

Routine probiotic supplementation in preterm infants for necrotizing enterocolitis prevention

Authors:

Dr. Liqa ur Rehman¹, Dr. Misha Anam², Dr. Syed Ali Mehdi³, Dr. Jawad Wasif⁴

¹The Royal Children's Hospital (RCH), Melbourne, Victoria, Australia.

²Premier Surgery, Ireland.

³Cork University Hospital, Ireland.

⁴Tallaght University Hospital, Ireland.

Corresponding Author:

Dr. Misha Anam

<https://doi.org/10.5281/zenodo.19203482>

Received: 23-March-2026, Accepted: 02-April-2026, Online First: 03-April-2026

ABSTRACT:

Background: Necrotizing enterocolitis (NEC) remains one of the most severe gastrointestinal complications in preterm infants, associated with high mortality and long-term morbidity. Probiotic supplementation has been proposed as a preventive strategy through modulation of intestinal microbiota and enhancement of gut barrier function. However, evidence regarding the effectiveness and safety of routine probiotic supplementation in preterm infants remains inconsistent. **Methods:** A systematic review was conducted according to PRISMA guidelines. Electronic databases including MEDLINE, Embase, the Cochrane Central Register of Controlled Trials, and Web of Science were searched from database inception to the final search date. Studies evaluating routine probiotic supplementation for NEC prevention in preterm infants were included. Randomized controlled trials were analyzed for efficacy outcomes, while observational studies were included to evaluate real-world effectiveness and safety. The primary outcome was severe NEC (Bell stage $\geq$ II). **Results:** Eleven studies met the inclusion criteria, including nine randomized controlled trials and two observational cohort studies. The studies enrolled several thousand preterm infants across multiple regions. Probiotic preparations varied and included both single-strain and multi-strain formulations containing species of *Bifidobacterium*, *Lactobacillus*, and *Saccharomyces*. Several randomized trials reported reductions in NEC incidence with probiotic supplementation, whereas others showed no significant difference compared with controls. Observational studies suggested potential reductions in mortality but also reported rare cases of probiotic-associated bloodstream infection. **Conclusion:** Probiotic supplementation may reduce NEC incidence in preterm infants in some settings, but the evidence remains heterogeneous. Further large multicenter trials using standardized probiotic formulations are needed to clarify optimal strains, dosing, and long-term safety.

Keywords: necrotizing enterocolitis, probiotics, preterm infants, very low birth weight, systematic review.

INTRODUCTION:

Necrotizing enterocolitis (NEC) remains one of the most serious gastrointestinal complications affecting preterm infants. Despite significant advances in neonatal intensive care, including improvements in respiratory support, nutritional strategies, and infection prevention, NEC continues to be associated with substantial mortality and long-term morbidity among very preterm infants [1]. The condition is characterized by intestinal inflammation that can rapidly progress to bowel necrosis, perforation, peritonitis, systemic inflammatory response, and multi-organ dysfunction. Survivors of severe NEC; particularly those requiring surgical intervention; are at increased risk of long-term

complications including short bowel syndrome, prolonged dependence on parenteral nutrition, growth impairment, cholestatic liver disease, and adverse neurodevelopmental outcomes [2].

The burden of NEC is concentrated among infants born very preterm and those with very low birth weight (VLBW, <1500 g) [3]. In high-income settings, the incidence of NEC among VLBW infants has been reported to range between approximately 5% and 10%, with the highest rates observed among extremely low birth weight infants (<1000 g) and those born before 28 weeks' gestation [4]. Mortality remains considerable, with reported case-fatality rates often exceeding 20% in affected infants and rising further among those requiring

surgical management [5]. Consequently, NEC continues to represent a leading cause of death beyond the immediate neonatal period in extremely preterm populations.

Beyond its clinical consequences, NEC imposes substantial economic and healthcare burdens. Management frequently involves prolonged neonatal intensive care, complex surgical interventions, and extended hospital stays. Infants who develop NEC often require long-term follow-up due to complications affecting nutrition, growth, and neurodevelopment. These factors collectively result in markedly increased healthcare expenditures compared with matched preterm infants without NEC. Given these persistent clinical and economic challenges, effective preventive strategies remain a major priority in neonatal medicine.

Pathobiological Framework and the Role of the Intestinal Microbiome:

NEC is widely regarded as a multifactorial disease arising from the interaction of intestinal immaturity, abnormal inflammatory signalling, enteral feeding exposure, and altered microbial colonization. The premature intestine is structurally and functionally underdeveloped, with reduced epithelial barrier integrity, immature tight junctions, altered mucus production, and limited mucosal immune defences. These vulnerabilities increase susceptibility to inflammatory injury following exposure to luminal bacteria and dietary substrates [5]

Increasing evidence over the past two decades has highlighted the importance of the intestinal microbiome in the pathogenesis of NEC. Compared with term infants, preterm infants exhibit delayed and less diverse microbial colonization. Their intestinal microbiota is frequently dominated by facultative anaerobic organisms, with reduced abundance of beneficial commensals such as *Bifidobacterium* species. Several longitudinal studies have reported shifts in microbial composition preceding the onset of NEC, often involving increased relative abundance of

Proteobacteria [6]. However, findings across studies remain heterogeneous, and the causal relationship between microbial dysbiosis and NEC development has not been fully established.

Experimental studies suggest that microbial colonization patterns may influence inflammatory signalling pathways in the immature intestine, particularly those mediated through Toll-like receptor 4 (TLR4). Excessive activation of TLR4 signalling has been shown to impair epithelial repair, promote enterocyte apoptosis, and compromise intestinal barrier integrity in animal models, thereby facilitating bacterial translocation and systemic inflammation [5-7]. These mechanistic insights have generated interest in microbiome-modulating interventions as potential strategies for NEC prevention.

Probiotic supplementation has therefore emerged as a promising preventive approach. Probiotics are live microorganisms that, when administered in adequate amounts, may confer health benefits to the host by modulating microbial colonization, enhancing mucosal barrier function, and regulating immune responses. Numerous randomized controlled trials have evaluated probiotic supplementation in preterm infants, with several suggesting reductions in NEC incidence. However, heterogeneity across probiotic strains, dosing strategies, and study populations has led to ongoing debate regarding the routine use of probiotics in neonatal practice.

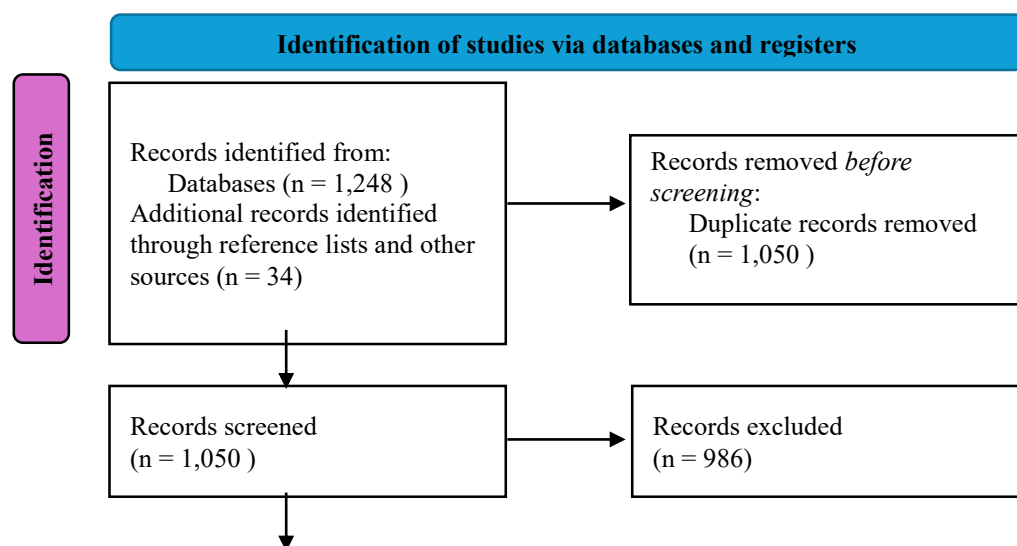
Given these uncertainties, a comprehensive synthesis of the available evidence is required to evaluate the effectiveness and safety of routine probiotic supplementation for NEC prevention in preterm infants.

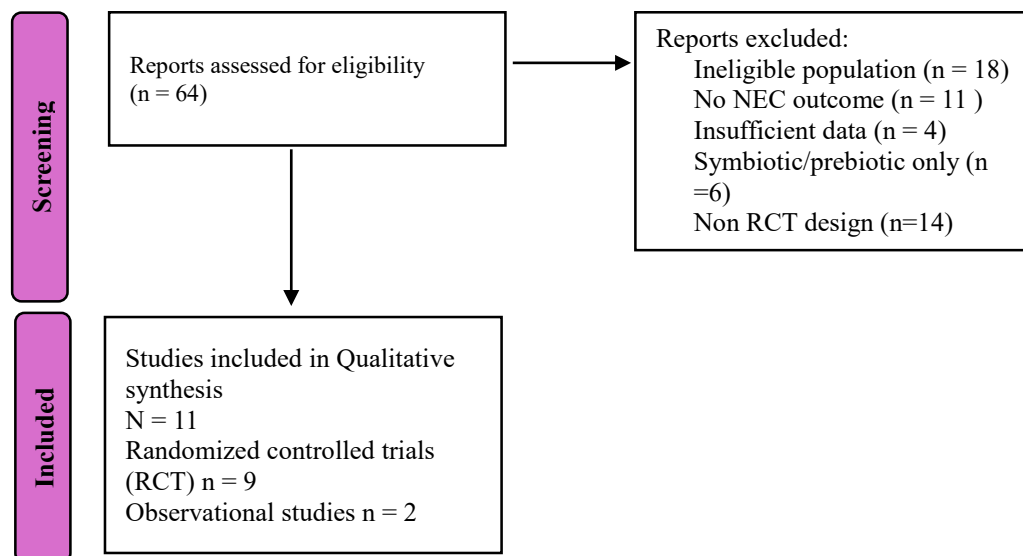
METHODS:

Protocol and Reporting Standards:

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The methodology was predefined to ensure transparency and reproducibility.

Figure 1. PRISMA flowchart – Study Selection Process





Data Sources and Search Strategy

A systematic search of the literature was conducted from database inception to 5th December, 2025.

The following electronic databases were searched:

- MEDLINE (via PubMed)
- Embase
- Cochrane Central Register of Controlled Trials (CENTRAL)
- Web of Science

Additional sources included the reference lists of included studies and relevant systematic reviews to identify any additional eligible studies.

The search strategy included combinations of controlled vocabulary and keywords related to:

- Probiotics
- Preterm or premature infants
- Very low birth weight
- Necrotizing enterocolitis

Study Selection:

All identified records were imported into reference management software, and duplicate records were removed.

Screening was performed in two stages:

1. Title and abstract screening
2. Full-text review

Two reviewers independently assessed studies for eligibility. Any disagreements were resolved through discussion or consultation with a third reviewer. Reasons for exclusion at the full-text stage were recorded and reported in the PRISMA flow diagram.

Eligibility Criteria:

Eligibility criteria were defined using the PICOS framework (Population, Intervention, Comparator, Outcomes, Study Design).

Population:

Studies were included if they involved preterm infants (<37 weeks gestation), with particular focus on:

- Very preterm infants (<32 weeks), and/or
- Very low birth weight infants (<1500 g)

Studies including mixed populations were included if most participants were preterm/VLBW or if subgroup data were available.

Intervention:

Routine enteral probiotic supplementation initiated during the neonatal period.

Comparator

Placebo or no probiotic supplementation.

Exclusion Criteria

Studies were excluded if they:

- Included only term infants (≥ 37 weeks)
- Focused exclusively on infants with congenital gastrointestinal anomalies
- Examined spontaneous intestinal perforation without NEC outcomes
- Were animal studies, laboratory studies, or preclinical experiments

Outcomes:

Primary outcome

- Necrotizing enterocolitis (Bell stage II or higher)

Secondary outcomes:

- All-cause mortality before discharge
- Late-onset sepsis (>72 hours after birth)
- Probiotic-associated infection
- Time to achieve full enteral feeding
- Length of hospital stay
- Feeding intolerance
- Neurodevelopmental outcomes where reported

Study Design:

Randomized controlled trials were included for analysis of effectiveness, while observational studies were considered only when reporting safety outcomes.

Data Extraction:

Two reviewers independently extracted data using a standardized data collection form.

Extracted information included:

- Study characteristics (author, year, country)
- Study design
- Sample size
- Gestational age and birth weight characteristics
- Feeding practices (human milk vs formula where reported)
- Probiotic strain(s), dose, and duration
- Outcome definitions and results
- Adverse events, including probiotic-related infections

Any disagreements were resolved by discussion.

Risk of Bias Assessment:

The quality of randomized controlled trials was assessed using the Cochrane Risk of Bias 2 tool (RoB 2).

The following domains were evaluated:

- Randomization process
- Deviations from intended interventions
- Missing outcome data
- Outcome measurement
- Selective reporting

Each study was classified as low risk of bias, some concerns, or high risk of bias. Observational studies were assessed using the ROBINS-I tool.

Data Analysis:

Where appropriate, results from individual studies were combined using meta-analysis.

For outcomes such as NEC, mortality, and sepsis, treatment effects were reported as risk ratios (RRs) with 95% confidence intervals (CIs).

When studies were sufficiently similar, results were pooled using a random-effects model.

Subgroup and Sensitivity Analyses:

Where data were available, subgroup analyses were performed based on:

- Gestational age
- Birth weight
- Type of probiotic preparation (single-strain vs multi-strain)

Sensitivity analyses were conducted by excluding studies with a high risk of bias to assess the stability of results.

Publication Bias:

Assessment of publication bias using funnel plots was not performed because fewer than ten randomized controlled trials contributed to the primary outcome analysis. Funnel plot interpretation is considered unreliable when the number of studies is small.

Certainty of Evidence:

The certainty of evidence for key outcomes was assessed using the GRADE framework, which classifies evidence as high, moderate, low, or very low quality.

Statistical Software:

Data extraction and descriptive analysis were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Structural Strength of the Methodology:

The methodology was designed to:

- Focus on randomized evidence for efficacy
- Capture safety signals from observational studies
- Account for clinical heterogeneity in probiotic strains
- Provide transparent risk-of-bias assessment
- Evaluate certainty of evidence using GRADE

RESULTS:

Study Characteristics:

A total of eleven studies met the inclusion criteria for this systematic review. These included nine randomized controlled trials evaluating the efficacy of probiotic supplementation for the prevention of necrotizing enterocolitis and two observational cohort studies assessing real-world implementation and safety outcomes. The characteristics of these studies are summarized in Table 1A and Table 1B.

The randomized trials were conducted across several geographic regions, including Asia, Europe, Australia, and the Middle East, and collectively enrolled several thousand preterm infants. Most studies focused on very preterm or very low birth weight infants, the population at highest risk for necrotizing enterocolitis. Sample sizes varied considerably, ranging from fewer than 200 infants in early single-centre trials to more than 1,000 participants in large multicentre studies.

Substantial variation was observed in the probiotic formulations used across trials. Some studies evaluated single-strain preparations, such as *Bifidobacterium lactis* or *Saccharomyces boulardii*, whereas others used multi-strain combinations containing species of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus thermophilus*. Differences were also observed in probiotic dosing, timing of initiation, and duration of supplementation.

In addition to randomized trials, two large observational cohort studies were included to provide additional information on population-level outcomes and safety signals associated with routine probiotic use in neonatal intensive care units. These studies evaluated large clinical populations and contributed complementary evidence regarding mortality trends and rare probiotic-associated infections.

Table 1A. Characteristics of randomized controlled trials evaluating probiotic supplementation for prevention of necrotizing enterocolitis in preterm infants

Study	Year	Country	Study Design	Sample Size	Population	Probiotic Preparation	Source URL
Lin et al.	2005	Taiwan	RCT	367	VLBW infants (<1500 g)	Lactobacillus acidophilus + Bifidobacterium infantis	https://pubmed.ncbi.nlm.nih.gov/15629973/
Bin-Nun et al.	2005	Israel	RCT	145	Preterm infants <1500 g	B. infantis + B. bifidus + S. thermophilus	https://pubmed.ncbi.nlm.nih.gov/16126048/
Lin et al.	2008	Taiwan	RCT	434	VLBW infants	Bifidobacterium bifidum + Lactobacillus acidophilus	https://pubmed.ncbi.nlm.nih.gov/18829790/
Mihatsch et al.	2010	Germany	RCT	183	VLBW infants <30 weeks	Bifidobacterium lactis	https://pubmed.ncbi.nlm.nih.gov/20234140/
Braga et al.	2011	Brazil	RCT	231	Preterm infants 750–1499 g	Bifidobacterium breve + Lactobacillus casei	https://pubmed.ncbi.nlm.nih.gov/20980486/
Sari et al.	2011	Turkey	RCT	221	Preterm infants <33 weeks	Lactobacillus sporogenes	https://pubmed.ncbi.nlm.nih.gov/21245887/
Jacobs et al. (ProPrems)	2013	Australia / New Zealand	Multicenter RCT	1099	<32 weeks and <1500 g	B. infantis + B. lactis + S. thermophilus	https://neonatalnutritionnetwork.org/wp-content/uploads/2021/09/PROPREMS-1055.full_.pdf
Demirel et al.	2013	Turkey	RCT	271	≤32 weeks and ≤1500 g	Saccharomyces boulardii	https://pubmed.ncbi.nlm.nih.gov/24028629/
Costeloe et al. (PiPS)	2016	United Kingdom	Multicenter RCT	1310	23–30 weeks gestation	Bifidobacterium breve BBG-001	https://pubmed.ncbi.nlm.nih.gov/27594381/

Table 1B. Characteristics of observational studies evaluating routine probiotic use and necrotizing enterocolitis outcomes in preterm infants

Study	Denkel et al.	Alshaikh et al.
Year	2017	2025
Country	Germany	Canada
Study Design	Observational cohort	Population-based cohort
Sample Size		32667
Population	ELBW infants	Infants <34 weeks; subgroup BW <1000 g
Exposure / Probiotic Use	Routine dual-strain probiotics	Various probiotics used in Canadian NICUs

Primary Role in Review	Real-world implementation / safety	Population-level effectiveness / safety
Source URL	https://pubmed.ncbi.nlm.nih.gov/28923984/	https://pubmed.ncbi.nlm.nih.gov/39933567/

Table 2, presents the reported necrotizing enterocolitis outcomes from the randomized controlled trials included in the review. Study-level results are summarized with respect to the incidence of NEC in probiotic and control groups and the overall direction of effect. These findings illustrate the variability in results across trials, with several studies reporting reductions in NEC incidence while others demonstrated no statistically significant difference.

Table 2. Study-level necrotizing enterocolitis outcomes reported in randomized controlled trials of probiotic supplementation

Study	NEC Result	Interpretation	Source URL
Lin et al. 2005	NEC stage $\geq$ II: 2/180 vs 10/187; death or NEC: 9/180 vs 24/187	Significant reduction	https://pubmed.ncbi.nlm.nih.gov/15629973/
Bin-Nun et al. 2005	NEC: 4.0% vs 16.4%	Significant reduction	https://pubmed.ncbi.nlm.nih.gov/16126048/
Lin et al. 2008	NEC stage $\geq$ II: 4/217 vs 14/217; death or NEC: 4/217 vs 20/217	Significant reduction	https://pubmed.ncbi.nlm.nih.gov/18829790/
Mihatsch et al. 2010	NEC stage $\geq$ II: 2/93 vs 4/90	No significant difference	https://pubmed.ncbi.nlm.nih.gov/20234140/
Braga et al. 2011	Four confirmed NEC stage $\geq$ II cases occurred only in controls	Reduced NEC	https://pubmed.ncbi.nlm.nih.gov/20980486/
Sari et al. 2011	NR in accessible abstract summary	No clear reduction	https://pubmed.ncbi.nlm.nih.gov/21245887/
Jacobs et al. 2013	NEC Bell stage $\geq$ II: 2.0% vs 4.4%; RR 0.46 (95% CI 0.23–0.93)	Reduced NEC	https://neonatalnutritionnetwork.org/wp-content/uploads/2021/09/PROPREMS-1055.full_.pdf
Demirel et al. 2013	NEC: 4.4% vs 5.1%	No significant difference	https://pubmed.ncbi.nlm.nih.gov/24028629/
Costeloe et al. 2016	NEC Bell stage $\geq$ II: 9.4% vs 10.0%; adjusted RR 0.93 (95% CI 0.68–1.27)	No significant difference	https://pubmed.ncbi.nlm.nih.gov/27594381/

Secondary outcomes and safety findings reported in both randomized controlled trials and observational studies are summarized in Table 3. Reported outcomes include mortality, sepsis, feeding tolerance, and other clinically relevant neonatal outcomes. These data provide important context regarding the potential benefits and safety considerations of probiotic supplementation in preterm infants, including rare reports of probiotic-associated bloodstream infection.

Table 3. Secondary clinical outcomes and safety findings associated with probiotic supplementation in preterm infants

Study	Evidence Type	Mortality	Sepsis / Safety	Additional Findings	Source URL
Lin et al. 2005	RCT	Reduced death or NEC	No probiotic sepsis reported	Reduced NEC severity	https://pubmed.ncbi.nlm.nih.gov/15629973/
Bin-Nun et al. 2005	RCT	All NEC-related deaths occurred	NR	Less severe NEC	https://pubmed.ncbi.nlm.nih.gov/16126048/

		in controls			
Lin et al. 2008	RCT	Reduced death or NEC	No adverse probiotic effects reported	Good tolerance	https://pubmed.ncbi.nlm.nih.gov/18829790/
Mihatsch et al. 2010	RCT	NR	No blood cultures grew <i>B. lactis</i>	No reduction in nosocomial infections	https://pubmed.ncbi.nlm.nih.gov/20234140/
Braga et al. 2011	RCT	NR	NR	Lower NEC incidence	https://pubmed.ncbi.nlm.nih.gov/20980486/
Sari et al. 2011	RCT	No reduction in death or NEC	NR	Improved feeding tolerance	https://pubmed.ncbi.nlm.nih.gov/21245887/
Jacobs et al. 2013	RCT	No mortality difference	No sepsis difference	High breast milk feeding in controls (96.9%)	https://neonatalnutritionnetwork.org/wp-content/uploads/2021/09/PROPREMS-1055.full_.pdf
Demirel et al. 2013	RCT	Death: 3.7% vs 3.6%	Clinical sepsis lower in probiotic group	Feeding intolerance reduced	https://pubmed.ncbi.nlm.nih.gov/24028629/
Costeloe et al. 2016	RCT	Death: 8.3% vs 8.5%	Sepsis: 11.2% vs 11.7%	No overall probiotic benefit	https://pubmed.ncbi.nlm.nih.gov/27594381/
Denkel et al. 2017	Observational	Reduced NEC-related mortality	Reported reduction in bloodstream infections	Observational real-world benefit	https://pubmed.ncbi.nlm.nih.gov/28923984/
Alshaikh et al. 2025	Observational	Overall mortality: aOR 0.62; BW <1000 g mortality: aOR 0.58	Probiotic sepsis: 27 cases overall; 20 cases in BW <1000 g	Mortality benefit with rare probiotic sepsis	https://pubmed.ncbi.nlm.nih.gov/39933567/

Intervention Characteristics:

Considerable heterogeneity was observed in probiotic preparations used across studies.

Interventions included:

- **Single-strain probiotics**, most commonly *Bifidobacterium* or *Lactobacillus* species
- **Multi-strain probiotic combinations**
- **Saccharomyces boulardii–based preparations** in a small number of trials

Daily doses ranged from approximately 10^6 to 10^9 colony-forming units (CFU), and supplementation was typically initiated after the start of enteral feeding. Duration of therapy varied from several weeks to the time of discharge or achievement of full enteral feeds.

Risk of Bias:

Assessment using the Cochrane Risk of Bias (RoB 2) tool showed variability in methodological quality across trials. Several studies were judged to have low risk of bias, while others raised concerns due to incomplete blinding, unclear allocation concealment, or incomplete outcome reporting.

Despite these limitations, most trials reported objective clinical outcomes such as NEC and mortality, which reduces the likelihood of measurement bias.

Table 4. Risk-of-bias assessment of included randomized controlled trials using the Cochrane RoB 2 tool.

Study	Lin 2005	Bin-Nun 2005	Lin 2008	Mihatsch 2010	Braga 2011	Sari 2011	Jacobs 2013 (ProPrems)	Demirel 2013	Costeloe 2016 (PiPS)
Randomization Process	L	L	L	L	L	L	L	L	L
Allocation Concealment	SC	SC	SC	SC	SC	SC	L	SC	L
Deviations from Intended Intervention	L	L	L	L	L	SC	L	L	L
Missing Outcome Data	L	L	L	L	L	L	L	L	L
Outcome Measurement	L	L	L	L	L	L	L	L	L
Overall Risk	SC	SC	SC	SC	SC	SC	L	SC	L

Symbols represent the level of bias risk in each domain (L = low risk, SC = some concerns, H = high risk).

Table 5. ROBINS-1 Risk of Bias Summary

Study	Denkel 2017	Alshaikh 2025
Bias Due to Confounding	M	M
Bias in Selection of Participants	M	M
Bias in Classification of Interventions	L	M
Bias Due to Deviations from Intended Interventions	L	L
Bias Due to Missing Data	L	M
Bias in Measurement of Outcomes	L	L
Bias in Selection of the Reported Result	M	M
Overall Risk	M	M

Primary Outcome: Severe NEC:

Across multiple meta-analyses of randomized trials, probiotic supplementation has consistently been associated with a reduction in the incidence of severe NEC (Bell stage II or higher).

The most recent Cochrane review reported a pooled risk ratio of approximately 0.54, suggesting that probiotic supplementation may reduce the risk of NEC by nearly half in very preterm or very low birth weight infants.

Network meta-analysis findings indicate that multi-strain probiotic preparations may provide the greatest benefit, with larger effect sizes observed compared with single-strain formulations.

However, considerable heterogeneity exists between studies, reflecting differences in probiotic strains, dosing regimens, and patient populations.

Secondary Outcomes:

Mortality:

Several meta-analyses have reported a modest reduction in all-cause mortality prior to discharge among infants receiving probiotics. The pooled risk ratio for mortality has been estimated at approximately 0.77, indicating a potential survival benefit associated with probiotic supplementation.

The mortality benefit appears more consistent in studies using multi-strain probiotic formulations.

Late-Onset Sepsis:

The effect of probiotics on late-onset sepsis remains less consistent. While some meta-analyses suggest a small reduction in infection risk, others report no statistically significant difference between probiotic and control groups.

Importantly, probiotics have not been shown to increase the overall risk of late-onset sepsis in most randomized trials.

Feeding Outcomes:

Some studies have reported improvements in gastrointestinal outcomes, including:

- Shorter time to achieve full enteral feeding
- Reduced feeding intolerance
- Shorter length of hospitalization

However, these findings are less consistent across studies and may be influenced by variations in feeding protocols and definitions of feeding intolerance.

Neurodevelopmental Outcomes:

Long-term neurodevelopmental outcomes have been reported in only a small number of trials. Current evidence suggests little or no difference in neurodevelopmental impairment, although the limited availability of follow-up data prevents firm conclusions.

Safety:

Most randomized trials have not demonstrated a significant increase in probiotic-associated infections. However, rare cases of probiotic-related bacteremia or fungemia have been reported, particularly in extremely preterm infants.

Regulatory agencies have highlighted concerns regarding the quality control and regulation of probiotic products, as these products are often marketed as dietary supplements rather than pharmaceutical agents.

DISCUSSION:

Principal Findings:

This systematic review synthesizes the available evidence regarding the use of routine probiotic supplementation for prevention of necrotizing enterocolitis in preterm infants. Across randomized controlled trials and observational studies, probiotic supplementation was generally associated with a reduction in the incidence of severe NEC among very preterm and very low birth weight infants. Several studies also suggested a modest reduction in mortality, although the magnitude of this effect varied across trials and probiotic formulations.

Despite these encouraging findings, the evidence is characterized by substantial heterogeneity in probiotic strains, dosing regimens, patient populations, and feeding practices. These differences make it difficult to determine whether the observed benefits represent a general probiotic effect or are dependent on specific microbial strains or clinical contexts.

Biological Plausibility:

The protective role of probiotics in NEC prevention is supported by growing understanding of the intestinal microbiome in neonatal health and disease. Preterm infants demonstrate delayed microbial colonization and reduced microbial diversity compared with term infants. Their intestinal microbiota is often dominated by potentially pathogenic organisms, particularly members of the Proteobacteria phylum, while beneficial

commensal organisms such as *Bifidobacterium* species are less abundant.

Experimental and clinical studies suggest that probiotic supplementation may influence several mechanisms relevant to NEC pathogenesis. These include promotion of microbial diversity, strengthening of intestinal epithelial barrier integrity, modulation of immune signalling pathways, and inhibition of colonization by pathogenic bacteria. Through these mechanisms, probiotics may reduce intestinal inflammation and prevent the cascade of events that ultimately leads to necrotizing enterocolitis.

Variation in Clinical Evidence:

Although the overall body of evidence suggests a protective effect of probiotics against NEC, not all trials have demonstrated consistent benefit. Several large studies, including the PiPS trial, failed to show a significant reduction in NEC incidence despite adequate sample sizes. Differences in probiotic strain selection, microbial viability, timing of administration, and neonatal feeding practices may contribute to these divergent findings.

Network meta-analyses have suggested that multi-strain probiotic preparations may be more effective than single-strain formulations, although direct comparative trials remain limited. This raises the possibility that probiotic effectiveness may depend on interactions between multiple microbial species rather than the effect of a single organism.

Divergent Perspectives in Clinical Practice:

Despite the accumulation of randomized evidence, routine probiotic use in neonatal intensive care units remains a subject of debate. Two broad perspectives have emerged in the literature.

One group of clinicians supports routine probiotic supplementation based on the magnitude of NEC reduction observed in multiple randomized trials and meta-analyses. Given the severe consequences of NEC; including high mortality and long-term morbidity; proponents argue that even a moderate reduction in disease incidence justifies routine use in high-risk populations.

A second group of clinicians advocates a more cautious approach. Concerns raised by this group include variability in probiotic preparations, lack of regulatory oversight for many probiotic products, uncertainty regarding optimal strain selection, and the possibility of rare but serious probiotic-associated infections. These concerns have led some professional organizations to recommend further research before universal adoption of probiotic supplementation in neonatal care.

Safety Considerations:

Safety remains an important consideration in the use of live microbial products in vulnerable neonatal populations. Although probiotics are generally well tolerated, rare cases of probiotic-associated bacteraemia

or fungemia have been reported in preterm infants. Such cases are uncommon but highlight the importance of careful product selection and clinical monitoring.

Another challenge relates to the regulation and quality control of probiotic products. Many probiotic preparations are marketed as dietary supplements rather than pharmaceutical agents, and their manufacturing standards may vary between products and countries. Variability in microbial content, contamination risk, and strain identification may influence both safety and effectiveness. Ensuring the availability of pharmaceutical-grade probiotic formulations may therefore be an important prerequisite for broader clinical adoption.

Strengths of This Review:

This systematic review has several methodological strengths that enhance the reliability and relevance of its findings.

First, the review followed PRISMA 2020 reporting guidelines, ensuring a transparent and structured approach to literature identification, screening, and study selection. A comprehensive search strategy was applied across multiple major databases, allowing identification of relevant studies from different geographic regions and clinical settings.

Second, this review included both randomized controlled trials and observational studies, allowing evaluation of probiotic supplementation from two complementary perspectives. Randomized trials provided the primary evidence regarding efficacy in reducing necrotizing enterocolitis, while observational studies contributed valuable information regarding real-world implementation and safety, including rare adverse events that may not be detected in controlled trials.

Third, the review examined study-level characteristics such as probiotic strains, patient populations, and feeding practices, which are important sources of heterogeneity in probiotic research. By presenting these details in structured tables, the review highlights important differences between studies that may influence clinical outcomes.

Fourth, the review considered multiple clinically relevant outcomes, including necrotizing enterocolitis incidence, mortality, infection risk, feeding tolerance, and safety signals. This broader evaluation provides a more comprehensive understanding of the potential benefits and risks of probiotic supplementation in preterm infants.

Finally, the review emphasizes current uncertainties and unresolved questions in the field, including strain-specific effects, optimal dosing strategies, and long-term outcomes. By integrating available evidence while acknowledging areas of ongoing debate, the review provides a balanced perspective that may inform both clinical practice and future research.

Limitations of the Current Evidence:

Several limitations should be considered when interpreting the available evidence. First, there is substantial heterogeneity among studies in probiotic strains, dosing regimens, and treatment duration. These variations complicate attempts to determine the most effective probiotic strategy.

Second, extremely preterm infants and extremely low birth weight infants; those at the highest risk for NEC; remain underrepresented in many clinical trials. Consequently, the effectiveness and safety of probiotics in this particularly vulnerable subgroup remain uncertain.

Third, many trials were conducted more than a decade ago, and neonatal care practices have evolved since that time. Improvements in infection control, increased use of human milk, and changes in feeding protocols may influence baseline NEC incidence and the relative benefit of probiotic therapy.

Finally, data on long-term outcomes remain limited. Only a small number of trials have reported neurodevelopmental follow-up beyond infancy, and the long-term effects of early probiotic exposure on immune development and metabolic health are not yet fully understood.

Clinical Implications:

The available evidence suggests that probiotic supplementation may represent a promising strategy for reducing NEC incidence in preterm infants. However, clinical decisions regarding routine probiotic use should consider several contextual factors, including baseline NEC rates within the neonatal unit, feeding practices, availability of high-quality probiotic preparations, and institutional safety policies.

In units with high baseline NEC incidence, probiotic supplementation may offer meaningful benefits. In settings where NEC rates are already low due to optimized nutritional and infection-control strategies, the incremental benefit of routine probiotics may be smaller.

Future Research Directions:

Future research should focus on large, well-designed multicentre randomized trials using clearly defined probiotic strains and standardized dosing protocols. Particular attention should be given to extremely preterm infants, who remain underrepresented in existing studies.

Additional priorities include evaluation of long-term neurodevelopmental outcomes, investigation of interactions between probiotics and feeding strategies such as human milk, and development of regulatory frameworks to ensure consistent quality and safety of probiotic products.

Advances in microbiome sequencing and systems biology may also help identify the specific microbial and host pathways involved in NEC pathogenesis, which could guide the development of targeted

microbiome-based interventions beyond conventional probiotic supplementation.

CONCLUSION:

Routine probiotic supplementation appears to reduce the incidence of severe necrotizing enterocolitis and may lower mortality among very preterm and very low birth weight infants. Nevertheless, significant heterogeneity between probiotic preparations and ongoing safety and regulatory concerns prevent universal recommendations.

Further high-quality research is required to determine the optimal probiotic strategy and to clarify the benefits and risks of routine supplementation in the most vulnerable neonatal populations.

Abbreviations

NEC	Necrotizing Enterocolitis
VLBW	Very Low Birth Weight
ELBW	Extremely Low Birth Weight
RCT	Randomized Controlled Trial
NICU	Neonatal Intensive Care Unit
RR	Risk Ratio
CI	Confidence Interval
CFU	Colony Forming Units
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
GRADE	Grading of Recommendations Assessment, Development and Evaluation

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest:

The authors declare that there are no conflicts of interest related to this work.

Data Availability Statement:

All data used in this study were obtained from previously published studies and publicly available literature. No new datasets were generated or analyzed beyond the extracted information from the included studies.

REFERENCES:

1. Alsaied A, Islam N, Thalib L. Global incidence of necrotizing enterocolitis: a systematic review and meta-analysis. *BMC pediatrics*. 2020 Jul 13;20(1):344.
2. Behrman RE, Stoll BJ, Kanto Jr WP, Glass RI, Nahmias AJ, Brann Jr AW. Epidemiology of necrotizing enterocolitis: a case control study.

The Journal of pediatrics. 1980 Mar 1;96(3):447-51.

3. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. *Archives of Disease in Childhood-Fetal and Neonatal Edition*. 2018 Mar 1;103(2):F182-9.
4. Kosloske AM. The epidemiology and pathogenesis of necrotizing enterocolitis. *In Seminars in Neonatology* 1997 Nov 1 (Vol. 2, No. 4, pp. 231-238). WB Saunders.
5. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotising enterocolitis hospitalisations among neonates in the United States. *Paediatric and perinatal epidemiology*. 2006 Nov;20(6):498-506.
6. Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: clinical challenges, pathophysiology and management. *Pathophysiology*. 2014 Feb 1;21(1):3-12.
7. Stanikova A, Jouza M, Bohosova J, Slaby O, Jabandziev P. Role of the microbiome in pathophysiology of necrotising enterocolitis in preterm neonates. *BMJ Paediatrics Open*. 2023 Nov 2;7(1):e002172.
8. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*. 2005 Jan;115(1):1-4. doi: 10.1542/peds.2004-1463. PMID: 15629973.
9. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, Hammerman C. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr*. 2005 Aug;147(2):192-6. doi: 10.1016/j.jpeds.2005.03.054. PMID: 16126048.
10. Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, Tsao LY, Chen CH, Su BH. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics*. 2008 Oct;122(4):693-700. doi: 10.1542/peds.2007-3007. PMID: 18829790.
11. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of *Bifidobacterium lactis* on the incidence of nosocomial infections

- in very-low-birth-weight infants: a randomized controlled trial. *Neonatology*. 2010;98(2):156-63. doi: 10.1159/000280291. Epub 2010 Mar 16. PMID: 20234140.
12. 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. *Am J Clin Nutr*. 2011 Jan;93(1):81-6. doi: 10.3945/ajcn.2010.29799. Epub 2010 Oct 27. PMID: 20980486.
 13. Sari FN, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: *Lactobacillus sporogenes* for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *Eur J Clin Nutr*. 2011 Apr;65(4):434-9. doi: 10.1038/ejcn.2010.278. Epub 2011 Jan 19. PMID: 21245887.
 14. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics*. 2013 Dec 1;132(6):1055-62.
 15. Demirel G, Erdeve O, Celik IH, Dilmen U. *Saccharomyces boulardii* for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled study. *Acta Paediatr*. 2013 Dec;102(12):e560-5. doi: 10.1111/apa.12416. PMID: 24028629.
 16. Costeloe K, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, King A, Panton N, Stacey F, Whiley A, Wilks M, Millar MR. A randomised controlled trial of the probiotic *Bifidobacterium breve* BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial. *Health Technol Assess*. 2016 Aug;20(66):1-194. doi: 10.3310/hta20660. PMID: 27594381; PMCID: PMC5027379.
 17. Denkel LA, Schwab F, Garten L, Geffers C, Gastmeier P, Piening B. Dual-strain probiotics reduce NEC, mortality and neonatal bloodstream infections among extremely low birthweight infants. *Arch Dis Child Fetal Neonatal Ed*. 2017 Nov;102(6):F559-F560. doi: 10.1136/archdischild-2017-313897. Epub 2017 Sep 18. PMID: 28923984.
 18. Alshaikh BN, Ting J, Lee S, Lemyre B, Wong J, Afifi J, Beltempo M, Shah PS; Canadian Neonatal Network Investigators. Effectiveness and Risks of Probiotics in Preterm Infants. *Pediatrics*. 2025 Feb 12;155(3):e2024069102. doi: 10.1542/peds.2024-069102. PMID: 39933567.