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BACTERIOLOGICAL PROFILE OF EXPRESSED BREAST MILK AT THE NEWBORN UNIT OF MOI TEACHING AND REFERRAL HOSPITAL, ELDORET KENYA
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ABSTRACT

Background: **Expressed breast milk (EBM) is defined as milk removed from a mother's breast without an infant's mouth at her nipple. Mothers breast milk is not sterile and can be a vehicle for commensal and pathogenic microorganisms derived from the mother or the newborn unit environment leading to neonatal sepsis, a leading cause of neonatal mortality. Knowledge of the contaminants is of importance in trying to establish enhanced safety of the breast milk.**
Objectives: **The aim of this study was to ascertain the prevalence and common bacterial contaminants of EBM.**
Design: **A descriptive cross sectional**
Setting: **Newborn Unit of Moi Teaching and Referral Hospital (MTRH), Eldoret, Kenya.**
Participants: **The study randomly sampled 170 mothers with babies admitted at the Newborn Unit of MTRH, expressing breast milk for the purpose of feeding their newborns**
Interventions: **Collected breast milk samples were subjected to laboratory analysis, including culture, microbiology and biochemical tests.**
Outcome measures: **contamination was defined as the presence of any type of bacteria while acceptable contamination was defined as Colony Forming Units of less than $1.0x10^4$/ml.**
Results: **78.2% (n=133) of the EBM samples had bacterial contamination. The bacterial contaminants isolated include *Staphylococcus epidermidis* (54.9%), *Staphylococcus aureus* (42.1%), *Staphylococcus saprophyticus* (2.3%), and *Enterococcus sp.* (0.8%). The CFU/ml on all the samples were less than $1.0x10^4$/ml and, therefore, within acceptable limits. Multivariate analysis of both neonatal**

and maternal factors revealed, there was no statistically significant association with bacterial contamination of EBM (p>0.05).

Conclusion: **A significant proportion of the EBM at the NBU of MTRH has bacterial contamination but within acceptable limits. There was no statistically significant association between contamination and factors considered in this study.**

INTRODUCTION

Among the many neonatal morbid conditions, the three major contributors to the global burden of disease are premature birth, birth asphyxia, and neonatal infections (Lawn et al., 2005; Harrison and Goodman, 2015). Preterm deliveries and neonatal sepsis are the leading contributors to neonatal mortality in Kenya (Kenya National Bureau of Statistics [KNBS], 2010); Transmission of infections to the newborn can occur variably via breastfeeding and use of EBM. Exclusive breastfeeding is defined as an infant receiving only breast milk from its mother, or a *wet nurse* or using expressed breast milk.

Infants not breastfed show a higher incidence of and severity of diarrheal illness and high susceptibility to neonatal sepsis compared to their counterparts who are breastfed. Exclusive breastfeeding for the first six months of life is a global and equally a Kenyan guideline for infant feeding. Thereafter, in addition to breastfeeding, complementary feeding is introduced, and mothers are encouraged to breastfeed for up to two years or more (UNICEF, 2007).

Expression of breast milk is a common practice in a newborn unit (NBU) or neonatal intensive care unit (NICU). Mothers usually express their milk for nutrition of their newborns who are unable to breastfeed due to prematurity, low birth weight, sickness and/or other related newborn condition like cleft lip/cleft palate (Micah, Alexa and Zachary, 2016; Ray, 2012). Expressing breast milk by hand in the first days after birth is encouraged to boost breastfeeding rates among these poorly feeding newborns.

The use of EBM has been noted to provide both nutritional and immunological benefits if it is stored within the appropriate temperature range (Ezz et al., 2004). However, in randomized studies of contamination of EBM following breast cleansing, breast milk was still found to be contaminated both by pathogenic and non-pathogenic bacteria, (Boateng, 2011; Ezz, 2004). In a study on bacterial growth in EBM, bacterial isolates represented normal skin flora. (Cossey 2011)

Infections are a major contributor to newborn deaths in developing countries. There are an estimated four million neonatal deaths around the world each year. A third of this is due to severe infections, and around one million is due to neonatal sepsis or pneumonia alone (Lawn et al., 2005; UNICEF 2007).

In an evaluation of the bacteriologic quality of breast milk in a neonatal service in Belgium, it was found that more than half of the milk samples contained either more than acceptable levels of staphylococcus (46%) or had pathogenic contamination (7%), (Vervoort et al., 2007). Evaluation of contamination studies conducted by Boo et al. (2001) and Karimi et al. (2012) showed significant bacterial contamination of breast milk obtained by manual expression and breast pumps. In a study on effect of

educational intervention in decreasing mothers expressed breast milk bacterial contamination, it established a bacterial contamination rate of 25.4% before intervention which reduced to 8.2% after the intervention *Pseudomonas, E. coli, and klebsiella sp.* were among the most common bacteria causing contamination in breast milk.(Karimi et al 2012) Empirical evidence shows that breast milk, may contribute to infection if it contains pathogenic organisms that may contribute to newborn sepsis (Youssef, 2002) and hence, increase neonatal morbidity and mortality (Ray et al., 2012).

NICUs provide an opportunity for newborns to improve their survival outcomes (Harrison and Goodman, 2015) during that critical period of life. Conversely, the majority of people have not recognized EBM as a source of neonatal sepsis in susceptible infants (Youssef, 2002). Hence, knowledge and prevention of neonatal infections offers the best opportunity for reducing the under-five mortality amicably (Jennifer and Pinelli, 2005).

Even though breast milk has been shown to improve neonatal survival, its contamination during expression, storage or feeding of newborns may contribute to newborn sepsis and therefore, increase neonatal morbidity and mortality.

Knowledge of the contaminants, if any, is particularly of importance in trying to establish proper safety of the breast milk. Besides, mothers who are unable to feed their babies effectively require support and information on how to express their breast milk safely and effectively in order to maintain their baby's well-being. It is also recognized that the handling, preparation and storage of EBM in a hospital environment may present potential health risks (Cossey, 2011) if it is not done safely.

It is not yet established what proportion of EBM has bacterial contamination at the NBU of MTRH, Eldoret, Kenya and hence the aim of this study.

MATERIALS AND METHODS

Setting: We adopted a descriptive cross sectional design so as to determine the frequency of contamination as well as the specific microbial contaminants at a particular point in time at the NBU. The study was conducted from April 2015 to December 2015. Mothers with sick neonates at the NBU are usually housed at the post-natal wards within the main hospital wing. They are then required to attend to their newborns every three hours for the purposes of breastfeeding and expression of breast milk for those unable to breastfeed.

Further, these mothers are required to observe basic hygiene practices like hand washing and use of clean containers in handling the EBM. All of these are done under the supervision and guidance of trained nurses and clinical nutritionists. At the time of conducting this study, storage of EBM was not practiced. Any EBM that remained after the neonates are fed was usually discarded.

***Participants Selection*:** The study enrolled participants who comprised of mothers with newborn babies admitted at the NBU of MTRH during the study period. These mothers were also expressing breast milk (EBM) for the purpose of feeding the newborns. Ethical approval from the institutional and ethics committee of MTRH was obtained before the start of this study.

Simple random sampling was adopted in selecting the participants from the study population. The participants were then subjected to the informed consent questionnaire and inclusion/exclusion criteria.

The inclusion criteria encompassed mothers practicing expression of breast milk at the NBU and consenting to the study. Exclusion criteria included mothers, not consenting to providing EBM and mothers with mastitis

Sample Size Determination: The sample size was mainly computed using the finite population correction method. The study anticipated the proportion to be larger than 5% (n/N > 0.05%), hence according to Daniel (1999), we use the finite population correction as follows;

$$n = \frac{NZ^2{}_{\alpha/2} * p(1-p)}{d^2(N-1) + Z^2_{\alpha/2}(p(1-p))}$$

Where;

$n =$ the anticipated sample size with finite population correction

$N =$ 312 (anecdotal data)

$Z_{\alpha/2} = 1.96$

$p =$ 0.588.We used the union of probabilities from objective one and two from this study at University of Nairobi. (Ajusi et al,1989)

$d = 5\%$

Calculating sample size yields the following figure:

$$n = \frac{(312 \times 1.96^2) \times (0.588 \times 0.412)}{(0.05^2 \times 312) + (1.96^2 \times 0.588 \times 0.412)},$$

$$n = \frac{290.363}{1.7082}$$

Hence, we had a sample size of $n \cong 170, subjects$

Sample Collection and Labeling: Participants expressed breast milk by manual method (use of clean hands), 6-8 milliliters (ml) of EBM into sterile containers, under the supervision of a trained research assistant. The samples were uniquely identified only by serial numbers.

Microbiological Analysis and Cultures: Collected samples were then transferred safely in a standard sample/specimen carrier to the MTRH microbiology laboratory where analysis of the type of bacteria and bacteria load was done at time zero (not later than an hour of collection). The processing, incubation and culture preparation was done in conjunction with the laboratory technologist conversant with microbiological analysis.

The EBM samples with their unique serial numbers were cultured at time zero after collection. The culture media used included chocolate agar, nutrient agar and MacConkey agar. Chocolate agar was for isolating fastidious organisms (Haiden et al., 2016); nutrient agar to isolate non-fastidious organisms (Haiden et al., 2016; Cossey et al., 2011); and mackonkey was used to differentiate between lactose and non-lactose fermenting organisms (Cossey et al., 2011)

The processed samples were then incubated for 24 hours at 37 degrees centigrade. Thereafter, the cultures were read for possible growth. After incubation, those with culture growth were examined microscopically after gram staining. Biochemical tests, including catalase and coagulase testing were then subjected to the sample for further microbial isolation.

Colony Forming Units (CFU) Determination: The CFU was determined by the spread-plate method count. EBM samples were spread over count medium and incubated for 24 hours at 37^0 C. The dilutions used were of 1:10, 1:100, and 1:1000 ratios, thus,

$$\text{CFU} = \frac{\text{Number of colonies x}}{\text{Test volume}} \text{ Dilution Factor}$$

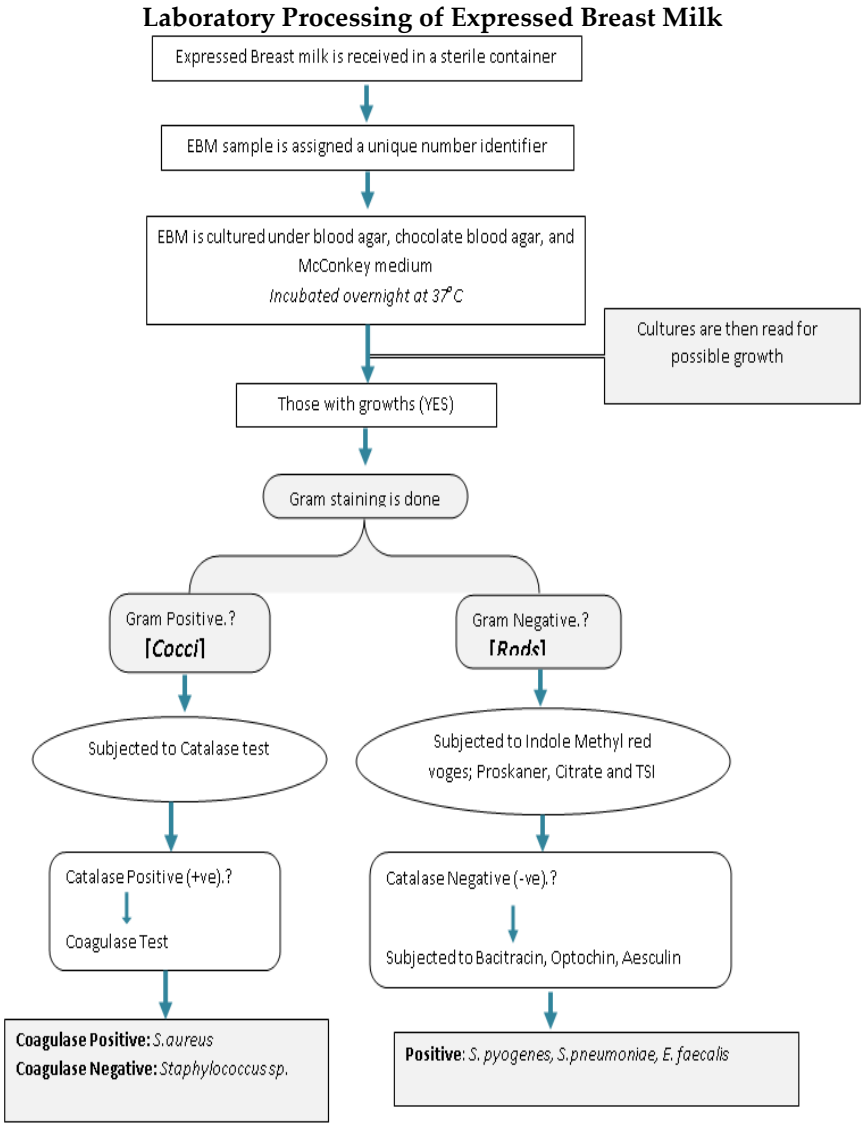

Figure 1: Laboratory testing of EBM

Data Management and Statistical Analysis: The hard copy data was translated into an SPSS (Vs. 21) database while ensuring accuracy and completeness. Socio-demographic and clinical profiles of the study participants were summarized using descriptive statistics and graphs (See subsequent sections). Categorical variables are summarized as frequencies and percentages while the continuous variables are summarized using measures of central tendencies. Data was analyzed using version 9.3 at 95% confidence interval and a significant P value of <0.005. Chi -square test

and fishers exact test were used to test for associations among categorical variables.

Ethical Considerations: The study was conducted after seeking approval from the Institutional Research and Ethics Committee (IREC) of Moi University and MTRH. Informed consent from study participants were then obtained prior to engagement or enrollment sort. Participation in the study was voluntary and the participants were free to leave at any time.

RESULTS

Socio-Demographic characteristics of neonates: Majority (62.9%, n=107) of the neonates had a birth weight <2500g while 37.1% (n=63) had a birth weight >2500g (Table 1). (54.7%) were born earlier than 37 weeks gestation while 45.3% (n=77) were born at or more than 37 weeks gestation. On mode of delivery, (80.6%, n=137) were delivered through spontaneous vaginal delivery (SVD), followed by 18.8% who were delivered by emergency cesarean section [EMCS] (Table 1)

Table 1

Characteristics of Neonates

Characteristic	Frequency (n)	Percentage (%)
Sex		
Male	99	58.2
Female	71	41.8
Total	170	100
Birth weight		
<2500g	107	62.9
>2500g	63	37.1
Total	170	100
Gestation at birth		
<37 weeks	93	54.7
>37 weeks	77	45.3
Total	170	100
Mode of delivery		
SVD	137	80.6
AVD	1	0.6
EMCS	32	18.8
Total	170	100

Socio-Demographic characteristics of mothers: Most mothers had attained some level of basic education - up to secondary (Table 2),while a majority did not have formal employment, hence, they were housewives (77%, n=131), meaning they were available most of the time for the purpose of breastfeeding on demand (Table 2).

Table 2

Socio-demographic of mothers

Parameter	Frequency (n)	Percentage (%)
Age of mothers		
<18 years	8	4.7
18-29	119	70
>29 years	43	25.3
Total	**170**	**100**
Level of education		
Primary	53	31
Secondary	62	36
Tertiary	55	33
Total	**170**	**100**
Occupation		
Housewife	131	77
Self employed	24	14
Formal employment	15	9
Total	**170**	**100**

Proportion of EBM that is contaminated with bacteria: 78.2% (n =133) of EBM collected from the NBU had Microbial isolates (Figure 2).

Bacterial microorganisms that contaminate EBM at the NBU: The bacterial contaminants included *Staphylococcus epidermidis* (54.9%), *Staphylococcus aureus* (42.1%), *Staphylococcus saprophyticus* (2.3%), and *Enterococcus sp* (0.8%) (Figure 3). Of note is that despite the contamination, the colony forming units (CFU/ml) were within acceptable limits for EBM i.e. $<1.0X10^4$/ml. (Table 3).

Table 3

Plate count of bacteria in EBM

CFU/ML	n	Frequency (%)
0	34	13.9
0.1 - 0.2 x 10^1	109	44.5
0.2 - 0.3 x 10^2	50	20.4
0.3 - 0.4 x 10^3	52	21.4

DISCUSSION

Socio-Demographic characteristics of neonates: The results are similar to findings by Harrison and Goodman (2015) on the demographics of neonates in NICU, which established that most of the admissions were due to prematurity (30%) and birth asphyxia (22%). Similarly, Karimi et al. (2011) also concluded that lower birth weight (<2500gm) and lower gestational age were statistically significant factors for admission to NICU. The mode of delivery can be a risk factor for neonatal survival (Micah, Alexa and Zachary, 2016) as it has an implication on neonatal wellbeing and survival after birth. For example, Lawn et al. (2005) established that a baby delivered through assisted vertex delivery (AV) is more like to develop asphyxia and thus, end up in the NBU. Similarly, studies by Cossey (2011) and Karimi et al. (2012) also observed that a significant majority of the newborns admitted to the NICU were delivered through non cesarean section mode.

Proportion of EBM that is contaminated with bacteria: This is more or less in tandem with other studies on microbial contamination of EBM. Boateng et al. (2011) found a prevalence of 97.1% microbial contamination of EBM. Lee et al. (2004) in bacteriological screening of expressed breast milk in Chinese women found a contamination rate of 63% in majority of the samples.

Contamination can be attributed to improper hand washing; contamination during the process of expressing the EBM; and contamination in the containers used to feed the neonates.

Bacterial microorganisms that contaminate EBM at the NBU: The organisms in this study were comparable to what was found in various other studies. Boateng et al (2011) isolated Staphylococcus epidermidis (82.4%), *Klebsiella pneumoniae* (20.6), Acinetobacter *sp.* (14.7%), *Staphylococcus aureus* (10%), Candida yeast (2.5%), and *Pseudomonas sp* (2.5%). Karimi et al. (2012) isolated Klebsiella (13.5%), *Staphylococcus epidermidis* (12.5%), Enterobacter (11%), *Esccherichia coli* (7.5%), and *Staphylococcus aureus* (2.5%). Heikkila and Saris (2003) isolated *S.aureus* (1.8%), *S. epidermidis* (50%), and *Enterococcus Sp.* (4.1%).

The bacterial isolates in this study, which are also largely comparable to the other studies elsewhere, are commonly found on the skin. They have been associated with EBM contamination in a number of the various settings.

The EBM colony forming units were all within acceptable limits of 1.0X104/ml, comparable to most similar studies (Table 3). Studies by Boateng et al. (2011), and Lee et al. (2004) have also reported levels of $<1x10^4$ CFU/ml and $<1x10^3$ CFU/ml respectively. Another similar cross sectional study by Karimi et al. (2012) reported CFU's of between $1x10^4$/ml and $1x10^5$/ml.

Factors associated with bacterial contamination of EBM: Bacterial contamination of EBM was analyzed against some maternal and neonatal parameters. The maternal factors analyzed include mode of delivery, maternal age, and occupation (Table 4). Delivery by emergency caesarean section (EMCS) and spontaneous vaginal delivery (SVD) had no odds to have contamination in the EBM. However, the statistical significance for EMCS and SVD was p=1.00. Besides, on the age of the mother, compared to those who are <18, those between18-29 had 1.212 times higher odds and those with 30- 45 had 1.229

times higher odds for EBM contamination (OR 1.212, 95% CI: 0.229 - 6.417; OR 1.229, 95% CI: 0.210 -7.178). Still, the observations were not statistically significant (p=0.80). (95% CI: 0.229 - 6.417 and 95% CI 0.210 - 7.178 [Table 4]. Some cross sectional studies by Lee et al. (2004) and Ray et al. (2012) have also looked at maternal hand hygiene practices and demonstrated some association with EBM contamination.

Table 4

Association between maternal factors and bacterial contamination

Variable	**Category**	**N**	**Percent**	**Exp (B) Od**	**95% C.I. For Exp(B)**		**Sig (P)**
					Lower	**Upper**	
Mode Of Delivery	AVD	1	0.6				
	EMCS	32	18.8	0.000	0.000	.	1.0
	SVD	137	80.6	0.000	0.000	.	1.0
Mother's Age	<18	8	4.7				
	18-29	119	70.0	1.212	0.229	6.417	0.821
	30-45	43	25.3	1.229	0.210	7.178	0.819
Occupation	Housewife	131	77.0				
	Self Employed	24	14.0	1.729	0.149	19.997	0.661
	Formal Employment	15	9.0	1.116	0.321	17.345	0.721

Neonatal factors analyzed against contamination of EBM included birth weight (<2500g and >2500g); sex (Female and Male); gestational age (<37 weeks and >37 weeks), diagnosis in the ward (neonatal sepsis, meconium aspiration syndrome, small for gestation) (Table 4.3). The Odds Ratio for gestational age at 37 weeks (OR 0.779, 95% CI: 0.319 - 1.900) and >37 weeks (OR 1.295, 95% CI: 0.354 - 4.377) reveal no statistical significance difference between the periods (p>0.005). However, the odds for female newborns were (OR 1.272, 95% CI: 0.587 - 2.753, p=0.542).

Similarly, these results were neither statistically significant (p>0.05) for birth weight and the admitting diagnosis. Birth weight OR 1.537, 95% CI: 0.613 - 3.850. Admitting diagnosis OR 2.103 95% CI: 0.604 - 7.325 (neonatal sepsis); OR 0.949 95% CI: 0.157- 5.733 (MAS); OR 1.063 95% CI: 0.195 - 5.785 (SGA); OR 1.646 95% CI: 0.430- 6.303 (birth asphyxia) and OR 0.629 95% CI: 0.160 - 2.476 (others).

Table 5

Association between neonatal characteristics and bacterial contamination

Variable	**Category**	**N**	**%**	**Exp (B) Od**	**95% C.I. For Exp (B)**		**Sig.(P)**
					Lower	**Upper**	
Gestational Age	<37 Week	93	54.7				
	37weeks	52	30.6	0.779	0.319	1.900	0.583
	>37 Weeks	25	14.7	1.295	0.354	4.377	0.696
Sex	Male	99	58.2				
	Female	71	41.8	1.272	0.587	2.753	0.542
Birth Weight	<2500g	107	62.9				
	>2500 G	63	37.1	1.537	0.613	3.850	0.359
Diagnosis	Prematurity	85	50.0				
	Sepsis	30	17.6	2.103	0.604	7.325	0.243
	MAS	10	5.9	0.949	0.157	5.733	0.955
	SGA	9	5.3	1.063	0.195	5.785	0.944
	Birth Asphyxia	19	11.2	1.646	0.430	6.303	0.467
	Others	17	10.0	0.629	0.160	2.476	0.507

A randomized study by Heikkila and Saris (2003) showed that the commensal staphylococci showed inhibitory activity against *Staphylococcus aureus*. Such inhibitory activity could limit the growth and, therefore, the number of bacterial colonies. The other reason may be explained by the inhibition of the microbial growth by the immune substances found in human breast milk, for example, lactoferrin, phagocytes and lysozymes (Nwet et al. 2016; Jennifer and Pinelli, 2005).

CONCLUSION

Three quarters of EBM produced by mothers at the NBU of MTRH had bacterial contamination. However, the colony forming units (CFU/ML) were within acceptable limits for EBM (less than $1.0x10^4$/ml and, therefore, safe for newborn feeding. The prevalent contaminating bacterial agents included *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Enterococcus faecalis.* Lastly, there was no statistically significant association between the maternal factors and newborn parameters in the propagation of bacteria to EBM.

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