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Role of Probiotic Supplementation in the Prevention of Necrotising Enterocolitis in Preterm Neonates - A Randomised Controlled Trial

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Abstract

Background: Necrotising enterocolitis is a leading cause of death and long-term morbidity among preterm neonates, and intestinal dysbiosis is central to its pathogenesis. Meta-analytic evidence supports probiotic supplementation for prevention, but data from Indian units, where the background incidence, feeding practices and pathogen profile differ, remain limited. This trial evaluated probiotic supplementation for the prevention of necrotising enterocolitis in preterm neonates.

Methods: One hundred and fifty neonates of gestational age below 34 weeks and birth weight below 1800 g were randomised to receive a multi-strain probioticpreparation containing Lactobacillus and Bifidobacterium species or an identical placebo, administered once daily with feeds from the establishment of enteral feeding until 34 weeks corrected age or discharge. The primary outcome was necrotising enterocolitis of modified Bell stage II or above. Secondary outcomes included stage III disease, culture-positive late-onset sepsis, time to full enteral feeds, mortality and duration of stay.

Results: Necrotising enterocolitis of stage II or above occurred in 5 of 75 neonates (6.7 percent) receiving probiotics and 14 of 75 (18.7 percent) receiving placebo (relative risk 0.36, 95 percent CI 0.14 to 0.94, p = 0.024), with a number needed to treat of 9. Stage III disease occurred in 1 and 6 neonates respectively (p = 0.115). Full enteral feeds were attained earlier with probiotics (11.4 versus 14.2 days, p = 0.001). Late-onset sepsis and all-cause mortality did not differ significantly. No case of probiotic sepsis occurred.

Conclusion: Probiotic supplementation significantly reduced the incidence of necrotising enterocolitis of stage II or above and shortened the time to full enteral

feeds, without demonstrable effect on sepsis or mortality in a trial not powered for those outcomes. The findings support the Cochrane conclusion while underlining that product quality assurance is the principal barrier to implementation in India.

Keywords: Enterocolitis, Necrotizing; Probiotics; Infant, Premature; Infant, Very Low Birth Weight; Gastrointestinal Microbiome; Sepsis

Introduction

Necrotising enterocolitis is the commonest acquired gastrointestinal emergency of the newborn and remains among the leading causes of death beyond the first week of life in preterm neonates. Its incidence is inversely related to gestational age and birth weight, and among very low birth weight infants it affects between five and ten percent in most reported series. Mortality among those requiring surgery approaches thirty percent, and survivors carry a substantial burden of long-term morbidity comprising intestinal stricture, short bowel syndrome with dependence on parenteral nutrition, growth failure and adverse neuro developmental outcome. The condition therefore consumes a disproportionate share of neonatal intensive care resources relative to its incidence.

The pathogenesis is multi factorial but converges on a common sequence. Prematurity of the intestinal barrier, with immature tight junctions, deficient mucin production, reduced immunoglobulin A secretion and an exaggerated inflammatory response mediated through toll-like receptor 4, renders the preterm intestine vulnerable. Onto this substrate is superimposed an abnormal pattern of bacterial colonisation. Whereas the term breastfed infant acquires a micro biome dominated by Bifidobacterium and Lactobacillus species, the preterm neonate in intensive care is colonised by a restricted flora dominated by Enterobacteriaceae and other potentially pathogenic organisms, a pattern promoted by caesarean delivery, delayed and formula-based feeding, broad-spectrum antibiotic exposure and the intensive care environment itself. This intestinal dysbiosis is now regarded as central to pathogenesis rather than incidental to it, and it provides the rationale for attempting to modify the micro biome directly.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. The mechanisms proposed in this context are several and plausibly act together: competitive exclusion of pathogenic organisms from mucosal binding sites, production of short-chain fatty acids and bacteriocins that inhibit pathogen growth, enhancement of tight junction integrity and mucin production, down regulation of the toll-like receptor 4 mediated inflammatory cascade, and promotion of secretory immunoglobulin A production. Each of these has experimental support.

The clinical evidence base is unusually large. Successive Cochrane reviews have addressed this question, and the version by Sharif and colleagues published in 2020, incorporating 56 trials and 10,812 infants, reported that probiotics may reduce the risk of necrotising enterocolitis with a relative risk of 0.54 and a 95 percent confidence interval of 0.45 to 0.65, alongside reductions in all-cause mortality and late-onset invasive infection.[1] The subsequent 2023 update reaffirmed these conclusions while applying the GRADE framework to the certainty of the evidence.[2] Earlier versions of the same review, by AlFaleh and Anabrees, had reached similar conclusions and had argued that the evidence strongly supported a change in practice.[3] The systematic review of Dermyshi and colleagues, examining specific

strains and high-risk populations, likewise supported a preventive effect.[4] Few interventions in neonatology rest on a comparable weight of randomised evidence.

Despite this, adoption has been uneven and the controversy is genuine rather than merely conservative. Three objections recur. First, the trials have used a wide variety of preparations, strains, doses and durations, and the evidence does not identify which specific formulation is optimal; a benefit demonstrated for one multi-strain product does not necessarily transfer to another. Second, probiotic organisms are live and have caused invasive disease in preterm neonates, with reported cases of Lactobacillus and Bifidobacterium sepsis and, most seriously, a fatal case of gastrointestinal mucormycosis attributed to a contaminated product, which prompted regulatory action in the United States. Third, probiotic preparations in many jurisdictions, including India, are marketed as food supplements rather than as pharmaceuticals and are consequently subject to far less rigorous quality assurance, so that the content of viable organisms in a purchased product may differ substantially from that stated on the label. Position statements from professional bodies have reflected this tension, generally acknowledging the efficacy evidence while emphasising product quality and the need for informed parental consent.[5]

Indian data are relevant for reasons beyond mere local applicability. The incidence of necrotising enterocolitis, the pattern of antimicrobial resistance among neonatal pathogens, rates of exclusive breast milk feeding, the availability of donor human milk and unit-level feeding protocols all differ substantially from the settings in which most of the constituent trials were performed, and each of these is a determinant of the outcome under study. Agarwal and colleagues examined the effect of oral Lactobacillus on enteric microflora in low birth weight Indian neonates, establishing feasibility in this setting.[6] Larger Indian trials remain relatively few. The present randomised, double-blind, placebo-controlled trial was therefore undertaken to evaluate the role of probiotic supplementation in the prevention of necrotising enterocolitis in preterm neonates in an Indian tertiary care setting.

Aims and Objectives

The trial aimed to determine whether routine supplementation with a multi-strain probiotic preparation reduced the incidence of necrotising enterocolitis in preterm neonates cared for in an Indian neonatal intensive care unit. The primary objective was to compare the incidence of necrotising enterocolitis of modified Bell stage II or above between neonates of gestational age below 34 weeks and birth weight below 1800 g randomised to receive a multi-strain probiotic preparation and those randomised to receive an identical placebo.

The secondary objectives were to compare the incidence of severe necrotising enterocolitis of modified Bell stage III, the incidence of surgical intervention for necrotising enterocolitis, the incidence of culture-positive late-onset sepsis occurring after 72 hours of life, and all-cause mortality before discharge between the two groups. Further objectives were to compare feeding-related outcomes comprising the time to attainment of full enteral feeding, the number of days on which feeds were withheld or interrupted, the duration of parenteral nutrition, the incidence of feed intolerance and the time to regain birth weight, and to compare the duration of hospital stay and weight gain velocity. The final objective was to record the safety of the intervention, in particular the occurrence of any invasive infection

attributable to a probiotic organism, and to document adherence to the supplementation protocol.

Materials and Methods

Study design and setting

This prospective, randomised, double-blind, placebo-controlled trial was conducted in the neonatal intensive care unit of the Department of Paediatrics, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya District, Karnataka, over an eighteen-month period from January 2024 to June 2025.

Approval of the Institutional Ethics Committee was obtained before the commencement of enrolment and the trial was registered prospectively with the Clinical Trials Registry of India. Written informed consent was obtained from a parent or legal guardian of every neonate, and the consent process explicitly addressed the live nature of the organisms administered and the theoretical risk of probiotic sepsis. The trial was conducted in accordance with the principles of the Declaration of Helsinki and with the National Ethical Guidelines for Biomedical and Health Research Involving Children issued by the Indian Council of Medical Research, and is reported in accordance with the CONSORT statement.

Participants

Neonates were eligible if the gestational age assessed by the new Ballard score was below 34 completed weeks, the birth weight was below 1800 g, and enteral feeding had been commenced or was expected to commence within the first seven days of life. Written informed consent from a parent or legal guardian was mandatory.

Neonates were excluded if there was a major congenital malformation, particularly any gastrointestinal anomaly including gastroschisis, omphalocele, intestinal atresia or Hirschsprung disease; congenital cyanotic heart disease or a haemodynamically significant patent ductus arteriosus at the time of screening; established necrotising enterocolitis or suspected necrotising enterocolitis at screening; severe perinatal asphyxia with hypoxic ischaemic encephalopathy of stage II or above; known or suspected immunodeficiency; a central venous catheter in situ at the time of intended commencement, given the theoretical risk of probiotic translocation; short bowel syndrome or prior gastrointestinal surgery; an anticipated inability to receive enteral feeds for more than seven days; moribund status at admission with an expectation of death within 48 hours; and refusal of parental consent.

Sample size calculation

The sample size was calculated for the primary outcome, the incidence of necrotising enterocolitis of modified Bell stage II or above. On the basis of unit records for the preceding two years the incidence in the eligible population was taken as 18 percent, and a relative risk reduction of approximately two thirds was anticipated on the basis of the pooled estimate reported in the Cochrane review, giving an expected incidence of 6 percent in the intervention group.

The formula for the comparison of two independent proportions was applied:

- n per group $= (Z_{1-\alpha/2} + Z_{1-\beta})^2 \times [p_1(1 - p_1) + p_2(1 - p_2)] / (p_1 - p_2)^2$
- Substituting $Z_{1-\alpha/2} = 1.96$, $Z_{1-\beta} = 0.84$ for 80 percent power, $p_1 = 0.18$ and $p_2 = 0.06$:
- $n = (2.80)^2 \times [0.1476 + 0.0564] / (0.12)^2$
- $n = 7.84 \times 0.2040 / 0.0144$
- $n = 1.5994 / 0.0144 = 111.1$

This calculation indicated that 112 neonates per group would be required. Recruitment of 224 neonates was not achievable within the study period at this centre, and the

sample size was therefore set at 75 per group, giving 150 in total. It must be stated explicitly that the trial was consequently underpowered for the primary outcome as originally framed, with a power of approximately 62 percent to detect the anticipated difference, and that this limitation applies with still greater force to every secondary outcome. This is addressed in the discussion.

Randomisation and blinding

Neonates were allocated in a 1:1 ratio using a computer-generated block randomisation sequence with variable block sizes, stratified by gestational age above and below 30 weeks and by birth weight above and below 1200 g. Allocation was concealed in sequentially numbered, sealed, opaque envelopes prepared by a person not otherwise involved in the trial.

The probiotic and placebo sachets were identical in appearance, weight, colour and odour, were supplied in identically labelled containers coded A and B, and were dispensed by the unit pharmacist who took no further part in the conduct of the trial. The treating team, the bedside nursing staff, the parents, the investigator recording outcomes and the radiologist reporting abdominal radiographs were all blinded to allocation. The code was broken only after completion of data analysis.

Intervention

Neonates in the intervention group received a multi-strain probiotic preparation containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum and Bifidobacterium bifidum, providing not fewer than one billion colony-forming units per dose, administered once daily mixed with expressed breast milk or formula. Neonates in the placebo group received an identical volume of maltodextrin carrier without viable organisms, administered in the same manner. Supplementation commenced with the first enteral feed and continued daily until 34 weeks corrected gestational age, discharge, or death, whichever occurred first. Supplementation was withheld on any day on which enteral feeds were withheld and resumed when feeding recommenced. The probiotic product was obtained from a single manufacturer in a single batch where possible, and the manufacturer certificate of analysis for viable organism content was verified for each batch used.

All neonates in both groups received identical standard care in accordance with unit protocol, comprising a preference for expressed breast milk with formula used only where maternal milk was unavailable, standardized feeding advancement at 20 to 30 mL per kilogram per day, minimal enteral nutrition where clinically indicated, antenatal corticosteroid administration where the opportunity arose, restrictive use of empirical antibiotics with review at 48 hours against culture results, and standard infection control measures.

Outcome definitions

The primary outcome was necrotising enterocolitis of modified Bell stage II or above, diagnosed on the basis of clinical features together with radiological confirmation by pneumatosis intestinalis, portal venous gas or free intraperitoneal gas, with all abdominal radiographs reported by a radiologist blinded to allocation. Stage III denoted advanced disease with clinical instability and either intestinal perforation or the need for surgical intervention.

Secondary outcomes comprised necrotising enterocolitis of stage III, surgical intervention for necrotising enterocolitis, culture-positive late-onset sepsis defined as the growth of a recognised pathogen from a blood culture obtained after 72 hours of life in a neonate with clinical features of sepsis, all-cause mortality before

discharge, the time to full enteral feeding defined as the tolerance of 150 mL per kilogram per day for 48 consecutive hours, the number of days on which feeds were withheld, the duration of parenteral nutrition, feed intolerance defined as gastric residuals exceeding half the previous feed volume with abdominal distension leading to interruption of feeding, the time to regain birth weight, weight gain velocity in grams per kilogram per day, and the duration of hospital stay. Safety outcomes comprised any blood culture growing a Lactobacillus or Bifidobacterium species and any other adverse event judged possibly attributable to the study product.

Statistical analysis

Data were entered in Microsoft Excel and analysed using IBM SPSS Statistics version 26.0 on an intention-to-treat basis, every randomised neonate being analysed in the allocated group. Normality was assessed by the Shapiro-Wilk test. Normally distributed continuous variables were expressed as mean with standard deviation and compared by the independent samples Student t test, and non-normally distributed variables as median with interquartile range and compared by the Mann-Whitney U test. Categorical variables were expressed as frequencies with percentages and compared by the Pearson chi-square test, with the Fisher exact test where any expected cell frequency was below five.

The effect on the primary outcome was expressed as a relative risk with a 95 percent confidence interval, together with the absolute risk reduction and the number needed to treat. Prespecified subgroup analyses by gestational age stratum, birth weight stratum and feeding type were performed. A two-tailed p value below 0.05 was considered statistically significant.

Results

Participant flow and baseline characteristics

One hundred and seventy-four preterm neonates were assessed for eligibility. Twenty-four were excluded, of whom eight had major congenital malformations, six had a central venous catheter in situ at screening, four were moribund at admission, three had a haemodynamically significant patent ductus arteriosus and three parents declined consent. One hundred and fifty neonates were randomised, 75 to the probiotic group and 75 to the placebo group, and all were included in the intention-to-treat analysis. Four neonates in the probiotic group and five in the placebo group died before completion of the intervention period and were analysed for all outcomes accrued to the point of death.

The two groups were comparable at baseline. The mean gestational age was 30.8 ± 2.2 weeks in the probiotic group and 31.1 ± 2.1 weeks in the placebo group (t = 0.85, p = 0.395), and the mean birth weight was 1342 ± 286 g and 1368 ± 294 g respectively (t = 0.55, p = 0.584). Twenty-six neonates (34.7 percent) and 24 neonates (32.0 percent) were born before 30 weeks (χ^2 = 0.12, p = 0.727). Forty-two neonates (56.0 percent) and 40 neonates (53.3 percent) were male (χ^2 = 0.11, p = 0.742). A complete course of antenatal corticosteroids had been received by 48 mothers (64.0 percent) and 46 mothers (61.3 percent) respectively (χ^2 = 0.11, p = 0.736). Exclusive expressed breast milk feeding was achieved in 44 neonates (58.7 percent) and 42 neonates (56.0 percent) (χ^2 = 0.11, p = 0.740). Baseline characteristics are presented in Table 1.

Primary outcome

Necrotising enterocolitis of modified Bell stage II or above occurred in 5 of 75 neonates (6.7 percent) in the probiotic group and 14 of 75 neonates (18.7 percent) in

the placebo group. The relative risk was 0.36 with a 95 percent confidence interval of 0.14 to 0.94 (χ^2 = 5.11, p = 0.024). The absolute risk reduction was 12.0 percentage points with a 95 percent confidence interval of 1.6 to 22.4 percentage points, corresponding to a number needed to treat of 9 with a 95 percent confidence interval of 4 to 62.

The median age at onset of necrotising enterocolitis was 14 days with an interquartile range of 10 to 21 in the probiotic group and 12 days with an interquartile range of 8 to 18 in the placebo group, a difference that did not reach significance (U = 28.0, p = 0.446). Pneumatosis intestinalis was the confirmatory radiological sign in 4 of 5 and 11 of 14 cases respectively, portal venous gas in 1 and 2, and free intraperitoneal gas in 0 and 1. Primary outcome data are set out in Table 2.

Secondary outcomes

Necrotising enterocolitis of stage III occurred in 1 neonate (1.3 percent) in the probiotic group and 6 neonates (8.0 percent) in the placebo group, a difference that did not reach statistical significance (Fisher exact test, p = 0.115), and surgical intervention was required in 1 neonate (1.3 percent) and 5 neonates (6.7 percent) respectively (Fisher exact test, p = 0.209). Mortality attributable to necrotising enterocolitis occurred in 1 neonate (1.3 percent) and 4 neonates (5.3 percent) (Fisher exact test, p = 0.367).

Culture-positive late-onset sepsis occurred in 12 neonates (16.0 percent) in the probiotic group and 18 neonates (24.0 percent) in the placebo group, a difference that did not reach statistical significance (χ^2 = 1.49, p = 0.222). All-cause mortality before discharge was 4 neonates (5.3 percent) and 8 neonates (10.7 percent) respectively (χ^2 = 1.44, p = 0.230).

Feeding outcomes favoured the probiotic group. Full enteral feeding was attained at 11.4 ± 4.2 days in the probiotic group compared with 14.2 ± 5.6 days in the placebo group (t = 3.46, p = 0.001). The median number of days on which feeds were withheld was 2 with an interquartile range of 1 to 4 and 4 with an interquartile range of 2 to 7 respectively (U = 1948.5, p = 0.002). The duration of parenteral nutrition was 8.6 ± 4.4 days and 11.2 ± 5.8 days (t = 3.09, p = 0.002), and feed intolerance occurred in 18 neonates (24.0 percent) and 31 neonates (41.3 percent) (χ^2 = 5.13, p = 0.024). The time to regain birth weight was 10.8 ± 3.6 days and 12.4 ± 4.2 days (t = 2.50, p = 0.014), and weight gain velocity was 14.6 ± 3.8 and 13.2 ± 4.1 g per kilogram per day (t = 2.17, p = 0.032). The duration of hospital stay was 24.6 ± 10.2 days and 28.4 ± 12.6 days (t = 2.03, p = 0.044). Secondary outcomes are detailed in Table 3.

Subgroup analyses

Prespecified subgroup analysis indicated that the effect on the primary outcome was numerically greater in the more immature and smaller strata, in which the baseline risk was highest. Among the 50 neonates born before 30 weeks, necrotising enterocolitis occurred in 3 of 26 (11.5 percent) in the probiotic group and 8 of 24 (33.3 percent) in the placebo group (relative risk 0.35, 95 percent confidence interval 0.10 to 1.16, p = 0.062), while among the 100 neonates born at 30 weeks or later it occurred in 2 of 49 (4.1 percent) and 6 of 51 (11.8 percent) respectively (relative risk 0.35, 95 percent confidence interval 0.07 to 1.64, p = 0.174). Neither subgroup comparison reached significance, which is expected given the reduced numbers, and the test for interaction was not significant (p = 0.994), indicating no evidence that the relative effect differed by gestational age stratum.

Among the 86 neonates receiving exclusive expressed breast milk, necrotising enterocolitis occurred in 2 of 44 (4.5 percent) and 7 of 42 (16.7 percent) in the probiotic and placebo groups respectively, and among the 64 receiving any formula it occurred in 3 of 31 (9.7 percent) and 7 of 33 (21.2 percent). The test for interaction was again not significant (p = 0.612). Subgroup data appear in Table 4.

Safety

No blood culture in either group grew Lactobacillus or Bifidobacterium species at any point during the study. No case of fungaemia occurred. Abdominal distension not amounting to feed intolerance was recorded in 14 neonates (18.7 percent) in the probiotic group and 19 neonates (25.3 percent) in the placebo group ($\chi^2 = 0.97$, p = 0.324), and vomiting in 9 neonates (12.0 percent) and 13 neonates (17.3 percent) ($\chi^2 = 0.86$, p = 0.353). No neonate discontinued the study product because of an adverse event. Adherence, defined as receipt of the study product on at least 80 percent of eligible feeding days, was achieved in 69 neonates (92.0 percent) in the probiotic group and 71 neonates (94.7 percent) in the placebo group. The certificate of analysis was verified for each of the three batches used, and the viable organism content was within the manufacturer specification on each occasion. Safety and adherence data are presented in Tables 5 and 6.

Tables

Table 1: Baseline demographic and perinatal characteristics of the two groups (N = 150)

Variable	Probiotic (n = 75)	Placebo (n = 75)	Test statistic	p value
Gestational age, weeks (mean ± SD)	30.8 ± 2.2	31.1 ± 2.1	t = 0.85	0.395
Gestational age <30 weeks, n (%)	26 (34.7)	24 (32.0)	$\chi^2 = 0.12$	0.727
Birth weight, g (mean ± SD)	1342 ± 286	1368 ± 294	t = 0.55	0.584
Birth weight <1200 g, n (%)	28 (37.3)	26 (34.7)	$\chi^2 = 0.11$	0.735
Male sex, n (%)	42 (56.0)	40 (53.3)	$\chi^2 = 0.11$	0.742
Small for gestational age, n (%)	21 (28.0)	19 (25.3)	$\chi^2 = 0.14$	0.711
Caesarean delivery, n (%)	46 (61.3)	44 (58.7)	$\chi^2 = 0.11$	0.740
Complete antenatal corticosteroids, n (%)	48 (64.0)	46 (61.3)	$\chi^2 = 0.11$	0.736
Apgar score <7 at 5 minutes, n (%)	13 (17.3)	15 (20.0)	$\chi^2 = 0.18$	0.674
Respiratory distress syndrome, n (%)	44 (58.7)	46 (61.3)	$\chi^2 = 0.11$	0.740
Surfactant administered, n (%)	26 (34.7)	28 (37.3)	$\chi^2 = 0.11$	0.735
Age at first enteral feed, days (mean ± SD)	2.4 ± 1.2	2.6 ± 1.3	t = 0.98	0.329
Exclusive expressed breast milk, n (%)	44 (58.7)	42 (56.0)	$\chi^2 = 0.11$	0.740
Empirical antibiotics at birth, n (%)	52 (69.3)	54 (72.0)	$\chi^2 = 0.13$	0.718
Early-onset sepsis, n (%)	9 (12.0)	11 (14.7)	$\chi^2 = 0.23$	0.632

SD, standard deviation. Randomisation was stratified by gestational age above and below 30 weeks and by birth weight above and below 1200 g.

Table 2: Primary outcome: necrotising enterocolitis of modified Bell stage II or above (N = 150)

Outcome	Probiotic (n = 75)	Placebo (n = 75)	Effect estimate (95% CI)	p value
NEC stage II or above, n (%)	5 (6.7)	14 (18.7)	RR 0.36 (0.14–0.94)	0.024
Absolute risk reduction, percentage points	—	—	12.0 (1.6–22.4)	—
Number needed to treat	—	—	9 (4–62)	—
Stage distribution, n				
Stage IIA	3	6	—	—
Stage IIB	1	2	—	—
Stage IIIA	1	3	—	—
Stage IIIB	0	3	—	—
Age at onset, days, median (IQR)	14 (10–21)	12 (8–18)	—	0.446
Confirmatory radiological sign, n				
Pneumatosis intestinalis	4	11	—	—
Portal venous gas	1	2	—	—
Free intraperitoneal gas	0	1	—	—

NEC, necrotising enterocolitis; CI, confidence interval; RR, relative risk; IQR, interquartile range. All abdominal radiographs were reported by a radiologist blinded to allocation.

Table 3: Secondary clinical and feeding outcomes (N = 150)

Outcome	Probiotic (n = 75)	Placebo (n = 75)	Test statistic	p value
NEC stage III, n (%)	1 (1.3)	6 (8.0)	Fisher exact	0.115
Surgery for NEC, n (%)	1 (1.3)	5 (6.7)	Fisher exact	0.209
Death attributable to NEC, n (%)	1 (1.3)	4 (5.3)	Fisher exact	0.367
Culture-positive late-onset sepsis, n (%)	12 (16.0)	18 (24.0)	$\chi^2 = 1.49$	0.222
All-cause mortality before discharge, n (%)	4 (5.3)	8 (10.7)	$\chi^2 = 1.44$	0.230
Time to full enteral feeds, days (mean ± SD)	11.4 ± 4.2	14.2 ± 5.6	t = 3.46	0.001
Days feeds withheld, median (IQR)	2 (1–4)	4 (2–7)	U = 1948.5	0.002
Duration of parenteral nutrition, days (mean ± SD)	8.6 ± 4.4	11.2 ± 5.8	t = 3.09	0.002
Feed intolerance, n (%)	18 (24.0)	31 (41.3)	$\chi^2 = 5.13$	0.024
Time to regain birth weight, days (mean ± SD)	10.8 ± 3.6	12.4 ± 4.2	t = 2.50	0.014
Weight gain velocity, g/kg/day (mean ± SD)	14.6 ± 3.8	13.2 ± 4.1	t = 2.17	0.032
Duration of hospital stay, days (mean ± SD)	24.6 ± 10.2	28.4 ± 12.6	t = 2.03	0.044
Retinopathy of prematurity requiring treatment, n (%)	5 (6.7)	7 (9.3)	$\chi^2 = 0.36$	0.548
Bronchopulmonary dysplasia at 36 weeks, n (%)	9 (12.0)	12 (16.0)	$\chi^2 = 0.50$	0.478

NEC, necrotising enterocolitis; SD, standard deviation; IQR, interquartile range. The trial was powered for the primary outcome only; the absence of significant differences in the outcomes above should not be interpreted as evidence of no effect.

Table 4: Prespecified subgroup analysis of the primary outcome (N = 150)

Subgroup	Probiotic n/N (%)	Placebo n/N (%)	Relative risk (95% CI)	p	p interaction
Gestational age					0.994
<30 weeks	3/26 (11.5)	8/24 (33.3)	0.35 (0.10–1.16)	0.062	
≥30 weeks	2/49 (4.1)	6/51 (11.8)	0.35 (0.07–1.64)	0.174	
Birth weight					0.874
<1200 g	3/28 (10.7)	9/26 (34.6)	0.31 (0.09–1.02)	0.038	
≥1200 g	2/47 (4.3)	5/49 (10.2)	0.42 (0.09–2.05)	0.281	
Feeding type					0.612
Exclusive breast milk	2/44 (4.5)	7/42 (16.7)	0.27 (0.06–1.25)	0.062	
Any formula	3/31 (9.7)	7/33 (21.2)	0.46 (0.13–1.61)	0.209	
Overall	5/75 (6.7)	14/75 (18.7)	0.36 (0.14–0.94)	0.024	

CI, confidence interval. Subgroup analyses were prespecified but the trial was not powered for them; these results are descriptive and hypothesis-generating only. No test for interaction approached significance.

Table 5: Safety outcomes and adverse events (N = 150)

Safety outcome	Probiotic (n = 75)	Placebo (n = 75)	Test statistic	p value
Blood culture growing Lactobacillus species, n (%)	0 (0.0)	0 (0.0)	—	—
Blood culture growing Bifidobacterium species, n (%)	0 (0.0)	0 (0.0)	—	—
Fungaemia, n (%)	0 (0.0)	1 (1.3)	Fisher exact	1.000
Abdominal distension not meeting intolerance criteria, n (%)	14 (18.7)	19 (25.3)	$\chi^2 = 0.97$	0.324
Vomiting, n (%)	9 (12.0)	13 (17.3)	$\chi^2 = 0.86$	0.353
Loose stools, n (%)	11 (14.7)	8 (10.7)	$\chi^2 = 0.55$	0.459
Spontaneous intestinal perforation, n (%)	1 (1.3)	2 (2.7)	Fisher exact	1.000
Discontinuation due to adverse event, n (%)	0 (0.0)	0 (0.0)	—	—
Any serious adverse event judged product-related, n (%)	0 (0.0)	0 (0.0)	—	—

The absence of probiotic sepsis in 75 exposed neonates is consistent with the rarity of the event but provides little power to exclude a risk of clinical importance. Neonates with a central venous catheter in situ at the intended time of commencement were excluded from the trial.

Table 6: Adherence to the study protocol and product verification (N = 150)

Parameter	Probiotic (n = 75)	Placebo (n = 75)	Test statistic	p value
Adherence ≥80% of eligible feeding days, n (%)	69 (92.0)	71 (94.7)	$\chi^2 = 0.42$	0.516
Median doses received, median (IQR)	26 (18–34)	27 (19–35)	U = 2694.0	0.671
Duration of supplementation, days (mean ± SD)	25.8 ± 9.4	26.4 ± 9.8	t = 0.38	0.703
Supplementation stopped at 34 weeks corrected age, n (%)	58 (77.3)	56 (74.7)	$\chi^2 = 0.14$	0.708
Supplementation stopped at discharge, n (%)	13 (17.3)	14 (18.7)	$\chi^2 = 0.05$	0.831
Supplementation stopped due to death, n (%)	4 (5.3)	5 (6.7)	Fisher exact	1.000
Number of product batches used	3	3	—	—
Batches with verified certificate of analysis, n (%)	3 (100.0)	—	—	—
Batches within specified viable organism content, n (%)	3 (100.0)	—	—	—
Lost to follow-up before discharge, n (%)	0 (0.0)	0 (0.0)	—	—

SD, standard deviation; IQR, interquartile range. The probiotic preparation contained Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum and Bifid obacterium bifidum providing not fewer than one billion colony-forming units per dose.

Discussion

This randomised, double-blind, placebo-controlled trial found that supplementation with a multi-strain probiotic preparation reduced the incidence of necrotising enterocolitis of modified Bell stage II or above from 18.7 percent to 6.7 percent, a relative risk of 0.36 and a number needed to treat of 9, and shortened the time to full enteral feeding by approximately three days. No case of invasive infection attributable to a probiotic organism occurred.

The magnitude and direction of this effect are consistent with the substantial body of pooled evidence. The Cochrane review by Sharif and colleagues, incorporating 56 trials and 10,812 infants, reported a relative risk of 0.54 with a 95 percent confidence interval of 0.45 to 0.65, together with reductions in all-cause mortality and late-onset invasive infection.[1] The 2023 update reaffirmed these findings within a GRADE framework.[2] Earlier iterations of the same review by AlFaleh and Anabrees reached comparable conclusions and argued that the evidence base already supported a change in clinical practice.[3] The point estimate obtained here, at 0.36, is more favourable than the pooled estimate, and it is important to recognise that this is what would be expected from a small single-centre trial by virtue of random variation alone; the confidence interval, extending from 0.14 to 0.94, comfortably includes the pooled estimate and the present result should be read as consistent with it rather than as evidence of a larger effect.

The failure to demonstrate significant reductions in stage III disease, in late-onset sepsis or in all-cause mortality requires plain statement rather than explanation away. All three outcomes moved in the direction of benefit, with stage III disease occurring in one against six

neonates and mortality in four against eight, but none reached statistical significance.

The reason is straightforward and was foreseeable at the design stage: the trial recruited 150 neonates when the sample size calculation for the primary outcome indicated 224, and the power for outcomes rarer than the primary outcome is correspondingly lower still. The absence of statistical significance for these outcomes is therefore uninformative and must not be reported or cited as evidence that probiotics do not affect mortality or sepsis, particularly given that the pooled evidence, with far greater power, does demonstrate such effects.[1,2]

The improvement in feeding outcomes is a consistent and clinically meaningful finding.

Attainment of full enteral feeds three days earlier, with a corresponding reduction in the duration of parenteral nutrition, carries implications beyond convenience, since parenteral nutrition is itself associated with central line-associated bloodstream infection, cholestasis and prolonged hospitalisation. The plausible mechanism involves improved intestinal motility and reduced feed intolerance consequent upon a more favourable microbial environment. The reduction in feed intolerance from 41.3 percent to 24.0 percent supports this interpretation.

Whether the feeding benefit is mediated wholly through the reduction in necrotising enterocolitis or represents an independent effect cannot be determined from these data, since the cases of necrotising enterocolitis themselves contribute prolonged feed interruption to the group means.

The safety findings are reassuring but must not be overstated. No case of probiotic sepsis occurred in 75 exposed neonates, which is consistent with the rarity of the event but provides essentially no power to exclude a risk of clinical importance; an event occurring in one per thousand exposures would very likely be absent from a sample of this size. The published reports of Lactobacillus and Bifidobacterium bacteraemia in preterm neonates, and the fatal case of gastrointestinal mucormycosis attributed to a contaminated preparation, remain the appropriate basis for caution, and the exclusion of neonates with a central venous catheter in situ at commencement was a deliberate design feature intended to mitigate the translocation risk. Position statements have consistently emphasised these considerations alongside the efficacy evidence.[5]

This brings the discussion to what is, in the Indian context, the decisive practical issue. Probiotic preparations in India are regulated as food supplements rather than as pharmaceuticals, and the viable organism content of a marketed product may differ materially from the label claim.

The efficacy demonstrated in the pooled literature attaches to specific strains at specific doses, and a benefit shown for one preparation cannot be assumed to transfer to another with a different strain composition or with an unverified viable count. In this trial the certificate of analysis was verified for each batch, which is a minimum requirement and one that many units would find difficult to implement routinely.

The conclusion that follows is not that probiotics should be adopted universally in Indian neonatal units, but that they should be adopted only where the product can be quality-assured, and that regulatory reform to bring neonatal probiotic preparations under pharmaceutical rather than food standards would be the single measure most likely to translate this evidence into safe practice.

The subgroup findings are consistent with the general principle that absolute benefit is greatest where baseline

risk is highest, with the incidence in the placebo group reaching 33.3 percent among neonates born before 30 weeks. Neither subgroup comparison achieved significance and the tests for interaction were far from significant, so these analyses should be regarded as hypothesis-generating and descriptive only. Reporting them as evidence that probiotics work better in one subgroup than another would not be supportable.

Limitations

The principal limitation is that the trial was underpowered. The sample size calculation indicated 112 neonates per group and 75 were recruited, giving approximately 62 percent power for the primary outcome and considerably less for every secondary outcome. That the primary outcome nonetheless attained significance reflects an observed effect larger than that assumed in the calculation, which may partly represent chance. The trial was conducted at a single centre, and the incidence of necrotising enterocolitis, the feeding practices, the rate of breast milk availability and the pathogen profile of this unit determine the applicability of the findings elsewhere. A single probiotic preparation was tested, and the findings cannot be extrapolated to other strain combinations, doses or manufacturers. The intervention continued only to 34 weeks corrected age, and no comment can be made on the effect of longer supplementation. Stool microbiome analysis was not performed, so the proposed mechanism was not verified and it is not known whether the intervention achieved the intended colonisation.

Follow-up ended at discharge, so neurodevelopmental outcome, which is arguably the outcome of greatest importance to families, was not assessed. Finally, although allocation was concealed and blinding was maintained, necrotising enterocolitis of stage II depends partly on clinical judgement in the assembly of the diagnostic picture, and complete elimination of ascertainment bias cannot be guaranteed even with blinded radiological reporting.

Conclusion

Supplementation with a multi-strain probiotic preparation containing Lactobacillus and Bifid obacterium species reduced the incidence of necrotising enterocolitis of modified Bell stage II or above from 18.7 percent to 6.7 percent in preterm neonates below 34 weeks of gestation and 1800 g of birth weight, with a number needed to treat of 9. Full enteral feeding was attained approximately three days earlier, feed intolerance was less frequent, the duration of parenteral nutrition was shorter and hospital stay was reduced. No case of invasive infection attributable to a probiotic organism occurred.

These findings are consistent with, and add modestly to, the large body of randomised evidence synthesised in successive Cochrane reviews. Two qualifications must accompany them. First, the trial was underpowered, recruiting 150 of the 224 neonates indicated by the sample size calculation, and the absence of significant differences in stage III disease, late-onset sepsis and mortality reflects that limitation rather than an absence of effect. Second, and more consequentially for practice in India, the efficacy demonstrated in this and other trials attaches to a specific preparation of verified viable organism content, whereas probiotic products in India are regulated as food supplements and their actual content may not match the label. Probiotic supplementation can therefore be recommended for preterm neonates in units able to verify product quality and to exclude neonates at particular risk of translocation, and the regulatory reclassification of

neonatal probiotic preparations would do more than any further trial to make this evidence safely usable. Larger multicentre Indian trials with neurodevelopmental follow-up and stool microbiome analysis remain warranted.

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