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ORIGINAL RESEARCH

Expressed Breast Milk Contamination in Neonatal Intensive Care Unit

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Background: The health benefits of breastfeeding are well known. However, some ill babies including those admitted to the neonatal intensive care unit (NICU) cannot be directly breastfed. In this situation, expressed breast milk (EBM) can be used. However, breast milk is not always sterile and may be contaminated by many microorganisms. EBM contamination is probably attributed to improper technical and hygienic factors and may pose significant threats to the newborn baby. The present study aimed to document the prevalence of EBM contamination in NICU and to uncover the relevant risk factors.

Subjects and Methods: The study included 118 mothers who could express breast milk for their own neonates admitted to the NICU. A checklist was used to document the steps the mothers followed during expression of milk and all steps of handling until the EBM reached the NICU. A 1 mL sample of EBM was obtained and sent to the microbiology laboratory within 20 minutes. Data obtained from the present study are expressed as number and percentage or mean ± standard deviation (SD). Statistical calculations were computed using SPSS 25.

Results: In the present study, 106 (89.8%) out of the assessed 118 EBM samples were contaminated. Hygienic factors related to EBM contamination included hand only wash, possible recontamination of hands during turning taps off, lack of using cotton pads or cloth piece on nipple and breast cleaning by water only. Other factors related to EBM contamination included container cleaning by water only, fresh milk refrigeration after > 4 hours, adding freshly expressed warm breast milk to refrigerated milk expressed earlier in the same day, milk transport in plastic bags with ice packs and longer transportation time. In the contaminated samples, the most commonly isolated organisms included *Staphylococcus aureus* (55.7%),*Staphylococcus epidermidis* (21.7%) and *Enterobacter* (11.6%).

Conclusion: The present study identified bacterial contamination in about 90% of EBM samples delivered to NICU infants. Factors related to EBM contamination include hygienic, storage and transport factors.

Keywords: breastfeeding, expressed breast milk, NICU

Introduction

Human breast milk is generally considered as the best source of nutrition for newborns and infants. As a worldwide public health recommendation, infants should be exclusively breastfed for the first six months of life to achieve optimal growth, development and health. In preterm infants, feeding with human milk has been found to reduce the rate of infectious complications including late onset sepsis[1] and necrotizing enterocolitis (NEC).[2] In addition, use of human milk was associated with better cognitive development at 2

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years,[3] lower rate of hospital readmissions and improved cardiovascular outcomes.[4,5]

However, when newborns are admitted to the neonatal intensive care unit (NICU), lactating mothers have to express their breast milk to feed their babies. Because breast milk is not always sterile, microorganisms can multiply when milk is not handled properly.[6] This may constitute a significant clinical challenge particularly in those vulnerable babies.[7]

Staphylococcus aureus, including MRSA, β-hemolytic *Streptococci, Pseudomonas* species, *Klebsiella* species, *Proteus* species and *Enterobacteria* are frequently identified in EBM and may place the infant at risk of infection.[8–10] Interestingly, Boo et al[11] hypothesized increased use of EBM in NICU may be related to increased risk of NEC. They noted that more than 90% of NEC cases occurred in infants on enteral feeding. Susceptible organisms include *Enterobacteria* and other gram-positive and gram-negative bacteria. In addition, some reports identified an association between breast milk *Enterobacter* and neonatal sepsis.[12,13] Moreover, the study of Mammina et al[14] suggested a link between use of EBM in the NICU and nosocomial colonization by imipenem-resistant *Pseudomonas aeruginosa.*

To increase the percentage of premature newborns who benefit from expressed breast milk (EBM), we need to ensure the appropriate handling of expressed milk till it reaches the baby and to perform bacteriological screening of milk to identify possible pathogens.[6] Studies assessing the impact of breast expression practices on EBM contamination in the NICU setting shows inconsistent conclusions.[15–17]

The present study aimed to evaluate the steps of maternal handling of EBM leading to the presence of contamination.

Subjects and Methods

The present study was conducted at Suez Canal University Hospitals in the period from January through December, 2019. The study protocol was approved by the local ethical committee of Faculty of Medicine, Suez Canal University (Ref.103/18) and informed consent was obtained from all participants in accordance with the Declaration of Helsinki on clinical research involving human subjects.

Mothers of preterm neonates consecutively admitted to NICU were included in the study if they could provide sufficient amount of EBM for their own babies. EBM is the human milk obtained through squeezing of the breast either manually or mechanically by aid of pumps. Those who had local breast infection, nipple bleeding or systemic infection were excluded. Before milk expression, experienced nurses instructed all mothers about the appropriate hygiene and technique for manual and pump expression (Comfort Manual breast pump, Philips Avent, England).

A checklist was used to document the steps the mothers followed during expression of milk and handling until the EBM reached the NICU. Once EBM was delivered to the NICU, mothers were asked to fill the checklist to document their hygienic and handling practices.

A sample (1mL) of EBM was obtained from every mother and sent to the microbiology laboratory within 20 minutes. In the lab, a sterile swab was taken and cultured on blood and MacConkey agar and incubated for 24–48 hours at 37°C temperature. Then, the plates were examined. If bacterial colonies appeared, the quantity of isolated bacteria was counted and gram-stained to identify the type of bacteria. Chemical reactions were used if necessary. Milk samples were defined as contaminated if culture showed $\geq 10^4$ staphylococcal cfus/mL or any Gram-negative organisms or *enterococci*.[18,19] Also, sample was considered contaminated if it contained $\geq 10^5$ cfus/mL of skin commensals (*Staphylococcus epidermidis*).[20]

Collected data were analyzed using SPSS statistical software version 23 (IBM, Chicago, USA). Categorical data was expressed as numbers and percentages, while numerical variables were expressed as mean and standard deviation. Categorical variables were compared by chi-square or Fisher's exact test, while numerical variables were compared using student *t* test. The differences were considered significant when p value is <0.05.

Results

The present study was conducted on milk samples obtained from 118 mothers of consecutive preterm babies admitted to the NICU. The included mothers had an age of 28.3 ± 6.9 years. It was found that 106 samples (89.8%) out of the assessed 118 EBM samples were contaminated. Comparison between contaminated and non-contaminated samples revealed significant association between EBM contamination and hygienic behavior. Hygienic factors related to EBM contamination included hand only wash, possible recontamination of hands during turning taps off without, lack of using cotton pads or cloth piece on nipple and breast cleaning by water only (Table 1).

Other factors related to EBM contamination included container cleaning by water only, fresh milk refrigeration after > 4 hours, adding freshly expressed warm breast milk

Table 1 Comparison Between Mothers with Breast Milk Contamination and Mothers without Regarding Hand, Breast and Pump Hygiene

	Contaminated EBM n=106	Non-Contaminated EBM n=12	p
Hand hygiene			
Finger nails short	68 (64.2)	12 (100.0)	0.16*
Jewelry removed	74 (69.8)	8 (66.7)	0.98*
Hand wash	92 (86.8)	12 (100.0)	0.93*
Hand wash with			
- Water only	44 (47.8)	4 (33.3)	0.67*
- Soap and water	48 (52.2)	8 (66.7)	
Washed parts			
- Hand only	39 (84.8)	-	<0.001*
- Hand, under nails and forearms	7 (15.2)	12 (100.0)	
Tool of hand drying			
- Disposable paper towels	24 (26.1)	8 (66.7)	0.11#
- Clean cloth towel	40 (43.5)	4 (33.3)	
- No drying	28 (30.4)	-	
Turning taps off without recontamination of hands	2 (2.2)	6 (50.0)	0.003*
Breast hygiene			
Using cotton pads or cloth piece on nipple	46 (43.4)	12 (100.0)	0.011*
Breast cleaning	60 (56.7)	2 (16.6)	0.09*
Breast cleaning by			
- Water only	32 (52.8)	-	0.013#
- Soap and water	18 (30.2)	-	
- Wipes	10 (17.0)	2 (100.0)	
Pump hygiene			
Type of expression			
- Hands	40 (37.7)	8 (66.7)	0.21*
- Manual breast pump	66 (62.3)	4 (33.3)	
Pump wash time			
- Rinse well in cold water after use	52 (78.8)	4 (100.0)	0.3*
- At end of the day	14 (21.2)	-	
Cleaning method			
- Water only	52 (78.8)	-	0.061*
- Water and liquid soap	14 (21.2)	4 (100.0)	

Notes: Data expressed as number and percent. Statistical analysis was achieved using Fisher's exact test (*) or chi-square test (#).

to refrigerated milk expressed earlier in the same day, milk transport in plastic bags with ice packs and longer transportation time (Table 2).

In the contaminated samples, the isolated organisms included *Staphylococcus aureus* ($\geq 10^4$ cfus/mL) in 59 samples (55.7%), methicillin-resistant *Staphylococcus aureus* ($\geq 10^4$ cfus/mL) in 2 samples (1.9%) and *Staphylococcus epidermidis* ($>10^5$ cfus/mL) in 23 samples (21.7%). Other isolates included *Enterobacter* in 12 samples (11.6%), *Actinobacteria* in 5 samples (4.7%), and *Klebsiella pneumonia* in 5 samples (4.7%) (Table 3).

Table 2 Comparison Between Mothers with Breast Milk Contamination and Mothers without Regarding Place of Expression, Milk Container, Storage and Transport Characteristics

	Contaminated EBM n=106	Non-Contaminated EBM n=12	p
Place of expression			
Home	68 (64.2)	8 (66.7)	0.98*
NICU	38 (35.8)	4 (33.3)	
Type of container			
Glass	18 (17.0)	-	0.76#
Special container for baby food	78 (73.6)	12 (100.0)	
Plastic container designed for general household use	10 (9.4)	-	
Container cleaning method			
Water only	68 (64.2)	-	<0.001#
Water and liquid soap	22 (20.8)	-	
Boiling	16 (15.1)	12 (100.0)	
Container drying method			
Clean cloth towel	16 (15.1)	-	0.76#
Paper tissue	80 (75.5)	12 (100.0)	
None	10 (9.4)	-	
Container Storage			
Keep in room temp	72 (67.9)	10 (83.3)	0.66*
Keep in refrigerator	34 (32.1)	2 (16.7)	
Need for milk storage	68 (64.2)	8 (66.7)	0.98*
Fresh milk refrigeration			
Within 4 hrs.	20 (29.4)	8 (100.0)	0.014*
> 4 hrs.	48 (70.6)	-	
Duration of fresh milk refrigeration			
48 hrs.	54 (79.4)	8 (100.0)	0.16*
> 48 hrs.	14 (20.6)	-	
Adding freshly expressed warm breast milk to refrigerated milk expressed earlier in the same day	58 (85.3)	2 (25.0)	0.024*
Cooling milk in the fridge before transporting	44 (66.0)	6 (66.7)	0.68*
Milk transport means			
Plastic bag with ice packs	50 (74.3)	-	0.009*
Plastic bag with no ice packs	18 (25.7)	8 (100.0)	
Transportation time (min.) mean ± SD	39.4 ± 16.6	18.8 ± 7.5	0.013§

Notes: Data expressed as number and percent or mean and standard deviation (SD). Statistical analysis was achieved using Fisher's exact test (*), chi-square test (#) or *t* test (§).

Table 3 Isolated Microorganisms from Contaminated EBM Samples (n=106)

Isolated Organisms	n (%)
Staphylococcus aureus	59 (55.7)
Staphylococcus epidermidis	23 (21.7)
Methicillin-resistant *Staphylococcus aureus*	2 (1.9)
Enterobacter	12 (11.6)
Actinobacteria	5 (4.7)
Klebsiella pneumoniae	5 (4.7)

Discussion

In the present study, the majority (89.8%) of EBM samples were contaminated with bacterial growth. The most commonly isolated organisms included *Staphylococcus aureus* (55.7%), *Staphylococcus epidermidis* (21.7%) and *Enterobacter* (11.6%). In comparison, Karimi et al[15] found that 85% of samples were infected, and dominant microorganisms were *Klebsiella* (13.7%) followed by *S. epidermidis* (12.5%). In another study, microbiological testing of EBM identified bacterial growth in 75% of samples.[16] The relatively high rate of EBM contamination in the present study is attributed to the multiple gaps in the processes of milk expression, storage and transport. It is important to note that in spite of the fact that mothers were instructed about the technical and hygienic aspects of breast milk expression, the compliance rate was not satisfactory. This may be explained by maternal anxiety and stress related to the postpartum period. Also, NICU admission was reported as a risk factor for maternal stress and anxiety.[21] Another important factor that may contribute to the high rate of EBM is the relatively hot and humid weather in Egypt in most months of the year.

Of note, MRSA was the least prevalent isolated organism in our study group. Interestingly, Behari et al[22] found that MRSA can be passed from mother to preterm infant through contaminated breast milk, even in the absence of maternal infection.

In our study, improper hand washing was associated with EBM contamination. This is consistent with the conclusions of Steele,[23] who noted that hand washing with aseptic techniques is valuable in preservation of bacterial growth to acceptable levels.

Another factor related to EBM contamination is lack of appropriate breast hygiene. In support of these findings, Rodríguez[24] suggested that maternal breast skin may be the source of breast milk contamination.

EBM contamination may be related to other storage and transport factors. In our work, boiling the containers was significantly associated with less milk contamination. In accordance with these findings, Eglash et al[25] recommended boiling infants' feeding items, especially for infants in NICU. Also, we found that bacterial growth was significantly associated with refrigeration of expressed milk after more than 4 hours. Likewise, Ukegbu et al[26] found that bacterial load was higher in breast milk samples stored at room temperature for up to 9 hours compared to those immediately refrigerated.

In addition, we found that bacterial growth was significantly associated with mixing the freshly expressed warm breast milk with refrigerated one expressed earlier in the same day. Actually, many studies have indicated that fresh milk should be cooled before adding to cold milk in the fridge and that stored milk should be rotated using first-in-first-out (FIFO) principles with the oldest milk being used first.[23]

Moreover, we noted that the mean transportation time of the contaminated EBM was significantly longer than that of sterile milk. As other studies reported, with both time and varying temperatures, components in human milk are decreased in potency, while the growth of pathogens is increased.[25,26]

Findings of the present study may have significant implications. The study found a high prevalence of EBM contamination. This problem should be addressed by adoption of more strict measures to control transmission of infection. In addition, the study raises concern about the possible hazards related to use of EBM. Probably, there is a need for clear and evidence-based recommendations to guide the care-givers' decisions on use of EBM particularly in vulnerable babies like those admitted to ICU.

The current study reported the Egyptian experience with EBM contamination in the ICU setting and tried to document the relevant risk factors in detail. However, the study is not without limitations. It was conducted at a single center, which limits the generalizability of its findings. Moreover, the study did not assess the relation between EBM contamination and neonatal outcome.

Conclusion

Conclusively, the present study identified bacterial contamination in about 90% of EBM samples delivered to NICU infants. Factors related to EBM contamination include hygienic, storage and transport factors.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

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