



STUDY OF BACTERIOLOGICAL PROFILE IN PRE PASTEURIZED HUMAN MILK IN HUMAN MILK BANK

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ABSTRACT **Introduction:** Human Breast milk provides all the nutrients for infant growth. Both colostrum & breast milk provide commensal & potentially probiotic bacteria to the infant gut that play a vital role in the colonization of the healthy infant gut. When the mother's own milk is unavailable, pasteurized human donor breast milk from Human Milk Banks is second choice. Human milk collected in Human Milk Bank can be contaminated during milking, collecting and handling methods. This study will be helpful in finding out various organisms present in pre-pasteurized human milk and thus can aid in formulating policy for milk collection.

AIMS & Objectives: To estimate the bacteriological flora present in pre-pasteurized human milk donated in Human Milk Bank.

Results: Bacteriological profile of our study showed that maximum pre-pasteurized milk samples grew (MSSA) Methicillin sensitive staphylococcus aureus (48%) followed by samples with no growth (40%).

Conclusion: This will help in establishing policy for the mother's milk so as to improve the quality of donor milk in Human milk bank setting which will help to reduce infant mortality

KEYWORDS : Human Milk Bank, Infant Feeding, Bacteriological profile, Donor Milk.

INTRODUCTION:

Human Breast milk provides all the nutrients, vitamins, and minerals that are required for infant growth. Therefore, to lead a healthy life doctors advise largely for exclusive breast-feeding for first 6 months of life. Both colostrum and breast milk provide commensal and potentially probiotic bacteria to the infant gut, such as staphylococci, streptococci, bifidobacteria, and lactic acid bacteria that play an important role in the colonization of the healthy infant gut.

When the mother's own milk is unavailable for the sick, hospitalized new born, pasteurized human donor breast milk from Human Milk Banks is second choice. Human milk collected in Human Milk Bank can be contaminated during milking, collecting and handling methods.

This study will be helpful in finding out various organism present in pre-pasteurized human milk and thus can aid in formulating policy for milk collection.

REVIEW OF LITERATURE:

The study in Journal of Food Protection, Vol.51, June 1988 shows that there is high bacteriological content in human breast milk and the risk associated with this can be reduced with breast feeding under good hygiene practices and by doing proper sanitary practices during collection, handling and milking (1).

- According to one of the studies done the most common and abundant bacterial species in their milk samples were *St. epidermidis*, *St. Lactarius*, and *St. hominis*.
- According to one of the studies by Shiao-wen Li et al it was found that in milk samples from mothers *Streptococcaceae*, *Staphylococcaceae*, and *Pseudomonadaceae* bacterial families were predominantly found (2)
- The results found in International Journal of Dairy Science Year: 2015 | Volume: 10 | Issue: 5 | Page No.: 206-218 suggests that Good hygiene practices during milking and subsequent handling of milk are essential to reduce the risk of contamination in the milk processing plant (3).
- One more study by Landers S and Updegrave K. 22% of mothers milk did not grow detectable bacteria on routine cultures, while before pasteurization the organism grown in mother's milk were lactose-fermenting GNR, lactose-fermenting GNR, non-lactose-fermenting GNR, oxidase-positive GNR, *Staphylococcus*,

Enterococcus, methicillin-resistant *S. aureus*. Maximum amongst which were *Staphylococcus aureus*. The majority of pooled milk samples grew two or more organisms. (4)

- One more study by Poonam H Singh, Vaishali Chaudhari et al "Bacteriological analysis of donor human milk in milk bank in an Indian setting" shows, the most common organisms isolated in pre-pasteurized samples were Gram-positive bacilli (51.89%), coagulase-negative staphylococci (CONS, 44.96%), and only 2% were no growth (5).

AIMS AND OBJECTIVES:

To estimate the bacteriological flora present in pre-pasteurized human milk donated in Human Milk Bank.

MATERIAL AND METHODS:

- Study Type :- It was a cross-sectional and descriptive analytical research study.
- Sample Size :- Total 50 sample.
- Sampling :- Universal non-probability sampling method has been used.
- Study Duration :- 2 months
- 1st month : Data has been collected.
- 2nd month :- Data analysis has been done.
- Human Selection Criteria :- Study had included, mother's donating milk in Milk Bank.

LACTATION CULTURE PROTOCOL:

<p>Cleansing the nipple</p> <ol style="list-style-type: none"> 1) Place a towel in the patient's lap prior to irrigation. 2) Prior to milk expression, irrigate the nipple with sterile saline. 3) Blot the nipple with sterile gauze after irrigation. 4) Cleanse each nipple with an alcohol wipe. Allow alcohol to dry. 5) Remove gloves and clean hands. 6) Apply clean gloves. 	<p>Maintain aseptic environment with hand hygiene and wearing gloves. Use hand sanitizer between gloving to prevent contamination.</p>
<p>Milk expression</p> <ol style="list-style-type: none"> 1. Ask the patient if she would prefer to manually express milk herself, or have the provider express the milk sample. 2. The patient or provider should clean hands prior to milk expression and put on gloves. 3. Position the dominant hand in a "C" shape, with the pads of the thumb and fingers about 1.5 inches behind the nipple. 4. Push straight back into chest wall. 5. Roll thumb and fingers forward to express milk. 6. Allow the first few drops to fall onto the towel. 7. Express 5-10 cc of "mid-stream milk" into sterile cup. 8. Label cup with patient label and left or right breast. 9. Repeat on opposite side. 10. Place in lab biohazard transport bag. 	<p>Some patient may not feel comfortable having a provider perform manual expression. Discuss this with patient prior to beginning the milk culture process. Obtain separate samples for the left and right breast. The micro lab will pool the samples to assure the same volume of sample from each breast.</p>

METHOD OF COLLECTION OF SAMPLE:

- 1) Cleansing the nipple as per protocol
- 2) Milk expression under all aseptic precaution.
- 3) The samples collected will be sent to microbiology laboratory for culture.

- Data Collection: Consent of mothers has been taken as a standard protocol in milk bank
- Milk expression had done either manually or with the help of breast pump.
- The data on sociodemographic characteristic- age in months, weight, socioeconomic status, education had been collected.
- The samples collected were sent to microbiology laboratory for culture. Culture reports were collected and studied accordingly.
- Confidentiality: - This study was in full compliance with voluntary participation of subjects, obtaining written consent in the regional language, explanation of the purpose of the study and confidentiality of information.

Statistical Analysis: -

Data was entered in Microsoft office.

Ethical Consideration:

Milk collection was a non-invasive method. Volume of human milk used for culture was 1ml.

Ethical Approval: -

Ethical clearance has been obtained from the Institutional Ethical Committee.

Consent taken from donor mother in milk bank was applicable for the study.

PROFORMA:

- Name of mother:
- Age:
- IPD no:
- Education status: Primary/ High School/ SSC/ HSC/ Graduate/ Post graduate:
- Socioeconomic status: Lower/Middle/Upper:
- Parity:
- Inborn or Outborn:
- Mother received Antibiotics: Yes/ No:
- Place of Delivery in case of outborn::
- Type of Delivery: Vaginal/ LSCS:
- Date and time of Milk collection:
- Method of milk collection: Manual / Breast pump:
- Date and time of collected milk send for culture study:
- Date of culture report:
- Organism isolated-

Sensitivity pattern of Antibiotic.:

Sensitive to Ampicillin/ Amoxyclavunate / Gentamicin/ Amikacin/ Cefotaxime/ Ceftriaxone/ Ceftazidime/ Meropenem/ Linezolid/ Vancomycin/ Ciprofloxacin/ Netilmicin:

OBSERVATION AND RESULTS:

DEMOGRAPHIC DATA:

- In terms of age.
- In terms of education.
- In terms of infant weight
- In terms of organisms isolated in microbiological examination.

Table no.1 showing Age Distribution of Mothers.

AGE OF MOTHERS(years)	NUMBER OF MOTHERS	PERCENTAGE
15-20	18	36
21-25	17	34
26-30	11	22
31-35	4	8
>35	0	0

Bar diagram no.1 showing age distribution of mothers.

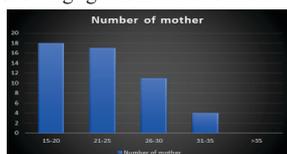


Table no.2: showing education profile of mothers.

EDUCATION	NUMBER OF MOTHERS	percentage
Illiterate	04	8
Primary	10	20
Secondary	08	16
SSC	15	30
HSC	12	24
Graduate	01	2

Pie diagram no.2 showing education profile of mothers.

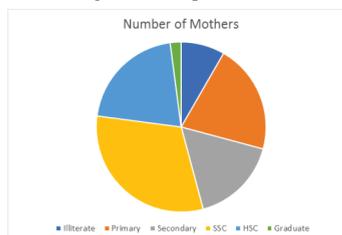


Table no.3 showing number of mothers according to weight of the babies.

Weight of infant(kg)	Number of infants	percentage
2-2.5	23	46
2.5-3	27	54

Pie chart no.3 showing numbers of mothers according to weight of babies.

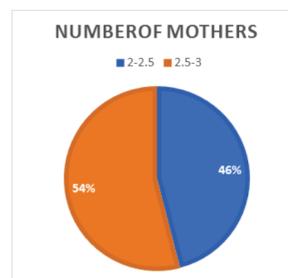
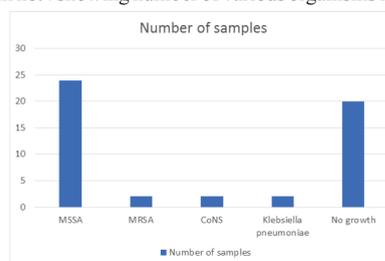


Table no.4 showing name & percentage of the organisms isolated on bacteriological examination in given samples.

S. No	Name of organism	Number of samples positive	Percentage
1	MSSA	24	48
2	MRSA	2	4
3	CONS	2	4
4	Klebseilla	2	4
5	No growth	20	40

Bar diagram no.4 showing number of various organisms isolated



DISCUSSION:

Demographic profile in our study shows that maximum donors were of the age group of 19-20 years, followed by the mothers in age group of 20 to 25 years.

Education profile of mothers in our study revealed that, maximum mothers were educated till SSC and HSC, and 4% were illiterate.

Maximum mothers who donated milk for our study had weight of their babies in the range of 2.5 to 3 kg followed by 2 to 2.5 kg

Bacteriological profile of our study as evident from the above results show that maximum samples grew Methicillin sensitive staphylococcus aureus (48%) followed by samples with no growth (40%). Other organisms which were grown were Methicillin resistant Staphylococcus aureus (4%), Coagulase negative staphylococcus

aureus (4%) and Klebsiella pneumoniae (4%).

Other study by Landers S and Updegrave K 22% of mothers milk did not grow detectable bacteria on routine cultures, while before pasteurization the organism grown in mothers milk were lactose-fermenting GNR, lactose-fermenting GNR, non-lactose-fermenting GNR, oxidase-positive GNR, Staphylococcus, Enterococcus, methicillin-resistant S. aureus. Maximum amongst which were Staphylococcus aureus. The majority of pooled milk samples grew two or more organisms(4). These results were comparable with our study but in our study we had 40% samples as no growth

One more study by Poonam H Singh, Vaishali Chaudhari et al "Bacteriological analysis of donor human milk in milk bank in an Indian setting" shows, the most common organisms isolated in pre-pasteurized samples were Gram-positive bacilli (51.89%), coagulase-negative staphylococci (CONS, 44.96%), and only 2% were no growth(5). These results were not comparable with our study as our study had 40% sample as no growth and our study had only 4% growth of Gram negative organisms.

Thus, it is evident that very limited data is available on the study of pre-pasteurized mothers' milk. Also, there is no concrete evidence on effect of demographic profile and education status on quality of pre-pasteurized mothers' milk.

CONCLUSION:

1. Demographic profile in our study shows that maximum donors were of the age group of 19-20 years, followed by the mothers in age group of 20 to 25
2. Education profile of mothers in our study revealed that, maximum mothers were educated till SSC and HSC, and only 4 % were illiterate.
3. Maximum mothers who donated milk for our study had weight of their babies in the range of 2.5 to 3 kg followed by 2 to 2.5 kgs
4. Bacteriological profile of our study as evident from the above results show that maximum pre pasteurised milk samples grew (MSSA) Methicillin sensitive staphylococcus aureus (48%) followed by samples with no growth (40%). Other organisms which were grown were (MRSA) Methicillin resistant Staphylococcus aureus (4%), (CoNS) Coagulase negative staphylococcus aureus (4%) and Klebsiella pneumoniae (4%).
5. A wide variety of bacteria contaminate human milk from donor mothers. Most milk samples grew MSSA. Although normal skin flora was cultured typically from milk samples, there were also many pre pasteurization milk samples contaminated with pathogenic organisms. Holder pasteurization can be an effective means to remove any detectable bacteria from samples of donor human milk.
6. Thus, this study can help us to educate mothers and families to improve the quality of human milk.
7. The Government of India is establishing the lactation management centres at various health care levels (like comprehensive Lactation Management Centre (CLMC) at Medical colleges/Large District Hospitals, Lactation Management Unit (LMU) at District Hospitals/Sub District Hospitals and District Hospitals/Sub District All Delivery points), the results of this study can be extrapolated to formulate guidelines for lactation management at these levels of health care, which can help to reduce neonatal mortality in long term.
8. Ultimately the breast milk remains the best source of nutrition for infants for several reasons. Doctors and primary health care givers also advocate largely for exclusive breast feeding, at least for first 6 months of life to reduce infant mortality rate. This will help in establishing policy for the mother's milk so as to improve the quality of donor milk in Human milk bank setting which will help to reduce infant mortality.

Limitations:

- 1) This study has limitation in terms of its quantity of samples and time period so for better results it can be done for at least 1 year with maximum number of samples.
- 2) Since the climate of India is variable this study should be done in different geographical area and in different seasons.
- 3) Also, there is scope for the study of variation in quality of milk donated by mothers having preterm versus term versus post term babies. The scope of this study can be increased to study this effect.

SUMMARY:

1. The given study has been done to estimate the bacteriological flora present in pre pasteurised human milk donated in Human Milk Bank.
2. It is cross sectional research study including 50 number of samples with duration of 2 months (from 12th august 2019 to 12th October 2019).
3. Method of collection of sample includes
 - Cleansing the nipple as per protocol
 - Milk expression under all aseptic precaution
 - The samples collected will be sent to microbiology laboratory for culture.
4. The data on sociodemographic characteristics of mothers (age, education status of mothers, weight of babies) is collected and their consent has been taken according to standard protocol.
5. Ethical clearance has been obtained from the Institutional Ethical Committee.
6. Bacteriological profile of our study as evident from the above results show that maximum pre pasteurised milk samples grew (MSSA) Methicillin sensitive staphylococcus aureus (48%) followed by samples with no growth (40%). Other organisms which were grown were (MRSA) Methicillin resistant Staphylococcus aureus (4%), (CoNS) Coagulase negative staphylococcus aureus (4%) and Klebsiella pneumoniae (4%).
7. Although normal skin flora was cultured typically from milk samples, contamination with pathogenic organisms has also found with mainly by MSSA.
8. With the help of this study we can educate mothers and families to improve the quality of human milk and can aid in reducing infant mortality rate.
9. This study can be extrapolated to formulate guidelines for lactation management at various centres in order to reduce neonatal mortality rate.
10. This will help in establishing policy for the mother's milk so as to improve the quality of donor milk in Human milk bank setting which may help to reduce infant mortality.

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