

## Bacterial Contamination of Expressed Breast Milk in Neonatal Intensive Care Unit

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Article information	Abstract
<p>Article history: Received: 24 Nov 2011 Accepted: 25 Dec 2011 Available online: 5 Nov 2012 ZJRMS 2013; 15(4): 48-52</p> <p>Keywords: Bacterial contamination breast milk NICU</p> <p>*Corresponding author at: Department of Pediatrics, Shahid Sadoughi University of Medical Sciences, Yazd,Iran. E-mail: drmehrankarimi@yahoo.com</p>	<p><b>Background:</b> The milks expressed from the mothers' breast might be infected during squeeze, storage and/or transmission. The infection level has been reported as different in various studies up to 97 percent. The main purpose of this study is to determine the infection level and its relevant organisms as well as to specify drug allergy of the expressed milks from the mothers with their infant admitted to NICU ward.</p> <p><b>Materials and Methods:</b> In this study, among the expressed milks from 80 mothers, were cultured each in an amount of 0.5-1cc and antibiotic discs selected for every strain was placed.</p> <p><b>Results:</b> The results indicate that 85 percent of samples were infected and dominant microorganisms were firstly <i>Klebsiella</i> (13.7%) and then <i>S. epidermidis</i> (12.5%). In addition, 95% of Gram negative bacteria strains were susceptible to imipenem. The most effective antibiotic on isolated staphylococci was ceftizoxime (46.6% resistance). The colony count in 32.4% gram negative bacteria and in 66.7% gram positive bacteria was between <math>10^4</math> to <math>10^5</math> CFU/ml and the remaining was above <math>10^5</math> CFU/ml (<math>p=0.02</math>). Furthermore, there was no significant relationship between bacterial infection of the expressed milks with the site of milk expressing (house or hospital), mode of expressing (by pump or hand), storage duration and the mother's demographic characteristics including age and/or literacy.</p> <p><b>Conclusion:</b> The studies show that infection prevalence in the milk samples was 85%; the most common infection factor was <i>Klebsiella</i> and then <i>S. epidermidis</i> that is indicative of high prevalence of hospital infection (nosocomial infection) in the infants ward.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

World Health Organization (WHO) and American Academy of Pediatrics (AAP) recommend strongly breastfeeding compared to all other types of milk [1]. Using breastfeeding for preterm and high-risk infants brings potential advantages [2]. Breastfeeding may play a protective role against otitis media, pneumonia, diarrhea, enterocolitis, necrotizing enterocolitis and sepsis [3].

Infants' risk of infection due to breast milk contamination is very low. Among the organisms which might be transmitted from mother to her infant, there are Human T Lymphotropic Virus Type I (HTLV Type 1) in endemic areas and HIV Type 1. However, pasteurization (in 62.5°C for half an hour) will not reduce such microbial contaminations [4]. Since the breast milk is not sterile, in case it is expressed and stored inappropriately, there will be the possibility of microorganisms to affect. Strict compliance with health issues at the time of expressing, transmission, storage and breastfeeding is of great importance and will prevent exogenous contamination. Contrary to dried milk, there is still no legal standard on

how to make use of the expressed breast milk [5]. Prematurity of infants and the related clinical conditions prevent the infant to suck effectively the mother's breast. Therefore, it is required to squeeze mother's breast milk and store it in the infants' ward that might cause the expressed milk to be infected in such path [6]. Two main factors in the infants' infection are hospital environments and the mothers themselves.

Neonatal infections which are caused in hospital as acquisitive are called nosocomial infections. Most of the infections are early onset type which is transmitted during delivery. The infections which usually display their symptoms from the first 48-72 hours after delivery are considered as nosocomial ones. However, there are some exceptions including late onset infections with organisms acquired from mother's genital organ such some *streptococcus* infections of group B and nosocomial infections acquired from delivery room where the related symptoms are appeared immediately after birth [8].

Any kind of pathogen may colonize infants, personnel or family in NICU and may be transmitted to the infant by

direct or indirect contact through infected materials and devices including intravenous fluids, medicines, disinfectants, respiratory equipment and etc. Meanwhile, *staphylococcus epidermidis* may play an important role in developing Nosocomial infections in NICUs [9] the present study was carried out in Yazd city.

### Materials and Methods

This study is a descriptive and cross-sectional investigation and the population under study consisted of 80 mothers whose infants were admitted to NIUC ward at Shahid Sadooghi Hospital. Demographic data including mothers' age and literacy was collected. The sample size was calculated as 80 persons selected by simple random sampling.

NICU nurses have taken an amount of 0.5-1cc (in excess of quantity required by infant) from the expressed milk which is stored in this ward at a time before the infant's feeding and have sent it to microbiology laboratory within 20 minutes. The requirement of the samples for entry into the study was included cases whose infants were kept in NICU ward.

The sample sent to microbiology lab was fully cultured using a loop with a volume of 0.001 ml on an EMB blood agar environment and then was incubated inside the incubator with a 37°C temperature for 24 hours. In case the bacteria was grown, the quantity of isolated bacteria was counted and were identified through Gram staining and conducting common biochemical tests including catalase, coagulase and oxidase differential tests. To measure antibiotic susceptibility, disc diffusion standard method and preparation of bacterial suspension with turbidity equivalent to half McFarland turbidity tube in Muller Hinton agar were applied. The samples were incubated for 18-24 hours in an incubator of 37°C, then inhibition zones around antibiotics were determined and compared with the standard table in terms of being susceptible, resistant and intermediate.

Milk samples was considered as infected in which mesophilic bacteria colony counts were higher than  $10^4$  CFU/ml or they appeared to be grown by pathogens such as *Shigella*, *Klebsiella*, *haemolyticus* group B, *Streptococcus*, *Staphylococcus aureus* and *pseudomonas species* in the medium.

Necessary data so collected were fed in computer and then were analyzed after codification using SPSS-17 statistical software and applying adequate statistical tests. In this study, descriptive statistical methods and  $\chi^2$  (chi-square tests), *t*-test were utilized. Additionally, to report the results of the study, the confidence level was considered as 95%.

### Results

The results showed that among total 80 samples of the study, 68 ones (85%) were infected and other 12 samples (15%) were uninfected. The frequency of the bacteria isolated from the milk samples included in terms of the bacteria type respectively as *Klebsiella* (13.7%), *S. epidermidis* (12.5%), *Enterobacter* (11.2%), *Escherichia*

*coli* (7.5%), *Pseudomonas aeruginosa* (6.2%), *Acinetobacter* (5%), *S. saprophyticus* (3.7%) and *Citrobacter* and *S. aureus* (each 2.5%), and 16 samples had more than one type of microorganism. Among the aforesaid 16 samples from which more than one type of microorganism was isolated, 14 cases (17.5%) included two types of microorganism and the other 2 cases (2.5%) had three types (Table. 1).

In determination of drug resistance of Gram negative bacteria isolated from the contaminated milks, we found that 95% of Gram negative bacteria strains were susceptible to imipenem. 71.5% of Gram negative bacteria strains were resistant to cephalosporins and 62.1% of them to aminoglycosides. The drug resistance of Gram negative bacteria isolated from the infected milks showed that the most effective antibiotic on isolated staphylococci was ceftizoxime so that 35% of staphylococcus strains were susceptible to ceftizoxime. The *S. epidermidis* and *S. aureus* were resistant to *vancomycin* respectively as 90% and 50%.

In respect of total colony count per sample, among 68 contaminated samples, one type of organism has been infection factor in 52 samples including 22 cases (42.3%) having  $10^4$  to  $10^5$  CFU/ml and the remaining 30 cases (57.7%) having more than  $10^5$  CFU/ml colonies. Among the samples with two or more types of bacteria, 14 cases had two types of bacteria including one at least as pathogen; the quantity of these 14 samples was greater than  $10^4$  and the remaining was above  $10^5$  CFU/ml. Also, two milk samples had 3 types of bacteria including one at least pathogen and the colony count of these two samples is above  $10^5$  CFU/ml. As Gram positive and Gram negative bacteria were separated, in 32.4% of cases of Gram negative bacteria, the colony count was  $10^4$  to  $10^5$  and in 66.7% of cases of Gram positive bacteria, there were  $10^4$ - $10^5$  colonies ( $p=0.02$ ). In respect of milk storage duration, it was determined that the samples kept in refrigerator for one hour or less were 82.3% infected, the samples kept in refrigerator for 1-3 hours were 90.9% infected and about the cases kept more than 3 hours in refrigerator, there was 100% infection ( $p=0.3$ ) (Table 2).

The results of this study showed that there is no significant relationship between the infection prevalence of the expressed milks from the mothers and some variables including mother's literacy, milk expressed methods (by pump or hand), place of milk collection (hospital and/or house).

**Table 1.** Frequency of the bacteria isolated from the expressed milks in terms of microorganism type

Type of organisms	Frequency (%)
S. Saprophyticus	3(3.7%)
S. Epidermidis	10(12.5%)
Citrobacter	2(2.5%)
Acinetobacter	4(5%)
S. Aureus	2(2.5%)
Enterobacter	9(11.2%)
Klebsiella	11(13.7%)
Escherichia coli	6(7.5%)
Pseudomonas aeruginosa	5(6.2%)
Two organism	14(17.5%)
Three organism	2(2.5%)
Negative growth	12(15%)
Total	80(100%)

**Table 2.** Frequency of bacterial infection in the squeezed milks in terms of the milk storage duration

Milk storage duration	≤ 1 hour Frequency (%)	1 to 3 hour Frequency (%)	≥ 3 hour Frequency (%)
Contaminated	51(82.3)	10(90.9)	7(100)
Non contaminated	11(17.7%)	1(9.1)	0
Total	62(100)	11(100)	7(100)

## Discussion

The findings achieved in this study showed that 85% of the milk samples related to the milks expressed from the mothers whose infants are admitted to NICU ward have been contaminated (the colony count is above  $10^4$  CFU/ml). In our investigation, the type of microorganism included respectively *Klebsiella* (13.7%), *S. epidermidis* (12.5%) and *Enterobacter* (11.2%).

Though in some studies like the one conducted by NG et al. those milks are considered as contaminated in which pathogen bacteria would have been grown or the colony count would be greater than  $10^4$  CFU/ml, in most of investigations, the colony count higher than  $10^4$  has been treated as contamination like what we have done in our study. The results of different studies have indicated such prevalence even up to 97percent [13].

Rozolen et al. have concluded evaluating 90 milk samples of 32 mothers that the uttermost microorganisms isolated from contaminated samples consisted of *Klebsiella* and coagulase negative *Staphylococcus* [14] which is consistent with the results of our investigation. Carroll et al. showed in their evaluation of 207 milk samples expressed from 70 mothers admitted to hospital that contamination prevalence was 97% most of which was related to coagulase negative *staphylococcus* and *Streptococcus viridans* (82%), *Enterobacter* (7%) and *Staphylococcus aureus* (6%) [13]. The results of an investigation carried out by el-Mohandes et al. on 108 milk samples from 40 mothers, showed an contamination prevalence of 87.5% in which the most infection factors consisted respectively of organisms including *S. epidermidis* (82%) and *Acinetobacter* (9%) [12]. Assessment the works carried out in this field indicates that frequency distribution of microorganisms as contamination factor vary in the hospitals of different countries; however, in most of studies, some bacteria including *S. epidermidis*, *S. aureus*, *Enterobacter*, Group B *Streptococcus*, *Escherichia coli* and *Klebsiella* [2, 3, 13-15].

In our study, among 11 milk samples with *klebsiella* as contamination factor, no cases of resistance to imipenem antibiotic disc has been reported and there was just three cases of resistance to amikacin. Meanwhile, in respect of Gram negative bacteria as an infection factor of expressed milks, the least drug resistance was related to imipenem antibiotic disc in a way that just 5.4% of the *Enterobacter* and *Escherichia coli* strains were resistant to imipenem. Though in-vivo results are different from in-vivo ones, it may be argued that in case the neonatal sepsis due to the milk contamination by these factors, imipenem can be used as an appropriate drug to treat such infants.

In our study on the positive Gram bacteria, the uttermost drug resistance was related to cefixime antibiotic disc (93.3%). Though resistances to ampicillin and gentamycin antibiotic discs exist in a high percentage of isolated Gram negative bacteria species (resistance to ampicillin: 83.7%, resistance to gentamicin: 54% and averagely 68.8%), following up the condition of the infants hospitalized in NICU showed that no death has been reported as an effect of neonatal infection in cases where the milk culture was positive. This issue might be due to prescription of a broad spectrum of antibiotics at the beginning of the infants' admittance to the NICU.

The results of our study show that among 52 samples of mothers' milk, just one type of microorganism was grown; 22 samples (42.3%) had colony counts higher than  $10^4$  and lower than  $10^5$  CFU/ml and other 30 samples (57.7%) had colony count higher than  $10^5$  CFU/ml that is suggesting to severe contamination in the samples under our study. In the investigation conducted by el-Mohandes et al. 38% of contaminated samples had more than 30,000 CFU/ml in which the highest infection was related to *S. epidermidis* [2].

Vervoort et al. showed in their examination on 584 milk samples expressed from 176 mothers whose infants were hospitalized in NICU that 7% of the samples had pathogen organisms and 46% of them had more than  $10^4$  coagulase negative *Staphylococci*. One of the factors that may cause milk contamination is to use pump (by hand or electric) or expressing the breast by hand [6]. The results of our investigation indicate that 84.2% of the samples squeezed by hand and 86.9% of the samples squeezed by pump have been infected showing no significant difference. Pittard et al. argued that there has been no difference between the levels of contamination in the milks squeezed by two mechanical and handy ways [11].

The investigation performed by Tyson et al. suggest that contamination developed by coliforms and Gram negative bacilli which are resistant to gentamicin in the cases where the milk is squeezed by pump at home have been more than those where squeezed by hand [16].

Furthermore, Boo et al. indicated in their study that the breast milk is contaminated when it is squeezed by pump at home compared to the time when it is squeezed by hand. They showed that there will be no distinct difference between the contamination from pump or hand when the milk is squeezed in the hospital [17].

In general, using electric pumps for expressing the milk is more preferable to handy pumps and/or squeezing by hand [1]. The place where mother squeezes her breast may be effective. In our study, 94.7% of contaminated cases are those where the milk sampling was made at home and 81.9% in the hospital. A research conducted by Rozolen et al. indicated that the milk squeezed at home, may be inappropriate for feeding the infants hospitalized in Brazil's NICUs, if it is not pasteurized [14]. The investigation performed by Boo et al. showed that home and hospital are effective on the milk contamination where it is squeezed by handy or mechanical methods [17]. Meanwhile, Davidson et al. argued in their investigation that the milks expressed at home had a

higher infection compared to those squeezed at the hospital [18].

Duration of the milk storage may be effective on its risk to be contaminated; as the results of our study shows, all the samples stored more than 3 hours are contaminated while the level of such contamination in those samples stored less than one hour is 82.3 percent. An investigation carried out by Igumbor suggest that storage duration should not be more than 24 hours for the squeezed breast milks which are stored at refrigerator temperature of 4-10°C, 8 hours for those stored at room temperature of 15-27°C and 4 hours for those stored at 30-38°C temperature. A research conducted by Olowe et al. showed that there is no significant difference between the rate of bacteria in milks within 24 hours after storage in refrigerator with the primary time of storage in refrigerator and they argued that if the milk is prevented to be severely infected at the time of squeeze, it can be stored in refrigerator for 24 hours and be used for infants feeding with a good safety [15]. According to a study performed by Moulin et al. the milk squeezed from the mother's breast may be used for feeding infants up to 9 hours at room temperature. In addition, Ajusi et al. showed in their investigation that the milk squeezed from mothers' breast can be stored for 8 hours at room temperature for infants' consumption [17].

In comparison, there has been no accordance between the aforesaid studies and ours; because in our investigation, all the samples stored more than 3 hours were infected and this issue is contrary to the results of such similar studies. In an investigation carried out by Rozolen et al. there were no relationship between the infection of the expressed milks and demographic characteristics of mothers including their age [14]. The results achieved by his study is consistent with our investigation so that in age group 18 years or less 75% of samples, in age group 18-35 years 84.8% and in age group above 35, 90% of the samples were infected.

The present study indicated that 86.7% of the milk samples were squeezed from illiterate mothers, 90% of the samples were taken from the mothers had primary to diploma education and 78.8% of them from the mothers with a diploma education or higher. In this respect, there was no significant relationship between the milk contamination and the level of mother's literacy.

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Rozolen et al. showed in their study that demographic characteristics and socio-economic situation of mothers was not relevant to the contamination of the milks squeezed from their breast [14]; the results of this study are consistent with what we concluded in our investigation.

Among the limitations of this study, we may suggest to ones occurred in preparation of a variety of necessary antibiotic discs for Gram negative and Gram positive bacteria during the project implementation. Meanwhile, somewhere in the study, some inadequate discs had been used for Gram positive and Gram negative bacteria that was modified and reevaluated. Comparing the results of our study with those of other investigations, it is found that contamination prevalence in the milks squeezed from mothers' breast was relatively high, but in terms of the organism type acquired from the culture medium, our results is approximately consistent with those of most studies.

Our investigation indicates that contamination prevalence in the milk samples was 85% and the most common contamination factor of the milks squeezed from mother's breast in NICU was *Klebsiella* and then *S. epidermidis*; this issue suggest to high possible prevalence of nosocomial infection in NICU.

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

No conflict.

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