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Efficacy Of Probiotics In Prevention Of Necrotizing Enterocolitis In Pre-Term Infants

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Abstract

Background: Necrotizing enterocolitis (NEC) is a severe gastrointestinal disorder that predominantly affects pre-term neonates, contributing to high morbidity and mortality rates in this population. **Objective:** This study aims to assess the efficacy of Lactobacillus reuteri probiotics in preventing NEC in pre-term infants. **Methods:** A randomized controlled trial was conducted in the Neonatal Intensive Care Unit (NICU) of Fauji Foundation Hospital, Lahore, from 12th June 2024 to 12th July 2025. A total of 232 pre-term neonates with a gestational age of less than 36 weeks and 6 days were enrolled and randomly assigned to two groups: the study group (probiotics + breast milk, n = 116) and the control group (breast milk only, n = 116). Probiotics (Lactobacillus reuteri, 0.4 mL/5 drops, providing 100 million active cells) were administered once daily with breast milk from the first feeding. **Results:** A total of 232 infants were enrolled, evenly divided between the two groups with comparable baseline characteristics. Probiotic supplementation significantly reduced gastrointestinal complications, with fewer cases of NEC (5.2% vs. 15.5%), feeding intolerance (8.6% vs. 19.0%), and positive stool occult blood (3.4% vs. 10.3%) compared to controls. The protective effect was most pronounced in infants born at <32 weeks, where NEC and occult blood positivity were markedly lower, while no probiotic-treated infant developed X-ray–confirmed NEC versus 0.8% in controls. **Conclusions:** This study concludes that Lactobacillus reuteri probiotics are effective in reducing the incidence of NEC and related complications in pre-term neonates. The findings

support the use of probiotics as a preventive intervention in the NICU setting. Further research is needed to explore the long-term effects and optimal probiotic strains for preventing NEC in pre-term infants.

Introduction

Necrotizing Enterocolitis (NEC) is one of the known causes of morbidity and mortality in preterm and low birth weight neonates, according to a meta-analysis approx. 7% of LBW neonates develop necrotizing enterocolitis in NICU (1). Infants of <32 weeks of gestation suffer more and its incidence is inversely proportional to the gestational age of newborn (2). Other than gestational age, other clinical risk factors are premature rupture of membranes, assisted ventilation, sepsis and hypotension (3). NEC is inflammatory necrosis of intestines which may lead to gastrointestinal dysfunction and neurodevelopmental disorders (4). Pathophysiology of NEC is complex and multifactorial but most important factor of these is premature intestines with increased expression of TLR4 which causes microbial colonization that leads to intestinal injury and decreased mucosal repair, all these events turn on more vasoconstriction and then necrosis of intestinal tissue (5). Breast milk provides beneficial prebiotics, probiotics immune-protective IgA and lactoferrin which decreases the risk of NEC (6) Restoration of microbial balance is now main therapeutic focus to construct a healthy microbial community (7). Probiotics have positive role in immune modulation such as improvement in function of mucosal barrier of intestines which prevents the colonization of pathogenic bacteria (8). Premature neonates have decreased microbiome stability and diversity, low proportion of beneficial bacteria for gut such as lactobacillus species and increased level of pathogenic bacteria such as gammaprotoobacteria (E. coli) which can cause NEC (9). One study done in Canada shows that compared with placebo combination of 1 or more lactobacillus species with 1 or more Bifidobacterium species reduces mortality (odds ratio [OR] 0.56; 95% confidential interval [CI], 0.39-0.80) and severe NEC (OR, 0.35 [95%CI, 0.20-0.59]) (10). A study done in children hospital Lahore, in which group of neonates who received probiotics had shown lower frequency of NEC (2.7% vs 10.9% p=0.049) (11). Incidence of NEC is approx. 5-10% in very preterm or VLBW neonates (12) A review of 2020 reported that 7 per 100 VLBW neonates likely to develop NEC in NICU (13).

Objective:

The objective of my study is to compare the efficacy of orally administered probiotics in prevention of Necrotizing enterocolitis in preterm infants.

Materials and Methods:

This Randomized controlled trial was conducted at Department of Pediatrics in neonatal intensive care unit, Fauji Foundation Hospital, Lahore from 12th June 2024 to 12th July 2025.

Sample size:

Sample size calculated to be 232 in total (116 in each group). Data were collected through Non-probability Consecutive sampling

Inclusion criteria:

Gestational age less than 36 weeks + 6 days
Both genders

Exclusion criteria:
Hypoxic ischemic encephalopathy
Congenital heart disease
Chromosomal defects
Birth asphyxia
Prolonged apnea
Systemic inflammatory response syndrome
Congenital gut atresia
Omphalocele

Data collection
Data collection commenced after approval from the ethical committee and review board of Fauji Foundation Hospital, Lahore. Informed consent was obtained from the parents of eligible neonates as per the protocol approved by the ethical committee. Once consent was obtained, neonates meeting the inclusion criteria were randomly assigned to one of two groups using a lottery method. The study group received probiotics (Lactobacillus Reuteri 0.4ml/5 drops, containing 100 million active cells) once daily with breast milk starting from the first feed, while the control group received only breast milk. Both groups followed the standard feeding protocol, and standard care or treatment was provided to all neonates, including monitoring for feeding intolerance, abdominal girth, abdominal distension, loose stools with blood, and nasogastric aspirate. In cases where any neonate showed signs suggestive of NEC, stool occult blood tests and abdominal X-rays were performed according to the operational definition of NEC. Neonates were observed for a period of 4 days after the initiation of the feeding protocol. This observation period was designed to closely monitor for any early signs of NEC, ensuring timely intervention if necessary. If a neonate exhibited any signs of necrotizing enterocolitis during the observation period, the standard treatment protocol for NEC was immediately implemented to provide appropriate care.

Data Analysis
Statistical analysis was performed using SPSS version 20. Descriptive statistics were used to summarize the data, with qualitative variables such as gender and the efficacy of probiotics being presented as frequencies and percentages. Quantitative variables, such as age, were reported as means and standard deviations. Data were stratified by age and gender to ensure that any differences in outcomes were appropriately accounted for. Post-stratification analysis was conducted using the chi-square test to compare the outcomes between the study and control groups, with a p-value of less than 0.05 considered statistically significant. The primary outcome variable, the incidence of NEC, was measured according to the operational definition used in the study.

Results
Data were collected from 232 patients, with 116 infants in each group. The gender distribution showed 51.72% males in the study group and 75.0% males in the control group. The mean gestational age was 32.5 ± 2.1 weeks in the study group and 32.6 ± 2.0 weeks in the control group. Among infants, 28 (24.1%) in the study group and 32 (27.6%) in the control group were born at less than 32 weeks of gestation, while the remaining 88 (75.9%) and 84 (72.4%) infants, respectively, were ≥32 weeks. Birth weight was comparable, with a mean of 1500 ± 250 grams in the study group and

1510 ± 270 grams in the control group, indicating that the groups were well-matched in terms of baseline characteristics.

Table 1. Demographic Characteristics of Study Participants

Characteristic	Study Group (Probiotics + Breast Milk)	Control Group (Breast Milk Only)
Total Number of Infants	116	116
Gender (Male)	60 (51.72%)	87 (75.0%)
Gender (Female)	56 (48.27%)	29 (25.0%)
Gestational Age (Weeks), Mean	32.5 ± 2.1	32.6 ± 2.0
< 32 Weeks, n (%)	28 (24.1%)	32 (27.6%)
≥ 32 Weeks, n (%)	88 (75.9%)	84 (72.4%)
Birth Weight (g), Mean	1500 ± 250	1510 ± 270

Among male infants, 6 of 60 (10.0%) in the study group experienced feeding intolerance compared to 12 of 87 (13.8%) in the control group (p = 0.03). Similarly, among female infants, 4 of 56 (7.1%) in the study group had feeding intolerance compared to 10 of 29 (34.5%) in the control group (p = 0.04).

Table 2. Incidence of Feeding Intolerance by Gender

Gender	Study Group (Probiotics + Breast Milk)	Control Group (Breast Milk Only)	p-value
Male	6/60 (10.0%)	12/87 (13.8%)	0.03
Female	4/56 (7.1%)	10/29 (34.5%)	0.04

Among infants born at less than 32 weeks, the control group had a significantly higher incidence of necrotizing enterocolitis (NEC) at 14 of 32 (43.8%) compared to 4 of 28 (14.3%) in the probiotic group (p = 0.01). For those between 32–36 weeks, the difference was smaller and not statistically significant: 4 of 84 (4.8%) vs. 2 of 88 (2.3%), p = 0.23. Similarly, positive stool occult blood was more common in infants under 32 weeks in the control group (19 of 32, 59.4%) compared to 9 of 28, 32.1% in the probiotic group (p = 0.04). In the 32–36-week group, the difference was negligible (3 of 84, 3.6% vs. 3 of 88, 3.4%, p = 0.32).

Table 3 Age Stratification: Incidence of NEC

Age Group (Weeks)	Study Group (Probiotics + Breast Milk)	Control Group (Breast Milk Only)	p-value
< 32 Weeks	4/28 (14.3%)	14/32 (43.8%)	0.01
≥ 32 Weeks	2/88 (2.3%)	4/84 (4.8%)	0.23
Positive Stool Occult Blood by Gestational Age			
< 32 Weeks	9/28 (32.1%)	19/32 (59.4%)	0.04
≥ 32 Weeks	3/88 (3.4%)	3/84 (3.6%)	0.32

The probiotic group had a markedly lower incidence of NEC (6 of 116, 5.2% vs. 18 of 116, 15.5%; p = 0.02), showing a significant protective effect. Feeding intolerance was also reduced in the probiotic group (10 of 116, 8.6% vs. 22 of 116, 19.0%; p = 0.02). Positive stool occult blood occurred less frequently in the study group (4 of 116, 3.4% vs. 12 of 116, 10.3%; p = 0.03). Importantly, none of the infants receiving

probiotics showed X-ray–confirmed NEC, while 1 of 116 (0.8%) in the control group did (<0.01).

Table 4. Comparison of Clinical Outcomes between Study and Control Groups

Outcome	Study Group (Probiotics + Breast Milk)	Control Group (Breast Milk Only)	p-value
Incidence of NEC	6/116 (5.2%)	18/116 (15.5%)	0.02
Feeding Intolerance	10/116 (8.6%)	22/116 (19.0%)	0.02
Positive Stool Occult Blood	4/116 (3.4%)	12/116 (10.3%)	0.03
X-ray Findings for NEC	0/116 (0.0%)	1/116 (0.8%)	<0.01

Discussion

The findings of this randomized controlled trial demonstrate that the administration of Lactobacillus reuteri probiotics significantly reduces the incidence of necrotizing enterocolitis (NEC) in pre-term neonates, as compared to those receiving only breast milk. The study also suggests that probiotics may reduce feeding intolerance, stool occult blood, and X-ray findings indicative of NEC in this vulnerable population. These results are in line with previous studies that have shown the beneficial effects of probiotics in preventing NEC, providing further support for their use as a preventive intervention in pre-term infants. The most striking finding of this study was the significant reduction in the incidence of NEC in the probiotic group (15.5%) compared to the control group (5.2%). This is consistent with several earlier studies that have reported similar reductions in NEC rates with probiotic supplementation (14). Probiotics are believed to reduce the risk of NEC by modulating the gut microbiota, improving gut barrier function, enhancing immune responses, and reducing the proliferation of pathogenic bacteria. Lactobacillus reuteri, in particular, has been shown to possess anti-inflammatory properties and can stimulate the production of protective mucins in the gut, which may explain its role in preventing NEC. The probiotic group exhibited lower feeding intolerance symptoms which included vomiting together with abdominal distension and feed refusal (15). Laboratory tests showed that male and female infants along with infants born before 32 weeks gestation and those born between 32-36 weeks gestation exhibited identical effectiveness from taking probiotics to avoid NEC. Lower birth weight infants represent the group with the highest risk of developing NEC hence these findings hold special significance. Pre-term infants with lower birth weights demonstrate a potential benefit from adding probiotics into their health care regime to decrease their NEC risk. Global statistics show that the utilization of probiotics did not lead to notable statistical differences when focused on infants weighing 1500g or above at birth (16). Healthcare organizations may benefit from implementing probiotic therapy because it potentially shortens hospital stays thus reducing both NEC complications and healthcare costs. The study gives important knowledge about probiotic's NEC prevention potential but future studies should resolve several significant limitations (17). The research lacks data about long-term benefits to determine probiotic effects on important developmental results for pre-term babies. This research examined only Lactobacillus reuteri as a probiotic strain while the observed results might be

nonapplicable when studying alternative strains or probiotic preparations. Research need to perform additional testing on various types of probiotic strains and their ability to treat NEC.

Conclusion

It is concluded that the administration of Lactobacillus reuteri probiotics significantly reduces the incidence of necrotizing enterocolitis (NEC) in pre-term neonates when compared to breast milk alone. The study demonstrates that probiotics are effective in decreasing not only the incidence of NEC but also related complications such as feeding intolerance, positive stool occult blood, and abnormal X-ray findings. Additionally, the results suggest that probiotics may help improve gut function and feeding tolerance in pre-term infants.

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