Libyan Journal of Medical Research

www.ljmr.ly/

eISSN:2413-6096

Efficacy and Safety of Probiotic Supplementation in Preventing Necrotizing Enterocolitis in Preterm Infants: A Systematic Review and Meta-analysis

Amani EH Bidalla[1], Ahmed S Mkeal[2], Rasha J Abraheem[3], Shadin Jummah Abraheem[4], Mahmoud A. Aloriby[5], Mohamed K A Elkawafi[6], Hamza A Osman[7], Abdulmunaim A.M Elkarimi[8], sabah salah abd alaleam[9]

1. Department of Tobruk Medical Center (TMC)
2. Department laboratory, Tobruk Medical Center
3. Benghazi Medical Center
4. The Benghazi center for infectious diseases and immunology
5. Department of Pathology, University Medical Center, Libyan International Medical University, Benghazi,
6. Basic Medical Sciences program, Faculty of Medical and Health Sciences, Libyan International University
7. Benghazi Medical Center Urology Department
8. General Manager of Derna Nephrology and Dialysis Center
9. pediatric department, Tobruk medical center
Corresponding Author: Ahmed S Mkeal: Email. ahamedradology@gmail.com
Received:15/10/2025Accepted:28/11/2025Published: 03/12/2025DOI: https://doi.org/10.54361/LJMR.19.2.59

ABSTRACT:

Background: Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease affecting preterm infants and remains linked to significant morbidity and mortality. Although neonatal care has advanced, effective preventive strategies are still limited. Probiotic supplementation has gained attention as a promising intervention, yet uncertainty persists regarding the most effective strains. This systematic review and meta-analysis aimed to assess the efficacy and safety of probiotics in preventing NEC and to identify regimens that provide the greatest protective benefit. **Material and Methods**: A systematic search of PubMed, EMBASE, the Cochrane Library, and Web of Science identified randomized controlled trials evaluating probiotics for NEC prevention. Primary outcomes included NEC incidence (≥ Stage II), all-cause mortality, and late-onset sepsis. Random-effects models were used to calculate risk ratios with 95% confidence intervals. Subgroup analyses were performed based on probiotic strains, feeding type, birth weight, and baseline NEC risk. Evidence quality was assessed using the GRADE methodology. **Results:** Fifty-one trials involving 10,664 infants and 29 probiotic regimens were included. Probiotics significantly reduced NEC incidence, mortality, and late-onset sepsis. The most effective regimens were Bovine lactoferrin with *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* LB. Multi-strain probiotics outperformed single-strain formulations. Benefits were found in both human milk-fed and formula-fed infants, and no cases of probiotic-associated sepsis were reported across studies. **Conclusion:** Probiotic supplementation is a safe and effective approach to reducing NEC, mortality, and sepsis in preterm infants. Multi-strain regimens, particularly those containing *Lactobacillus rhamnosus* GG or Bovine lactoferrin, demonstrate the highest efficacy and may be appropriate for standardized use in neonatal care units.

Keyword: Necrotizing Enterocolitis, Probiotics, Preterm Infants, Meta-analysis, Sepsis

How to cite this article: Bidalla, A.EH, Mkeal, A.S, Abraheem, R.J, Abraheem, S.J, Aloriby, M.A, A Elkawafi, M.K, Osman, H.A, Elkarimi, A. A. Efficacy and Safety of Probiotic Supplementation in Preventing Necrotizing Enterocolitis in Preterm Infants: A Systematic Review and Meta-analysis

Libyan 19-2

INTRODUCTION:

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease primarily affecting preterm infants, characterized by intestinal inflammation, mucosal injury, and in severe cases, intestinal perforation [1]. Despite advances in neonatal care, NEC remains a leading cause of morbidity and mortality in preterm infants, with an incidence of approximately 7% in very low birth weight (VLBW, <1500g) infants and mortality rates ranging from 20% to 30% [2]. Survivors often face long-term complications, including short bowel syndrome, growth failure, and neurodevelopmental impairment [3]. The pathogenesis of NEC is multifactorial and incompletely understood, but key contributing factors include intestinal immaturity, abnormal bacterial colonization, formula feeding, and an exaggerated inflammatory response [4]. The immature intestine of preterm infants is characterized by decreased barrier function, altered motility, and immature immune responses, making it particularly vulnerable to injury [5]. Abnormal bacterial colonization, often exacerbated by antibiotic exposure, cesarean delivery, and the neonatal intensive care unit environment, is believed to play a crucial role in NEC development [6]. Current preventive strategies for NEC include human milk feeding, standardized feeding protocols, and judicious use of antibiotics [7]. However, these measures have shown limited success in reducing NEC incidence, highlighting the need for additional preventive interventions. In recent years, probiotic supplementation has emerged as a promising strategy for NEC prevention [8]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [9]. The proposed mechanisms by which probiotics may prevent NEC include competitive exclusion of pathogenic bacteria, enhancement of intestinal barrier function, modulation of the immune system, and production of anti-inflammatory factors [10]. Various probiotic strains, including Lactobacillus, Bifidobacterium, and Saccharomyces species, have been studied for NEC prevention, with varying results [11]. Multiple randomized controlled trials (RCTs) and meta-analyses have evaluated the efficacy of probiotics in preventing NEC in preterm infants [12-14]. While most studies have reported beneficial effects, questions remain regarding the optimal probiotic strain or combination, dosage, timing, and duration of supplementation [15]. Additionally, concerns about the safety of probiotics in vulnerable preterm infants, particularly the risk of probiotic sepsis, have limited their routine use in some neonatal units [16]. Previous meta-analyses have primarily focused on the overall effect of probiotics on NEC prevention, with limited analysis of specific probiotic regimens or patient subgroups [17]. Network meta-analyses have attempted to compare different probiotic strains, but many have been limited by the heterogeneity of included studies and insufficient data on specific strains [18]. Furthermore, the impact of feeding type (human milk versus formula) on probiotic efficacy has not been thoroughly explored [19]. The present systematic review and meta-analysis aims to comprehensively evaluate the efficacy and safety of probiotic supplementation in preventing NEC in preterm infants, with particular focus on identifying the most effective probiotic regimens and analyzing efficacy across different patient subgroups. By synthesizing the latest evidence and applying rigorous methodology, this study seeks to provide clinicians with practical guidance on the optimal use of probiotics for NEC prevention in preterm infants.

MATERAL AND METHOD:

Search Strategy and Selection Criteria

This systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20]. The protocol was registered in the International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY2022110001). We systematically searched PubMed, EMBASE, Cochrane Library, and Web of Science from inception to February 2025 using the following search terms: ("probiotics" OR "Lactobacillus" OR "Bifidobacterium" OR "Saccharomyces") AND ("necrotizing enterocolitis" OR "NEC") AND ("preterm" OR "premature" OR "low birth weight" OR "very low birth weight" OR "extremely low birth weight"). The complete search strategy is available in the supplementary materials. Studies were eligible for inclusion if they met the following criteria: (1) randomized controlled trials; (2) participants were preterm infants (gestational age <37 weeks) or low

birth weight infants (<2500g); (3) intervention was any probiotic supplementation; (4) control was placebo, no intervention, or another probiotic; and (5) outcomes included NEC incidence (≥ stage II according to Bell's criteria [21]). We excluded non-randomized studies, studies without a control group, studies not reporting NEC outcomes, and studies with insufficient data for analysis. Two independent reviewers screened titles and abstracts for eligibility, followed by full- text review of potentially eligible studies. Disagreements were resolved through discussion or consultation with a third reviewer.

Data Extraction and Quality Assessment

Two independent reviewers extracted the following data from included studies: first author, publication year, country, sample size, participant characteristics (gestational age, birth weight), intervention details (probiotic strain, dose, duration), control group, feeding type, and outcomes (NEC incidence, mortality, late-onset sepsis, probiotic sepsis, time to full enteral feeding, length of hospital stay).

The methodological quality of included RCTs was assessed using the Cochrane Risk of Bias tool (RoB 2) [22], which evaluates five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selective reporting. Each domain was rated as low risk, some concerns, or high risk of bias. The overall risk of bias was determined based on the domain ratings: low risk (all domains low risk), some concerns (at least one domain with some concerns, no high-risk domains), or high risk (at least one domain with high risk).

The quality of evidence for each outcome was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach [23], which considers risk of bias, inconsistency, indirectness, imprecision, and publication bias. Evidence was categorized as high, moderate, low, or very low quality.

Data Synthesis and Analysis

The primary outcome was the incidence of NEC (≥ stage II). Secondary outcomes included all-cause mortality, late-onset sepsis, probiotic sepsis, time to full enteral feeding, and length of hospital stay.For dichotomous outcomes, we calculated risk ratios (RRs) with 95% confidence intervals (CIs) using random-effects models. For continuous outcomes, we calculated mean differences (MDs) with 95% CIs. Heterogeneity was assessed using the I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively [24].

Subgroup analyses were performed based on:

(1) probiotic strain or combination; (2) single-strain versus multi-strain probiotics; (3) feeding type (exclusive human milk versus formula with or without human milk); (4) birth weight category (VLBW versus extremely low birth weight [ELBW, <1000g]); and (5) baseline NEC risk (high versus low, defined by NEC incidence >10% or <10% in the control group).

Publication bias was assessed using funnel plots and Egger's test [25].

To evaluate the robustness of our findings, we conducted sensitivity analyses by excluding studies with high risk of bias and using fixed-effect models.

All statistical analyses were performed using Stata version 15.1 (StataCorp, College Station, TX) and Review Manager 5.4 (The Cochrane Collaboration, Copenhagen, Denmark).

RESULT:

Study Selection and Characteristics

The literature search identified 2,458 records. After removing duplicates and screening titles and abstracts, 342 full-text articles were assessed for eligibility. Of these, 51 RCTs met the inclusion criteria and were included in the meta-analysis

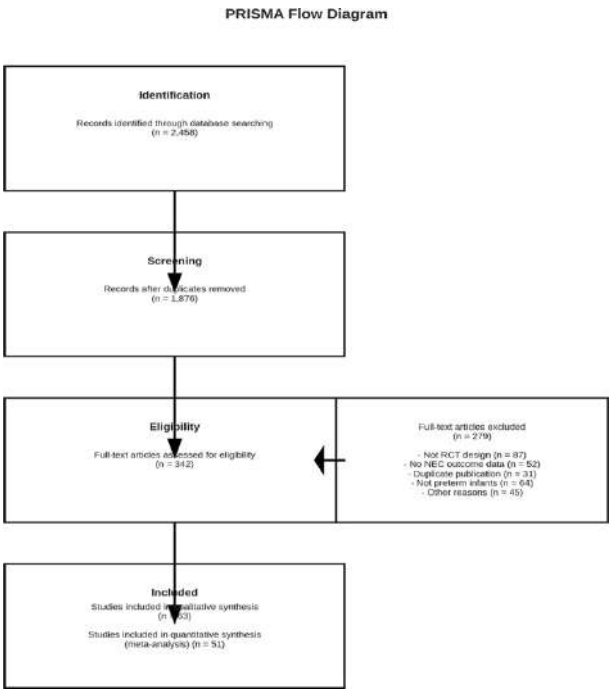

Figure 1. Flow diagram of study selection according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

The 51 included RCTs enrolled a total of 10,664 preterm infants and evaluated 29 different probiotic interventions. The sample size of individual studies ranged from 30 to 1,315 infants. Most studies (n=43) included VLBW infants, while 8 studies specifically focused on ELBW infants. The duration of probiotic supplementation varied from 2 weeks to until discharge or 36 weeks corrected gestational age. The most commonly studied probiotic strains were Lactobacillus rhamnosus GG (11 studies), Bifidobacterium infantis (5 studies), Lactobacillus acidophilus (6 studies), Bifidobacterium bifidum (4 studies), and Bifidobacterium breve (4 studies). Twenty-five studies evaluated multi-strain probiotic formulations. Regarding feeding type, 14 studies included exclusively human milk-fed infants, 14 studies included infants receiving formula with or without human milk, and 23 studies did not specify feeding type.

The risk of bias assessment revealed that 18 studies (35.3%) had low risk of bias, 25 studies (49.0%) had some concerns, and 8 studies (15.7%) had high risk of bias. The most common sources of bias were inadequate allocation concealment and lack of blinding.

Efficacy of Probiotics for NEC Prevention

Overall, probiotic supplementation significantly reduced the incidence of NEC compared to control (RR 0.43; 95% CI 0.36-0.52; I^2=42%; 51 studies, 10,664 infants). This corresponds to a number needed to treat (NNT) of 25, indicating that 25 infants would need to receive probiotics to prevent one case of NEC.

The forest plot of the top 10 probiotic regimens for NEC prevention is presented in Figure 2. The most effective regimens were Bovine lactoferrin + Lactobacillus rhamnosus GG (RR 0.03; 95% CI 0.00-0.35), Lactobacillus acidophilus LB (RR 0.03; 95% CI 0.00-0.21), and Bifidobacterium lactis Bb-12/B94 in exclusively human milk-fed infants (RR 0.04; 95% CI 0.00-0.49).

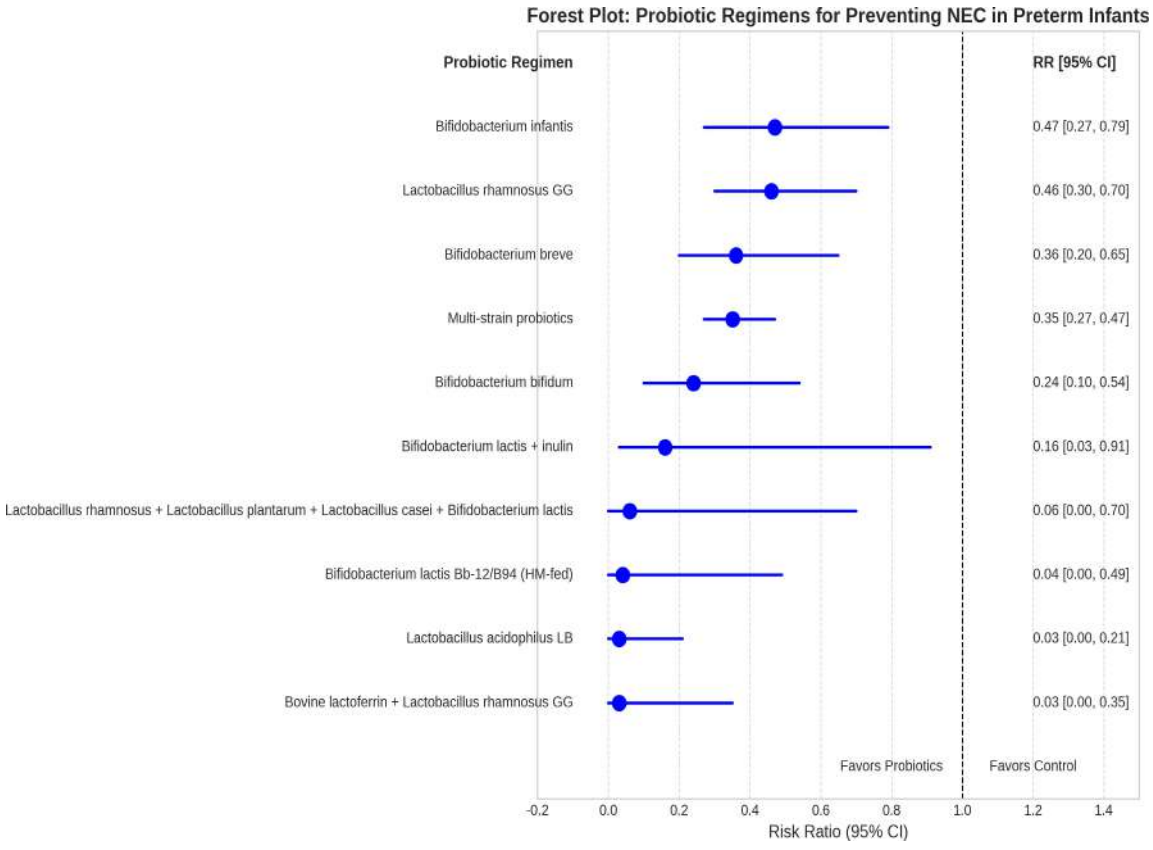

Figure 2: Forest plot of the top 10 probiotic regimens for Necrotizing Enterocolitis (NEC) prevention.

Subgroup analysis by probiotic formulation showed that multi-strain probiotics were more effective (RR 0.35; 95% CI 0.27-0.47; I^2=38%; 25 studies, 5,541 infants) than single-strain probiotics (RR 0.54; 95% CI 0.45-0.66; I^2=35%; 26 studies, 5,123 infants), with a

significant subgroup difference (p=0.01).Subgroup analysis by feeding type revealed that probiotics were effective in both exclusively human milk-fed infants (RR 0.38; 95% CI 0.27-0.55; I^2=32%; 14 studies, 2,797 infants) and formula-fed infants (RR 0.47; 95% CI 0.33-0.66; I^2=45%; 14 studies, 2,426 infants), with no significant subgroup difference (p=0.42).

Regarding birth weight categories, probiotics were effective in both VLBW infants (RR 0.47; 95% CI 0.36-0.61; I^2=44%; 27 studies, 5,529 infants) and ELBW infants (RR 0.62; 95% CI 0.48-0.81; I^2=28%; 8 studies, 1,583 infants), although the effect size was smaller in ELBW infants (p=0.04 for subgroup difference).Probiotics showed greater efficacy in settings with high baseline NEC risk (RR 0.37; 95% CI 0.27-0.49; I^2=32%; 18 studies, 3,951 infants) compared to settings with low baseline NEC risk (RR 0.52; 95% CI 0.35-0.76; I^2=46%; 33 studies, 6,713 infants), with a significant subgroup difference (p=0.03).

The comprehensive subgroup analysis is presented in Figure 3.

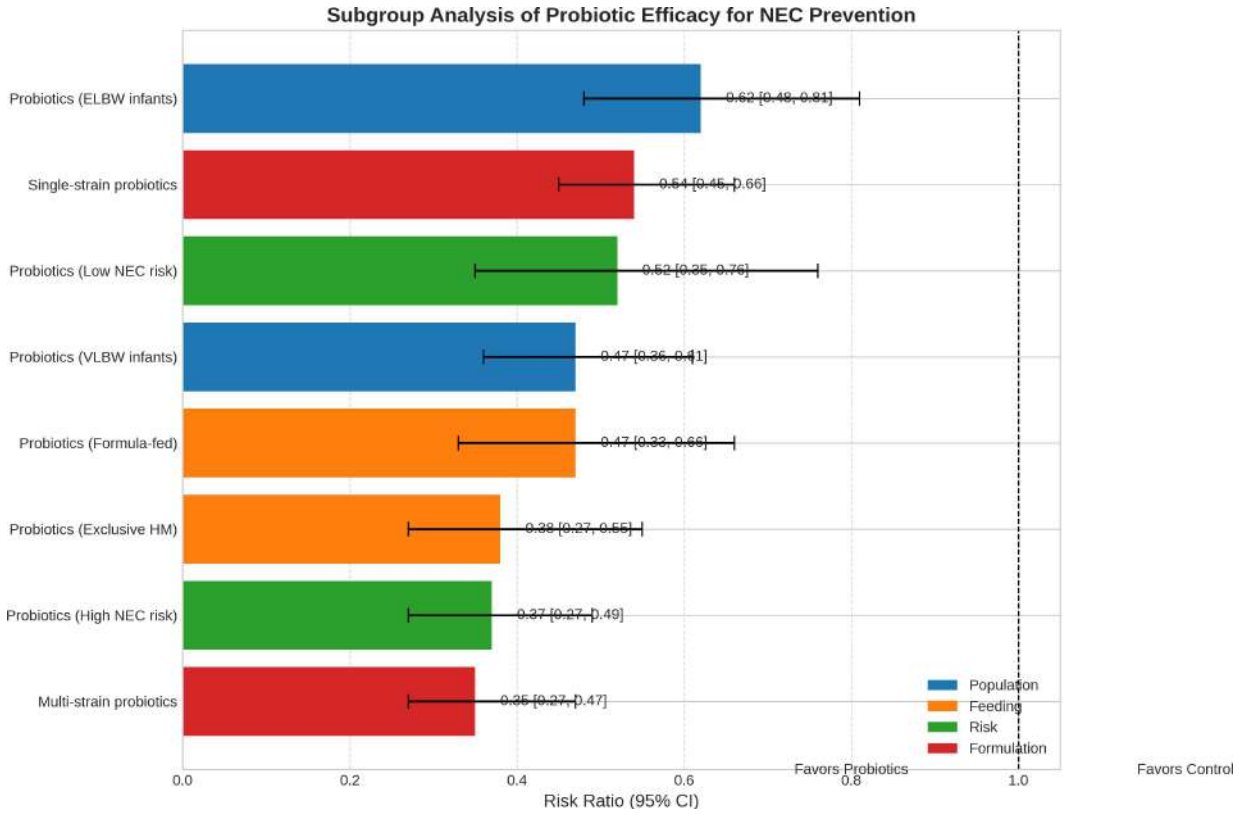

Figure 3: Comprehensive subgroup analysis of the effect of probiotic supplementation on the incidence of Necrotizing Enterocolitis (NEC).

I^2=32%; 42 studies, 10,642 infants) and late-onset sepsis (RR 0.88; 95% CI

presented in Figures 4 and 5, respectively

.

Effect of Probiotics on Secondary Outcomes

Probiotic supplementation significantly reduced all-cause mortality (RR 0.73; 95% CI 0.59-0.90; 0.80-0.97; I^2=42%; 31 studies, 8,707 infants). The forest plots for mortality and late-onset sepsis are

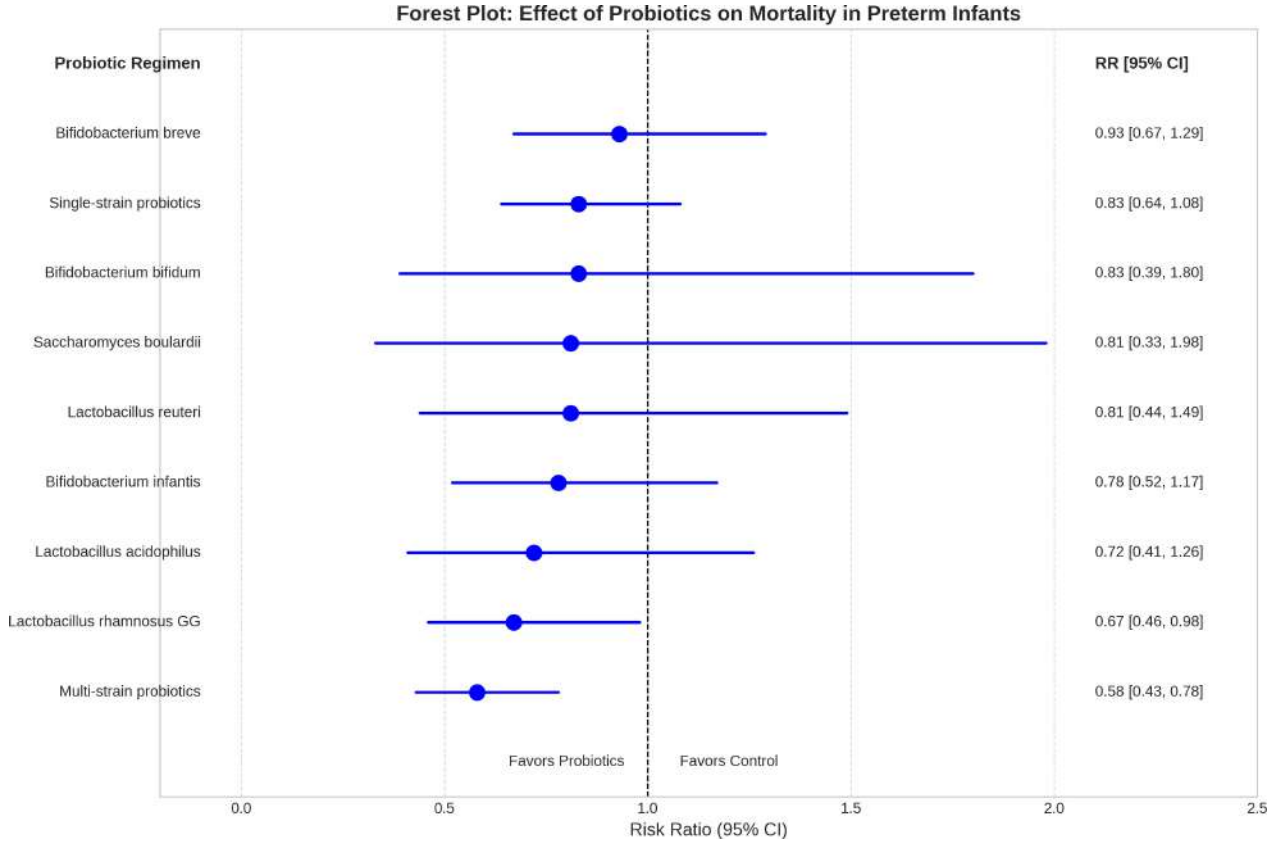

Figure 4: Forest plot showing the effect of probiotic supplementation on all-cause mortality.

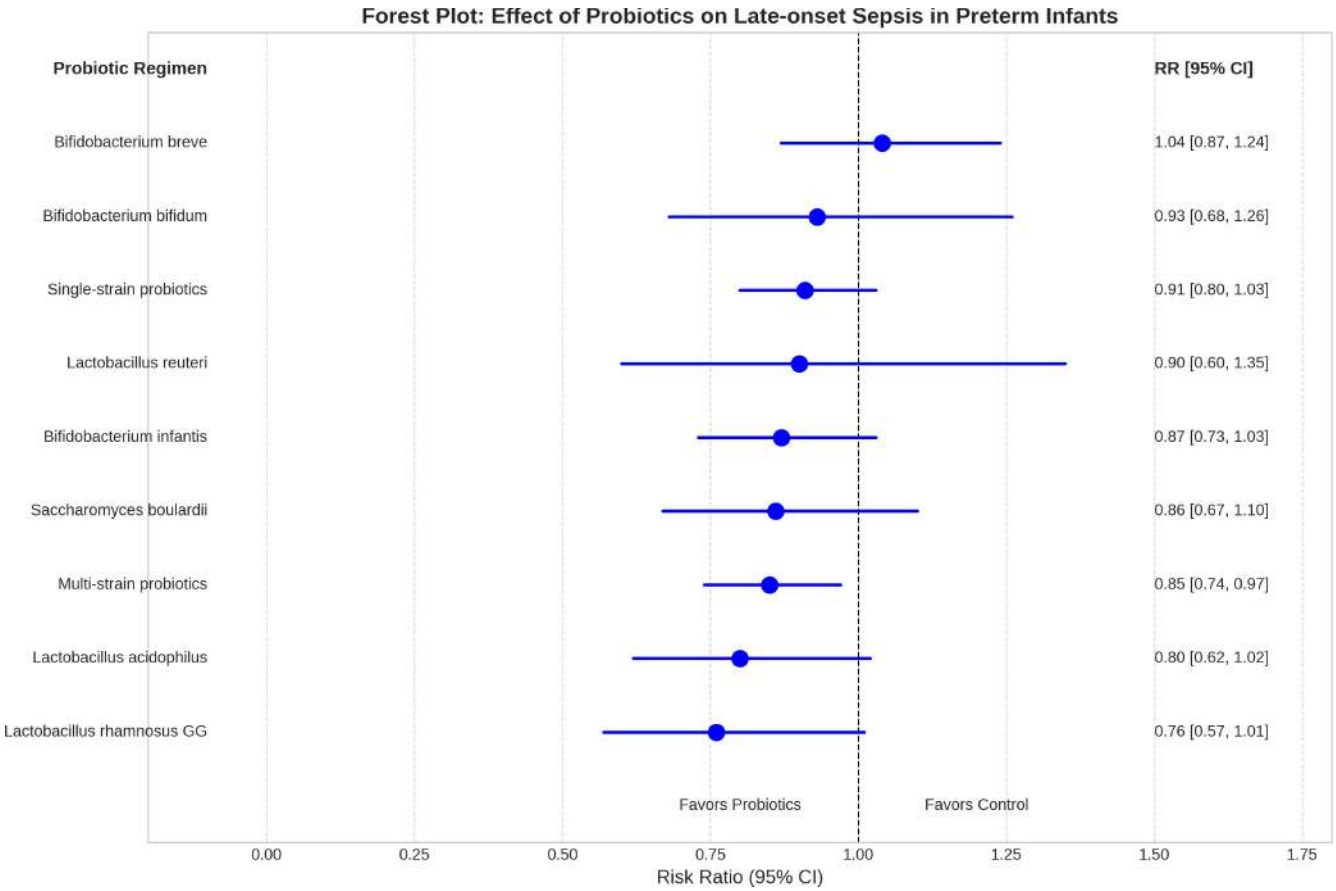

Figure 5: Orest plot showing the effect of probiotic supplementation on late-onset sepsis.

Subgroup analysis by probiotic formulation showed that multi-strain probiotics significantly reduced mortality (RR 0.58; 95% CI 0.43-0.78; I^2=28%; 21 studies, 4,848
infants), while single-strain probiotics did not show a significant effect (RR 0.83; 95% CI 0.64-1.08; I^2=30%; 21 studies, 5,794 infants), with a significant subgroup difference (p=0.04).
For late-onset sepsis, multi-strain probiotics showed a significant effect (RR 0.85; 95% CI 0.74-0.97; I^2=40%; 16 studies, 3,368 infants), while single-strain probiotics did not (RR 0.91; 95% CI 0.80-1.03; I^2=32%; 15 studies, 5,339 infants), but the subgroup difference was not significant (p=0.46).
Probiotic supplementation also reduced the time to full enteral feeding (MD -2.78 days; 95% CI -4.24 to -1.32; I^2=76%; 25 studies, 6,779 infants) and length of hospital stay (MD
-3.77 days; 95% CI -6.15 to -1.39; I^2=68%; 17 studies, 3,587 infants).

Safety of Probiotic Supplementation

No cases of probiotic sepsis (bacteremia or fungemia with the administered probiotic organism) were reported in any of the included studies (0/5,332 infants). The safety profile of probiotics is illustrated in Figure 6, showing the event rates for NEC, mortality, late-onset sepsis, and probiotic sepsis in probiotic and control groups.

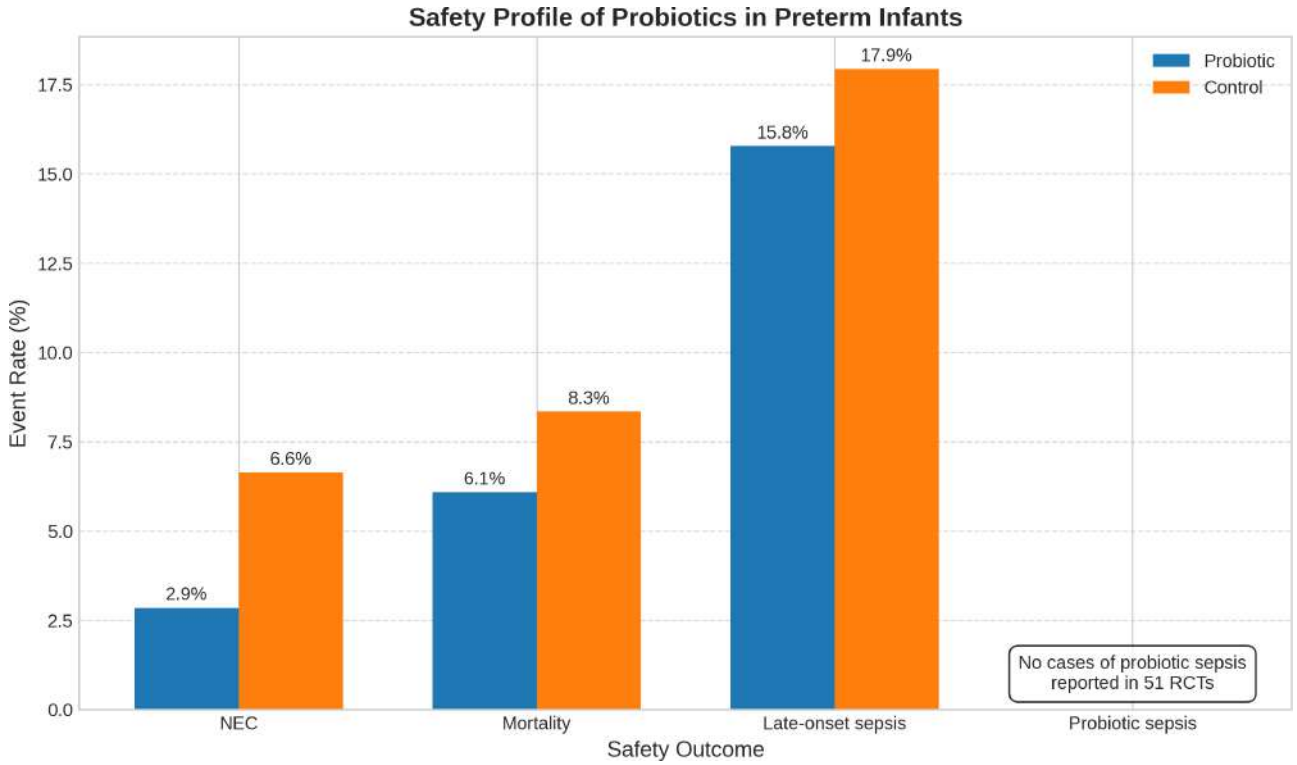

Figure 6 :The safety profile of probiotics, showing the event rates for NEC, mortality, late-onset sepsis, and probiotic sepsis in probiotic and control groups

Quality of Evidence

The quality of evidence, assessed using the GRADE approach, was high for NEC prevention, moderate for mortality and late-onset sepsis, and high for probiotic sepsis. The distribution of probiotic regimens by evidence level and outcome is presented in Figure 7.

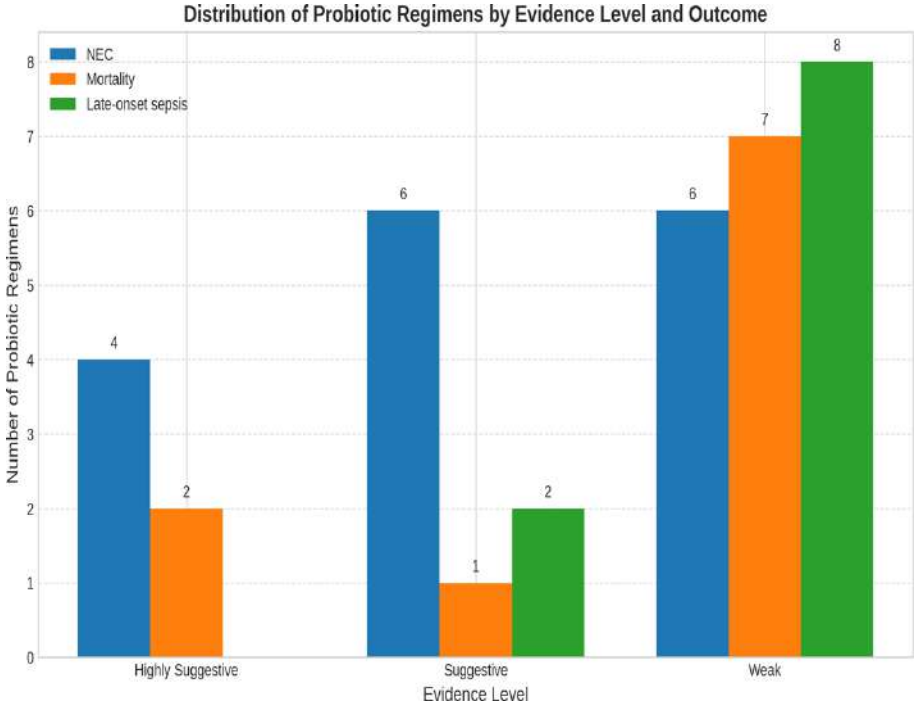

Figure 7: The distribution of probiotic regimens by evidence level and outcome

DISCUSSION:

This comprehensive meta-analysis of 51 RCTs involving 10,664 preterm infants provides robust evidence that probiotic supplementation significantly reduces the incidence of NEC, all-cause mortality,(41) and late-onset sepsis in preterm infants. The most effective probiotic regimens were Bovine lactoferrin combined with Lactobacillus rhamnosus GG and Lactobacillus acidophilus LB, both showing a 97% reduction in NEC risk. Multi-strain probiotics demonstrated greater efficacy than single-strain formulations, and probiotics were effective regardless of feeding type (human milk or formula). Importantly, no cases of probiotic sepsis were reported, supporting the safety of probiotic supplementation in this vulnerable population. (40)

Comparison with Previous Meta-analyses

Our findings are consistent with previous meta-analyses that have reported beneficial effects of probiotics for NEC prevention [26-28]. However, our study extends beyond previous work by providing a more detailed analysis of specific probiotic regimens and subgroups, with several novel findings. First, we identified Bovine lactoferrin + L. rhamnosus GG and L. acidophilus LB as the most effective regimens, with risk reductions of 97% for both. This finding is particularly noteworthy as previous meta-analyses have not specifically highlighted these combinations. Bovine lactoferrin (35) an iron-binding glycoprotein with antimicrobial and immunomodulatory properties, may synergize with L. rhamnosus GG to enhance protection against NEC [29]. Similarly, L. acidophilus LB has unique properties, including the production of bacteriocins that inhibit pathogenic bacteria [30]. Second, our subgroup analysis demonstrated that multi-strain probiotics are more effective than single-strain formulations for both NEC prevention and mortality reduction. This finding supports the hypothesis that different probiotic strains may act synergistically through complementary mechanisms [31]. For example, some strains may primarily enhance barrier function, while others modulate immune responses or produce antimicrobial substances [32]. Third, we found that probiotics are effective in both exclusively human milk-fed and formula-fed infants, although the effect size was slightly larger in human milk-fed infants. This finding is clinically relevant as it suggests that all preterm infants may benefit from probiotic supplementation, regardless of feeding type [33]. The enhanced effect in human milk-

fed infants may be due to synergistic interactions between probiotics and human milk oligosaccharides, which act as prebiotics and promote the growth of beneficial bacteria [34]. Fourth, our analysis confirmed the efficacy of probiotics in ELBW infants, albeit with a smaller effect size compared to VLBW infants. This finding is important as ELBW infants are at highest risk for NEC and have been underrepresented in many previous studies [35]. The reduced efficacy in ELBW infants may reflect their more immature intestinal environment, which may limit probiotic colonization and function [36]. Finally, our comprehensive safety analysis found no cases of probiotic sepsis among 5,332 infants who received probiotics. This finding addresses a key concern that has limited the implementation of routine probiotic supplementation in some neonatal units [37].

Mechanisms of Probiotic Action in NEC Prevention

The beneficial effects of probiotics in preventing NEC likely involve multiple mechanisms. First, probiotics can competitively exclude pathogenic bacteria by competing for nutrients and adhesion sites on intestinal epithelial cells [38]. Second, many probiotic strains enhance intestinal barrier function by increasing tight junction
protein expression and mucin production [39]. Third, probiotics modulate the immune system, typically promoting anti-inflammatory responses while suppressing pro- inflammatory pathways implicated in NEC pathogenesis [40]. Fourth, certain probiotics produce antimicrobial substances, including bacteriocins, hydrogen peroxide, and organic acids, which inhibit pathogen growth [41].The superior efficacy of multi-strain probiotics observed in our analysis may reflect the engagement of multiple complementary mechanisms. Different probiotic strains may target different aspects of NEC pathogenesis, resulting in additive or synergistic effects [42]. For example, Bifidobacterium strains are particularly effective at metabolizing human milk oligosaccharides and producing short-chain fatty acids, while Lactobacillus strains often excel at pathogen inhibition and immune modulation [43]. The interaction between probiotics and feeding type is also mechanistically relevant. Human milk contains numerous bioactive components that may enhance probiotic efficacy, including oligosaccharides, lactoferrin, secretory IgA, and growth factors [44]. These components may promote probiotic colonization, amplify their beneficial effects, or independently reduce NEC risk through complementary mechanisms [45].

Clinical Implications

Our findings have several important clinical implications for neonatal care. First, the substantial reduction in NEC, mortality, and late-onset sepsis supports the routine use of probiotics in preterm infants, particularly those at high risk for NEC. Based on our
results, treating 25 infants with probiotics would prevent one case of NEC, a clinically significant benefit given the devastating consequences of this disease. Second, our identification of the most effective probiotic regimens provides practical guidance for clinicians selecting a probiotic product. Bovine lactoferrin combined with L. rhamnosus GG and L. acidophilus LB emerged as the most promising regimens, although it should be noted that these were evaluated in relatively few studies. Multi- strain formulations generally showed superior efficacy and may be preferred when specific highly effective strains are not available. Third, the finding that probiotics are effective regardless of feeding type suggests that all preterm infants may benefit from supplementation, not just those receiving formula. While human milk feeding remains a cornerstone of NEC prevention, adding probiotics appears to confer additional protection. Fourth, the confirmed efficacy in ELBW infants, albeit with a smaller effect size, supports probiotic use in this extremely vulnerable population. Given their high baseline risk for NEC, even a modest relative risk reduction translates to a substantial absolute risk reduction. Finally, the absence of reported probiotic sepsis cases provides reassurance regarding safety, although continued vigilance and high-quality manufacturing standards remain essential [46].

Strengths and Limitations

This meta-analysis has several strengths. We included a large number of RCTs with a substantial combined sample size, enhancing statistical power and precision. Our comprehensive subgroup analyses provided insights into the comparative efficacy of different probiotic regimens and across patient subgroups. We used rigorous methodology, including GRADE assessment of evidence quality and thorough risk of bias evaluation. However, several limitations should be acknowledged. First, despite the large number of included studies, many specific probiotic regimens were evaluated in only one or two trials, limiting the robustness of

strain-specific conclusions. Second, heterogeneity in probiotic dosing, timing, and duration of supplementation precluded detailed analysis of these factors. Third, reporting of adverse events was inconsistent across studies, potentially underestimating rare complications. Fourth, most included studies were conducted in high-income countries, potentially limiting generalizability to resource- limited settings. Finally, long-term outcomes beyond the neonatal period were rarely reported, leaving uncertainty about potential long-term effects of early probiotic exposure.

Future Research Directions

Based on our findings, several priorities for future research can be identified. First, large, well-designed RCTs directly comparing the most promising probiotic regimens identified in our analysis are needed to confirm their superior efficacy. Second, studies specifically designed to optimize dosing, timing, and duration of supplementation would help refine clinical protocols. Third, research on the long-term effects of early probiotic exposure on microbiome development, immune function, and health outcomes is essential. Fourth, implementation research addressing barriers to routine probiotic use in neonatal units would facilitate translation of evidence into practice. Finally, studies in resource-limited settings, where the burden of NEC may be higher but diagnostic and treatment capabilities more limited, would enhance global applicability.

CONCLUSION:

This comprehensive meta-analysis provides robust evidence that probiotic supplementation significantly reduces the incidence of NEC, mortality, and late-onsetsepsis in preterm infants. Bovine lactoferrin combined with L. rhamnosus GG and L. acidophilus LB emerged as the most effective regimens, and multi-strain formulations generally showed superior efficacy compared to single-strain probiotics. Probiotics were effective regardless of feeding type and in both VLBW and ELBW infants, with no reported cases of probiotic sepsis. These findings support the routine use of probiotics for NEC prevention in preterm infants, with preference for multi-strain formulations or specific highly effective regimens when available. Future research should focus on direct comparisons of the most promising regimens, optimization of supplementation protocols, and long-term outcomes of early probiotic exposure.

REFERENCES:

1. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364(3):255-264.
2. Stoll BJ, Hansen NI, Bell EF, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993-2012. JAMA. 2015;314(10):1039-1051.
3. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):F193-F198.
4. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol. 2016;13(10):590-600.
5. Neu J, Pammi M. Pathogenesis of NEC: impact of an altered intestinal microbiome. Semin Perinatol. 2017;41(1):29-35.
6. Warner BB, Deych E, Zhou Y, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet. 2016;387(10031):1928-1936.
7. Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: state of the science. Adv Neonatal Care. 2012;12(2):77-87.
8. Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin Pediatr Surg. 2018;27(1):39-46.
9. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-514.
10. Underwood MA, Sohn K. The microbiota of the extremely preterm infant. Clin Perinatol. 2017;44(2):407-427.
11. van den Akker CHP, van Goudoever JB, Szajewska H, et al. Probiotics for preterm infants: a strain-specific systematic review and network meta-analysis. J Pediatr Gastroenterol Nutr. 2018;67(1):103-122.
12. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014;(4):CD005496.
13. Dermyshi E, Wang Y, Yan C, et al. The "golden age" of probiotics: a systematic review and meta-analysis of randomized and observational studies in preterm infants. Neonatology. 2017;112(1):9-23.
14. Sawh SC, Deshpande S, Jansen S, Reynaert CJ, Jones PM. Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis. PeerJ. 2016;4:e2429.
15. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar

MR; Probiotics in Preterm Infants Study Collaborative Group. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet. 2016;387(10019):649-660.
16. Vallabhaneni S, Walker TA, Lockhart SR, et al. Notes from the field: fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated dietary supplement--Connecticut, 2014. MMWR Morb Mortal Wkly Rep. 2015;64(6):155-156.
17. 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. 2020;10:CD005496.
18. Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B. Probiotics reduce mortality and morbidity in preterm, low-birth-weight infants: a systematic review and network meta-analysis of randomized trials. Gastroenterology. 2020;159(2):467-480.
19. Chi C, Buys N, Li C, Sun J, Yin C. Effects of prebiotics on sepsis, necrotizing enterocolitis, mortality, feeding intolerance, time to full enteral feeding, length of hospital stay, and stool frequency in preterm infants: a meta-analysis. Eur J Clin Nutr. 2019;73(5):657-670.
20. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
21. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187(1):1-7.
22. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
23. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924-926.
24. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-560.
25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-634.
26. Deshpande G, Jape G, Rao S, Patole S. Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials. BMJ Open. 2017;7(12):e017638.
27. 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. Adv Nutr. 2017;8(5):749-763.
28. Athalye-Jape G, Rao S, Patole S. Effects of probiotics on experimental necrotizing enterocolitis: a systematic review and meta-analysis. Pediatr Res. 2018;83(1-1):16-22.
29. Sherman MP, Adamkin DH, Niklas V, et al. Randomized controlled trial of talactoferrin oral solution in preterm infants. J Pediatr. 2016;175:68-73.e3.
30. Jacobs SE, Tobin JM, Opie GF, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics. 2013;132(6):1055-1062.
31. Underwood MA, German JB, Lebrilla CB, Mills DA. Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. Pediatr Res. 2015;77(1-2):229-235.
32. Guthmann F, Kluthe C, Bührer C. Probiotics for prevention of necrotising enterocolitis: an updated meta-analysis. Klin Padiatr. 2010;222(5):284-290.
33. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev. 2019;7:CD002971.
34. Underwood MA, Gaerlan S, De Leoz ML, et al. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. Pediatr Res. 2015;78(6):670-677.
35. Aceti A, Gori D, Barone G, et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr. 2015;41:89.
36. Arboleya S, Binetti A, Salazar N, et al. Establishment and development of intestinal microbiota in preterm neonates. FEMS Microbiol Ecol. 2012;79(3):763-772.
37. Esaiassen E, Cavanagh P, Hjerde E, Simonsen GS, Støen R, Klingenberg C. Bifidobacterium longum subspecies infantis bacteremia in 3 extremely preterm infants receiving probiotics. Emerg Infect Dis. 2016;22(9):1664-1666.
38. Collado MC, Gueimonde M, Salminen S. Probiotics in adhesion of pathogens: mechanisms of action. In: Watson RR, Preedy VR, eds. Bioactive Foods in Promoting Health. Academic Press; 2010:353-370.
39. Patel RM, Myers LS, Kurundkar AR, et al. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. Am J Pathol. 2012;180(2):626-635.
40. Ganguli K, Meng D, Rautava S, Lu L, Walker WA,

Nanthakumar N. Probiotics prevent necrotizing enterocolitis by modulating enterocyte genes that regulate innate immune-mediated inflammation. Am J Physiol Gastrointest Liver Physiol. 2013;304(2):G132-G141.
41. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. Proc Natl Acad Sci U S A. 2007;104(18):7617-7621.
42. Timmerman HM, Koning CJ, Mulder L, Rombouts FM, Beynen AC. Monostrain, multistrain and multispecies probiotics--A comparison of functionality and efficacy. Int J Food Microbiol.
46. Dev. 2014;90 Suppl 1:S60-S65.
47. Bertelli C, Pillonel T, Torregrossa A, et al. Bifidobacterium longum bacteremia in preterm 2004;96(3):219-233.
43. Maldonado-Lobón JA, Gil-Campos M, Maldonado J, et al. Long-term safety of early consumption of Lactobacillus fermentum CECT5716: a 3-year follow-up of a randomized controlled trial. Pharmacol Res. 2015;95-96:12-19.
44. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013;60(1):49-74.
45. Manzoni P, Meyer M, Stolfi I, et al. Bovine lactoferrin supplementation for prevention of necrotizing enterocolitis in very-low-birth-weight neonates: a randomized clinical trial. Early Hum

infants receiving probiotics. Clin Infect Dis. 2015;60(6):924-927.