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MICROBIOLOGICAL PROFILE OF DONOR HUMAN MILK PRE AND POST PASTEURIZATION

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ABSTRACT

Introduction: Human Breast milk is considered a pillar of child survival worldwide as it is both immunologically and nutritionally ideal for infants, especially in case of premature infants or in resource-limited settings. Therefore, Human Milk Bank is seen to play a critical role and becomes an an essential component of newborn health.

Material and method: Donor mothers were screened and counselled before expressing breast milk. The donor human milk (DHM) was then pooled and mixed into different batches. Samples were sent for pasteurization to microbiology laboratory. In the microbiology laboratory milk samples are processed as per the national guidelines. Pre and post pasteurization colony counts were reported. In case of post-pasteurization, if colony count <103 sample is acceptable but if colony count > 105 sample is discarded. Low culture positivity seen in the post pasteurization sample confirms the presence of a good and efficient human milk banking system in the hospital.

Results: Among the samples -59.7% of the pre-pasteurization and none of the post-pasteurization samples were culture positive. The most predominant bacteria isolated were Coagulase negative staphylococcus (31.3%) followed by Staphylococcus aureus (29.1%) and Micrococcus (28.7%). Among gram negative isolates most commonly isolated organism was Klebsiella spp (2.8%).

Conclusion: An effective HMB system is a pre-requisite for ensuring infants' access to a safe, high-quality and sustainable supply of DHM when MOM is unavailable. Thus we should aim at making them an integral part of NICUs to obtain maximum benefit for the vulnerable neonates.

KEYWORDS : Human Breast milk, pasteurization, donor human milk,

INTRODUCTION

Human Breast milk is considered a pillar of child survival worldwide as it is both immunologically and nutritionally ideal for infants, especially in case of premature infants or in resource-limited settings. [1] Inadequate breastfeeding contributes to an estimated 11.6% of child deaths worldwide and about 8 00 000 deaths annually. World Health Organization (WHO)[2] and American Academy of Paediatrics [3], recommend DHM (Donor Human Milk) as the next best option whenever MOM (mother's own milk) is not available. According to the United Nations' 'International Covenant on Economics, Social and Cultural Rights and the Declaration and Convention on the Rights of the Child', access to a safe, high-quality supply of DHM is part of a child's human right to optimal nutrition .[4]

Human milk has been identified as a fundamental source of bioactive components including microbes that may contribute to neonatal gut colonisation and development of immunity and maturation during the crucial early stages of development. [5]Premature neonates and infants are at an increased risk of developing sepsis, respiratory distress, retinopathy of prematurity, neurodevelopment delays and necrotizing enterocolitis (NEC) which in turn is associated with increased mortality. Studies have reported that formula-fed infants have 6 to 20 times higher risk of experiencing NEC compared with breast milk-fed infants [6]

This is where a Human Milk Bank plays a critical role and becomes an an essential component of newborn health. A human milk bank (HMB) is a service established for recruiting human milk donors, collecting DHM, and then pasteurization, screening, storage and distribution of the milk to meet infants' specific needs. [7-10]

A National Human Milk Bank and Lactation Counselling Centre was opened at Lady Hardinge Medical College (LHMC) in June 2017 with the purpose of improving infant survival, supporting breastfeeding and also act as a teaching, training and demonstration site for other milk banks. The centre facilitates collection, pasteurization, microbiological testing and safe storage of Donor Human Milk. Vatsalya – Maatri Amrit Kosh" was established in collaboration with the Norwegian government, Oslo University and Norway India Partnership Initiative (NIPI).

MATERIALAND METHODS:

A prospective study was conducted at Lady Hardinge Medical College from January 2018 to December 2019 to evaluate the efficacy of pasteurization being done at the HMB.

The donor mothers were counselled, history, informed consent was taken and serological screening (for HIV, Hep B, Hep C and syphilis) was done. Milk samples were collected according to a standard protocol and were promptly transported to the laboratory under refrigerated conditions. Mothers express milk either manually or by using a breast pump.

The donor human milk (DHM) is then pooled and mixed into different batches. Before pasteurization 1 ml of sample is collected in a universal sterile container from each of the pooled batches. Pasteurization is done by Holder's method. Following pasteurization 1 ml samples from each of the batches are collected in universal sterile containers. These samples are then sent to microbiology laboratory for culture testing.

Microbiological screening:

In the microbiology laboratory milk samples are processed as per the national guidelines.[11] As per the guidelines a dilution of 1:10 is made by adding 9 ml of normal saline to 1 ml of milk sample. These dilutions are then plated on blood agar and mac conkey agar using a 0.01 ml calibrated loop. The plates are incubated at 37 C for 18-24 hours and then checked for any bacterial growth. If growth is present, colony count is done. No growth is acceptable in the post-pasteurization sample. The whole batch is discarded if it does not meet acceptable bacteriological standards. In the pre-pasteurization sample, however the following criteria is followed:

If colony count $<10^3$ – sample is acceptable
If colony count $> 10^5$ – sample discarded
If colony count 10^3 to 10^5 - sample used only if skin commensals are present [11]

A heavily contaminated sample with certain bacteria like *E.coli* and *S. aureus* is discarded as they can produce enterotoxins and heat stable enzymes that remain unaffected by pasteurization. Any colony count above 10^4 cfu/ml in case of these bacteria is unacceptable.[11]

Identification:

Any growth found on the culture plates was processed in the microbiology for identification. Gram staining was done and

depending on the finding further testing was done. For gram positive cocci – slide and tube coagulase was done. A cefoxitin disc was used by disc diffusion method for Methicillin Resistant Staphylococcus aureus screening. Biochemical tests namely- TSI, citrate, mannitol, urease, oxidase, indole and motility were done for identification of gram negative bacilli as per standard protocol. [12] Final identification was also done on Vitek ™.

Preservation and storage of DHM:

The DHM is stored only after a negative report from the microbiology laboratory. The donor human milk is then sealed and labelled with the batch ID, pool number, date of freezing and expiry date. It can then be stored at -20 C for upto 3-6 months. This processed donor human milk (PHDM) can be taken out from the freezer on FIFO (first in first out) basis and dispensed whenever a requisition comes from the physician.

RESULTS:

A total of 480 DHM samples were received in the microbiology laboratory- 466 pre and 466 post pasteurization samples for a period of 24 months (January 2018 to December 2019). Among the pre-pasteurization samples 278 out of 466 were culture positive (59.5%). The organisms isolated were Micrococcus (28.7%), followed by Staphylococcus aureus (29.1%) and Coagulase negative staphylococcus (31.3%). (Table 1) The colony count in each of these cases was between 10 to 10^2 cfu/ml. Post pasteurization each of these 446 DHM samples were found to be culture negative. All post pasteurization samples were culture negative.

Table1.Organisms isolated from pre- pasteurized Donor Human Milk.

Organism	Number	Percentage
MSSA	51	18.3%
MRSA	30	10.7%
CONS	87	31.3%
Micrococcus	80	28.7%
E. coli	2	0.7%
Klebsiella spp.	8	2.8%
Acinetobacter spp.	2	0.7%
ASB	18	6.5%
TOTAL	278	59.7%

DISCUSSION:

Human Breast Milk plays a critical role in the growth, development and building up the pre-term infant immunity since they contain a variety of factors, such as immunoglobulins, inmunocompetent cells, fatty acids, polyamines, oligosaccharides, lysozyme, lactoferrin, and antimicrobial peptides. In addition, breast milk is an important and continuous source of commensal bacteria, including staphylococci, streptococci, and lactic acid bacteria, to the infant gut. In the present study we have studied the microbiological profile of the DHM and the effectiveness of the pasteurization process.

In our study 59.7% of the pre-pasteurization and none of the post-pasteurization samples were culture positive in comparison to another study done in India by in Rajasthan (2017) by Gupta N et al[13] reporting 81% and 22% in pre and post pasteurization respectively. Culture negative post pasteurization samples confirms a good pasteurization technique and effective storage and transport protocol at the centre.

Human breast milk is in itself a source of commensal bacteria that may colonise the infant gut. However, in most studies the predominant culturable bacterial populations have been identified as Staphylococcus. [5] In our study S. aureus was isolated from 29.1% of the samples which is higher than other studies[14,15, 16] - Caroll et al (UK,1979), Serafini AB et al (Brazil, 2003) and Wen Li et al (Taiwan, 2017) ranging between 6.2% to 12.2%. However higher prevalence rate of 40% was reported from Brazil by Colaco W et al[17]. An Indian study done by Gupta N et al [13] reported a similar isolation rate of Staphylococci (25%) . Presence of Staphylococcus aureus can be explained by contamination from a secondary source, such as the skin and nasal fossa, or unsatisfactory sanitary conditions of the utensils being used in the process. [15]

In our study we have reported coagulase negative staphylococcus from 31.3% of the milk samples which is low as compared to other studies [13,18,19] - done in Rajasthan (2017) by Gupta N et al, 2017 (43%), Gujrat (2016) by Singh P et al (44.6%) and Brazil (1998) by Almeida et al (51.7%). Other researchers [15,20,19] outside India have also found coagulase positive Staphylococcus in similar proportions of human milk samples -28.1% by Nikodemuz et al (1986) and 29% by Almeida et al(1998) , both done in Brazil. Lower rates (20.6%) have been reported by Serafini AB [15] et al (2003) from Brazil.

Micrococcus was isolated from 17.1% of our pre-pasteurization milk samples. The high rate of isolation of these commensal bacteria could be because of inadequate cleaning of areola, lack of hand hygiene or the lactation consultant not guiding the donor mothers properly. Presence of Aerobic spore bearers (3.8%) indicates towards environmental contamination, stressing on the need of effective disinfection strategies to tackle such problems.

Among the Enterobacteriaece, coliforms have been commonly reported from human milk samples and since their presence may indicate faecal contamination they are considered to be particularly important in bacteriological control of HMBs.[15] Research studies [13,15,21,16] have isolated coliform microorganisms from 23% (Gupta N et al, India 2017), 21.1% (Serafini et al, Brazil 2003), 8.48% (Novak et al, Brazil 2001)and 4.5% (Wen Li et al, Taiwan 2017)of the milk samples. We however reported a lower rate of coliforms isolation in (3.5%) of the milk samples. Regular cleaning of breast pumps, its tubing and milk storage containers are important factors in reducing the colonization of such organisms in them.

Studies conducted in early 1920 s showed the presence of streptococci in human milk and since then numerous studies have detected various species of *Streptococci (mitis and salivarius)* likely transferred from the infant's oral mucosa[22]. In the recent years also authors Hunt *et al.* (2011,USA) and Cabrera-Rubio *et al* (2012, Spain) . have reported genera associated with the oral cavity such as *Streptococcus.* [23,24] In our study however Streptococci was not isolated from any of the milk samples which could be due to low level of colonization of Streptococci among neonates. This can be supported by a study done at LHMC by Shah D et al[25] in 2014 and other Indian studies[26] reporting low colonization rates of Streptococci (3.2% and 6.7% respectively).

Pseudomonas has been reported as a dominant member of the human milk microbiota in several studies including Ward *et al.* (Canada, 2013)[27] and Wen Li et al (Taiwan, 2017) [16] accounting for 61% and 14% of the milk samples. A study done in India (2017) by Gupta N et al[13] reported Pseudomonas in 6% of the raw milk samples. In our study however Pseudomonas was not reported. This confirms the use of good practices being followed in the milk bank during milk expression and collection over the years.

All the post pasteurization samples in our study were culture negative, whereas post- pasteurization contamination reported by other Indian studies [13,28] have been found to be 22% and 3.6%(Gupta N et al and Kumar N et al respectively). A study done in Brazil by Serafini et al[15] reported a high contamination rate of 50.6% (25.7% of these were molds and yeasts).

Standard practices as given by National guidelines [11] are being followed in the Lactation centre starting from donor selection, serological screening, lactation counselling, proper expression of breast milk, storage, microbiological screening and finally distribution. Thus the low level of presence of micro-organism in the post-pasteurization samples in comparison to different studies indicate good pasteurization and infection control practice being followed at the milk bank in our hospital.

Lacunae:

Gut anaerobic flora was not included in this study. Also the mode of delivery of the donor mother could not be included in the study as donor milk was pooled from different mothers irrespective of their health or delivery status. Thus correlation of the results with the type of delivery and its impact on the breast milk flora could not be studied. Microbiome analysis not done, due to lack of sequence and the high cost involved in it. Microbiome will actually determine the level of colonization of human milk.

CONCLUSION:

Comprehensively evaluating the human milk microbiota will give us better insight to its signi cance and activity in relation to the developing infant gut microbiota and health. [5] In order to do that we need efficient HMB sytems in the hospitals- which is a pre-requisite for ensuring infants' access to a safe, high-quality and sustainable supply of DHM

when MOM is unavailable. Health policies in our country should aim at opening more HMBs and making them an integral part of NICUs to obtain maximum benefit for the vulnerable neonates.

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