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The Effect of Educational Intervention on Decreasing Mothers' Expressed Breast Milk Bacterial Contamination Whose Infants are Admitted to Neonatal Intensive Care Unit

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ABSTRACT

Background: Various reasons accounted for the infection of infants kept at Neonatal Intensive Care Unit (NICU). Expressed breast milk may be regarded as a source of infection in infants. This study investigated the source of bacterial contamination and the impact of educational interventions on the contamination of mothers' expressed breast milk (EBM) level whose infants are hospitalized at NICU.

Methods: Fifteen mothers used to express breast milking their infants admitted at NICU, involved in this study was conducted during October 2011-March 2012. Samples taken from hands, breast, pumps, breast milk, and milk storage containers and therefore 244 samples were prepared by sterile cotton swab and cultured on Blood Agar and EBM. After presenting enough training to mothers, cultures of the same positions were carried out again. Only those samples proved infected that number of their bacterial colonies exceeded 104 cfu / ml or even there was a growth of pathogenic organism.

Results: Before intervention 80% of mothers had infected by at least one sample that reduced to 36% after the intervention. Before intervention 25.4% of samples were contaminated; however after intervention, it reduced to 8.2%. The main source of contamination was milk containers and pumps; moreover, *Pseudomonas*, *E-coli*, and *Klebsiella* were among the most common bacteria of samples' contamination.

Conclusion: The possibility of EBM contamination was relatively high but educational interventions might reduce the risk of prevalence.

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Introduction

Breast milk is the best nutrition for growing infants since it has the capability to meet all their nutritional needs. Moreover this physiological liquid owns great advantages for infants' health and growth of term and preterm infants[1]. Breast milk is related to lower manifestation of Necrotizing Enterocolitis (NEC) and diarrhea within infancy and also lower manifestation of inflammatory bowel disease (IBD), type 2 diabetes and obesity in adulthood period[2].

Regarding nutrition of premature infants with breast milk there are noticeable advantages reported about infant's immunity system, development of neuro-sensory and digestive system; moreover, many reports revealed that it reduces morbidity related to premature infants' infection of those enjoying breast milk. Some studies also showed positive effects of breast milk comparing to formula with respect to evolution of neuromotor, intelligent quotient and strength of vision' development. In addition, in breast milk infants, amount of re-hospitalization after being discharge is lower [3,4]. Evidences show that exclusive breast milk within early 4 months would protect infants against atopic dermatitis and wheezing of early infancy [5]. Breast milk comparing formula may lessen NEC outbreak 7 times. The protection effect of breast milk is

related to its inflammatory components (like cytokines and growth factors) as well as prebiotics and probiotics that play a role in adjusting intestinal microflora. Studies confirmed that breast feeding both lessen NEC prevalence and its extent too; moreover, freezing and warming of mothers' expressed breast milk would not destroy its protective effect [6].

Breast milk is not sterile and may act as a mean to transfer commensal and pathogen microorganisms that taken from mother or Neonatal Intensive Care Unit (NICU) environment. Human milk may potentially transfer infectious agents that usually cause late onset sepsis especially in pre-term and very low birth weight (VLBW) infants. Transferring of some pathogens like *Staphylococcus aureus*, group B *Streptococcus*, *Escherichia Coli*, *Pseudomonas*, *Klebsiella*, *Serratia*, *Salmonella* and *Cytomegalovirus* is reported through mother's milk. Amongst one of the most worrisome is HIV.

Today, necessary hygiene measures are suggested during collecting, transferring, preservation and feeding of mother's milk at NICU to assure that breast milk is biologically safe and perfect in respect to nutritional and immunological features[7].

It was previously supposed that breast milk causing resistance against any bacteria existed in the milks; hence screening of EBM was obsolete. However, some reports indicate appearance of infant's sepsis and NEC in this regard. This completely challenged the belief that EBM even with pathogens are thoroughly harmless [8]. Because of various reasons, infants kept at NICU may suffer from infection; yet, considering that almost most of them are premature and their immune system is not evolved completely, outbreak of infection may cause mortality.

Considering with the susceptibility of infants admitted in NICU to serious infections and also possible morbidity and mortality this study was done to investigate outbreak of EBM bacterial contamination among mothers whose infants hospitalized at NICU. In addition we tried to find the sources of contamination and define the effect of educational intervention with respect to contamination reduction.

Methods

After confirmation of university institutional Ethics Committee (certification number: 27106), this Quasi-experimental study, was done on 50 mothers used EBM to feed their infants admitted at NICU of Shahid Sadoughi Hospital, Yazd, Iran during May-December 2010. Samples were prepared from a mother, extracted in a laboratory by assistance of technician from hands, breast, pump, milk container, and also breast milk. Sample size estimation was based on α=0.05 and ß error=0.8 and based on previous studies S=2. To get the sample, some mother milk's drops were expressed directly into sterile container and cultured using sterile swap; other samples were also prepared using sterile swap.

Samples were cultured in a blood agar and EMB medium and then incubated at 37°C for 24 h; next isolated colonies got identified and their type of bacterium and number of colony were determined. Samples accounted as contaminated only if numbers of bacteria colonies were more than 10^4 cfu/ml or even growth of a pathogen organism was observed.

Then, mothers were educated by the help of an intern about the hygiene of gathering, maintenance, and infants' nutrition procedure; next day cultures were again gathered from the same locations and mothers.

Educational topics were extracted in consultation with two health educators and two pediatricians and its validity was confirmed by two health care specialists, with its reliability determined by piloting on a group of 15 participants (Alpha=0.720). These mothers were not included in main study. Then after being summing up by the intern released to mothers in a face-to-face manner. In learning procedures emphasis was being put over the following issues: the necessity of hand washing with water and soap before each EBM feeding, daily showering if possible otherwise breast washing with clean water, and remember not using soap or any other disinfectant solution; moreover embarking on labeling milk gathering containers and inserting infant's name over; not applying joint express milking instruments, joint milk gathering containers, and joint handy or electrical pumps. Mothers were educated to use pre-boiled wide-mouthed glass or container when express breast milking with hand and use blunt–wall like container e.g. plastic dishes for milk preservation. Moreover, required instructions were learnt about the quality of EBM preservation, manner of dish washing and its accessories.

After gathering and performing necessary controls, data feed to SPSS 17 statistical software and analyzed using frequency, percentage, and Fisher exact statistical test. Hence, level of confidence defined as 95% for reporting the study' results and *P* value <0.05 was observed statistically significant.

Results

Totally 50 women were included in the study. The mean age of cases was 24.3 ±4.52 years, ranging between 16 and 38 years. Thirty one women (62%) were primigravids and 19 (38%) of them were multigravids. About 16% of them had academic degrees, 46% were graduates from high school, and 38% had primary or guidance school education. Before intervention 62 samples (25.4%) were contaminated; however, after intervention this fact reduced to 20 samples (8.2%) (Table 1) (*P*<0.001).

Table 1: Distribution of EBM bacterial contamination before and after intervention (*P*<0.001) (Fisher Exact Test)

	Before intervention		After intervention	
Sample	**Number**	**Percent**	**Number**	**Percent**
Contaminated	62	25.4	20	8.2
Non-contaminated	182	74.6	224	91.8
Total	244	100.0	244	100.0

The most bacterial contamination, before intervention, was related to samples derived from collecting milk containers (70%); this reduced to 26% after intervention. Moreover, samples derived from collection pumps showed that before intervention 20 samples (45.5%) were contaminated that reduced to 7 samples (15.9%) after intervention. In addition, with respect to other cases, intervention procedure caused cases of bacterial contamination reduced. Before intervention, the lowest amount of contamination related to samples derived from mothers' milk, i.e. only two cases of samples had bacterial contamination; however, no contamination observed in this regard after intervention. Moreover, interventions were capable of reducing contamination outbreak in all the cases (Table 2).

Table 2: Distribution of EBM bacterial contamination before and after intervention based on the sampling source (Fisher Exact Test)

Sample		Before intervention		After intervention		*P* value
		Number	Percent	Number	Percent	
Mothers' hand	Contaminated	3	6.0	0	0.0	0.490
	Non-contaminated	47	94.0	50	100.0	
Mothers' breast	Contaminated	4	8.0	0	0.0	0.170
	Non-contaminated	46	92.0	50	100.0	
Milk container	Contaminated	35	70.0	13	26.0	<0.001
	Non-contaminated	15	30.0	37	74.0	
Milk pump	Contaminated	20	45.5	7	15.9	0.005
	Non-contaminated	24	54.5	37	84.1	
Sum	Contaminated	62	25.4	20	8.2	<0.001
	Non-contaminated	182	74.6	224	91.8	
Total		224	100.0	244	100.0	

Regarding type of organisms separated before intervention, *Pseudomonas* and *E. coli* in 24 samples (9.8%), *Pseudomonas* in 18 cases (7.4%) and *E. coli* in 16 cases (6.5%) were cultured in mediums. After intervention following contaminations reported: 12 samples (4.9%) *Pseudomonas*, 6 samples (2.5%) *E. coli*, and 2 samples (0.8%) mixture of *Pseudomonas* and *E. coli* (Table 3).

Table 3: Comparison of different bacterial growth before and after the intervention based on sampling source (descriptive analysis); in samples of gram positive cocci there was no sample with bacteria colonies more than 10^4 cfu/ml

Type of microorganism	Hand N (%)		Breast N (%)		Pump N (%)		Milk N (%)		Container N (%)		Total N (%)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Pathogen												
Pseudomonas	1 (2.0)	-	4 (8.0)	-	6 (13.6)	4 (9.1)	-	-	7 (14.0)	8 (16.0)	18 (7.4)	12 (4.9)
E. coli	1 (2.0)	-	-	-	7 (15.9)	3 (6.8)	1 (2.0)	-	7 (14.0)	7 (6.0)	16 (6.5)	6 (2.5)
Klebsiella	-	-	-	-	3 (6.8)	-	-	-	1 (2)	-	4 (6.1)	-
Mixed (*Pseudomonas* &*E coli*)	-	-	-	-	4 (9.1)	-	-	-	20 (40)	2 (4)	24 (9.8)	2 (0.8)
Non- pathogen												
Gram positive cocci	29 (58.0)	23 (46.0)	33 (66.0)	23 (46.0)	8 (18.2)	6 (13.6)	17 (34.0)	11 (22.0)	7 (14.0)	23 (46.0)	94 (38.5)	86 (35.2)
Yeast	4 (8.0)	3 (6.0)	10 (20.0)	12 (24.0)	3 (6.8)	5 (11.4)	12 (24.0)	9 (18.0)	5 (10.0)	8 (16.0)	34 (13.9)	37 (15.2)
Negative culture	15 (30.0)	24 (48.0)	3 (6.0)	15 (30.0)	13 (29.5)	26 (59.1)	20 (40.0)	30 (60.0)	3 (6.0)	6 (12.0)	54 (22.1)	101 (41.4)
Total	50 (100)	50 (100)	50 (100)	50 (100)	44 (100)	44 (100)	50 (100)	50 (100)	50 (100)	50 (100)	244 (100)	244 (100)

In samples of gram positive cocci there was no sample with bacteria colonies more than 10^4 cfu/ml.

With respect to relation of samples contamination with the age of mother, before intervention, the highest percent of contamination (83.3%) was related to more than 35 year old group and the lowest one (27.7%) to less than 25 year old group (*P* =0.78). After intervention the highest percentage of samples contamination belonged to more than 35 year old group (at least one contaminated sample in 50% of mothers) and the lowest percentage of contamination belonged to 25- 35 year old group (at least one contaminated sample in 33.3% of mothers) (*P*=0.73).

Before intervention, the highest percentage of bacterial contamination belonged to under-diploma group (at least one contaminated sample in 84.6% of mothers) and the lowest contamination to above-diploma group (at least one contaminated sample in 66.7% of mothers) (*P* =0.51). After intervention the highest percentage of samples contamination belonged to under-diploma group (at least one contaminated sample in 46.2% of mothers) and

the lowest contamination belonged to above-diploma group (at least one contaminated sample in 11.1% of mothers) (P =0.16).

Discussion

Todays it is proved that breast milk has many advantages for infants; however, it may be accompanied by some problems like the possibility of milk contamination by different bacteria occurring during EBM feeding.

The results of present study showed that before intervention 80% of mothers had infected by at least one sample that reduced to 36% after the intervention. Before intervention 25.4% of samples were contaminated; however after intervention, it reduced to 8.2% (P < 0.001). The main source of contamination was milk containers and pumps; moreover, *Pseudomonas*, *E-coli*, and *Klebsiella* were among the most common bacteria of samples' contamination. The results of various studies report contamination outbreak differently, even up to 97% (9). Moreover, after intervention *Pseudomonas* (4.9%) and *E.coli* (2.5%) were also considered as the most common cause of contamination.

Frequency of microorganism that cause EBM contamination in different hospitals of various countries is not the same; however, most of them reported that following bacteria are considered as the main factors for EBM contamination: *S. epidermidis, S. aurous, Enterobacter,* group B strep., *E. coli* and *Klebsiella* [9-13].

EBM contamination outbreak was 85% and the most common bacteria that cause contamination were *Klebsiella* (13.7%), *S. epidermidis* (12.5%), *Enterobacter* (11.2%), *E. coli* (7.5%) and *P. aeruginosa* (6.2%) [14]. By evaluation of 207 milk samples pertaining to 70 hospitalized mothers, contamination outbreak was 97% and it mostly related to negative staph. coagulase and *St. viridans* (82%), *Enterobacter* (7%), and *S. aurous* (6%) [9]. The results of El-mohandes et al. showed that contamination outbreak was 87.5%; which *S. epidermidis* and *Acinetobacter* had highest rate respectively [10].

In present study *Pseudomonas* and *E. coli* had the highest rate of outbreak that is contrary to the most studies results. The reason for this difference may be stemmed from this fact that in other studies only contamination of expressed milk breast samples were studied; however, in our study beside milk sample, hand and mother breast, as well as pump and milk container contamination are also covered. This helped us to have a broader picture on the possible contamination that could occur during process of milking to its consumption by infant. Moreover, in our study the highest rank of contamination belongs to milk containers and pumps that regarding the type of separated microorganism form culture mediums like *Pseudomonas*; this may be an indicator of hospital acquired contamination.

The quality of sampling and transfer of samples to laboratory is a problem in many studies, in such a manner that possibility of contamination is high during transportation. To prevent this case in our study, mothers were guided directly to the laboratory and their milk sample was directly transferred into medium in order to prevent secondary contaminations. Moreover, samples of mothers' hand and breast were taken without using transferring containers. Different types of sampling method in our study with others may be the reason of some differences about bacteria causes of contamination. As in present study we tried to prevent possible confounding bias that could make over or under estimation of bacterial contaminations.

Before intervention, 4% of hand samples, 8% of breast samples, and 2% of EBM samples were contaminated; however, after intervention none of them were contaminated (Table 3). This shows that mothers' hand, breast, and milk have little role in the bacterial contamination of EBM and the most important role in this regard may be due to containers and pumps; hence before intervention, samples gathered from pumps and containers showed contamination of 45.5% and 70% respectively. This case, after intervention, reduced meaningfully to 15.9% and 26% respectively. Pumps and containers in environment of hospital are more vulnerable than other sources because of their nature as process of their washing is an important issue that can effect on the extent of their contamination. The results show that educational interventions, although not enough, play effective role in reduction of pumps and containers' contamination. Of course even after intervention the amount of contamination was still considerable and this shows more needed interventions; for example in this study we did not focused on sterilization procedures or bloating containers or pumps in hypocolorite solutions. Possibly this procedure can help to decrease the contamination more.

Our results showed that there was no significant relation between the amount of bacterial contamination of EBM and mothers' age and education, in accordance with results of Karimi and Rozolen's study. They found that there was no significant relation between mothers' demographic characters including age and education at one side and amount of EBM bacterial contamination at the other side [12,14]. Schanler et al. also found that there was no relation between microbial colony count of EBM and the age, race, education of mother, mother–infant skin contact and neonatal sepsis; their results are also similar with our outcomes [15].

Li Ma et al. regarding evaluation of cleaning and disinfecting protocol efficiency, concluded that washing with water and soap and next with water alone is the most effective method of cleaning and reduced pathogen load between 3.7 to 3.1 times. Immersing in hypochlorite solution for 30 minutes also cause reduction of 3.7 times of pathogens load. This results show that intervention carry-

ing out in our study is also comparable to the above mentioned case [16].

We did not have any other intervention except education like boiling milk containers or bloating them in hypocholorite solution. Future study should focus on the effects of such interventions and their possible additive effects to mothers` education to decrease contamination of milk. Of course it is possible that some mothers did not have enough collaboration. We tried to decrease the bias of this problem by strict education and checking the mothers by remembering them and checking with themselves that what they did. However in some cases this problem was inevitable and this was a shortage in our study.

Conclusion

The bacterial contamination of EBM in this hospital is high and it may be reduced by carrying out educational intervention. Moreover, the main source of contamination is milk containers and collecting pumps; yet role of mothers' hand, breast, and EBM in this regard is low. *Pseudomonas, E. coli*, and *Klebsiella* are among the most bacteria affecting EBM contamination and this issue probably shows a high rate of hospital acquired contamination outbreak in this hospitals.

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Conflict of interest statement

Authors had no conflict of interest to declare.

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