VOLUME - 11, ISSUE - 08, AUGUST - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Medical Microbiology

A STUDY ON BACTERIOLOGICAL PROFILE IN DONOR HUMAN MILK, PRIOR AND SUBSEQUENT TO PASTEURIZATION.

Dr. N. Sowmiya MBBS, Govt. Rajaji Hospital, Madurai.

Dr. S. Radhakumari MD (Microbiology), Professor,Department of Microbiology, Madurai Medical College, Madurai.

Dr. G. Manjula* M.D (Microbiology), Assisstant professor, Institute of Microbiology, Madurai Medical College, Madurai. *Corresponding Author

ABSTRACT Human milk is the gold standard to protect the infant by stimulating immune function development and enhancing growth & development of tissues [1,8,18]. Feeding with sterile donor milk if mother's milk is unavailable, will lower the complications in preterm infants [14]

Manual expression may not be preferred by all donors and better method is using sterile 'Pump'[4]. The bacteria present in contaminated milk may be responsible for mild diarrhea, meningitis and even death[17]. All the milk in the human milk bank must be properly collected, stored, pasteurized. Holder pasteurization (62.5 C for 30 minutes) reduces the bacterial contamination to minimal levels that pose no risk to the recipient.

Bacterial contamination of donor human milk in milk bank before and after holder pasteurization was assessed.In 30 collected expressed milk, 21 samples (70%) showed bacterial growth. Among this, 18(60%) were CoNS , 2(6.67%) were Escherichia coli and 1(3.33%) was Staphylococcus aureus. In 20 samples of pasteurized milk, there was no growth for 19 (95%) samples.Main contaminant of pump expressed milk in present study is skin commensal followed by Gram negative bacilli and staphylococcus aureus.

KEYWORDS : Human milk, Milk bank, Pasteurisation

INTRODUCTION:

Human milk is the gold standard to protect the infant by stimulating immune function development, anti-inflammatory , antimicrobial activity and enhancing growth & development of tissues [1,8,18]. Feeding with sterile donor milk if mother's milk is unavailable, will lower the complications like necrotizing enterocolitis(NEC) in preterm infants [14].

Surplus milk from mothers who are feeding their own babies in the neonatal intensive care unit may be suitable for donor milk.

The Guideline Development Group(GDG) made recommendations for the expression, sterilization and storage of human milk . Different method of expression techniques affect the composition of the milk. GDG recognised that manual expression may not be preferred by all donors and better method is using sterile 'Pump'[4]. The bacteria present in contaminated milk may be responsible for mild diarrhea, meningitis and even death[17].So all donors must undergo a vigorous screening process for infections such as tuberculosis, syphilis, HIV, hepatitis B similar to that used for donating blood and all the milk in the human milk bank must be properly collected, stored, pasteurized.

Bacterial contaminants, such as low virulence skin commensals , coagulase-negative staphylococci, Micrococci and bacteria with greater virulence, such as *S. aureus* and *E. coli*, originate from skin or other sources. High levels of these bacteria causes significant health problem in neonates [4].

Holder pasteurization (62.5°C for 30 minutes) reduces the bacterial contamination to minimal levels that pose no risk to the recipient. But any milk with very high levels should be discarded because pasteurization may not reduce the levels to acceptable amounts, and may not destroy any bacterial toxins.

The present paper helps to guide whether the particular donor mother continues the donation process , proper & better method of cleaning the breast before the expression and the value of pasteurization in the reduction of bacterial load.

AIMS AND OBJECTIVES:

Aims and objectives of the present study is

1. To detect bacterial contamination of donor human milk in milk bank before and after holder pasteurization.
2. To accentuate the process of pasteurization in destroying the bacteria present in the expressed milk, donated to human milk bank.
3. To identify the donor of human milk contaminated with bacteria, and enlightening the donor about the aseptic procedures in expressing the breast milk.

MATERIALS AND METHODS:

After getting institutional ethical clearance and informed consent from donor mother , milk samples were collected from each donor and second sample was collected after pasteurization of expressed milk.

Study Type: Prospective study.

Study Period: 2 months

Study Population:

1. Breast milk obtained during expression before pasteurization.
2. Pasteurized breast milk.

Sample Size:

1.30 samples of breast milk expressed from the individual lactating donor mother.
2.20 samples of breast milk after pasteurization.

Study Area:

1. Breast milk bank in Neonatal Intensive Care Unit ward in Govt.Rajaji Hospital.
2. Institute of Microbiology,Madurai medical College.

Inclusion Criteria:

1. Expressed milk.
2.Pasteurized milk.

Exclusion Criteria: Milk expressed less than 100 ml.

Specimen Collection:

Donor were instructed to clean the breast thoroughly with soap and water. Before the expression of milk, the breast and

nipple was cleaned with cotton soaked in hot water. Then the milk was expressed into the sterile bottle using sterile expression pump.

Sample 1:
From that sterile bottle, 1ml of milk was collected in a 2ml sterile Laxbro vials.

Sample 2:
After expression , this individual milks were sterilized by Holder's method of Pasteurization. From this pasteurized milk, 20 samples were collected in a sterile containers and transported to the microbiology lab immediately, where they were processed by inoculating on to Mac Conckey agar and blood agar plates using sterile, calibrated bacteriological loop. Mac Conckey agar was incubated at 37^{0}C for 24hours.

Blood agar plate was incubated in candle jar.

INTERPRETATION:
After 24hours all the incubated culture plates was screened for the bacterial growth. No colony growth was considered as non contaminated milk and if there is bacterial colony present ,calculation was done as follows:

Number of colonies in each plate was counted. Then the colony Forming Units was calculated by using the formula

If the loop delivers 0.01ml, the CFU is :
Number of colonies × 100 = Total colony forming units(CFU)

If the isolated bacteria belongs to pathogenic group like Enterobacteriacea/ Staphylococcus aureus and the colony forming unit is ≥ 10^4CFU/ml

And

For commensal bacteria like S.epidermiditis and Micrococci colony forming unit is

≥10^5 CFU/ml

that particular milk was considered as contaminated and it was discarded.

OBSERVATIONS AND RESULTS:
In 30 collected expressed milk, 9 samples (30%) no growth seen. Other 21 samples (70%) showed bacterial growth. Among this 21 samples, 18(60%) were CoNS , 2(6.67%) were Escherichia coli and 1(3.33%) was Staphylococcus aureus. In 20 samples of pasteurized milk, there was no growth for 19 (95%) samples.Only one sample(5%) was contaminated with CoNS.

Table:1 Culture Isolate For Expressed Milk:

S. NO	NAME OF THE BACTERIA	NUMBER OF ISOLATES	PERCENTAGE %
1	CoNS	18	60
2	Escherichia coli	2	6.67
3	Staphylococcus aureus	1	3.33
4	No Growth	9	30
	TOTAL	30	100

Table:2 Culture Report For Pasteurised Milk:

S. NO	NAME OF THE BACTERIA	NUMBER OF ISOLATES	PERCENTAGE %
1	No Growth	19	95
2	CoNS	1	5

DISCUSSION:
In present study in expressed milk, 70% is contaminated. It is in contrast with the,a research conducted by Knoop U, Matheis G, in German,in electronic pump expressed milk , the contamination rate is 11.5%[6] .But According to Mehran Karimi results indicate that 85 % of samples were infected[10] .A study by Roberto Sosa, Lewis Barness, out of 41 samples, no growth occur in 8 samples (19.5%) [16], but in our study 30% of samples showed no growth. In a molecular study by Shiao-Wen Li, Koichi Watanabe, the five most predominant bacterial families were *Streptococcaceae*(24.4%), Pseudomonadaceae (14.0%), Staphylococcaceae (12.2%),Lactobacillaceae (6.2%), and Oxalobacteraceae (4.8%)[19], in the present study skin commensal is the predominant among expressed milk 60%, E.coli is 6.67% and S.aureus is 3.33%.

In a study by Mehran Karimi dominant microorganisms were firstly Klebsiella (13.7%) and then S. epidermidis (12.5%)[10] and in the research by Roberto Sosa, Lewis Barness, CoNS in 33 samples (80%), Klebsiella in 2 samples (4.8%), Pseudomonas in one sample (2.4%) were isolated in unpasteurized breast milk[19]

According to Knoop U, Matheis G, Gram negative bacilli, beta-hemolytic Streptococci Group B and Staphylococcus aureus were found in unprocessed milk [6] .PO Ukegbu, AC Uwaegbute, told , the average colony counts were within acceptable limits (<10^4CFU/ml). Enterococcus faecalis, Escherichia coli and Staphylococcus aureus were the predominant bacteria isolated in the breast milk samples [12]. According to E O Igumbor .R.D Mukura in Central African study , there is growth of nonpathogenic skin commensal in unpasteurized milk and the best storage temperature is 0-4^0 C for 24 hrs [3]. But as in other studies , Klebsiella and haemolytic Streptococci are not isolated in our study.Main contaminant of pump expressed milk in present study is skin commensal followed by Gram negative bacilli and staphylococcus aureus. In a study by Nwankwo MU, Offor E, at room temperature the mature milk can be stored for 6 hrs, and the colostrum can be stored for 12 hrs without any contamination [11].

In the present study the expressed milk is stored in 4^{0}C for 24 hrs and after pasteurization it is stored at deep freezer -70^{0}C till it is used.

CONCLUSION:
The breast milk bank is useful for feeding the newborn babies without the supply from mother milk. It satisfy the nutritional requirement of babies and favours mental and physical growth. But we must be careful in supplying best quality of sterile milk.
1. In NICU, separate room should be available for breast milk bank with a separate partition for milk collection.
2.Expression pump should be used to collect the milk , which should be properly cleaned , dried and stored in closed shelf .
3. For each mother , separate collection bottle should be used . After the collection from one mother , it should be stored in refrigerator individually with label and if the same mother comes next day, the milk will be collected in the same bottle.
4. After the collection of more than 100 ml of milk from individual mother , it is pasteurized individually in separate stainless steel container by Holders method of pasteurization. Then the milk is stored in deep freezer , till its usage.
5.All the collection bottles should be cleaned properly and sterilized by autoclave.
6. The temperature in the refrigerator, deep freezer and autoclave should be checked daily for the supply of breast milk properly.
7.As it is new field , more research can be invited like molecular investigation , temperature variation in sterilization and cleaning method of breast before expression to establish proper guide line in Human Milk Bank.

SUMMARY:
- In unsterilized breast milk bacterial contamination is 70%.
- Skin commensal is common contaminant 60%, next is

Gram negative Bacilli E.coli 6.67%, S.aureus is seen in 3.33% of milk

- After pasteurization , only 5% of the milk is contaminated with Skin commensal.

From this efficacy of pasteurization in removing pathogenic and commensal organism is analysed.

1.Human breast milk bank is becoming mandatory in all tertiary care hospital. Only fewer journals are available and there is no standard guidelines for the proper techniques to be followed in the milk bank. At this juncture the present study will enlight, trigger most researches in future.

2. Through this research Donors can be trained for the proper techniques for:

- cleaning the breast before expression.
- To follow more sterile technique in milk expression , collection ,milk storage.

Overall this present study is to help the donors and the milk bank for providing a sterile breast milk to neonates, thereby preventing the complications.

REFERENCES:

1. Clare DA, Catignani GL, Swaisgood HE (2003) Biodefense properties of milk: the role of antimicrobial proteins and peptides. Curr Pharm Des 9:1239–1255.
2. C L Jones, R F Jennison, and S W D'Souza - Bacterial contamination of expressed breast milk , Br Med J. 1979 Nov 24; 2(6201): 1320–1322.
3. E O Igumbor,RD Mukura Storage of breast milk: Effect of temperature and storage duration on microbial growth- The Central African journal of medicine 46(9):247-51 · September 2000 with 482.
4. Guidelines established for milk banking by GDG.
5. Hayashi S, Kimura H, Oshiro M, Kato Y, Yasuda A, et al. Transmission of cytomegalovirus via breast milk in extremely premature infants. Journal of perinatology: The official journal of the California Perinatal Association 2011; 31(6): 440–45.
6. Knoop U, Matheis G, Schütt-Gerowitt H. [Bacterial contamination of pump-collected breast milk] PubMed. 1985 Aug;133(8):537-41.
7. Landers S, Updegrove K. Bacteriological screening of donor human milk before and after Holder pasteurization. Breastfeeding medicine: The official journal of the Academy of Breastfeeding Medicine 2010; 5(3): 117–21
8. Lawrence RM, Pane CA (2007) Human breast milk: current concepts of immunology.
9. Matsubara F, Sagara Y, Kato Y, Harada A, Koizumi A, et al. Detection of antibodies to Human T-cell leukemia virus types 1 and 2 in breast milk from East Asian women. Biological & Pharmaceutical Bulletin, 2014; 37(2): 311–14.
10. Mehran Karimi , Zia Eslami Bacterial Contamination of Expressed Breast Milk in Neonatal Intensive Care Unit the BMJ · January 2013 with 314 Reads.
11. Nwankwo MU[1], Offor E, Bacterial growth in expressed breast-milk.. Ann Trop Paediatr. 1988 Jun;8(2):92-5
12. PO Ukegbu, AC Uwaegbute, II Ijeh, AU Ukegbu Bacterial Load in Expressed and Stored Breast Milk of Lactating Mothers in Abia State, Nigeria- African Journal of Food, Agriculture, Nutrition and Development-Vol 13, No 4 (2013)
13. Rønnestad A, Abrahamsen TG, Medbø S, Reigstad H, Lossius K, Kaaresen PI, et al 2005, Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. Pediatrics 115(3): e269–e276.
14. Quigley M, Henderson G, Anthony M (2007) Formula milk versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev CD002971.
15. Rhodes J. Evidence-based recommendations for breast pumping hygiene. Neonatal Intensive Care 2011; 24(4): 20–23.
16. Roberto Sosa,; Lewis Barness, Bacterial Growth in Refrigerated Human Milk Article JAMA Paediatric -January 1987
17. Sarah Keim(2011)Breast milk sharing carries health risks from bacterial contamination:study.
18. Schanler R, Shulman RJ, Lau C (1999) Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula.
19. Shiao-Wen Li, Koichi Watanabe, Bacterial Composition and Diversity in Breast Milk Samples from Mothers Living in Taiwan and Mainland China Front Microbiol. 2017; 8: 965. Published online 2017 May 30.
20. Susan Landers and Kim Updegrove ;Bacteriological Screening of Donor Human Milk Before and After Holder Pasteurization an article in Breastfeeding Medicine 5(3):117-21 · June 2010
21. Thorley V 2000, Feeding Their Babies: Infant-Feeding Advice Received by Queensland Women in the Postwar Period, 1945– 1965. MA Thesis, University of Queensland, 2000
22. Tyler P 2006, Dépister de Tuberculose – Analyse d'Une Campagne Nationale en Australie, 40ème Congrès d'Histoire de la Medicine, International Society for the History of Medicine. 7 August