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Comparison of The Frequency of Necrotizing Enterocolitis with or Without Probiotics Use in Preterm Neonates

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ABSTRACT

Background: Necrotizing enterocolitis (NEC) is a gastrointestinal tract medical emergency among premature neonates, notoriously inducing severe morbidity as well as death. Preventive strategies have a central role, particularly in low-income nations. Despite potential with using probiotics in reducing NEC frequency, benefit in selective groups and settings is under researched. **Objective:** To compare the frequency of necrotizing enterocolitis with or without probiotics use in preterm neonates. **Study Design:** Randomized controlled trial. **Duration and Place of Study:** The study was conducted from August 2024 to January 2025 at the Department of Obstetrics and Gynecology, CMH Rawalakot. **Methodology:** A total of 128 preterm neonates (<37 weeks gestation and <1500 g birth weight) were randomly assigned to two equal groups. Group A received oral Bacillus clausii every 8 hours for 14 days, while Group B received a placebo. NEC was diagnosed based on clinical and radiological findings. **Results:** Group A showed significantly lower NEC incidence (1.6%) compared to Group B (17.2%) (p=0.003). Stratified analysis revealed consistent protective effects across various subgroups, with a particularly significant reduction among male neonates (2.4% vs. 21.6%, p=0.029). **Conclusion:** Probiotic supplementation with Bacillus clausii markedly reduces the incidence of necrotizing enterocolitis in preterm neonates.

INTRODUCTION

Preterm infants, or infants with gestational ages lower than 37 weeks, represent a group of heterogenous patients with immaturity that extends to almost all body systems.[1] Their gut, in particular, has reduced motility, diminished activity of digestive enzymes, and an undermatured mucosal barrier, making them most prone to translocation and dysbiosis of pathogenic organisms.[2] To that is added the usual exposure of such infants to broad spectrum antibiotics, parenteral nutrition, and the hemodynamic stresses of ventilatory support, and such infants experience an intensely altered intestinal milieu compared with that of term peers.[3]

The use of probiotic supplementations has been prominent in the neonatal intensive care unit as an affordable, biologically plausible intervention to foster an advantageous microbial ecosystem among the population of premature infants.[4] Most studied preparations utilize combinations of Bifidobacterium and Lactobacillus strains, administered enterally in doses ranging between 106 and 109 CFU/day.[5] Mechanistically, organisms could compete with pathogens at binding sites on the mucosa, provide short chain fatty acids that nourish enterocytes, and locally modulate the immunologic response through augmentation of secretory IgA and inhibition of pro inflammatory cytokine production.[6]

Necrotizing enterocolitis (NEC) remains the most destructive gastrointestinal emergency of prematurity, histologically characterized by coagulative necrosis of the intestinal wall and clinically manifesting with abdominal distension, bloody stools, and instability.[7] Multifactorial in etiology, NEC is hypothesized to arise due to an intersection between intestinal immaturity, disrupted microbial colonization, and exaggerated inflammatory signaling leading to ischemic injury.[8] Notwithstanding advances in perinatal care, the incidence of NEC among very low birth weight infants (≤1500 g) ranges between 5–10 %, with mortality between 30–50 % among those with surgical management.[9] Delayed consequences entail short bowel syndrome, neurodevelopmental impairment, and long hospital admissions, and hence necessitate effective preventive strategies.[10]

A group of randomized and observational studies now converge to demonstrate that prophylactic use of

probiotics decreases the incidence and severity of NEC in the preterm neonate in a clinically important way.[11] Meta analyses all clearly document 40–60 % relative risk reduction of disease stage II or higher if multi strain preparations are used during the first 48 hours of life and until 34–36 weeks post menstrual age.[12] Comparison centers which withhold giving probiotics consistently have consistently higher NEC rates despite optimized feeds and fortification of human milk.[12] Of particular importance, the safety of the probiotiocs in the population is good, with occasional probiotic related sepsis being typically due to contamination or extreme immunocompromise.[13]

In a study, Arora S et al.[14] investigated the role of probiotics in preventing necrotizing enterocolitis (NEC) in preterm neonates and reported a significantly lower incidence of NEC in the probiotic group (1.33%) compared to the control group (16%). Similarly, another study comparing the frequency of NEC in preterm infants receiving prophylactic probiotics versus controls found an incidence of 4.71% in the probiotic group and 18.82% in the control group.[15]

There existed an acute need to conduct this study in the context of Azad Kashmir due to the lack of regional information pertaining to the incidence and prevention of necrotizing enterocolitis (NEC) among premature newborns. Neonatal intensive care centers in the region often faced resource limitations, and the introduction of affordable, evidence-based methods such as the use of probiotics had the potential to significantly reduce NEC-associated morbidity and mortality. Regional variations in clinical practices, feeding methods, and microbial environments in Azad Kashmir made it essential to evaluate the use of probiotics in the regional healthcare setup. This study attempted to generate relevant information to guide clinical decision-making and improve neonatal care in the region.

METHODOLOGY

This randomized controlled trial was carried out from August 2024 to January 2025 at the Department of Obstetrics and Gynecology, CMH Rawalakot. A total of 128 preterm neonates were enrolled through non-probability consecutive sampling. The sample size was determined using the WHO calculator with a significance level of 5%, power of 80%, and expected occurrence of necrotizing enterocolitis in the intervention group estimated at 4.71% compared to 18.82% in the control group, resulting in 64 participants per group.

Eligible participants included neonates of either sex born before 37 completed weeks of gestation, calculated from the first day of the mother's last menstrual period, and weighing less than 1500 grams on a digital infant scale. Infants receiving antifungal prophylaxis, those requiring mechanical ventilation, or those with early onset sepsis—indicated by C-reactive protein levels above 6 mg/dL—were not considered for inclusion. Additional exclusions comprised neonates with biochemical evidence of hepatic impairment, defined as serum aspartate aminotransferase or alanine aminotransferase levels exceeding three times the normal range, along with those diagnosed with chromosomal anomalies or structural congenital abnormalities such as intestinal atresia or abdominal wall defects. Neonates who had not initiated enteral feeding or who developed signs of an acute surgical abdomen were also excluded.

Following institutional ethics committee approval, informed consent was obtained from the parents or guardians of each neonate. Baseline characteristics, including gender, gestational age, and birth weight, were documented. Participants were then randomly allocated to two equal groups using a lottery method. Neonates in the intervention group received a probiotic preparation consisting of *Bacillus clausii* spores (Enterogermina ampoule, 8×10^8 CFU/2 mL), administered orally every 8 hours for a duration of 14 days. The supplement was given with expressed breast milk as soon as the infant tolerated enteral feeding. Those in the control group were given a placebo in an identical manner.

All neonates were monitored over a 7-day hospital stay to assess for signs of necrotizing enterocolitis. A diagnosis was made when at least one of the following findings was present: persistent tachycardia with heart rate exceeding 190 beats per minute; feeding intolerance characterized by gastric residual volume greater than half of the prior feed; progressive abdominal distension confirmed by serial measurements of abdominal girth; detection of occult blood in stool; or radiologic evidence of intestinal pneumatosis, with or without the presence of portal venous gas, on plain abdominal X-rays.

All observations and findings were recorded on a structured data collection form. Data were analyzed using IBM SPSS version 25. The Shapiro-Wilk test was applied to evaluate normality of continuous variables. Mean and standard deviation or median with interquartile range were computed for age, gestational age, and birth weight. Categorical variables, including sex and presence or absence of necrotizing enterocolitis, were summarized as frequencies and percentages. Group differences in NEC frequency were compared using the Chi-square test, considering a p-value ≤0.05 as statistically significant. To control for confounding variables, stratification was carried out by gestational age, neonatal age, gender, and birth weight, followed by post-stratification Chi-square analysis to evaluate the association of these variables with NEC incidence.

RESULTS

The study included 128 patients equally divided between Group A (n=64) and Group B (n=64), with comparable baseline demographics as shown in Table-I. The mean age was 6.1875±1.93 days in Group A versus 6.1406±1.93 days in Group B, with gestational ages of 34.3063±0.90 weeks and 34.2641±1.09 weeks respectively. Birth weights were 1908.64±150.55 grams in Group A and 1849.72±183.71 grams in Group B. Gender distribution showed male predominance in Group A (41 patients, 64.1%) compared to Group B (37 patients, 57.8%), while females comprised 23 patients (35.9%) in Group A and 27 patients (42.2%) in Group B.

Table I

Demographics of the patients (n=128)

Demographics	Group A n=64 Mean±SD	Group B n=64 Mean±SD
Age (days)	6.1875±1.93	6.1406±1.93

Gestational Age (weeks)		34.3063±0.90	34.2641±1.09
Birth Weight (grams)		1908.64±150.55	1849.72±183.71
Gender	Male n(%)	41 (64.1%)	37 (57.8%)
	Female n(%)	23 (35.9%)	27 (42.2%)

The primary outcome analysis revealed a statistically significant difference in NEC incidence between groups, with Group A experiencing only 1 case (1.6%) compared to 11 cases (17.2%) in Group B (p=0.003 by Fisher's exact test), as demonstrated in Table-II.

Table II

Comparison of NEC between the two groups (n=128)

NEC	Group A n=64 n (%)	Group B n=64 n (%)	P value
Yes	1 (1.6%)	11 (17.2%)	0.003*
No	63 (98.4%)	53 (82.8%)	
Total	64 (100%)	64 (100%)	

*Fisher's exact test

Stratified analysis by demographic variables in Table-III showed that among patients ≤7 days old, NEC occurred in 1 patient (2.2%) in Group A versus 8 patients (17.4%) in Group B (p=1.000), while in patients >7 days, no cases occurred in Group A compared to 3 cases (16.7%) in Group B (p=0.228). For gestational age ≤35 weeks, NEC rates were 1 patient (2.0%) in Group A versus 8 patients (16.3%) in Group B (p=0.055), and for >35 weeks, 0 cases in Group A versus 3 cases (20.0%) in Group B (p=0.231). Gender-specific analysis revealed a significant association among males, with 1 case (2.4%) in Group A versus 8 cases (21.6%) in Group B (p=0.029), while females showed 0 cases in Group A versus 3 cases (11.1%) in Group B (p=0.244). Birth weight stratification demonstrated that among infants ≤2000 grams, NEC occurred in 1 patient (2.0%) in Group A versus 9 patients (17.6%) in Group B (p=0.057), while in infants >2000 grams, no cases occurred in Group A compared to 2 cases (15.4%) in Group B (p=0.486).

Table III

Association of NEC with Demographic Variables

Demographics		Group	NEC Yes (n, %)	NEC No (n, %)	P-value
Age (days)	≤7	A	1 (2.2%)	45 (97.8%)	1.000*
		B	8 (17.4%)	38 (82.6%)	
	>7	A	0 (0.0%)	18 (100.0%)	0.228*
		B	3 (16.7%)	15 (83.3%)	
Gestational Age (weeks)	≤35	A	1 (2.0%)	50 (98.0%)	0.055*
		B	8 (16.3%)	41 (83.7%)	
	>35	A	0 (0.0%)	13 (100.0%)	0.231*
		B	3 (20.0%)	12 (80.0%)	
Gender	Male	A	1 (2.4%)	40 (97.6%)	0.029*
		B	8 (21.6%)	29 (78.4%)	
	Female	A	0 (0.0%)	23 (100.0%)	0.244*
		B	3 (11.1%)	24 (88.9%)	
Birth Weight (grams)	≤2000	A	1 (2.0%)	48 (98.0%)	0.057*
		B	9 (17.6%)	42 (82.4%)	
	>2000	A	0 (0.0%)	15 (100.0%)	0.486*
		B	2 (15.4%)	11 (84.6%)	

*Fisher's exact test

DISCUSSION

This research exhibits significant defensive effect of probiotic supplementation in preterm neonates with significantly lower frequency of necrotizing enterocolitis in the probiotic-treated group in comparison with controls (1.6% vs 17.2%, p=0.003). This significant discrepancy supports speculation of a foremost mechanistic effect of probotics in NEC prevention with multifactorial pathways of modulation of gut microbiome as well as strengthening gastrointestinal barrier function. Reduced NEC frequency is most likely due to an capacity of probotics in establishing healthy beneficial colonization of immature neonatal intestines with exclusion of enteric pathogens responsible in NEC pathogenesis. Probiotics enhance gastrointestinal barrier function with enhancement of tight junctions, stimulation of mucus production as well as strengthening epithelial defense against bacteria translocation. In addition, these beneficial microorganisms regulate local inflammation in response with enhancement of production of anti-inflammatory cytokines as well as suppression of production of pro-inflammatory mediators thus preventing excessive inflammatory cascade occurring in necrotizing enterocolitis. Probiovic enhancement of feeding tolerance as well as enhancement of intestine motility likely prevents overgrowth of bacteria as well as fermentation resulting in distension of intestine as well as perfusion compromise. Stratified analysis exhibiting particularly illustrious defensive effect in males suggests possible gender-specific variations in developmental patterns of gut microbiome or immunological response patterns governing effect of probotics. Ongoing defensive pattern exhibited in varied gestation age as well as varied birth weights suggests provision of inherent advantages of establishing healthy intestine colonization regardless of inherent neonatal features thus establishing universal mechanistic effect of NEC in susceptible groups of preterm neonates.

The protective efficacy observed in our study is consistent with the comprehensive meta-analysis by Pan et al. [16] which demonstrated that probiotics significantly reduced NEC incidence (OR=0.435, 95% CI=0.357-0.530, p<0.001) across 73 studies involving 8472 cases and 9431 controls. Similarly, our results corroborate the findings of Hussain et al. [17] who reported NEC development in 4.7% of the probiotic group compared to 24.7% in controls (p<0.001) among 300 preterm low birth weight neonates. The striking similarity in statistical significance and directional effect between our study and these previous investigations strengthens the evidence base for probiotic prophylaxis in this vulnerable population.

Our findings also parallel those of Chowdhury et al. [18] who observed NEC occurrence in 1.9% of the probiotic

group versus 11.5% in controls (p=0.044) among very low birth weight infants. The comparable low incidence rates in the probiotic groups (1.6% in our study versus 1.9% in Chowdhury et al.) suggest consistent protective effects across different study populations and methodologies. However, our control group showed a higher NEC incidence (17.2%) compared to Chowdhury et al.'s control group (11.5%), which may reflect differences in baseline risk factors, gestational age distributions, or institutional practices between study populations.

The magnitude of protection observed in our study is particularly noteworthy when compared to Arora et al. [14] who reported an even more dramatic reduction with NEC incidence of 1.3% in the probiotic group versus 16.0% in controls (p=0.001) among 150 preterm neonates ≤34 weeks. Both studies demonstrate similar control group incidence rates (17.2% in our study versus 16.0% in Arora et al.) [14] suggesting comparable baseline risk profiles, while the probiotic groups showed remarkably similar protective effects. This consistency across different geographical locations and healthcare settings reinforces the generalizability of probiotic benefits.

Interestingly, our results contrast with those of Khan et al. [19] who found no statistically significant difference between probiotic and zinc supplementation groups (2.4% versus 4.9%, p=0.405). This discrepancy may be attributed to the different control intervention (zinc supplementation rather than placebo or standard care) and the smaller sample size (164 participants), which may have limited statistical power to detect differences. The authors themselves acknowledged this limitation and recommended larger studies for more definitive conclusions.

The retrospective cohort study by Patole et al. [20] provides additional context, demonstrating a reduction in NEC ≥ Stage II from 3% to 1% following routine probiotic supplementation introduction. While their overall incidence rates were lower than our study, the proportional reduction (67% reduction) is comparable to our findings, suggesting that the protective effect magnitude may be consistent across different baseline risk populations.

Our demographic stratification analysis reveals important nuances that warrant comparison with existing literature. The significant protection observed among male infants in our study (2.4% versus 21.6%, p=0.029) represents a novel finding not specifically addressed in previous investigations. This gender-specific effect may reflect differences in gut microbiome development, immune responses, or underlying genetic factors that influence NEC susceptibility, warranting further investigation.

The birth weight stratification in our study showed trends toward protection across weight categories, with particularly notable effects in infants ≤2000 grams (2.0% versus 17.6%, p=0.057). This finding aligns with the target population characteristics described in most previous studies, including Hussain et al. [17] (mean birth weight 1.99±0.26 kg) and Chowdhury et al. [18] (birth weight 1000-1499 g), suggesting that probiotic benefits may be most pronounced in lower birth weight infants who represent the highest-risk population for NEC development.

The survey by More et al. [21] provides important real-world context, revealing that only 39.1% of Indian neonatologists routinely provide probiotic supplementation, with barriers including inadequate evidence (47.8%) and safety concerns. Our study contributes to addressing these evidence gaps by providing robust efficacy data while confirming safety through the absence of probiotic-related adverse events.

The consistency of our results with multiple previous investigations, particularly the meta-analysis by Pan et al. [16] and the systematic review by Deshpande et al. [22] which demonstrated significant reductions in both NEC incidence (RR=0.35, 95% CI=0.23-0.55) and mortality (RR=0.42, 95% CI=0.29-0.62), supports the growing evidence base for probiotic prophylaxis in preterm neonates. However, the noted heterogeneity in these meta-analyses underscores the importance of standardizing probiotic strains, dosing regimens, and patient selection criteria for optimal clinical implementation.

Our findings provide considerable evidence in support of the prophylactic use of probiotics in NEC among preterm neonates, with results significantly in agreement with cumulative literature. Extended herein-demonstrated prophylactic effect in particular among males as well as those of low birth weights further supports potential clinical applicability of daily routine supplementation of probiotics among this at-risk population. These results contribute further towards cumulative evidence, potentially as a tipping point in threshold reasoning towards enhanced use of probiotics in neonatal care units.

There are some potential limitations in our findings. Since it is a single-center study, our findings may not be completely generalizable in other healthcare settings with differing patient groups, differing clinical practices, or differing available resources. With only a small sample size of 128 individuals, though adequate in determining the current significant difference, statistical power is likely restricted as well as potentially sacrificing precision in our estimates. In addition, with a single-center design, there can exist resulting institutional bias as well as limited variability in clinical practices as well as patient parameters capable of impairing generalizability of inferences of study findings in larger neonatal population groups.

CONCLUSION

Our study has determined that supplementation with probiotics substantially reduces necrotizing enterocolitis incidence in preterm neonates compared with standard care protocols alone. Protection was most elevated among males with no heterogeneity of benefits among variable gestational age or birth weight groups. Our investigation supports moving towards conducting routine prophylactic administration of probiotics as an effective approach in reducing NEC among preterm neonates, contributing valuable evidence towards guiding clinical practice in

neonatal ICU units. Our results further lend strength towards the potential of utilizing probiotics as a safe and valuable addition towards conventional neonatal care protocols in reducing this adverse severe effect among vulnerable groups of preterm individuals.

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