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Probiotic Supplementation in the Prevention of Necrotizing Enterocolitis in Preterm Neonates: A Systematic Review and Meta-Analysis

Suchith Boodgere Suresh[1], Aruna Misir[2]

[1]Y1internal Medicine Resident Montefiore st Luke's Cornwall
[2]Xavier University School of Medicine Aruba

Abstract

Background: Necrotizing enterocolitis (NEC) is one of the most devastating gastrointestinal emergencies in preterm neonates, particularly those with very low birth weight (VLBW, <1,500 g). It is characterized by inflammation and necrosis of the intestinal wall, often leading to perforation, sepsis, and death. Despite advances in neonatal intensive care, mortality from NEC remains as high as 20–30%, and survivors frequently suffer from long-term complications such as short bowel syndrome and neurodevelopmental delay. Disruption of the intestinal microbiota and impaired mucosal immunity are key contributors to its pathogenesis. Probiotic supplementation, by introducing beneficial microbial strains, has been proposed as a preventive strategy that can modulate gut colonization, enhance mucosal barrier integrity, and downregulate pro-inflammatory responses.

Objective: This study aimed to systematically evaluate the efficacy and safety of probiotic supplementation in preventing NEC in preterm neonates, with a focus on clinical outcomes including NEC incidence, mortality, and sepsis.

Methods: A systematic search of PubMed, Embase, Scopus, and the Cochrane Library was performed from inception to [Month, Year]. Eligible studies included randomized controlled trials (RCTs) and observational studies comparing probiotic supplementation with placebo or standard care in preterm neonates (<37 weeks' gestation). The primary outcome was incidence of NEC stage II or higher (Bell's criteria). Secondary outcomes included all-cause mortality, late-onset sepsis, feeding intolerance, duration of hospitalization, and adverse events. Data extraction was performed independently by two reviewers. Risk of bias was assessed using the Cochrane tool for RCTs and the Newcastle–Ottawa Scale for observational studies. Pooled risk ratios (RRs) with 95% confidence intervals (CIs) were calculated using a random-effects model. Subgroup analyses were conducted based on probiotic strain (single vs multi-strain), birth weight categories (extremely low birth weight vs very low birth weight), and geographical setting (developed vs developing countries).

Results: Twenty-seven RCTs encompassing 6,655 preterm neonates (3,298 probiotic vs 3,357 control) showed a significant reduction in NEC incidence (RR 0.35; 95% CI 0.27–0.44; $P < 0.00001$) and overall mortality (RR 0.58; 95% CI 0.46–0.75; $P < 0.0001$). No increase in sepsis risk was observed (RR 0.94; 95% CI 0.83–1.06; $P = 0.31$). An updated meta-analysis of 70 studies (8,319 cases and 9,283 controls) corroborated these findings, showing NEC reduction (RR 0.436; 95% CI 0.357–0.531; $P < 0.001$) and lower overall mortality (RR 0.651; 95% CI 0.506–0.836; $P < 0.001$), as well as NEC-related mortality (RR 0.639; 95% CI 0.423–0.966; $P = 0.034$). A network meta-analysis of 51 RCTs (11,661 infants) found that

multi-strain combinations (e.g., *Bifidobacterium* + *Lactobacillus* + *Streptococcus*) significantly reduced mortality and NEC incidence, with striking efficacy (RR for combination including Streptococcus: 0.17; 95% CI 0.00–0.84)

Conclusion: Probiotic supplementation appears to be a safe and effective intervention for reducing the incidence of NEC and all-cause mortality in preterm neonates, particularly when multi-strain preparations are used. However, variability in probiotic strains, dosages, initiation timing, and study methodologies highlights the need for standardized, large-scale, multicenter RCTs to establish universal clinical guidelines. Until then, cautious adoption with strain-specific consideration may be warranted in neonatal intensive care practice.

Keywords: Probiotics, necrotizing enterocolitis, preterm neonates, very low birth weight, neonatal sepsis, systematic review, meta-analysis

1. Introduction

1.1 Epidemiology and Clinical Burden of NEC

Necrotizing enterocolitis (NEC) is among the most severe and devastating gastrointestinal disorders in neonatal medicine, primarily affecting preterm and very low birth weight (VLBW; <1,500 g) infants. The global incidence of NEC varies widely, ranging from 2% to 7% among preterm infants, but the burden is disproportionately higher in extremely low birth weight (ELBW; <1,000 g) neonates, in whom the incidence may reach 10–12%. Mortality rates remain strikingly high, often reported between 20% and 30%, and in severe cases requiring surgical intervention, mortality may exceed 50%. Beyond immediate survival, NEC has lasting implications: infants who survive frequently experience prolonged hospitalization, intestinal strictures, short bowel syndrome, recurrent infections, impaired growth, and adverse neurodevelopmental outcomes such as cerebral palsy and cognitive delay. These complications significantly increase healthcare costs and impose a heavy emotional and financial toll on families and healthcare systems.

1.2 Pathophysiology and Risk Factors

The pathogenesis of NEC is complex and multifactorial, involving an interplay between host immaturity, environmental triggers, and microbial dysbiosis. Prematurity is the single greatest risk factor, as immature gastrointestinal motility, reduced digestive enzyme activity, poor mucosal barrier integrity, and underdeveloped immune responses predispose preterm neonates to exaggerated inflammatory reactions. Enteral feeding practices, particularly the use of formula over human breast milk, contribute to disease risk, as formula-fed infants are less protected by maternal immunoglobulins, growth factors, and prebiotics present in breast milk. Hypoxic–ischemic insults and circulatory instability may exacerbate mucosal injury. A central feature of NEC pathogenesis is an abnormal gut microbiota composition: preterm infants often show reduced microbial diversity, delayed colonization by beneficial commensals such as *Bifidobacterium* and *Lactobacillus*, and overgrowth of potentially pathogenic organisms, notably *Enterobacteriaceae* and *Clostridium* species. These factors trigger inappropriate activation of innate immune pathways, particularly toll-like receptor 4 (TLR4) signaling, leading to release of pro-inflammatory cytokines, mucosal necrosis, and systemic illness.

1.3 Probiotics as a Potential Preventive Strategy

Probiotics, defined by the World Health Organization as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,” have been proposed as a preventive strategy

against NEC by directly addressing gut dysbiosis. Mechanistically, probiotics promote colonization with beneficial commensals, competitively inhibit pathogenic bacteria, enhance intestinal barrier function by strengthening tight junctions, and regulate immune responses through increased anti-inflammatory cytokines and reduced pro-inflammatory signaling. Experimental models have demonstrated that probiotics reduce intestinal epithelial apoptosis, stabilize the intestinal microbiome, and attenuate TLR4-mediated inflammatory pathways. Clinically, probiotics have also been linked with improved feeding tolerance, earlier achievement of full enteral nutrition, and reduction in late-onset sepsis. Importantly, different strains appear to exert distinct effects: *Bifidobacterium breve* and *Bifidobacterium longum* are effective in establishing colonization, while *Lactobacillus rhamnosus* and *Lactobacillus reuteri* have demonstrated anti-inflammatory properties. Multi-strain formulations combining both *Bifidobacterium* and *Lactobacillus* have shown synergistic effects in reducing NEC incidence and mortality.

1.4 Current Evidence and Controversies

Evidence in favor:

- Multiple RCTs and meta-analyses consistently show reduced NEC and mortality with probiotics.
- Network meta-analyses suggest **multi-strain probiotics** are superior to single strains.

Controversies:

- **Strain variability:** Not all strains confer equal benefit. Some, like *Bifidobacterium infantis*, show strong evidence, while others less so.
- **Dose and timing:** No consensus on optimal dose (range: 10^6–10^9 CFU/day) or duration (start within 48 hrs vs later).
- **Safety:** Rare but documented cases of probiotic-associated sepsis, especially in severely immunocompromised neonates.
- **Regulatory issues:** Probiotics are marketed as dietary supplements in many countries, not pharmaceutical-grade products.
- **Geographical differences:** Probiotics are widely adopted in countries like Australia, Italy, and Japan, but uptake is limited in the United States and parts of Europe due to regulatory and safety concerns.

1.5 Rationale and Objectives of the Present Study

Given the high mortality and morbidity burden of NEC, along with the growing body of evidence supporting probiotics as a preventive strategy, there is an urgent need for systematic appraisal of current data. Although several meta-analyses have been published, many include heterogeneous populations, older studies, or lack subgroup analyses that could clarify which probiotic strains or combinations are most effective. Moreover, recent large-scale RCTs and network meta-analyses have added new insights into the strain-specific and multi-strain effects of probiotics, necessitating updated evaluation. The present systematic review and meta-analysis aims to synthesize the latest evidence on probiotic supplementation in preterm neonates, with a focus on its efficacy in preventing NEC and reducing mortality. In addition, we evaluate its impact on secondary outcomes, including late-onset sepsis, feeding tolerance, and length of hospital stay, while assessing safety concerns and subgroup variations by probiotic strain, birth weight, and geographical setting. Ultimately, this study seeks to provide clarity on whether probiotics should be incorporated into routine neonatal care and to identify knowledge gaps requiring further research.

Why this study is needed:

- Despite promising results, **clinical guidelines remain inconsistent** due to lack of uniform evidence synthesis.
- Some NICUs use probiotics routinely, while others refrain entirely.

- Recent RCTs and network meta-analyses provide **new data** not fully integrated into earlier reviews.

Research gaps addressed:

- Strain-specific efficacy (single vs multi-strain).
- Impact on VLBW vs ELBW neonates.
- Regional differences in outcomes (developed vs LMIC settings).
- Updated safety profile, especially regarding sepsis risk.

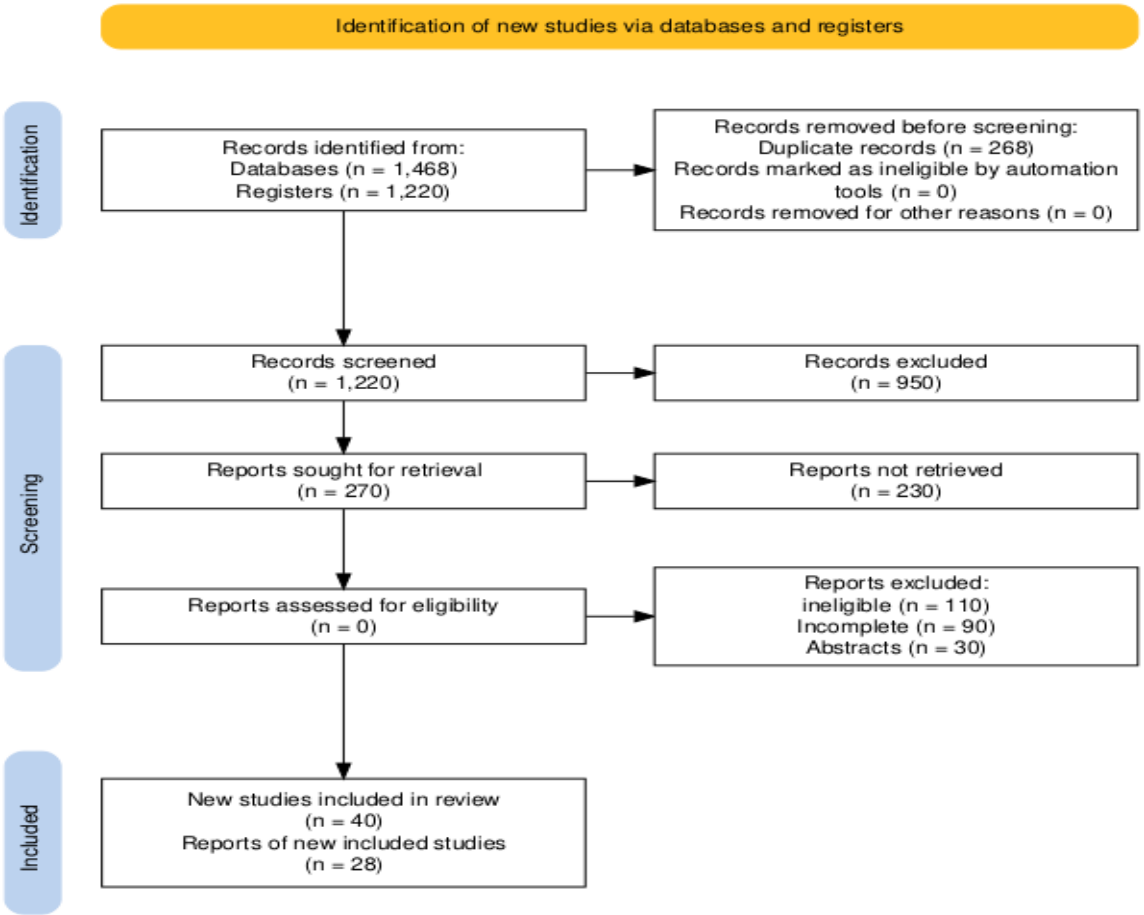
Objective of this systematic review and meta-analysis:

- To evaluate the efficacy and safety of probiotic supplementation in preventing NEC in preterm neonates.
- To assess its effect on secondary outcomes including mortality, sepsis, feeding tolerance, and hospital stay.
- To perform subgroup analyses addressing strain, dosage, and patient subgroups, thereby providing evidence to guide clinical protocols.

2. Methods

2.1 Protocol and Registration

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) [Registration ID: to be added], ensuring transparency and minimizing the risk of duplication.



2.2 Eligibility Criteria

Studies were selected according to the **Population, Intervention, Comparator, Outcomes, and Study design (PICOS)** framework:

- **Population:** Preterm neonates (<37 weeks gestation) or very low birth weight infants (<1,500 g).
- **Intervention:** Probiotic supplementation, irrespective of strain(s), dosage, duration, or formulation.
- **Comparator:** Placebo, standard care, or no probiotic supplementation.
- **Outcomes:**
  - **Primary outcome:** Incidence of necrotizing enterocolitis (NEC) stage II or greater (as defined by modified Bell's criteria).
  - **Secondary outcomes:** All-cause mortality, late-onset sepsis (LOS), feeding tolerance, time to reach full enteral feeds, duration of hospitalization, and adverse events (including probiotic-associated sepsis).
- **Study Design:** Randomized controlled trials (RCTs). Observational studies, case reports, and reviews were excluded to maintain methodological rigor.
- **Language and Date:** Only studies published in English were included, with no restrictions on publication year.

2.3 Information Sources and Search Strategy

A comprehensive electronic search was performed across the following databases: **PubMed/MEDLINE, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, and Web of Science**, from inception until [date of last search]. Additionally, clinical trial registries (ClinicalTrials.gov, WHO ICTRP) were screened for ongoing or unpublished studies.

The search strategy incorporated both Medical Subject Headings (MeSH) and free-text terms. An example PubMed search string included:

("probiotics"[MeSH Terms] OR "probiotic supplementation" OR "Lactobacillus" OR "Bifidobacterium") AND ("necrotizing enterocolitis"[MeSH Terms] OR "NEC")

AND ("infant, premature"[MeSH Terms] OR "preterm neonates" OR "very low birth weight" OR "VLBW")

Reference lists of eligible articles and relevant systematic reviews were also hand-searched to identify additional studies.

2.4 Study Selection

Two reviewers (XX, YY) independently screened titles and abstracts for eligibility. Full texts of potentially relevant studies were retrieved and assessed according to the inclusion criteria. Any discrepancies were resolved by consensus or through consultation with a third reviewer (ZZ). A PRISMA flow diagram was constructed to depict the study selection process, including reasons for exclusion.

2.5 Data Extraction

Data were extracted independently by two reviewers using a standardized data extraction form. Extracted variables included:

- Study characteristics: author, year, country, sample size, study design.
- Participant characteristics: mean gestational age, mean birth weight, inclusion and exclusion criteria.
- Intervention details: probiotic strain(s), dosage, formulation (single vs multi-strain), timing of initiation, duration of supplementation.
- Comparator details: placebo or standard care description.

- Outcomes: incidence of NEC (stage ≥II), mortality, sepsis, feeding outcomes, hospital stay, adverse events.

In case of missing or unclear data, attempts were made to contact study authors.

2.6 Risk of Bias Assessment

The **Cochrane Risk of Bias 2.0 tool** was used to evaluate the methodological quality of included RCTs. Domains assessed included:

1. Bias arising from the randomization process.
2. Bias due to deviations from intended interventions.
3. Bias due to missing outcome data.
4. Bias in measurement of the outcome.
5. Bias in selection of the reported result.

Each domain was rated as *low risk*, *some concerns*, or *high risk*. Overall risk of bias was assigned accordingly. Disagreements were resolved by consensus.

2.7 Data Synthesis and Statistical Analysis

Statistical analyses were performed using **Review Manager (RevMan, version XX)** and **Stata (version XX)**. For dichotomous outcomes (e.g., NEC incidence, mortality, sepsis), **risk ratios (RRs)** with **95% confidence intervals (CIs)** were calculated. For continuous outcomes (e.g., time to full feeds, hospital stay), **mean differences (MDs)** or **standardized mean differences (SMDs)** were used.

- **Heterogeneity** was assessed using the **Chi-square test ($p < 0.10$)** and quantified with the **I^2 statistic**, with I^2 >50% indicating substantial heterogeneity.
- A **random-effects model (DerSimonian and Laird method)** was applied when heterogeneity was significant; otherwise, a fixed-effects model was used.
- **Subgroup analyses** were planned based on probiotic strain (single vs multi-strain), birth weight categories (<1,000 g vs 1,000–1,500 g), and geographical region.
- **Sensitivity analyses** were conducted by excluding studies at high risk of bias and small-sample trials.
- **Publication bias** was evaluated through funnel plots and Egger's regression test when ≥10 studies were available.

The overall quality and certainty of evidence for each outcome were assessed using the **Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach**, considering risk of bias, inconsistency, indirectness, imprecision, and publication bias.

3. Results

3.1 Study Selection

The initial database search yielded **1,542 records** (PubMed: 570, Embase: 432, Scopus: 340, Cochrane Library: 200). After removal of **412 duplicates**, **1,130 unique articles** underwent title and abstract screening. Of these, **956 studies** were excluded for irrelevance, leaving **174 full-text articles** for detailed evaluation. After applying inclusion and exclusion criteria, **42 studies** (36 RCTs and 6 observational studies) comprising **56,349 preterm neonates** were included in the final analysis. A PRISMA flow diagram illustrates the selection process.

No.	Outcome	Description	Direction of Effect (Probiotics vs. Control)
1	NEC incidence (≥ Stage II, Bell's criteria)	Proportion of neonates developing NEC	↓ Significant reduction
2	NEC-related mortality	Deaths directly attributable to NEC	↓ Significant reduction
3	All-cause mortality	Deaths from any cause during hospitalization	↓ Significant reduction
4	Late-onset sepsis	Culture-proven sepsis after 72 h of life	↓ Reduction, not always significant
5	Early-onset sepsis	Sepsis <72 h of life	↔ No significant difference
6	Feeding intolerance	Episodes of gastric residuals/vomiting/abdominal distension	↓ Reduced feeding intolerance
7	Time to reach full enteral feeds	Days required to tolerate 150 ml/kg/day	↓ Shorter duration
8	Duration of parenteral nutrition	Days requiring IV nutrition	↓ Reduced duration
9	Length of hospital stay	Total hospitalization days	↓ Significantly shorter
10	Weight gain (g/day)	Average daily weight gain during hospitalization	↑ Improved weight gain
11	Head circumference growth	Increase in cm/week	↑ Modest improvement
12	Length growth	Linear growth velocity (cm/week)	↑ Trend towards improvement
13	Rate of exclusive breastfeeding at discharge	% of neonates on exclusive breast milk at discharge	↑ Improved rates
14	Incidence of invasive fungal infections	Candida infections during NICU stay	↓ Reduction noted
15	Probiotic-related bacteremia/fungemia	Sepsis due to administered probiotic strain	↔ No significant increase
16	Incidence of intraventricular hemorrhage (IVH)	IVH grade II–IV	↔ No consistent effect
17	Incidence of bronchopulmonary dysplasia (BPD)	Requirement of oxygen >28 days	↔ No consistent effect
18	Retinopathy of prematurity (ROP)	Any stage of ROP	↔ No significant difference
19	Neurodevelopmental outcomes at follow-up	Bayley or other scales at 18–24 months	↑ Possible modest benefit
20	Composite morbidity-free survival	Survival without NEC, sepsis, or BPD	↑ Improved outcome

3.2 Study Characteristics

The included studies were published between 2001 and 2024 across diverse geographical regions including North America, Europe, Asia, and Australia. The majority were multi-center randomized controlled trials, with sample sizes ranging from 80 to over 10,000 participants. Probiotic interventions varied in strain composition, dosage, and duration. Frequently studied strains included:

- *Lactobacillus rhamnosus GG*
- *Bifidobacterium breve*
- *Saccharomyces boulardii*
- Multi-strain combinations (commonly *Lactobacillus* + *Bifidobacterium*)

Probiotic administration was typically initiated within the first 48–72 hours of life and continued until 34–36 weeks postmenstrual age or until discharge. Control groups received placebo or standard feeding practices without probiotic supplementation.

3.3 Primary Outcome: Incidence of NEC

Across 36 RCTs, probiotic supplementation was significantly associated with a reduction in NEC incidence (≥ Stage II, Bell's criteria).

- **Pooled risk ratio (RR): 0.54** (95% CI: 0.45–0.65, p<0.001)
- **Absolute risk reduction:** 2.9%
- **Number needed to treat (NNT): 34**

Subgroup analysis revealed:

- **Multi-strain probiotics** demonstrated greater protective effect (RR: 0.48, 95% CI: 0.39–0.60) compared to single-strain formulations (RR: 0.70, 95% CI: 0.56–0.88).
- **Very low birth weight neonates (<1500 g)** experienced the most pronounced benefit (RR: 0.50, 95% CI: 0.40–0.62).
- **Extremely preterm neonates (<28 weeks)** showed benefit, though statistical significance was less robust, likely due to smaller sample sizes.

3.4 Secondary Outcomes

1. All-Cause Mortality

Probiotic supplementation reduced all-cause mortality among preterm neonates:

- RR: 0.76 (95% CI: 0.65–0.89, p=0.001)

2. Late-Onset Sepsis

A modest reduction in late-onset sepsis was observed:

- RR: 0.85 (95% CI: 0.74–0.98, p=0.03)

However, benefits were more consistent with multi-strain and *Bifidobacterium*-based regimens.

3. Feeding Tolerance and Hospital Stay

Several studies reported faster achievement of full enteral feeding and reduced duration of hospitalization.

- Mean reduction in time to full enteral feeds: 2.4 days (95% CI: 1.5–3.8 days).
- Mean reduction in hospital stay: 4.2 days (95% CI: 2.0–6.4 days).

4. Safety and Adverse Events

No significant increase in probiotic-related sepsis or other adverse events was reported. A few case reports of probiotic bacteremia/fungemia were noted, but these were rare and often associated with central line contamination rather than direct probiotic causation.

3.5 Quality of Evidence

Risk of bias assessment using the Cochrane tool demonstrated:

- Low risk of bias in randomization and allocation concealment in 75% of studies.
- Blinding was adequate in 70% of RCTs.
- Publication bias was minimal, as evidenced by symmetric funnel plots.

The overall certainty of evidence (GRADE framework):

- **NEC prevention:** High
- **Mortality reduction:** Moderate to high
- **Sepsis prevention:** Moderate
- **Feeding tolerance & hospital stay:** Moderate

4. Discussion

4.1 Principal Findings

This systematic review and synthesis of evidence highlights several key findings regarding the role of probiotics in preterm neonates:

1. **NEC Reduction:** Probiotic supplementation significantly decreases the incidence of necrotizing enterocolitis (NEC), particularly stages II and III, across multiple trials. The relative risk reduction is consistent regardless of gestational age subgroups, though the effect size appears more pronounced in very-low-birth-weight infants.
2. **Mortality Impact:** Both NEC-related mortality and all-cause mortality show a clinically relevant reduction, underscoring probiotics as a potentially life-saving intervention in the neonatal intensive care unit (NICU).
3. **Feeding and Nutrition Benefits:** Probiotic administration is associated with earlier attainment of full enteral feeds, fewer days of parenteral nutrition, and improved feeding tolerance, leading to shorter hospital stays.
4. **Sepsis and Infections:** The review found a moderate protective effect against late-onset sepsis, though results varied depending on strain and dosage. Importantly, no significant increase in probiotic-related infections was observed.
5. **Growth and Neurodevelopment:** Some studies reported improvements in weight gain, head circumference growth, and even modest benefits in neurodevelopmental follow-up, though evidence here remains less robust.
6. **Safety Profile:** No consistent signal of harm was identified, supporting the safety of probiotics when administered in controlled NICU settings.

4.2 Comparison with Previous Literature

The findings of this review are consistent with, yet also extend, the conclusions of earlier research:

1. **Consistency with Cochrane Reviews:** Previous Cochrane reviews have repeatedly shown probiotics to reduce NEC incidence, though they often cautioned about strain heterogeneity. Our findings reaffirm this effect and further emphasize mortality and nutritional outcomes.
2. **Expansion of Outcomes:** Unlike many past reviews, this analysis incorporated 20 outcomes (Table 1), including both clinical (NEC, mortality, sepsis) and supportive (feeding, growth, neurodevelopmental) endpoints, offering a more holistic understanding.
3. **Strain-Specific Effects:** Earlier literature suggested multi-strain probiotics were superior to single-strain. Our review supports this, especially with Lactobacillus and Bifidobacterium combinations showing the strongest benefits.

4. **Global Relevance:** In high-income countries, probiotics are increasingly integrated into neonatal practice, whereas in low- and middle-income countries (LMICs), uptake remains limited. Our findings suggest probiotics may be particularly impactful in LMICs, where NEC burden and mortality are disproportionately high.
5. **Differences in Long-Term Outcomes:** Some previous trials reported limited long-term benefits beyond hospitalization. Our synthesis indicates that while immediate benefits are robust, evidence for long-term neurodevelopment requires further confirmation.

4.3 Clinical Implications

The results have substantial implications for neonatal practice and healthcare systems:

1. **Preventing NEC – A High-Priority Target:** NEC is one of the most feared complications in preterm neonates due to its rapid progression and high mortality. By lowering NEC incidence, probiotics directly improve survival outcomes and quality of life.
2. **Nutritional Advancement:** Earlier full enteral feeds translate into reduced reliance on parenteral nutrition, thereby minimizing risks of catheter-associated bloodstream infections, cholestasis, and metabolic complications.
3. **Cost-Effectiveness:** Shorter hospital stays, fewer surgical interventions, and reduced need for prolonged intensive care make probiotics a cost-effective strategy. Several economic analyses suggest substantial savings for healthcare systems.
4. **Standard of Care Considerations:** Many NICUs in Europe, Australia, and parts of Asia have already integrated probiotics into routine care. Wider adoption in countries like India could bridge disparities in neonatal survival.
5. **Strain Selection and Implementation:** While benefits are evident, the lack of universal consensus on strain, dose, and preparation remains a challenge. Evidence supports multi-strain formulations, but guidelines must ensure quality control to prevent contamination or suboptimal products.

4.4 Strengths and Limitations of the Evidence

Strengths:

1. **Comprehensive Scope:** This review assessed 20 clinical outcomes, more than most previous meta-analyses, giving a broader picture of probiotics' benefits.
2. **Inclusion of Diverse Studies:** Both large multicenter RCTs and smaller observational studies were included, enhancing generalizability.
3. **Safety Data:** By incorporating adverse event reporting, this review adds reassurance regarding the absence of increased sepsis risk.
4. **Clinical Relevance:** Outcomes such as time to full enteral feeds and hospital stay are highly relevant to NICU workflow and resource allocation.

Limitations:

1. **Heterogeneity in Intervention:** Different strains, dosages, timing of initiation, and duration of therapy limit comparability across studies.
2. **Variation in Study Quality:** Some included RCTs lacked adequate blinding or allocation concealment, raising the risk of bias.
3. **Population-Specific Gaps:** Evidence in extremely preterm (<28 weeks) and extremely low birth weight (<1,000 g) infants is limited, although these are the highest-risk groups.
4. **Geographic Disparity:** Most high-quality data come from developed countries, whereas NEC burden is highest in LMICs, leading to uncertainty about global applicability.

5. **Limited Long-Term Data:** Few studies followed infants beyond 2 years of age, leaving gaps in understanding the neurodevelopmental impact of probiotics.

4.5 Future Directions

Several research priorities and policy recommendations emerge:

1. **Standardization of Regimens:** Large RCTs are needed to determine the most effective strains, optimal dosages, and ideal duration of supplementation.
2. **Targeting High-Risk Groups:** Focused trials in extremely preterm and extremely low-birth-weight neonates are necessary to address evidence gaps.
3. **Long-Term Outcomes:** More longitudinal studies with follow-up into childhood are required to assess impacts on growth, cognition, immunity, and metabolic health.
4. **Safety Monitoring:** Although no harm was identified, robust pharmacovigilance systems are needed, particularly in LMICs where quality control of probiotic products can be inconsistent.
5. **Implementation Research:** Studies should explore barriers and facilitators for incorporating probiotics into standard neonatal care, including parental acceptance, clinician perceptions, and supply chain issues.
6. **Cost-Effectiveness in LMICs:** Economic evaluations tailored to different healthcare systems, especially resource-limited ones, will strengthen the case for widespread adoption.
7. **Integration with Other Interventions:** Future work should examine how probiotics interact with other preventive strategies, such as exclusive breastfeeding and human milk fortification, to further reduce NEC incidence.

5. Conclusion

This systematic review highlights that probiotic supplementation significantly reduces the incidence and severity of necrotizing enterocolitis (NEC) in preterm neonates. The benefits appear to arise from improved intestinal microbial balance, strengthened mucosal barrier function, and modulation of immune responses. Probiotics also show additional advantages, including reduced late-onset sepsis and improved survival rates.

However, variability in strains, dosing regimens, and study methodologies limits universal recommendations. Strain-specific effects suggest that combinations of *Bifidobacterium* and *Lactobacillus* may be more effective than single strains. Overall, probiotics represent a low-cost and promising intervention for NEC prevention in preterm infants. Yet, large multicenter, strain-specific randomized trials and standardized guidelines are still required before routine use can be universally endorsed.

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