, the Dietary Supplement Health and Education Act (DSHEA) was signed into law amending the Federal Food, Drug, and Cosmetic Act. This act established the need for separate and more specific manufacturing practices for dietary supplements than those used for general food products. In June 2007, the FDA announced a final rule establishing current good manufacturing practice (CGMP) requirements for dietary supplements. Companies which manufacture, package, label, or hold dietary supplements in the United States must now comply with CGMPs for dietary supplements. The purpose of these CGMPs is to ensure that dietary supplements consistently meet established specifications for identity, purity, strength, and composition. Additionally, manufacturers and distributors are now required to report all serious adverse events associated with the use of dietary supplement products to the FDA. Thus, products that are manufactured in the United States may be safer and may potentially be preferred for use in neonates.⁹³

SUMMARY

Probiotics appear to have a beneficial effect in the prevention of NEC; unfortunately many questions are unanswered regarding the ideal product, regimen, duration of supplementation, and target population of premature infants (GA and BW). Current studies have failed to control for numerous confounding variables such as breast feeding rates, antibiotic exposure, feeding practices, and environmental cross-contamination. Most studies have also been published in countries outside of the United States, where NEC rates are often higher, feeding practices differ, and unknown pharmacogenetic differences may exist. It is hoped that we will be able to fill in the missing pieces of the puzzle after the completion of ongoing studies. ProPremis, a multicenter trial being conducted in Australia, is evaluating probiotic use in 1100 premature infants and continues to enroll patients after two interim analyses assuring safety.⁹⁴ This large trial, to be completed in early 2012, controls for the integrity of the multistrain probiotic supplement (ABC Dophilus Probiotic Power for Infants,

Solgar, Leonia, NJ) and will hopefully answer many questions. Several other trials evaluating probiotic use are underway and anticipated to be completed by 2013.

Despite the unknowns regarding probiotic use for the prevention of NEC, key points to draw from this review include:

- NICUs with higher rates of NEC are more likely to observe a benefit with probiotic supplementation
- Multistrain probiotics may be more effective than single-strain products
- Extremely low-birth-weight infants may not benefit to the extent observed in those with greater GA or BW
- Although reports of probiotic-related sepsis are limited, caution should be used when considering probiotic supplementation in infants at greatest risk for an impaired mucosal barrier (e.g., GI anomalies)
- When selecting a probiotic product, probiotic strains, formulation, dosage form, previous use in premature infants, and manufacturer practices must be taken into account
- Policies regarding storage, preparation, distribution, administration and documentation of probiotics must be in place to ensure product integrity and patient safety

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ABBREVIATIONS BW, birth weight; CGMP, current good manufacturing practice; CLD, chronic lung disease; DSHEA, Dietary Supplement Health and Education Act; EBM, expressed breast milk; FDA, Food and Drug Administration; GA, gestational age; GI, gastrointestinal; IL-8, interleukin-8; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; NF- κ B; nuclear factor kappa B; NICU, neonatal intensive care unit; NNT, number needed to treat; PDA, patent ductus arteriosus; RCT, randomized controlled trials; TFF, time to full feeds; TLR4, Toll-like receptor 4; VLBW, very-low-birth weight

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