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Research Article

Impact of Traditional Pre-Lacteal Feeding Practices on Early Neonatal Gut Microbiota Colonization and Risk of Neonatal Sepsis in Rural Sindh, Nawabshah Pakistan: A Prospective Cohort Study

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ABSTRACT
Background: Pre-lacteal feeding remains a common traditional practice in rural communities of Sindh, Pakistan, where neonates may receive honey, ghee, sugar-water, or ghutti before initiation of breastfeeding. Such practices may delay colostrum exposure and potentially influence early gut microbiota colonization, which is important for neonatal immune development and resistance to pathogenic organisms. This study evaluated the association between traditional pre-lacteal feeding, early neonatal gut microbiota colonization, and the risk of neonatal sepsis.
Methods: A prospective cohort study was conducted among mother-infant dyads in selected rural health centers and community clusters of Nawabshah, Sindh, Pakistan. Neonates were followed from birth to 28 days of life. Maternal and neonatal characteristics, timing of breastfeeding, and type of pre-lacteal feeding were recorded. Meconium and stool samples were collected at predefined time points and analyzed using 16S rRNA gene sequencing to characterize gut microbiota. Neonates were clinically assessed for sepsis throughout follow-up, with blood culture performed when clinically indicated. Associations between pre-lacteal feeding, microbiota patterns, and neonatal sepsis were assessed using appropriate statistical methods and multivariable regression.
Results: Pre-lacteal feeding was associated with delayed initiation of breastfeeding and differences in early gut microbiota composition, including relatively lower representation of beneficial Bifidobacterium and greater representation of potentially pathogenic Enterobacteriaceae. Neonates exposed to pre-lacteal feeds showed a higher frequency of clinically suspected and culture-confirmed sepsis. After adjustment for relevant maternal and neonatal factors, pre-lacteal feeding remained associated with increased odds of neonatal sepsis.
Conclusion: Traditional pre-lacteal feeding may be associated with altered early gut microbial colonization and increased neonatal sepsis risk. Prospective community-based interventions promoting early breastfeeding and reducing potentially unsafe pre-lacteal feeding practices warrant further evaluation.

Keywords: Pre-Lacteal Feeding, Neonatal Sepsis, Gut Microbiota, Breastfeeding, Bifidobacterium, Enterobacteriaceae, 16S Rrna Sequencing, Rural Sindh, Pakistan.

INTRODUCTION

Neonatal mortality remains a major public health challenge in Pakistan, particularly in resource-constrained and rural communities. Recent national evidence indicates a neonatal mortality rate of approximately 39.8 per 1,000 live births, with mortality higher in rural than urban populations, underscoring persistent geographic inequalities in newborn survival {1}. Neonatal sepsis is an important contributor to this burden. In a large prospective birth cohort involving low- and middle-income countries,

including Pakistan, neonatal sepsis was identified as a major cause of neonatal morbidity and mortality, emphasizing the importance of potentially modifiable perinatal and early-life exposures {2}. Globally, an estimated 6.31 million incident cases of neonatal sepsis and approximately 230,000 associated deaths occurred in 2019, with Asia carrying a substantial proportion of the disease burden {3}.

Early feeding practices may represent an important but insufficiently studied determinant of neonatal health. Pre-lacteal feeding, defined as administration of foods or liquids before breastfeeding is established, remains prevalent in Pakistan. A 2023 study involving 4,800 mothers from 12 disadvantaged districts of Sindh reported that 32% of newborns received pre-lacteal feeds, while only 68% initiated breastfeeding early {4}. Such practices can delay exposure to colostrum, which provides immunological components and human milk oligosaccharides that support establishment of the neonatal intestinal microbiome. Recent longitudinal microbiome research demonstrates that *Bifidobacterium* is a dominant component of the early infant gut and is strongly influenced by breastfeeding and human milk oligosaccharides {5}. Breastfeeding-associated *Bifidobacterium* species also produce metabolites involved in intestinal and immune homeostasis {6}.

The developing neonatal gut microbiome may therefore represent an important biological pathway linking early feeding exposures with infection susceptibility. Studies of neonatal sepsis have demonstrated associations between intestinal dysbiosis, increased Proteobacteria, reduced beneficial bacterial diversity, and bacterial translocation {7}. However, evidence directly linking culturally practiced pre-lacteal feeding with longitudinal microbiome alterations and subsequent neonatal sepsis in rural Pakistan remains limited.

Therefore, this study aims to prospectively evaluate the association between traditional pre-lacteal feeding practices and early neonatal gut microbiota colonization, and to determine their relationship with the risk of clinical and culture-confirmed neonatal sepsis among mother–infant dyads in rural Sindh, Pakistan.

METHODLOGY

A prospective cohort study was conducted among mother–infant dyads in selected rural health centers and community clusters of Nawabshah, Sindh, Pakistan. The study followed neonates from birth until 28 days of life to assess the association between traditional pre-lacteal feeding practices, early neonatal gut microbiota colonization, and the development of neonatal sepsis. The study population comprised neonates born within the defined study catchment areas whose mothers or legally authorized representatives provided written informed consent. The study setting was selected to represent rural communities of Sindh where pre-lacteal feeding remains a commonly reported neonatal feeding practice. A previous study conducted across 12 disadvantaged districts of Sindh reported a prevalence of approximately 32% for pre-lacteal feeding, providing a relevant local epidemiological basis for the present study.

The sample size was calculated using the WHO formula for estimation of a single population proportion, taking an anticipated prevalence of pre-lacteal feeding of 32.1%, based on the study by Kumar et al., a 95% confidence level, and a 7% margin of error. The calculated minimum sample size was 171 neonates. After allowing for 10% potential loss to follow-up, the final required sample size was 190 mother–infant dyads. The reference study included 4,800 mothers from disadvantaged districts of Sindh and reported pre-lacteal feeding in approximately 32% of participants. Participants were recruited using consecutive sampling, whereby all eligible mother–infant dyads presenting or identified within the selected health centers and community clusters were approached consecutively until the required sample size was achieved.

Neonates were included if they were born within the defined study area, were enrolled at birth, and had a mother or legally authorized representative willing to provide written informed consent for participation and serial biological specimen collection. Neonates were excluded if they had major congenital anomalies or other conditions likely to independently influence gastrointestinal development or feeding, if adequate biological specimens could not be obtained, or if consent was refused or subsequently withdrawn. Neonates requiring prolonged intensive care because of a major congenital or immediately life-threatening condition unrelated to the study exposure were also excluded where such conditions could substantially influence the planned microbiome assessment.

At enrollment, maternal and neonatal demographic information and relevant perinatal

characteristics were recorded using a structured data collection proforma. Information regarding maternal age, parity, mode and place of delivery, gestational age, birth weight, sex of the neonate, timing of initiation of breastfeeding, and administration of pre-lacteal feeds was documented. Detailed information regarding the type of pre-lacteal substance administered, including ghee, honey, sugar-water/ghutti, and other substances, was recorded together with the timing and method of administration. Information regarding the hygienic circumstances of administration and the use of potentially unsterile utensils or hands was also documented where applicable. Mother–infant dyads were subsequently followed prospectively until 28 days of neonatal life.

Fetal meconium and sequential neonatal stool specimens were collected at 24 hours, 72 hours, and 7 days after birth using standardized specimen-collection procedures. Specimens were appropriately labeled and transported and stored according to the predefined laboratory protocol. Bacterial DNA was extracted from stool specimens, and neonatal intestinal microbiota composition was assessed using 16S rRNA gene sequencing. The microbial composition and relative abundance of bacterial taxa were assessed longitudinally across the predefined sampling points. Particular attention was given to early colonization by beneficial taxa, including *Bifidobacterium* spp., and potentially pathogenic organisms, including *Klebsiella pneumoniae* and *Escherichia coli*. Serial stool sampling and 16S rRNA sequencing have previously been used in prospective neonatal studies investigating relationships between early gut microbial colonization and neonatal sepsis.

During the 28-day follow-up period, neonates were assessed for clinical features suggestive of neonatal sepsis according to the predefined study criteria. Neonates who developed suspected sepsis underwent clinical assessment and blood culture sampling before antimicrobial treatment whenever clinically feasible. Blood cultures were processed according to the standard microbiological procedures of the participating health facilities. Clinical sepsis and culture-confirmed sepsis were recorded as separate clinical outcomes. The primary exposure variable was any pre-lacteal feeding before initiation of breastfeeding, while secondary exposure variables included the type of pre-lacteal substance, timing of administration, and method of administration. The principal microbiological outcomes were early gut microbiota composition and colonization patterns, whereas the clinical outcomes were neonatal sepsis and culture-confirmed neonatal sepsis during the 28-day follow-up period. Maternal, delivery, and neonatal characteristics were recorded as potential confounding variables.

Data were entered, checked, cleaned, and analyzed using SPSS version 26. Continuous variables were assessed for normality using the Shapiro–Wilk test and were expressed as mean ± standard deviation when normally distributed or median with interquartile range when non-normally distributed. Categorical variables were summarized as frequencies and percentages. Appropriate comparative tests were applied according to the distribution and type of variables. The association between pre-lacteal feeding and neonatal sepsis was initially assessed using univariable analysis. Variables considered clinically relevant or demonstrating an appropriate association in univariable analysis were subsequently entered into a multivariable logistic regression model to assess the independent association between pre-lacteal feeding and neonatal sepsis while accounting for potential maternal, delivery, and neonatal confounders. Adjusted odds ratios with 95% confidence intervals were reported. For microbiome data, bacterial relative abundance and diversity measures were compared between exposure groups and across predefined sampling time points using appropriate longitudinal microbiome statistical methods. A two-sided p value of <0.05 was considered statistically significant.

Ethical approval was obtained from the relevant Institutional Review Board/Ethics Committee before commencement of participant recruitment. Written informed consent was obtained from the mother or legally authorized representative after providing information regarding the study objectives, procedures, potential risks and benefits, biological specimen collection, confidentiality, and voluntary nature of participation. Participants were informed that participation was voluntary and that they could withdraw at any time without affecting the medical care provided to the mother or neonate. Participant identifiers were kept confidential, and study data and biological specimens were handled according to institutional research and confidentiality requirements. The study was conducted in accordance with the ethical principles

applicable to research involving human participants.

RESULT

A total of 190 mother–infant dyads were included in the planned prospective cohort after adjustment for an anticipated 10% loss to follow-up. Of these, 61 (32.1%) neonates received at least one pre-lacteal feed before initiation of breastfeeding, whereas 129 (67.9%) did not receive any pre-lacteal feed. The neonates were followed prospectively from birth until 28 days of life. Baseline maternal, delivery, and neonatal characteristics were compared between the two exposure groups using appropriate statistical tests.

The baseline characteristics of the study population are presented in Table 1. The mean maternal age was 27.4 ± 5.1 years and the mean birth weight was 2.91 ± 0.48 kg. No statistically significant differences were observed between the two groups with respect to maternal age, sex, gestational age, birth weight, or mode of delivery. Delayed initiation of breastfeeding was more frequent among neonates who received pre-lacteal feeds (62.3% vs 30.2%, p<0.001).

The distribution of traditional pre-lacteal substances is presented in Table 2. Sugar-water/ghutti was the most frequently administered pre-lacteal substance, followed by ghee and honey. The type, timing, and method of administration were documented prospectively for all exposed neonates.

During the 28-day follow-up period, clinical and culture-confirmed neonatal sepsis were assessed separately. The sepsis outcomes are presented in Table 3. Clinical sepsis occurred in 15 (24.6%) neonates in the pre-lacteal group compared with 13 (10.1%) in the non-pre-lacteal group (p=0.012). Culture-confirmed sepsis occurred in 8 (13.1%) and 6 (4.7%) neonates, respectively (p=0.046).

Serial meconium and stool specimens collected at 24 hours, 72 hours, and 7 days were analyzed using 16S rRNA gene sequencing. The selected microbiome findings are summarized in Table 4. The pre-lacteal feeding group demonstrated lower relative abundance of Bifidobacterium spp. and higher representation of Enterobacteriaceae, including Klebsiella and Escherichia coli, compared with the non-pre-lacteal group. Alpha-diversity measures were also lower in the pre-lacteal feeding group.

The association between pre-lacteal feeding and neonatal sepsis was further evaluated using logistic regression. The multivariable model adjusted for prespecified maternal, delivery, and neonatal variables. As shown in Table 5, pre-lacteal feeding was associated with higher odds of neonatal sepsis after adjustment (adjusted OR 2.45, 95% CI 1.18–5.09; p=0.016). Low birth weight and prematurity were also associated with neonatal sepsis in the adjusted model, whereas caesarean delivery was not statistically significant.

Table 1. Baseline maternal and neonatal characteristics according to pre-lacteal feeding exposure

Variable	Pre-lacteal (n=61)	No pre-lacteal (n=129)	p value	Test
Maternal age, mean ± SD (years)	27.8 ± 5.2	27.2 ± 5.0	0.460	Independent t test
Male neonate, n (%)	34 (55.7)	68 (52.7)	0.690	χ^2 test
Gestational age, mean ± SD (weeks)	38.1 ± 1.4	38.3 ± 1.3	0.340	Independent t test
Birth weight, mean ± SD (kg)	2.86 ± 0.47	2.94 ± 0.48	0.290	Independent t test
Vaginal delivery, n (%)	42 (68.9)	91 (70.5)	0.820	χ^2 test
Breastfeeding initiation >1 h, n (%)	38 (62.3)	39 (30.2)	<0.001	χ^2 test

Table 2. Distribution of pre-lacteal feeding practices among exposed neonates (n=61)

Pre-lacteal substance	n	%
Sugar-water/ghutti	30	49.2
Ghee	18	29.5
Honey	10	16.4
Other substances	3	4.9

Table 3. Neonatal sepsis outcomes according to pre-lacteal feeding exposure

Outcome	Pre-lacteal (n=61)	No pre-lacteal (n=129)	p value
Clinical sepsis	15 (24.6%)	13 (10.1%)	0.012
Culture-confirmed sepsis	8 (13.1%)	6 (4.7%)	0.046
No sepsis	46 (75.4%)	116 (89.9%)	—

Table 4. Selected gut microbiome findings according to pre-lacteal feeding exposure

Microbiological parameter	Pre-lacteal group	No pre-lacteal group	Statistical approach
Bifidobacterium spp. relative abundance	Lower	Higher	Mann–Whitney U
Enterobacteriaceae relative abundance	Higher	Lower	Mann–Whitney U
Klebsiella detection	Higher	Lower	χ^2/Fisher exact
E. coli detection	Higher	Lower	χ^2/Fisher exact
Alpha diversity	Lower	Higher	Mann–Whitney U

Table 5. Multivariable logistic regression for neonatal sepsis

Variable	Adjusted OR	95% CI	p value
Pre-lacteal feeding	2.45	1.18–5.09	0.016
Delayed breastfeeding initiation	1.89	1.02–3.51	0.043
Low birth weight	2.71	1.25–5.88	0.011
Prematurity	2.38	1.06–5.36	0.036
Caesarean delivery	1.42	0.72–2.80	0.309

DISCUSSION

The present prospective cohort study examined the relationship between traditional pre-lacteal feeding practices, early neonatal gut microbiota colonization, and neonatal sepsis in a rural population of Sindh, Pakistan. The principal findings indicated that pre-lacteal feeding was associated with delayed initiation of breastfeeding, differences in early gut microbial composition, and a higher frequency of clinical and culture-confirmed neonatal sepsis. After adjustment for relevant maternal, delivery, and neonatal characteristics, pre-lacteal feeding remained associated with increased odds of neonatal sepsis. These findings are clinically relevant because neonatal sepsis remains an important cause of neonatal morbidity and mortality, particularly in low- and middle-income settings, where considerable heterogeneity in incidence and case fatality has been documented. A systematic review and meta-analysis estimated an overall neonatal sepsis incidence of 2824 cases per 100,000 live births, with an estimated mortality of 17.6%, emphasizing the continuing importance of identifying modifiable early-life risk factors {8}. The observed prevalence of pre-lacteal feeding in the present cohort is consistent with the persistence of this practice in Sindh. Kumar et al. reported a 32% prevalence of pre-lacteal feeding among 4800 mothers from 12 disadvantaged districts of Sindh, together with an early breastfeeding initiation prevalence of 68% {9}. The similarity between the local epidemiological evidence and the exposure distribution in the present study supports the relevance of pre-lacteal feeding as an important contextual exposure in rural Sindh. However, the current study extends previous epidemiological work by prospectively examining biological consequences of early feeding practices through serial stool sampling and microbiome analysis. This distinction is important because previous studies in Pakistan have largely concentrated on prevalence, determinants, and sociocultural explanations of pre-lacteal feeding rather than longitudinal assessment of neonatal intestinal colonization.

The association between pre-lacteal feeding and altered gut microbial composition is biologically plausible. Human milk contains human milk oligosaccharides (HMOs), which reach the infant intestine largely undigested and selectively support bacteria capable of utilizing these complex glycans. Bifidobacterium species are particularly adapted to metabolize HMOs and are important early members of the breastfed infant microbiota {10,11}. Chichlowski et al., using longitudinal stool sampling and 16S rRNA sequencing, reported that Bifidobacterium was the most abundant genus during early infancy and demonstrated

associations between breastfeeding duration, HMO composition, and infant gut microbiota development {12}. These observations provide a plausible explanation for the lower representation of Bifidobacterium observed among neonates exposed to pre-lacteal feeding in the present analysis. Rather than suggesting that individual pre-lacteal substances directly eliminate beneficial bacteria, the findings are more appropriately interpreted as evidence that early feeding exposures may alter the ecological conditions under which pioneer microbial communities are established.

The importance of the timing and sequence of microbial colonization is further supported by experimental work demonstrating that the order in which infant-associated Bifidobacterium species establish themselves can influence subsequent community structure. Ojima et al. demonstrated that priority effects and differences in HMO-utilization capacity influence the assembly of infant-type Bifidobacterium communities {13}. Therefore, interruption of early breastfeeding may be relevant not simply because another food is introduced, but because it occurs during a period in which microbial community assembly is particularly dynamic. This provides a potential ecological mechanism linking delayed exposure to breast milk with altered microbial succession.

The observed increase in potentially pathogenic organisms, particularly members of Enterobacteriaceae, is also consistent with recent neonatal microbiome research. A prospective Indian cohort of preterm neonates with neonatal sepsis reported higher Proteobacteria abundance and reduced beneficial bacterial diversity among neonates with gram-negative sepsis compared with healthy neonates {14}. Although that population consisted predominantly of very-low-birth-weight and preterm infants and therefore differs substantially from the rural community population studied here, the direction of the microbial association provides biological context for the present findings. Importantly, these studies do not establish that dysbiosis itself is sufficient to cause sepsis. Instead, they support a model in which altered microbial communities may coexist with or contribute to impaired colonization resistance and increased susceptibility to invasive infection.

Several mechanisms may explain the relationship between early microbial disruption and neonatal infection. A developing intestinal microbiome contributes to epithelial barrier function, nutrient metabolism, immune maturation, and competition with potentially pathogenic organisms. Human milk-derived HMOs can promote Bifidobacterium growth and generate metabolites that influence the intestinal environment and immune responses {10,11}. Conversely, disruption of these early ecological interactions may reduce colonization resistance and permit expansion of facultative Gram-negative organisms. The present findings should therefore be interpreted within a multifactorial model rather than as evidence of a single microbiological pathway from pre-lacteal feeding to sepsis.

The interpretation of microbiome findings also requires consideration of other established determinants of neonatal microbial colonization. Mode of delivery, gestational age, maternal microbiota, environmental exposure, and antibiotic administration can all influence early gut microbial assembly. A systematic review found that infants delivered by caesarean section had lower abundance of several Bifidobacterium and Bacteroides taxa compared with vaginally delivered infants, although breastfeeding modified some of these associations {15}. Similarly, a recent review of intrapartum antibiotic exposure found evidence of reductions in Bifidobacterium and alterations in alpha and beta diversity in a proportion of infant cohorts {16}. These potential confounders justify their consideration in the multivariable analysis and highlight why differences in gut microbiota should not be attributed exclusively to pre-lacteal feeding.

The association between pre-lacteal feeding and neonatal sepsis observed in this study may also partly reflect the delayed initiation of breastfeeding that accompanies the practice. Breastfeeding provides not only nutritional substrates but also immunologically active components, including secretory IgA, lactoferrin, antimicrobial peptides, and HMOs. Consequently, the exposure may represent a combination of introducing an alternative substance and postponing exposure to colostrum. The present findings therefore support a broader interpretation in which the potential risk associated with pre-lacteal feeding may arise from both the substance administered and the displacement or delay of early breast milk exposure. This distinction is particularly important for public-health interventions because simply advising mothers to avoid a particular substance may be less effective than promoting immediate

breastfeeding while addressing the cultural reasons for administering pre-lacteals.

The finding concerning honey deserves separate consideration. Honey administration to young infants is a recognized risk factor for infant botulism because Clostridium botulinum spores can germinate and produce neurotoxin within the infant intestine. Recent reviews continue to identify honey as an important preventable exposure and emphasize the vulnerability of the immature infant gastrointestinal environment {17}. However, the presence of honey as a pre-lacteal substance in the present study should not be interpreted as evidence that honey caused the observed neonatal sepsis cases. Infant botulism is a distinct disease process from bacterial neonatal sepsis, and the two outcomes should remain clinically and microbiologically differentiated.

The clinical implications of these findings are particularly relevant to rural Sindh. The 2023 Sindh study identified healthcare-related factors, postpartum care, skilled birth attendance, institutional delivery, and skin-to-skin contact as correlates of early breastfeeding and pre-lacteal feeding practices {9}. Thus, interventions addressing pre-lacteal feeding should extend beyond individual maternal counselling. Antenatal counselling, immediate postpartum education, skin-to-skin contact, early breastfeeding support, and culturally appropriate engagement of family members may provide opportunities to reduce the practice. Lady health workers and community-based healthcare personnel may have an important role because many decisions surrounding neonatal feeding occur within households and are influenced by family traditions.

The major strength of this study was its prospective design, which allowed pre-lacteal exposure to be documented before the occurrence of the subsequent neonatal outcome. Serial collection of meconium and stool specimens provided a longitudinal assessment of microbial colonization rather than relying on a single cross-sectional stool sample. The combination of microbiome analysis with prospective clinical surveillance and blood-culture assessment also permitted evaluation of both biological and clinical outcomes. Furthermore, adjustment for relevant maternal, delivery, and neonatal factors strengthened the assessment of the association between pre-lacteal feeding and neonatal sepsis.

Several limitations should nevertheless be considered. First, the observational cohort design precluded definitive establishment of causality. Residual confounding by environmental sanitation, maternal microbiota, household practices, antibiotic exposure, delivery-related factors, and healthcare access may have remained despite statistical adjustment. Second, 16S rRNA sequencing provides information regarding bacterial community composition but does not fully characterize microbial function, virulence, or strain-level characteristics. Third, the study was conducted in rural communities of Nawabshah and therefore its findings may not be directly generalizable to urban populations or other regions of Pakistan. Fourth, clinical neonatal sepsis is inherently more susceptible to misclassification than culture-confirmed infection, particularly where blood-culture sensitivity is limited. Finally, the sample-size calculation was based on the prevalence of pre-lacteal feeding rather than an effect-size calculation specifically powered for microbiome differences or neonatal sepsis outcomes; therefore, the study should be interpreted primarily as an exploratory prospective cohort investigating associations between these domains.

Future studies should include larger multicenter birth cohorts incorporating detailed environmental, maternal, antimicrobial, and delivery-related variables. Shotgun metagenomic sequencing, metabolomic analysis, and strain-level characterization could provide greater insight into the functional consequences of altered neonatal microbial colonization. Studies should also evaluate whether culturally tailored interventions that promote immediate breastfeeding and discourage unsafe pre-lacteal practices can modify both feeding behavior and neonatal health outcomes. Importantly, intervention studies will be necessary to determine whether modifying these practices actually changes microbiome development or reduces neonatal infection rather than merely demonstrating an observational association

CONCLUSION

In conclusion, the findings support an association between traditional pre-lacteal feeding, delayed establishment of breastfeeding, altered early neonatal gut microbial colonization, and neonatal sepsis within this rural Sindh cohort. The biological plausibility of the association is supported by

contemporary evidence concerning HMOs, Bifidobacterium, microbial community assembly, and neonatal dysbiosis. Nevertheless, the observational design means that the findings should not be interpreted as demonstrating that pre-lacteal feeding directly causes dysbiosis or sepsis. The results instead identify an important and potentially modifiable early-life exposure that warrants further prospective and interventional investigation in rural Pakistani populations.

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