JMHT: Journal of Medicine and Health Technology
Vol. 03, No. 01, May, 2026, pp. 27-36
ISSN: 3046-6865
DOI: 10.12962/j30466865.v3i1.2634

Enhancing Gut Health: Probiotic Interventions in Preterm Neonates with Necrotizing Enterocolitis

Rahmah Yasinta Rangkuti[1], Muhammad Nazhif Haykal[2], Erna Furaidah[1]
[1]Medical Professional Education Study Program, Institut Teknologi Sepuluh Nopember, Keputih, Sukolilo, Surabaya, 60111, Indonesia
[2]Medical Undergraduate Study Program, Institut Teknologi Sepuluh Nopember, Keputih, Sukolilo, Surabaya, 60111, Indonesia

Corresponding Author: rahmah.yasinta@its.ac.id

Abstract — Necrotizing enterocolitis (NEC) remains a significant challenge in neonatal care, particularly in preterm neonates, due to its high mortality and morbidity rates. Probiotics, defined as live microorganisms that confer health benefits to the host, have emerged as a promising intervention to enhance gut health and reduce the risk of NEC. This study aimed to evaluate the role and efficacy of probiotic interventions in preterm neonates with NEC by analysing existing literature and clinical studies. Data on the types of probiotics used, their mechanisms of action, dosage regimens, and clinical outcomes were extracted and analysed. The findings revealed that specific strains of probiotics, including *Lactobacillus* and *Bifidobacterium*, significantly reduced the incidence of NEC and associated mortality in preterm neonates. Probiotics were found to modulate gut microbiota, enhance mucosal integrity, and reduce systemic inflammation, thereby mitigating the pathogenesis of NEC. The study also highlighted variations in the efficacy of different probiotic strains and dosing strategies, indicating a need for standardized protocols. The results underscore the potential of probiotics as a preventive and therapeutic tool for NEC in preterm neonates. Future research should focus on identifying optimal probiotic strains, dosages, and long-term safety profiles to establish robust clinical guidelines and improve neonatal outcomes.

Keywords — *probiotics; necrotizing enterocolitis; preterm neonates; gut health; neonatal care.*

How to cite: Rangkuti RY, Haykal MN, Furaidah E. Enhancing gut health: probiotic interventions in preterm neonates with necrotizing enterocolitis. JMHT: Journal of Medicine and Health Technology. 2026;3(1):27-36. doi: 10.12962/j30466865.v3i1.2634.

Manuscript received 3 Feb. 2025; revised 1 Mar. 2026; accepted 9 Apr. 2026. Date of publication 4 May 2026.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency disease in preterm babies. This disease is characterized by inflammation, ischemia, and infection in the patient's abdominal area, which can be accompanied by perforation. While global estimates suggest that NEC affects approximately 5% of all infants born prematurely, incidence rates vary considerably between different countries. For instance, Japan reports relatively low rates of 1% to 2%, whereas incidence in Hong Kong has been documented as high as 28%. Within the clinical setting, NEC is responsible for 1% to 7% of all admissions to Neonatal Intensive Care Units (NICUs). In the United Kingdom, specifically within level 2 and 3 units, a 2% prevalence was reported during a one-year period in 2010. The true incidence is however unknown due to several cases of suspected early NEC that is initially managed in the same way. The aetiology of NEC is multifactorial, with low birth weight being a common risk factor [1].

Exposure to enteral feedings, low birth weight, and advanced gestational age may reveal the preterm gut's developmental immaturity and impaired ability to appropriately handle the entrance of microorganisms into the intestinal lumen. This puts the premature baby at risk for aberrant bacterial colonization, inadequately developed intestinal immunity, and uncontrolled intestinal inflammation. The significance of commensal or probiotic organisms in mediating the ongoing development of gut structure and function has been shown by laboratory data and clinical investigations [2]. Consequently, a growing number of studies have suggested using probiotic supplements to modify human diseases, particularly those originating in the gastrointestinal tract. The aim of this review is to consider the use of probiotics based on existing research.

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disorder predominantly affecting premature infants. Approximately 10% of infants born weighing less than 1,500 grams develop NEC, with a mortality rate ranging from 20% to 30%. Current treatment and prevention strategies for NEC are inadequate. Survivors often face significant long-term health issues, including short bowel syndrome, cholestatic liver disease, and impaired neurodevelopmental and growth outcomes. Factors such as prematurity, low birth weight, enteral feeding practices, and antibiotic exposure have been associated with the onset of NEC [3].

Typically, NEC presents in a premature infant who has been stable in the neonatal intensive care unit (NICU) but suddenly exhibits symptoms like abdominal distension, bilious vomiting, and bloody stools (hematochezia). In many cases, the condition rapidly progresses to systemic sepsis and cardiovascular collapse, indicating escalating intra-abdominal inflammation and necrosis of the intestines [4]. In severe instances of necrotizing enterocolitis (NEC), infants may rapidly deteriorate, exhibiting signs of shock and swiftly progressing to pneumoperitoneum, which can lead to death. A hallmark radiographic indicator of NEC is pneumatosis intestinalis, characterized by the presence of gas within the bowel wall. This phenomenon is believed to result from bacterial fermentation of substances within the intestinal lumen.

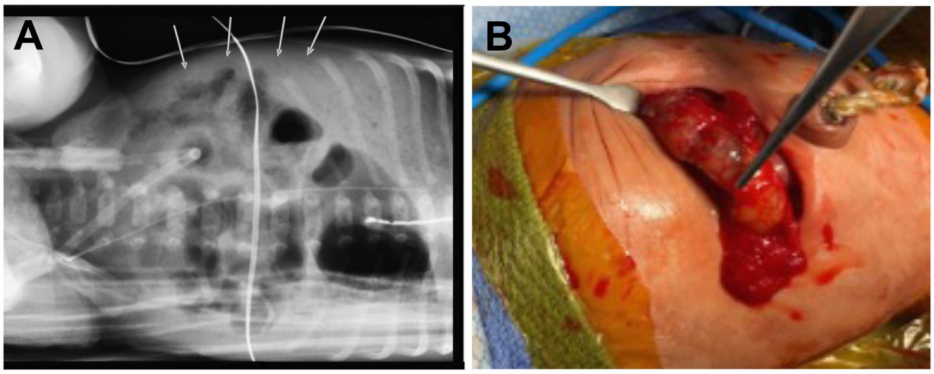

Fig. 1. Radiologic and clinical findings in necrotizing enterocolitis. A. Abdominal x-ray revealing the presence of pneumatosis intestinalis and intestinal dilation in a child with NEC; B. The intestinal findings in the patient in A, revealing the presence of intestinal ischemia and necrosis [4].

Surgical intervention is warranted in cases of necrotizing enterocolitis (NEC) presenting with pneumoperitoneum, peritonitis, or intestinal obstruction. Without prompt surgical intervention to remove necrotic bowel tissue, infants unresponsive to initial medical management often succumb to the disease. Mortality rates for infants requiring surgery are notably high, ranging from 30% to 50%. Survivors of surgical NEC are at risk for complications such as intestinal strictures, short bowel syndrome, and intestinal failure. The clinical stages of NEC are categorized using the Bell staging criteria, established in 1978 [4]. Stages 2a and above are considered severe and typically necessitate surgical treatment rather than solely medical therapy [1,5].

Although the precise etiopathogenesis of necrotizing enterocolitis (NEC) remains incompletely elucidated, it is widely recognized as a complex, multifactorial condition driven heavily by intestinal immaturity and microbial dysbiosis [6,7]. In a fully developed gastrointestinal tract, a robust and multi-dimensional mucosal barrier effectively contains luminal microbes, preventing the translocation of pathogens and averting inappropriate hyper-inflammatory cascades. Conversely, the premature neonatal intestine is intrinsically susceptible to severe inflammatory injury due to a combination of developmental deficits; these include heightened epithelial permeability, sluggish peristaltic motility, abnormal mucus layer composition, and deficient production of secretory IgA (sIgA). This compromised physical and immunological barrier is further exacerbated by the aberrant microbial colonization patterns typical of preterm infants in the neonatal intensive care environment [8]. When an immature, highly permeable gut encounters pathogenic dysbiosis, the risk of bacterial translocation into the lamina propria and systemic

circulation rises exponentially. Advanced molecular profiling emphasizes the critical role of this delicate microbial ecosystem in disease progression; specifically, an initial predominance of Firmicutes in the first week of life has been identified as a predisposing factor, which is then typically followed by a sudden, pathological bloom of Proteobacteria (such as Enterobacteriaceae) immediately prior to the clinical onset of NEC [4,7,9]. Ultimately, these ecological shifts highlight that maintaining intestinal microbial homeostasis is paramount in preventing the barrier failure and inflammatory cascade that characterize NEC pathogenesis [7].

Table. 1. Modified Bell's classification of NEC

Stage	Systemic Signs	Intestinal Signs	Radiographic Signs
I: Suspected NEC	Temperature instability, bradycardia, apnoea	Elevated gastric residuals, mild abdominal distention, occult blood in stool	Normal or mild ileus
IIA: Mild NEC	Similar with stage I	Prominent abdominal distention ± tenderness, absent bowel sounds, grossly bloody stools	Ileus, dilated bowel loops with focal pneumatosis
IIB: Moderate NEC	Mild acidosis, thrombocytopenia	Abdominal wall oedema and tenderness ± palpable mass	Extensive pneumatosis, early ascites, ± PVG
IIIA: Advanced NEC	Respiratory and metabolic acidosis, mechanical ventilation, oliguria, hypotension, DIC	Worsening wall oedema and erythema with induration	Prominent ascites, persistent bowel loop, no free air
IIIB: Advanced NEC	Vital sign and laboratory evidence of deterioration, shock	Evidence of perforation	Pneumoperitoneum

The pathogenesis of necrotizing enterocolitis (NEC) is driven by a convergence of risk factors, most notably extreme prematurity, enteral formula feeding, impaired intestinal perfusion (ischemia), and aberrant bacterial colonization. The interplay of these variables precipitates a rapid, overwhelming inflammatory cascade that culminates in the coagulative bowel necrosis characteristic of the disease. In severe presentations, this intense inflammation leads to multisystem organ failure, carrying a mortality rate that can reach up to 30%. Toll-like receptors (TLRs), and specifically TLR4, are critical mediators in initiating this hyper-inflammatory response. As pattern-recognition receptors, TLRs identify specific microbial ligands; TLR4, for example, is activated by lipopolysaccharide (LPS) present on the outer membrane of Gram-negative bacteria [8]. The pathological activation of TLR4 fundamentally disrupts the intestinal equilibrium between injury and repair. It actively drives mucosal injury by inducing enterocyte cell death through both apoptosis and necroptosis, while simultaneously suppressing compensatory mucosal healing mechanisms such as epithelial restitution and proliferation [4]. This profound epithelial damage compromises the mucosal barrier, facilitating the translocation of luminal bacteria into the lamina propria. Once translocated, these microbes can activate TLR4 receptors situated on mesenteric endothelial cells. This endothelial activation triggers severe local vasoconstriction secondary to a reduction in nitric oxide production, thereby profoundly exacerbating intestinal ischemia and subsequent tissue injury [4,10]. Furthermore, research indicates that intestinal TLR expression is tightly regulated by developmental stage. While fetal and highly premature enterocytes exhibit robust pro-inflammatory cytokine release upon LPS stimulation, this receptor responsiveness normally downregulates shortly after a full-term birth. Following TLR4 activation, the downstream signaling cascade culminates in the nuclear translocation of the transcription factor NF-κB, which drives the mass production of inflammatory cytokines. Consequently, the inherently high TLR4 expression and diminished regulatory inhibition in the premature gut predispose it to a baseline hyper-inflammatory state compared to the mature intestine, explaining the unique susceptibility of preterm neonates to NEC [4,8].

The conventional clinical management of necrotizing enterocolitis (NEC) relies primarily on supportive and reactive measures, encompassing strict bowel rest, empiric broad-spectrum antibiotic therapy, parenteral nutrition, and intensive cardiorespiratory monitoring. In advanced or fulminant presentations, surgical interventions such as peritoneal drainage or the resection of necrotic intestine become unavoidable [7]. While these life-saving interventions are the current standard of care, they are accompanied by profound iatrogenic drawbacks and long-term morbidities. Extensive bowel resection frequently precipitates short-bowel syndrome and chronic intestinal dysfunction. Furthermore, the consequent reliance on prolonged parenteral nutrition necessitates central venous catheterization, which inherently increases the risk of nosocomial bloodstream infections and cholestatic liver disease [1]. Collectively, these severe complications dramatically extend the duration of neonatal intensive care hospitalization and exponentially inflate healthcare expenditures [7]. Given the substantial limitations, high costs, and severe adverse effects associated with these traditional management strategies, there is a critical and pressing need to identify alternative, preventative therapeutic modalities such as microbiome modulation that can successfully mitigate the incidence of NEC while minimizing iatrogenic harm [7,11].

Probiotics: Mechanism of Action

The conceptual history of probiotics began in 1965 when Lilly and Stillwell initially characterized them as "growth-promoting factors". This definition was later refined in 2001 by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), which described probiotics as live microorganisms that, when consumed in sufficient quantities, provide a health advantage to the host. In clinical practice, the most prevalent probiotic genera are *Bifidobacterium* and *Lactobacillus*. The *Lactobacillus* genus encompasses a diverse range of species such as *L. acidophilus, L. rhamnosus, L. bulgaricus, L. reuteri*, and *L. casei*. Similarly, frequently utilized *Bifidobacterium* species include *B. animalis, B. infantis, B. lactis,* and *B. longum*. While these organisms have proven effective in managing conditions like acute diarrhea, medical consensus remains divided regarding which specific strains or combinations offer the highest efficacy for neonatal care [12]. Despite their use in clinical practice, uncertainty persists regarding the most effective probiotic types or specific strains [7].

Extensive clinical research has highlighted the potential of probiotics to mitigate the risk of necrotizing enterocolitis (NEC). Evidence suggests that a synergistic approach combining *Lactobacillus* and *Bifidobacterium* with breast milk results in a more significant decrease in NEC severity and occurrence than breast milk alone. This is particularly vital because the neonatal gut microbiome undergoes a rapid transformation immediately after birth, quickly outnumbering the infant's own eukaryotic cells. While term infants typically possess a microbiome rich in beneficial bacteria, preterm infants often suffer from dysbiosis characterized by high levels of pathogens and low levels of commensal species. Breast milk supports healthy colonization by providing complex oligosaccharides that function as prebiotics, nourishing beneficial microbes and facilitating the maturation of the gut-associated lymphoid tissue (GALT) and the epithelial barrier. Probiotic administration serves as a proactive strategy to correct these imbalances in preterm infants who are often exposed to hospital environments and frequent antibiotic use [3].

Experimental models, including both in vitro and animal studies, have further clarified the biological pathways through which commensal bacteria protect the fragile neonatal gut. These mechanisms provide a theoretical framework for understanding how probiotics can prevent the onset of NEC [13]. Preterm infants are inherently vulnerable due to physiological immaturity, which manifests as impaired barrier function, reduced leukocyte counts, and gut dysmotility, all of which prime the system for inflammation. Because fecal microbiota transplantation (FMT) presents significant practical and safety hurdles in neonates, probiotics have emerged as a more accessible and standardized method for optimizing the gut environment in high-risk infants [14].

Probiotics engage with the host via multiple unique biological pathways, including: (1) Genetic enhancement: Probiotics promote the overexpression of cytoprotective genes, thereby safeguarding intestinal cells from damage; (2) Inflammatory control: Probiotics facilitate the downregulation of pro-inflammatory gene expression. They accomplish this by inhibiting the activation of Toll-like receptor 4 (TLR4) and obstructing the subsequent activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); (3) Metabolic support: These advantageous bacteria generate butyrate and other short-chain fatty acids that act as a principal energy source for colonocytes; (4) Pathogen suppression: By lowering both pH levels and oxygen tension within the intestinal lumen, probiotics foster an environment that curtails the proliferation of detrimental Enterobacteriaceae; (5) Structural integrity: Probiotics are essential for the maturation and sustained functionality of the intestinal barrier; (6) Microbial competition: They directly compete with potentially pathogenic microbes for nutrients and adhesion sites; and (7) Immune modulation: Probiotics assist in regulating cellular immunity by preserving a healthy balance between T-helper 1 (Th1) and T-helper 2 (Th2) responses [7,15].

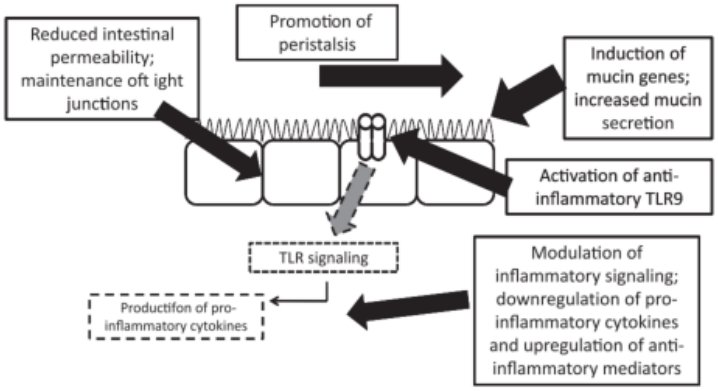

Fig. 2. Mechanism of action for probiotics [8]

Specific probiotic strains demonstrate unique protective properties. For instance, *Lactobacillus reuteri* possesses the ability to neutralize hydrogen peroxide, while *Bifidobacterium infantis* exhibits an inherent biological resistance to it [10]. Furthermore, *Bifidobacterium* species have been shown to dampen the production of pro-inflammatory cytokines like IL-6, IL-8, and IL-23 in animal models, while *Lactobacillus* species help lower TNF-α and IL-6 levels [7]. Research conducted by Samara et al. emphasized the vital ecological impact of specific microbes on early-life gut development, showing that a multi-strain probiotic formulation could effectively catalyze microbiome maturation to mirror that of full-term neonates while enhancing both immune and metabolic markers. This randomized trial focused on extremely preterm infants, investigating how a combination of one *Lacticaseibacillus* strain and four native *Bifidobacterium* strains influenced the microbiome's functional and compositional evolution. The study observed that consistent daily intake of this probiotic blend expedited the development of a stable, interconnected, and mature microbial community similar to that found in term infants. Through structural equation modelling, the investigators identified probiotics as a primary factor influencing the assembly of the microbiome. Within this framework, Bifidobacterium presence and specific fecal metabolites served as the most reliable indicators of this maturation process. Ultimately, this *Bifidobacterium*-led transformation was associated with the creation of an anti-inflammatory intestinal environment. This demonstrates that these bacterial strains function as "ecosystem engineers" that drive critical immunological and microbial advancements in the most vulnerable premature populations. [11].

The protective role of probiotics on intestinal structure has also been extensively documented in various animal studies. For instance, in an experimental model of acute colitis, the use of *Lactobacillus casei* was shown to significantly lower intestinal permeability. Notably, the treated subjects preserved healthy concentrations of the tight junction protein zonula occludens-1,

whereas untreated controls suffered from depleted levels. In a parallel fashion, research utilizing rat models of necrotizing enterocolitis (NEC) demonstrated that supplementing with *Bifidobacterium bifidum* acted as a safeguard for the ileum. This intervention effectively prevented the disruption and irregular expression of both adherens junction and tight junction proteins. Such findings suggest that probiotics reinforce the gut's physical defenses against the inflammatory damage characteristic of NEC [16,17].

Clinical Evidence on Probiotics for NEC Prevention

Recent studies have explored innovative probiotic delivery systems to prevent necrotizing enterocolitis (NEC). For instance, a single administration of *Lactobacillus reuteri* biofilm cultivated on biocompatible microspheres has been shown to significantly reduce both the incidence and severity of NEC, as well as decrease intestinal permeability in experimental models. This novel approach suggests potential future benefits in NEC prevention [3]. While single-strain probiotics have not demonstrated a significant impact on mortality rates, combinations of multiple probiotic species have been effective in reducing both the incidence of stage II–III NEC and associated mortality. This indicates that the protective effects are primarily due to the synergistic interactions among different probiotic strains [7]. Additionally, research comparing various probiotic strains found no significant differences in their effects on NEC incidence or in the elevation of fecal secretory immunoglobulin A (sIgA) levels. Notably, fecal sIgA concentrations were positively correlated with gestational age and body weight in very low birth weight preterm infants [18].

Although probiotic therapy has demonstrated substantial clinical efficacy, its benefits are currently reliant on continuous, daily administration, as standard supplementation largely fails to induce permanent alterations to the host's indigenous microbiome [20]. To circumvent the need for repetitive dosing, researchers such as Navarro et al. have investigated an innovative delivery mechanism: cultivating Lactobacillus reuteri as a biofilm adhered to biocompatible microspheres. Biofilms consist of complex cellular communities anchored to a surface and enveloped in a self-generated polymeric matrix composed of DNA, lipids, proteins, and oligosaccharides. In contrast to isolated, free-floating (planktonic) cells, bacteria structured within a biofilm demonstrate vastly superior resistance to harsh physiological stressors, including gastric acidity, antimicrobial agents, and host immune defenses. Ultimately, this specialized biofilm state not only significantly enhances the bacteria's ability to survive gastrointestinal transit and adhere to intestinal tissues, but it also more accurately replicates the natural microbial reservoirs found within the human gut [3].

Table 2. Studies evaluating the use of probiotic preparations to prevent NEC

Study (ref)	Year	Study target	N	Probiotic	Results
Dashti et al.	2014	Low birth weight infant	136	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus thermophilus, Bifidobacterium breve, and Bifidobacterium*	No benefit of probiotics for the prevention of NEC.
Sun J et al.	2017	<1500 g, and/or <32 weeks gestation	8998	Bifidobacterium infantis, Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus	In the probiotics group, incidence of NEC is reduced by 37%, sepsis by 37%, mortality by 20%
Robertson et al.	2020	<1500 g, and/or <32 weeks gestation	982	*Lactobacillus acidophilus and Bifidobacterium bifidum combination probiotics; L. acidophilus, B. bifidum, and B. longum subspecies infantis*	Incidence of NEC is higher in control group (7.5%) compared to probiotic group (3.1%)
Tobias et al.	2022	<1500 g	483	*Bifidobacterium longum subsp. infantis*	Incidence of NEC is higher in control group (11% (n=301)) compared to probiotic group (2.7% (n=182))
Zhou et al.	2023	Preterm infant	47 studies, 17332 neonates	*Lactobacillus GG, Lactobacillus acidophilus, Bifidobacterium infantis, Bifidobacterium bifidus, Streptococcus thermophilus (vary between studies)*	Probiotic exhibited a dramatical reduction in the risk of NEC, sepsis, and mortality.
Cripps et al.	2023	<32 weeks gestation, and <1500 g	805	*Bifidobacterium infantis, Bifidobacterium lactis, Streptococcus thermophilus*	Incidence of NEC is higher in control group (7.6%) compared to probiotic group (3.6%)

Extensive research underscores the prophylactic value of probiotics against necrotizing enterocolitis (NEC) in premature infants, though clinical outcomes depend heavily on specific treatment variables. A meta-analysis by Sun et al. demonstrated that probiotic supplementation reduced the incidence of NEC by 37% (95% CI: 0.51, 0.78) compared to control groups, despite inconsistencies in the bacterial strains used across the evaluated trials. Their findings suggest that probiotics achieve maximum efficacy when formulated with multiple strains, administered at a daily dosage of less than 10^9 CFU for under six weeks, and delivered via breast milk or formula [19]. The superior efficacy of multi-strain formulations is further supported by Jiang et al., whose meta-analysis revealed that while both mixed probiotics and single-strain Lactobacillus significantly decrease the rate of severe (stage II-III) NEC (RR = 0.39, 95% CI: 0.26–0.57; and RR = 0.53, 95% CI: 0.36–0.78, respectively), isolated use of Bifidobacterium or Saccharomyces fails to provide this same protective effect. Furthermore, Jiang et al. highlighted that only mixed probiotic regimens significantly reduce overall mortality (RR = 0.52, 95% CI: 0.34–0.80); single-strain therapies utilizing Lactobacillus, Bifidobacterium, or Saccharomyces alone do not yield a statistically significant reduction in death rates [7].

Broader investigations also support these benefits. A network meta-analysis of 47 double-blind randomized controlled trials involving 17,332 premature infants by Zhou *et al*. confirmed that dietary interventions utilizing probiotics significantly lower the risk of both NEC and sepsis compared to a placebo. Similarly, research by Cripps et al. showed a notable reduction in NEC incidence for infants receiving probiotics (3.6%) versus those who did not (7.6%), alongside decreased rates of mortality and late-onset sepsis—findings that are widely corroborated by various other studies [14]. Cripps *et al.* in their studies also showed that infants receiving probiotics had a lower risk of developing NEC compared with those that did not (32/419 (7.6%) vs. 14/386 (3.6%)) [20]. There was also a reduction in the late-onset sepsis rate and mortality rate. This outcome is also supported by many other studies [21,22]

Despite the overwhelming consensus that probiotics help prevent NEC in premature and low-birth-weight neonates, some literature presents contrasting outcomes. For instance, a study by Dashti et al. found no statistically significant difference in NEC incidence between infants receiving prophylactic probiotics and control subjects [23]. However, this discrepancy highlights the complexities of treating this vulnerable population; the authors acknowledged that their results were likely influenced by several confounding variables, including the specific probiotic strain and dosage utilized, concurrent antibiotic administration, and a generally low baseline incidence of NEC within their neonatal cohort during the study period.

A recent systematic review evaluating 60 trials and over 11,000 infants found that despite methodological flaws and small sample sizes in many studies, administering probiotics to very low birth weight and very preterm neonates can effectively reduce both mortality and the incidence of necrotizing enterocolitis (NEC). However, the treatment does not significantly alter neurodevelopmental outcomes or the overall risk of severe infections, and notably, it appears to offer little to no protective benefit against NEC, death, or severe infection for extremely premature or extremely low-birth-weight infants (under 1.0 kg) [24].

While meta-analyses generally show a reduction in late-onset sepsis with probiotic use, there are legitimate safety concerns regarding the introduction of live microorganisms to highly vulnerable infants. Documented case reports have linked specific strains including *Saccharomyces boulardii, Lactobacillus rhamnosus GG,* and *Bifidobacterium* to probiotic-induced sepsis in both preterm and term infants, particularly those with underlying conditions or those housed near treated patients. Because anaerobic blood cultures are not routinely performed, the true rate of probiotic sepsis may be underreported; nevertheless, the overall drop in clinical sepsis and mortality in major trials suggests that these infections are extremely rare. Beyond direct infection, there are three other major practical concerns. First, commercially available probiotics can be contaminated with pathogenic organisms due to a lack of stringent manufacturing controls. Second, product labels frequently misrepresent the actual bacterial strains and colony counts present, which can vary significantly between different lots of the same product. Finally, horizontal transmission (cross-colonization) frequently occurs in the NICU, resulting in probiotic bacteria colonizing the gastrointestinal tracts of adjacent, untreated infants [15,25].

Meta-analyses and systematic reviews consistently demonstrate that multi-strain probiotic formulations are superior to single-strain preparations in preventing NEC, reducing overall mortality, and enhancing weight gain in very-low-birth-weight (VLBW) infants [7], [19]. Specifically, combinations utilizing both Lactobacillus and Bifidobacterium species yield the most significant clinical benefits [18], [26]. The enhanced efficacy of multi-strain products is hypothesized to stem from synergistic interactions that provide broader colonization resistance and more robust immunomodulation than a single species can achieve alone [7], [25]. Conversely, several clinical trials using single strains have yielded mixed or negative results. For example, the large-scale PiPS trial utilizing *Bifidobacterium breve* BBG-001 failed to show a significant reduction in NEC incidence or sepsis [2], [26]. Similarly, the use of fungal probiotics, such as *Saccharomyces boulardii*, has not demonstrated a significant protective effect against NEC and carries a rare but notable risk of fungal sepsis [7,16,27].

The protective mechanisms of probiotics are highly strain-specific [26]. *Bifidobacterium infantis*: This species has emerged as a particularly potent candidate for NEC prophylaxis. *B. infantis* (such as the EVC001 strain) possesses a unique genetic capacity to completely metabolize human milk oligosaccharides (HMOs). This allows it to thrive in the breastfed infant gut, effectively outcompeting pathogenic bacteria like *Enterobacteriaceae* and *Clostridia* while producing anti-inflammatory metabolites that downregulate Toll-like receptor 4 (TLR4) signaling [21]. *Lactobacillus rhamnosus GG* (LGG) and *Lactobacillus reuteri*: These are also widely utilized and studied. LGG is noted for improving intestinal barrier function, upregulating cytoprotective genes, and exerting anti-inflammatory effects. L. reuteri has been shown to successfully modulate TLR4 and NF-κB signaling pathways while increasing the frequency of Foxp3+ regulatory T cells in the ileum, directly counteracting the hyper-inflammatory cascade of NEC [16,27,28].

The optimal dosage of probiotics for extremely vulnerable preterm infants remains an area of active investigation, with clinical trials utilizing a wide range of doses, typically between $1x10^8$ and $6x10^9$ colony-forming units (CFU) per day [9,15]. Interestingly, subgroup analyses from comprehensive meta-analyses suggest a "less is more" paradigm: daily dosages of $<10^9$ CFU were found to be statistically more effective at reducing the risk of NEC, lowering mortality, and decreasing the length of hospital stay compared to higher dosages ($\geq 10^9$ CFU). Researchers hypothesize that smaller doses (e.g., $1,5x10^9$ CFU/day or

less) may be optimal because they successfully colonize the gut and confer competitive exclusion without overwhelming the profoundly immature and compromised immune systems of VLBW infants [19].

Current Guidelines and Recommendations

Despite extensive research demonstrating the potential benefits of probiotics in preventing necrotizing enterocolitis (NEC), the routine administration of probiotics to preterm infants remains highly controversial. Consequently, current guidelines and recommendations from major professional societies vary significantly, reflecting a balance between the promising efficacy data and persistent concerns regarding product safety, strain specificity, and the lack of pharmaceutical-grade regulation [29].

In 2020, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) issued consensus-based guidelines regarding probiotic use in preterm infants. They emphasized that any probiotic product should adhere to current good manufacturing practices, hospital laboratories must be equipped to detect probiotic bacteraemia, and clinicians must share the potential risks and benefits with parents. The panel conditionally recommended administering *Lactobacillus rhamnosus GG* (LGG) at doses ranging from $1x10^9$ to $6x10^9$ colony-forming units (CFUs), or a combination of *Bifidobacterium infantis, Bifidobacterium lactis, and Streptococcus thermophilus* at doses of $3–3,5x10^8$ CFUs per strain, noting the low certainty of evidence. Concurrently, the American Gastroenterological Association (AGA) recommended specific probiotic strains for preventing necrotizing enterocolitis (NEC) in preterm infants, suggesting combinations such as *Lactobacillus rhamnosus* paired with *Bifidobacterium infantis*, or *Lactobacillus casei* paired with *Bifidobacterium breve*, based on moderate to high-level evidence. In contrast, the 2021 statement from the American Academy of Pediatrics (AAP) advised against universal probiotic administration in preterm infants, particularly those weighing less than 1 kg at birth, citing concerns over the absence of FDA-approved pharmaceutical-grade products in the U.S., potential risks like sepsis, and inconsistent efficacy and safety data [29]. Across these varying guidelines, a clear consensus emerges regarding how hospitals should handle probiotic administration if they choose to proceed.

Future Directions and Research Gaps

At present, the therapeutic scope of commercially available probiotics is largely confined to a small number of bacterial species and a single fungal strain. Moving forward, as diagnostic and sequencing technologies advance to better elucidate the complex differences in intestinal archaea and viruses (including both bacteriophages and human viruses) between states of health and disease, it is highly anticipated that the therapeutic landscape will expand to incorporate a diverse array of next-generation probiotic agents spanning multiple biological kingdoms. Furthermore, the clinical decision to implement routine probiotic protocols in neonatal intensive care units remains complex and highly individualized. This multifaceted decision must account for several institutional variables, primarily a center's existing baseline incidence of necrotizing enterocolitis (NEC) and the concurrent implementation of alternative prophylactic strategies, most notably the promotion of an exclusive human milk-based diet.

CONCLUSION

Preterm neonates with necrotizing enterocolitis (NEC) experience high rates of morbidity and mortality, leading to prolonged hospital stays and increased healthcare costs. Numerous studies suggest that probiotic treatment may reduce the risk of NEC in preterm infants. However, the optimal type, strain, and dosage of probiotics for NEC prevention remain subjects of debate. Further research is needed to better understand the effects of specific probiotic strains on high-risk neonatal populations. While promising evidence supports the use of probiotics to lower the risk of NEC in very low birth weight (VLBW) infants, no

specific probiotic has been conclusively identified as the most effective. Some studies highlight the potential benefits of *Lactobacillus* and *Bifidobacterium* species, but definitive conclusions cannot yet be drawn. Until robust data establishes the safest and most effective probiotic strains, dosages, and timing for administration, the implementation of probiotic prophylaxis in infants at risk for NEC will remain uncertain.

REFERENCES

[1] Thakkar HS, Lakhoo K. Necrotizing enterocolitis. Surgery (Oxford). 2016;34(12):617-620. doi: 10.1016/j.mpsur.2022.09.007

[2] Warner BB, Tarr PI. Necrotizing enterocolitis and preterm infant gut bacteria. InSeminars in Fetal and Neonatal Medicine. WB Saunders 2016;21(6):394-399.

[3] Olson JK, Rager TM, Navarro JB, Mashburn-Warren L, Goodman SD, Besner GE. Harvesting the benefits of biofilms: A novel probiotic delivery system for the prevention of necrotizing enterocolitis. Journal of pediatric surgery. 2016;51(6):936-941.

[4] Scheese DJ, Sodhi CP, Hackam DJ. New insights into the pathogenesis of necrotizing enterocolitis and the dawn of potential therapeutics. Semin pediatr surg. 2023;32(3):151309

[5] Sampath V, Martinez M, Caplan M, Underwood MA, Cuna A. Necrotizing enterocolitis in premature infants—a defect in the brakes? Evidence from clinical and animal studies. Mucosal immunology. 2023;16(2):208-220.

[6] Lau CS, Chamberlain RS. Probiotic administration can prevent necrotizing enterocolitis in preterm infants: a meta-analysis. Journal of pediatric surgery. 2015;50(8):1405-1412.

[7] Jiang T, Zhang H, Xu X, Li H, Yang J. Mixed probiotics decrease the incidence of stage II-III necrotizing enterocolitis and death: a systematic review and meta-analysis. Microbial pathogenesis. 2020;138:103794.

[8] Frost BL, Caplan MS. Necrotizing enterocolitis: pathophysiology, platelet-activating factor, and probiotics. Semin pediatr surg. 2013; 22(2):88-93.

[9] Martin CR, Walker WA. Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. Semin pediatr surg. 2008;32(2):127-137.

[10] Tian B, Zhang Y, Deng C, Guo C. Efficacy of probiotic consortium transplantation on experimental necrotizing enterocolitis. Journal of Surgical Research. 2022;279:598-610.

[11] Samara J, Moossavi S, Alshaikh B, Ortega VA, Pettersen VK, Ferdous T, Hoops SL, Soraisham A, Vayalumkal J, Dersch-Mills D, Gerber JS. Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. Cell host & microbe. 2022;30(5):696-711.

[12] Tarhani F, Nezami A. Role of probiotics in treatment of congenital heart disease and necrotizing enterocolitis. PharmaNutrition. 2019;8:100144.

[13] Chang HY, Lin CY, Chiau JS, Chang JH, Hsu CH, Ko MH, Lee HC. Probiotic supplementation modifies the gut microbiota profile of very low birth weight preterm infants during hospitalization. Pediatrics & Neonatology. 2024;65(1):55-63.

[14] Zhou J, Yang M, Wang F, Liu S, Hei M, Jiang M. Assessment of food supplements for the prevention of necrotizing enterocolitis in preterm neonates: A systematic review and network meta-analysis. Computers in Biology and Medicine. 2023;167:107601.

[15] Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin pediatr surg. 2018;27(1):39-46.

[16] Liu D, Shao L, Zhang Y, Kang W. Safety and efficacy of Lactobacillus for preventing necrotizing enterocolitis in preterm infants. International Journal of Surgery. 2020;76:79-87.

[17] Tobias J, Olyaei A, Laraway B, Jordan BK, Dickinson SL, Golzarri-Arroyo L, Fialkowski E, Owora A, Scottoline B. Bifidobacterium longum subsp. infantis EVC001 administration is associated with a significant reduction in the incidence of necrotizing enterocolitis in very low birth weight infants. The Journal of pediatrics. 2022;244:64-71.

[18] Gómez–Rodríguez G, Amador-Licona N, Daza-Benítez L, Barbosa-Sabanero G, Carballo-Magdaleno D, Aguilar-Padilla R, González-Ramirez E. Single strain versus multispecies probiotic on necrotizing enterocolitis and faecal IgA levels in very low birth weight preterm neonates: a randomized clinical trial. Pediatrics & Neonatology. 2019;60(5):564-569.

[19] Sun J, Marwah G, Westgarth M, Buys N, Ellwood D, Gray PH. Effects of probiotics on necrotizing enterocolitis, sepsis, intraventricular hemorrhage, mortality, length of hospital stay, and weight gain in very preterm infants: a meta-analysis. Advances in nutrition. 2017;8(5):749-763.

[20] Cripps EK, Dargaville PA, De Paoli AG. Impact of probiotic administration on the incidence of necrotising enterocolitis: A single-centre cohort study. Journal of Paediatrics and Child Health. 2023;59(5):760-765.

[21] Tobias J, Olyaei A, Laraway B, Jordan BK, Dickinson SL, Golzarri-Arroyo L, Fialkowski E, Owora A, Scottoline B. Bifidobacterium longum subsp. infantis EVC001 administration is associated with a significant reduction in the incidence of necrotizing enterocolitis in very low birth weight infants. The Journal of pediatrics. 2022;244:64-71.

[22] Robertson C, Savva GM, Clapuci R, Jones J, Maimouni H, Brown E, Minocha A, Hall LJ, Clarke P. Incidence of necrotising enterocolitis before and after introducing routine prophylactic Lactobacillus and Bifidobacterium probiotics. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2020;105(4):380-386.

[23] Dashti AS, Afjeh SA, Basiry A, Shirvani F, Seifi K, Taheri ZM. Prophylactic probiotics for prevention of necrotizing enterocolitis (NEC) in low birth weight neonates. Arch Pediatr Infect Dis. 2014;2:175-179.
[24] Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. Cochrane Database Syst Rev. 2023;7(7):CD005496.
[25] Underwood MA. Probiotics and the prevention of necrotizing enterocolitis. Journal of pediatric surgery. 2019;54(3):405-412.
[26] Johnson-Henry KC, Abrahamsson TR, Wu RY, Sherman PM. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. Advances in nutrition. 2016;7(5):928-37.
[27] Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial–. The American journal of clinical nutrition. 2011;93(1):81-86.
[28] Underwood MA. Impact of probiotics on necrotizing enterocolitis. Semin Perinatol. 2017;41(1):41-51. doi:10.1053/j.semperi.2016.09.017
[29] Barbian ME, Patel RM. Probiotics for prevention of necrotizing enterocolitis: Where do we stand?. Semin Perinatol. 2023;47(1):151689. doi:10.1016/j.semperi.2022.151689