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

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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

Peasteurized 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.,0.49% (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).

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.

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

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

REFERENCES


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CONCLUSION

Contamination rates in our study for both pre- and post-pasteurized samples are quite low as compared to other studies. Community collection of donor milk was also safe with regard to bacteriological contamination as strict donor screening protocol, Funding: None; Conflict of Interest: None Stated.

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