Doi: 10.32677/IJCH.2017.v04.i01.003

Original Article

Bacteriological analysis of donor human milk in milk bank in an Indian setting

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Received – 11 November 2016 Initial Review – 29 November 2016 Published Online – 09 January 2017

ABSTRACT

Background: Although human milk banking in India was started in 1984, limited data regarding bacteriological quality of milk are available. **Objective:** To find bacteriological contamination of milk samples in human milk bank. **Methods:** Retrospective analysis of milk samples from milk bank for the year 2009-2015 was done. Group A donor mothers were from postnatal or neonatal units of our hospital. Group B mothers were from milk donation camps organized at the community level. All milk samples were plated on blood agar for bacteriological analysis. Pre-pasteurized samples were analyzed for the year 2014-2015 only. **Results:** Total donor mothers were 3670. 1481 (40%) were from milk donation camp at the community level. 327 (9%) were preterm milk. 2.34% of post-pasteurized samples showed contamination as compared to 9.1% pre-pasteurized milk samples (p=0.002). Group A mothers showed a higher contamination rate as compared to Group B mothers in both pre-pasteurized (p<0.001) and post-pasteurized samples (p=0.006). The most common organisms isolated in pre-pasteurized samples were Gram-positive bacilli (51.89%), coagulase-negative staphylococci (CONS, 44.96%), and Gram-negative bacilli (3%). The most common organisms in post-pasteurized samples were Gram-positive bacilli (88.23%) and CONS (11.76%). No Gram-negative bacilli were isolated from post-pasteurized samples. **Conclusion:** Contamination rates in our study for both pre- and post-pasteurized samples are quite low as compared to other studies. Community collection of human milk was safe with regard to bacteriological contamination. Holder's method of pasteurization is effective in reducing contamination.

Key words: *Contamination, Donor human milk, Post-pasteurized sample, Pre-pasteurized sample*

Pasteurized donor human milk (PDHM) through milk bank is an alternative to mother's milk when needed [1,2]. The microbiological quality of PDHM is a public health issue as recipients may be susceptible to neonatal diseases if this process of pasteurization is compromised [2-6].

Worldwide, varied guidelines are being followed for pasteurization techniques, quality control, and cutoffs for pre- and post-pasteurized milk [7-9]. Studies have been done to see contamination rates, types of organisms, and an association of organisms isolated with the method of collection and source of collection [10-14]. The primary objective of the study was to find out overall contamination rate in our milk bank. The secondary objective was to compare contamination rate in two groups of donor mothers and to compare the bacterial isolates in pre- and post-pasteurized milk samples.

METHODS

Our milk bank was started in 2008, and we have been holding human milk collection camps in the community on specified days in addition to milk collected in our milk bank. This is a retrospective analysis of the data in our milk bank from 2009 to 2015. Consent of mothers was taken as a standard protocol. Since it is a retrospective analysis of data, ethical clearance was not taken.

Donor screening was done by detailed history, physical examination, and serological testing for human immunodeficiency virus, hepatitis B surface antigen, and venereal disease research laboratory. Donors who did not fulfill the eligibility criteria were excluded from donating milk. In a hospital setting, milk expression was done either manually or with the help of breast pump. In the community setting, only manual expression was done, and this milk was transported to milk bank maintaining cold chain within 3 h of collection.

After collection, all milk samples were pasteurized by Holder's method (62.5*C for 30 min), followed by rapid cooling. Aliquots of milk from each container were sent for culture after pasteurization. Milk was plated on blood agar for aerobic organisms. Milk samples were stored in a separate refrigerator till culture reports were received.

Routine testing of pre-pasteurized milk sample was not adopted. As a quality check measure, for years 2014 and 2015, the milk bank made a policy decision to send pre-pasteurized milk samples for culture, and policy was revised in 2015 to isolate the organism for both pre and post-pasteurized samples. This was in accordance with Human Milk Banking Association of North America (HMBANA) guidelines. In resource-poor countries, only post-pasteurization samples are sent to cut costs [8]. Colony count, anaerobic cultures, and fungal cultures were not done due to lack of facility.

As per HMBANA guideline, pre-pasteurized milk is unsafe for use under the following circumstances: (1) Total viable organism $>10^5$ colony forming unit (CFU)/ml, (2) $>10^4$ CFU/ml for *Enterobacteriaceae,* or (3) 10^4 CFU/ml for *Staphylococcus aureus*. No growth is acceptable in post-pasteurization microbiology cultures. In our study, colony count was not done, so pre-pasteurized milk samples which showed any growth were subjected to pasteurization for research purposes only but were not disbursed to any newborns. All post-pasteurized culture-positive samples were discarded.

Our study group comprised milk from two types of donor mothers: Group A and B. Group A mothers were eligible mothers from postnatal wards and mothers whose babies were admitted in the neonatal unit. Group B consisted of mothers fulfilling the eligibility criteria who donated at community level under the supervision of milk bank staff.

RESULTS

A total of 3670 mothers had donated milk for our human milk bank, out of which 1481 mothers were from milk donation camp organized at the community level, thus contributing 40% of milk collection. 1874 mother's milk was full-term and 327 was preterm milk. A total of 3455 post-pasteurized and 1569 pre-pasteurized milk samples were sent for bacteriological analysis. Culture positivity was 2.34% (81/3455) in post-pasteurized samples as compared to 9.1% (144/1569) positivity in pre-pasteurized samples (p=0.02) (Table 1).

Among post-pasteurized samples, Group A mothers showed 1.84% (64/2232) culture positivity as compared to Group B mothers, i.e.,e0.49% (17/1223) (p=0.06). A significant difference was also noted in pre-pasteurized samples between Group A and Group B mothers (18.4% vs. 1.7%, p<0.001).

Isolation of organism was done in the year 2015. 732 milk samples each from pre- and post-pasteurized samples were sent for organism isolation. Organisms were isolated from 129 pre-pasteurized and 17 post-pasteurized samples (Table 2). Gram-positive bacilli were predominant organisms isolated from both pre-)(51.93%) and post-pasteurized milk (88.23%). Coagulase-negative staphylococci (CONS) were the second most common organism among both the groups. Gram-negative bacilli were seen in only pre-pasteurized samples, of which 1 was Klebsiella, 1 was *Escherichia Coli,* and 2 were *Acinetobacter*. No post-pasteurized sample was positive for Gram-negative bacteria. There was a significant reduction in culture positivity rate in post-pasteurized samples (Table 2).

Table 1: Comparison of bacteriological positivity in pre-pasteurized and post-pasteurized milk samples

Milk samples	Number of samples	Positive culture samples (%)	p-value
Post-pasteurized samples			
Group A	2232	64 (1.84)	0.06
Group B	1223	17 (0.49)	
Total	3455	81 (2.34)	
Pre-pasteurized samples			
Group A	701	129 (18.4)	<0.001
Group B	868	15 (1.7)	
Total	1569	144 (9.1)	

Table 2: Organisms in pre-pasteurization versus post-pasteurization donor milk

Organisms	Pre-pasteurization (n=732) (%)	Post-pasteurization (n=732) (%)
Gram-positive bacilli	67 (51.93)	15 (88.23)
CONS	58 (44.96)	02 (11.76)
Klebsiella	01 (0.77)	00
Acinetobacter	02 (1.5)	00
E. coli	01 (0.77)	00
Total culture positive	129	17

E. coli: *Escherichia coli,* **CONS: Coagulase-negative staphylococci**

DISCUSSION

The present study reveals that contamination rate in pre-pasteurized milk was 9.1% and in post-pasteurized milk was 2.34% in our milk bank. Contamination rates were lower in community collection of milk as compared to the hospital-based collection (0.49% vs. 1.84%). Studies from different milk banks about microbiological contamination of milk have shown varying result depending on whether pre- or post-pasteurization samples were evaluated [13,10], method of collection of milk in the container was taken into consideration [12], or source of the collection was home-based samples or milk bank samples [11]. No data from India are available although milk banking in India was started in 1984 [15]. This study highlights the overall prevalence of contamination in pre- and post-pasteurized milk and contamination rates as per source of collection.

Keim et al. found that pre-pasteurized milk showed contamination rate of 91% in home-collected samples of breast milk, while those collected in milk bank showed a contamination rate of 2.5% [11]. Similarly, a study from China showed a contamination rate of 86% in pre-pasteurized samples of milk [16]. Serafini showed 70.1% contamination in pre-pasteurized milk from their milk bank [10]. In our study, the rate of contamination in pre-pasteurized milk was 9.1%. Post-pasteurization rates of contamination in studies have been reported as 75% [11], 50.7% [10], and 7% [13]. In our study, this rate was 2.34%. The higher rate of contamination in these studies was seen in unsupervised home-collected milk. While Keim et al. had seen contamination rate as low as 2.5% in samples collected in their milk bank.

The method of collection has been compared in a study by Lucas and Roberts in 1979. He reported that if milk was collected in vessels washed with plain detergent, the rate of contamination was higher for both pre- and post-pasteurized milk as compared to the collection in vessels cleaned with hypochlorite solution. Similarly, home-based milk collection had a higher contamination rate as compared to the hospital-based collection

(91% vs. 75%) [11]. In our study, the second group (mothers from whom milk was collected in the community on a prescribed day under the supervision of trained staff) had a contamination rate of only 0.49%, whereas hospital-based milk samples had contamination rate of 1.84%.

The higher contamination rates in the hospital setting can be explained by the following facts: First, our hospital caters to a low socioeconomic class of patients as it is a public hospital. These indoor mothers did not have access to the regular bathing facility during hospital stay, thus reflecting unsatisfactory hygienic status. Second, most of the mothers preferred milk expression by the pump in the hospital that could also be the reason for a higher rate of contamination. As described in a study by Tyson et al. [6], manual expression of milk is less likely to be contaminated as compared to milk collected by other methods. Contamination with coliforms and gentamicin-resistant Gram-negative rods has been reported with the use of breast pumps. Stringent precaution reduced but did not eliminate this contamination [6].

The types of organisms isolated in milk banks in different studies over the years, from 1978 to 2015 are discussed here. In 1978, Roberts and Severen from Northampton found high counts of *E. coli*, *S. aureus*, and toxigenic *S. aureus* in pre-pasteurized milk samples [14]. In 2003, Serafini et al. from Brazil reported organisms such as Enterococci (36%), molds and yeasts (31.6%), *S. epidermis* (20.59%), *S. aureus* (7.35%), and Streptococcus viridian (2.2%) from pre-pasteurized milk. The post-pasteurized milk in their study had organisms such as molds and yeasts (50.7%), *S. Lugdunensis* (20.6%), *S. aureus* (6.9%), and *Enterobacter* (4.1%) [10]. Landers and Updegrove in their study, reported *S. aureus* in 87%, lactose fermenting Gram-negative rods in 62%, and Enterococci in 16% in pre-pasteurized samples [13]. In contrast to above studies where the Gram-positive organism was predominant as compared to Gram-negative, Keim et al. reported a predominance of Gram-negative organisms in both hospital-based and home-based collection [11].

In our study, the common organisms isolated in pre-pasteurized milk were Gram-positive bacilli in 52%, CONS in 45%, and Gram-negative organisms in 3% samples. In post-pasteurization samples, Gram-positive bacilli were found in 88.23% and CONS in 11.76% samples. No Gram-negative organisms were isolated from post-pasteurization samples in our study. Gram-positive bacteria such as CONS in pre-pasteurized sample suggest poor skin hygiene; however, Holder's method of pasteurization was effective in reducing the contamination significantly

CONCLUSION

Contamination rates in our study for both pre- and post-pasteurized samples are quite low as compared to other studies. Community collection of human milk was also safe with regard to bacteriological contamination as strict donor screening protocol, and aseptic measures were followed. Holder's method of pasteurization is effective in reducing contamination significantly.

Our study has some limitations as it was a retrospective study. Second, due to lack of facility, colony count has not been done for the isolated organisms in the laboratory, and isolation of yeast and mold also has not been done. Third, as pre-pasteurization microbiological testing has been started for last 2 years, limited data for this pool are available.

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Funding: None; Conflict of Interest: None Stated.

How to cite this article: Singh PH, Surana AU, Chaudhari V. Bacteriological analysis of donor human milk in milk bank in an Indian setting. Indian J Child Health. 2017; 4(1):7-9.