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# Impact of breast milk expression practices on expressed breast milk contamination: cross-sectional study.

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## Abstract

### Background:

Expressed breast milk (EBM) is regarded as the best nutritional source for premature and sick babies admitted to neonatal intensive care units (NICUs). Nevertheless, poor hygiene standards during expression, storage, transportation, and handling of the EBM can increase the risk of infection from bacteria in the milk.

### Objectives:

1. To determine the prevalence of microbial contamination in expressed breast milk.
2. To identify the common microbial organisms isolated from expressed breast milk.
3. To assess maternal breast milk expression, storage, and transport practices.
4. To identify practices significantly associated with microbial contamination of expressed breast milk.

### Methods:

A hospital-based cross-sectional study was done for 12 months on 178 mothers of LBW premature neonates who were admitted to a tertiary care NICU. Those mothers having breast infection, nipple bleeding, or systemic infection were excluded from the study. The EBM samples were checked for hygiene, expression, storage, and transportation. One mL was cultured on blood and MacConkey agar plates.

### Results:

A cross-sectional study conducted in a hospital for 12 months included 178 mothers of LBW premature babies admitted to a tertiary care NICU. Mothers who had breast infection, nipple bleeding, or systemic infection were excluded from this study. The EBM samples were screened for hygiene, expression, storage, and transportation. One mL of the sample was inoculated on blood and MacConkey agar. The isolates were confirmed by the conventional method. The data were analyzed through SPSS 22.0.

### Conclusion:

A high prevalence of EBM contamination was observed among mothers of preterm neonates. Maternal nail hygiene and appropriate handling of storage containers were significant determinants of contamination.

### Recommendation

NICUs should strengthen standardized EBM expression, collection, storage, and transportation protocols. Mothers should receive regular education on hand hygiene, breast and equipment cleaning, safe storage, and timely milk delivery.

***Keywords:*** *Expressed breast milk, microbial contamination, neonatal intensive care unit, preterm neonates, breast milk hygiene, hand hygiene, breast milk handling, Staphylococcus aureus, infection prevention.*
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## Introduction

Breastfeeding is widely recognized as the optimal method of infant feeding and is one of the most effective interventions for improving child survival, growth, and development. Breast milk provides complete nutrition for infants during the first six months of life, supplying all essential nutrients, energy, bioactive factors, and immunological components required for healthy growth. It also contains antibodies,

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antimicrobial proteins, enzymes, hormones, and growth factors that protect infants against common childhood infections, particularly gastrointestinal and respiratory illnesses. The protective effects of breastfeeding extend beyond infancy, contributing to improved cognitive development, reduced risk of obesity and non-communicable diseases, and better educational and socioeconomic outcomes later in life. Furthermore, breastfeeding benefits mothers by reducing the risk of breast and ovarian cancers, promoting birth spacing through lactational amenorrhea, and facilitating postpartum recovery. [1,2]

The World Health Organization (WHO) and UNICEF recommend initiation of breastfeeding within one hour of birth, exclusive breastfeeding for the first six months of life, followed by the introduction of appropriate complementary foods while continuing breastfeeding up to two years of age or beyond. Despite these well-established recommendations, exclusive breastfeeding practices remain suboptimal worldwide. Recent estimates indicate that only about 44–46% of infants younger than six months are exclusively breastfed globally, with considerable regional and national variations. Although improvements have been observed over the past decade, many countries, including India, have yet to achieve the WHO global nutrition target for exclusive breastfeeding. [3,4]

Breastfeeding assumes even greater importance for preterm and critically ill neonates admitted to neonatal intensive care units (NICUs). Premature infants have immature gastrointestinal, immune, and metabolic systems, making them highly susceptible to infections, necrotizing enterocolitis, feeding intolerance, and poor growth. Human milk from mothers of preterm infants contains higher concentrations of immunological factors, secretory immunoglobulins, growth-promoting substances, and essential fatty acids, including docosahexaenoic acid (DHA), which support intestinal maturation, neurodevelopment, and immune protection. Consequently, human milk significantly reduces morbidity and mortality among preterm infants compared with formula feeding. [5]

However, many hospitalized neonates are unable to breastfeed directly because of prematurity or severe illness. In such circumstances, expressed breast milk (EBM) serves as the preferred alternative for providing the benefits of human milk. Although EBM remains the recommended source of nutrition, contamination may occur during milk expression, storage, transport, or handling because of inadequate hygienic practices or improper collection techniques. Contaminated EBM may expose vulnerable neonates to potentially pathogenic microorganisms, increasing the risk of healthcare-associated infections, prolonged hospitalization, and adverse clinical outcomes. Understanding the prevalence of EBM contamination and identifying modifiable risk factors are therefore essential for improving neonatal care practices and ensuring the safe administration of expressed breast milk in NICU settings.[6]

## Material and methods

### Study design

A hospital-based cross-sectional study was conducted to assess microbial contamination of expressed breast milk (EBM) and its association with maternal and milk-handling practices.

### Study Setting and Study Period

The study was conducted at **[Name of Tertiary Care Hospital]**, a tertiary care hospital located in **[City, State, Country]**. The hospital provides comprehensive medical, surgical, obstetric, paediatric, and neonatal services and has a Neonatal Intensive Care Unit (NICU) providing specialised care for preterm and low-birth-weight neonates. The study was conducted over 12 months, from **January to December 2023**.

### Participants

Study subjects comprised mothers of low birth weight premature neonates admitted to the NICU during the study period. Mothers willing to breastfeed and take part in the study were enrolled. The number of subjects was recruited consecutively till the desired sample size was reached.

Mothers with local breast infection, nipple bleeding, or systemic infection were excluded from the study.

### Sample size

The required sample size was calculated using the formula:

$$\mathbf{N = 4pq/l^2}$$

where $p$ was the expected prevalence of EBM contamination (90%), $q$ was $100 - p$ (10%), and $l$ was the allowable error, calculated as 5% of the prevalence (4.5%).

Thus:

$$\mathbf{N = 4 \times 90 \times 10 / (4.5)^2 = 177.77}$$

The calculated sample size was rounded to **178 mothers**.

### Participant selection

Eligible mothers were selected using consecutive sampling during the study period. Mothers who fulfilled the eligibility criteria and provided written informed consent were enrolled. Recruitment continued until 178 participants were included in the study.

### Bias

Selection bias was controlled to the minimum level through consecutive selection of eligible mothers during the study period. All the participants were screened against standardized criteria. Information bias was controlled

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through the use of a checklist for recording the information on hygiene, breast milk expression, storage, handling, and transport. The nurses gave standardized instructions for proper milk expression and hygiene. Standardized microbiological techniques were used for laboratory work.

## Data collection

Information about maternal hygiene and practices for expressing and handling EBM was gathered by a structured questionnaire after acquiring written informed consent. The questionnaire was used to evaluate the hygiene of hands, nails, jewelry, breasts, milk expression, sterilization of vessels and containers, and transportation of EBM to the NICU.

One milliliter of EBM sample was taken aseptically from each mother, and it was delivered to the microbiology lab within 20 minutes of sampling. A sterile swab was inoculated into blood agar and MacConkey agar. The culture plates were incubated at 37°C for 24 to 48 hours, followed by examination for bacterial growth.

The bacterial growth was quantified and identified by Gram staining and biochemical tests wherever necessary. EBM was regarded as contaminated if the culture showed growth of $\geq 10^4$ colony-forming units per mL of staphylococci, Gram-negative bacteria, or enterococci; or $\geq 10^5$ colony-forming units per mL of skin commensals like Staphylococcus epidermidis.

## Study variables

The dependent variable was microbial contamination of the expressed breast milk. Independent variables were mother's hand hygiene, nail hygiene, removing jewellery, breast hygiene, location of expressed milk, technique of expressed milk, method of cleaning the container used to express the milk, the storage container used and storage/transportation of the milk, which included using ice packs. Microbiological variables were type and number of microorganisms found in EBM.

## Statistical analysis

Data were recorded on an MS Excel spreadsheet and analysed using Statistical Package for the Social Sciences (SPSS), version 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables were reported in terms of frequency and percentage, and continuous variables in terms of mean and standard deviation. The appropriate tests were employed to establish any relationship between study variables, such as the chi-square test and Student's t-test where applicable. The statistical significance level was set at a p-value $<0.05$.

## Ethical considerations

Approval of ethical considerations was obtained from the institution's ethics committee before carrying out the study. All the mothers who took part in the study gave their written consent before participation. Participation in the study was voluntary, and all participants were told of the objective and methods of the study.

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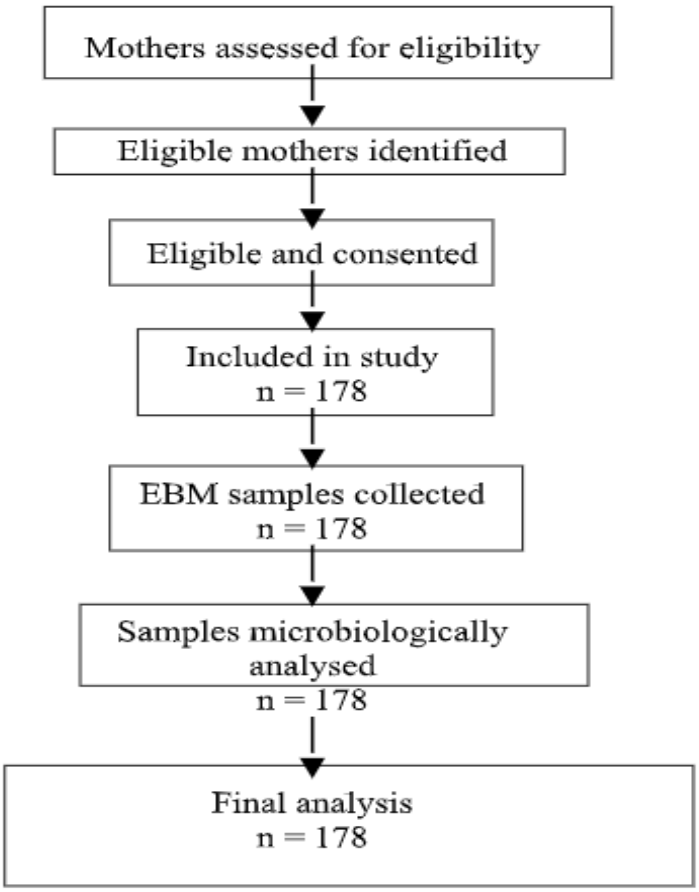
## Results

### Participant flow




During the study period from January to December 2023, eligible mothers of preterm neonates with low birth weight admitted to the NICU were identified. Participants were screened based on the inclusion and exclusion criteria established for the study. The eligible mothers, who agreed to take part in the study, provided informed consent and were recruited consecutively. A total of 178 mothers were recruited, and all 178 participants had their EBM samples collected and analyzed. None of the participants was excluded during recruitment due to lack of or poor-quality samples.

### Table 1. Association of hand hygiene practices with EBM contamination (n=178)

| n=178 | Total Frequency | (%) | Contaminated EBM | (%) | Non-Contaminated EBM | (%) | P-Value |
|---|---|---|---|---|---|---|---|
| **Hand Hygiene Finger nails removed (YES)** | 118 | 66.29 | 100 | 84.75 | 18 | 15.25 | * |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **NO** | 60 | 33.71 | 60 | 100.00 | 0 | 0.00 | 0.045 |
| **Jewelry removed (YES)** | 120 | 67.42 | 111 | 92.50 | 09 | 7.50 | * |
| **NO and wash (YES)** | 58 | 32.58 | 49 | 84.48 | 9 | 15.52 | 0.114 |
| | 104 | 58.43 | 96 | 92.31 | 08 | 7.69 | * |
| **NO** | 74 | 41.57 | 60 | 81.08 | 14 | 18.92 | 0.124 |
| **Breast Hygiene Using cotton pads on nipple** | | | 101 | | | | * |
| | 114 | 64.04 | | 88.60 | 13 | 11.40 | |

**Without regard to hand, breast hygiene.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (YES) | | | | | | | |
| Breast cleaning with water | 64 | 35.96 | 59 | 92.19 | 05 | 7.81 | 0.606 |
| **Place of Expression** | | | | | | | |
| NICU | 65 | 36.52 | 57 | 87.69 | 08 | 12.31 | * |
| PNC | 113 | 63.48 | 103 | 91.15 | 10 | 8.85 | 0.450 |
| **Type of container used** | | | | | | | |
| Glass | 20 | 11.24 | 20 | 100.00 | 0 | 0.00 | * |
| House Hold | 21 | 11.80 | 20 | 95.24 | 1 | 4.76 | 1 |
| Special | 137 | 76.97 | 120 | 87.59 | 17 | 12.41 | 0.471 |
| **Type of container cleaning method** | | | | | | | |
| Water | 117 | 65.73 | 117 | 100.00 | 0 | 0.00 | * |
| ter and Soap | 30 | 16.85 | 28 | 93.33 | 2 | 6.67 | 0.449 |
| Boiling | 31 | 17.42 | 15 | 48.39 | 16 | 51.61 | <0.001 |
| **Transport of Milk** | | | | | | | |
| Plastic Bag with Ice Pack | 67 | 37.64 | 64 | 95.52 | 3 | 4.48 | * |
| Plastic Bag | 111 | 62.36 | 96 | 86.486 | 15 | 13.51 | 0.715 |

***P-Value (1.0 - No Significance, >0.5 Not Significant, <0.5 statistically significant, <0.01 highly significant)***

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**Table 2. Association of Breast Hygiene and Milk-Handling Practices with EBM Contamination (n=178)**

| Total Samples (n =178) | STERILE | CULTURE |
|---|---|---|
| **178** | 18 (0.101%) | 160 (89.88%) |



This table shows that out of 178 samples (n = 178), only 18 (10.11%) expressed breast milk samples had no growth of any organism, and the remaining 160 (89.88%) samples showed growth of various organisms.

**Table 3 -Isolated Microorganisms from Contaminated EBM Samples (n=160)**

| Isolated Organisms | n (%) |
|---|---|
| ***Staphylococcus aureus*** | **85 (47.75%)** |
| ***Staphylococcus epidermidis*** | **40 (22.47%)** |
| ***Enterobacter*** | **20(11.23%)** |
| ***Actinobacteria*** | **03(1.68%)** |
| ***Klebsiella pneumoniae, Escherichia coli*** | **(3.37%).** |
| | **(3.37%).** |

Out of 178 (n=178) total samples, 125 (70.22%) showed Gram-positive cocci in EBM samples. Out of 125, 85 (47.75%) were *S. aureus* and 4 (22.47%) were *S. epidermidis*. A total of 32 (17.97%) samples had Gram-negative bacilli grown. Out of 32, 20(11.23%) were *Enterobacter,* followed by 06 where *K. pneumoniae* and *E. coli* (3.37%). Actinobacteria 3(1.68%).

## Discussion

The present study assessed microbial contamination in expressed breast milk (EBM) collected from 178 mothers of preterm neonates admitted to the Neonatal Intensive Care Unit (NICU) and postnatal care (PNC) ward. A high prevalence of contamination was observed, with 160 (89.88%) samples demonstrating bacterial growth, while only 18 (10.11%) samples showed no microbial growth. Maternal hygiene practices played a significant role in reducing EBM contamination. Removal of fingernails before milk expression showed a statistically significant association with lower contamination rates (p=0.045), emphasizing the importance of proper hand and nail hygiene. Similar observations have been reported by Heidi et al., who demonstrated that microorganisms harboured beneath fingernails contribute substantially to bacterial transmission.[7] Although mothers who removed jewellery and practiced handwashing exhibited lower contamination rates, these associations were not statistically significant in the present study. Nevertheless, these findings support existing evidence suggesting that comprehensive hand hygiene remains a fundamental component of infection prevention during breast milk expression.

Breast hygiene practices also influenced contamination rates. Mothers who used sterile cotton pads or cleaned the breast with water before milk expression generally demonstrated lower contamination; however, these differences were not statistically significant. Similar findings have been reported by Boo et al [8] and Ransome-Kuti et al.[9], indicating that while breast cleansing is advisable, it should complement rather than replace appropriate hand hygiene and aseptic milk handling practices.

The place of milk expression, whether in the NICU or PNC ward, did not significantly influence contamination rates, suggesting that environmental location alone is less important than adherence to standardized hygienic protocols. Likewise, although all samples stored in glass containers remained free from contamination, no statistically significant difference was observed between glass, household, and specialized storage containers. These observations indicate that proper cleaning, handling, and storage procedures may have a greater impact on microbial contamination than the type of container used.

The method of container cleaning demonstrated a significant association with contamination. Unexpectedly, containers cleaned by boiling showed higher contamination rates compared with those cleaned using water or soap and water ($p<0.001$). This finding may reflect contamination introduced during post-boiling handling, inadequate drying, or improper storage rather than the boiling process itself. Similar observations have been reported by White et al. [10], emphasizing that sterilization procedures must be followed by appropriate handling to maintain container sterility. Although transporting milk using insulated bags with ice packs resulted in lower contamination rates than transport

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without ice packs, the difference was not statistically significant. [11,12]

Microbiological analysis revealed that Gram-positive cocci were the predominant isolates, particularly *Staphylococcus aureus* and *Staphylococcus epidermidis*, followed by Gram-negative organisms including *Enterobacter*, *Klebsiella pneumoniae*, and *Escherichia coli*. These organisms are common skin and environmental flora and have been frequently reported in contaminated EBM, consistent with previous studies. Their presence suggests contamination primarily occurs during milk expression and handling.

Overall, the high prevalence of EBM contamination observed in this study underscores the need for standardized infection-control measures, comprehensive maternal education on hygienic milk expression techniques, appropriate container handling, and adherence to recommended storage and transport practices. Implementing evidence-based protocols within NICUs may substantially reduce microbial contamination and improve the safety of expressed breast milk for vulnerable preterm infants.

## Generalizability

The results obtained from this study could be used in tertiary NICUs for handling low birth weight and premature babies, especially those whose mothers store and express breast milk using evidence-based medicine. However, generalization of the study results could be difficult to some extent due to the fact that this study was conducted in one tertiary care hospital alone.

## Conclusion

The present study demonstrated a high prevalence of microbial contamination in expressed breast milk (EBM), with 89.88% of samples showing bacterial growth, predominantly Gram-positive cocci, particularly *Staphylococcus aureus and Staphylococcus epidermidis*, followed by Gram-negative organisms such as *Enterobacter* and *Klebsiella pneumoniae*. Among the various maternal and handling practices evaluated, proper nail hygiene showed a statistically significant association with reduced contamination, highlighting its importance during milk expression. Although handwashing, use of cotton pads, breast cleaning, place of milk expression, type of storage container, and transportation with ice packs were associated with lower contamination rates, these differences were not statistically significant. Interestingly, the method of container cleaning significantly influenced contamination, with boiled containers demonstrating higher contamination rates, suggesting that improper handling after sterilization may negate the benefits of boiling. Overall, the findings emphasize that strict adherence to evidence-based hygienic practices during milk expression, appropriate container handling, storage, and transport is essential to minimize microbial contamination and ensure the microbiological safety of expressed breast milk, particularly for vulnerable preterm infants admitted to neonatal intensive care units.

## Limitations

Limitations associated with this study include: being conducted using a cross-sectional design; this made it difficult to conclude the existence of any causality between maternal practices and contamination with EBM. It was carried out in one tertiary care center and, thus, might lack generalizability. Some of the hygiene and handling practices were self-reported; hence, they could have been influenced by memory/recall and social desirability bias.

## Recommendations

Procedures for standardisation of EBM communication and handling must be improved in NICUs. Regular training sessions along with practical demonstration regarding hygiene of hands, fingernails, breast and containers, and transportation of EBM can be provided to mothers. Surveillance of the practice of handling of EBM and microbiology of EBM will assist in identifying preventable sources of contamination. Further multi-centric studies are required to validate these results.

## Acknowledgement

The authors acknowledge the support of the participating mothers and the NICU staff who assisted with participant recruitment, EBM collection, and data collection. The authors also acknowledge the microbiology laboratory staff for their assistance with sample processing and identification of microbial isolates.

## List of Abbreviations

**EBM:** Expressed Breast Milk
**NICU:** Neonatal Intensive Care Unit
**LBW:** Low Birth Weight
**CFU:** Colony-Forming Unit
**SPSS:** Statistical Package for the Social Sciences
**IBM:** International Business Machines
**SD:** Standard Deviation
**$\chi^2$:** Chi-Square
**p:** *p*-value
**EBM:** Expressed Breast Milk

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