



Final Report

The effect of Pretoria Pasteurisation on bacterial contamination of hand-expressed human breast milk

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Table of contents	Page
Executive summary	1
Introduction	3
Methods	5
Results	7
Discussion	10
Conclusion	11
References	13

Executive Summary

One of the challenges facing the development of programs to reduce mother to child transmission of HIV in developing countries remains the problem of infant feeding. One of the alternative feeding methods under investigation for infants of HIV infected mothers is heat treatment of expressed breast milk by Pretoria Pasteurisation. This is a simple low cost method of heat treatment of expressed breast milk (EBM) which is suitable for use in a resource poor domestic setting and has been shown to inactivate HIV in the milk of infected women. The Pretoria Pasteurisation method is currently used in several hospitals for the feeding of premature infants born to HIV infected mothers, as well as by breast milk banks in underresourced areas of South Africa. Several studies have shown that EBM is commonly contaminated with bacteria. Bacterial contamination in EBM has been found to be associated with infections and even death in premature infants. There is often a delay between expression of breast milk (and/or pasteurization), and consumption by the infant and this raises concerns about the possibility of bacterial overgrowth in samples after Pretoria Pasteurisation. The objective of this study was to determine the effect of Pretoria Pasteurisation on commensal and pathogenic bacteria in hand-expressed human breast milk, and to determine the duration of time for which milk can safely be kept without refrigeration after Pretoria Pasteurisation.

Samples of milk were hand expressed by lactating women in the postnatal ward. The samples were split into control and pasteurized specimens. The pasteurized specimens underwent Pretoria Pasteurisation. All samples were stored at room temperature and were sampled for bacterial culture every four hours, up to 12 hours. Significant levels of bacterial contamination occurred in 59% of control and 7.8% of pasteurized samples. Five Pasteurised samples showed significant contamination. There is strong evidence that the contaminating organisms in these samples were introduced by handling after pasteurization. The 53 (91%)

pasteurized samples which had no contamination at 4 hour remained uncontaminated for the remainder of the standing period of 12 hours. 41% of control samples already had significant growth after standing at room temperature for 4 hours.

It can be concluded that Pretoria Pasteurisation kills pathogenic and commensal bacteria in hand-expressed breast milk. Expressed breast milk which has undergone Pretoria Pasteurisation can be kept without refrigeration for up to 12 hours with minimal probability of bacterial contamination provided that it is kept in the pasteurization container and is not handled.

Introduction

The prevalence of HIV seropositivity at sites taking part in a pilot program for the prevention of mother to child transmission of HIV in South Africa is 30%¹. Antiretrovirals have been shown to effectively reduce mother to child transmission of HIV during the peripartum period, however there still remains a 10-15% risk of postnatal transmission via breastfeeding². One of the challenges facing the development of programs to reduce mother to child transmission of HIV in developing countries remains the problem of infant feeding. Formula feeding is not only expensive but is not always feasible because of lack of the necessary facilities and in areas of high infant mortality can potentially increase infant morbidity and mortality, not only in infants born to HIV infected women but also in others due to a spill-over effect. Several alternative feeding methods for infants of HIV positive women have been explored. One of the the alternative methods devised is Pretoria Pasteurisation³. This is a simple low cost method of heat treatment of expressed breast milk (EBM) which is suitable for use in a resource poor domestic setting and has been shown to inactivate HIV in the milk of infected women⁴. The method uses the passive transfer of heat from water heated to boiling point which is poured into an aluminum pot, to milk contained in a glass jar which is placed into the hot water. Milk temperatures between 56°C and 62,5° C are maintained for approximately 15 minutes.

Pretoria Pasteurisation has been implemented in several settings in South Africa. It has been implemented in several neonatal units to feed preterm infants born to HIV infected women. Preterm infants are susceptible to infections and if formula fed, lose the protection provided by breast milk and are also at increased risk of potentially fatal outbreaks of necrotising enterocolitis. Special preterm formula milks are also expensive. Pretoria Pasteurisation is also being used in breast milk banks in resource poor areas of South Africa. For many reasons, including transportation time, there is often a delay between expression of breast milk and consumption by the infant. When expressing for a preterm infant, the mother

may express more milk than is needed for a feed and the options are then to discard the remaining milk or to store it for a later feed. The storage of EBM is particularly useful for women who are not able to stay on the premises. Mothers who work outside the home may also wish to express milk to be fed to their infant by a caretaker while they are at work, and some of these women may be without refrigeration facilities.

Several studies have shown that EBM is commonly contaminated with commensal or pathogenic bacteria^{5,6}. Human milk contains macrophages and other immune factors which provide substantial antibacterial and antiviral properties. Ajusti showed that unprocessed EBM can be safely kept at room temperature for eight hours before bacterial contamination reaches unacceptable levels⁷. In a local study, Delport found unacceptable levels of pathogenic bacteria in hand expressed breast milk⁸. There has been debate about the effects of these bacteria on the healthy term neonate and what level of contamination should be considered acceptable.^{9,10} Bacterial contamination does, however, present a substantial problem to preterm neonates. Pathogenic contamination in EBM has been found by several investigators to be associated with infections and even death in preterm infants^{6,11}. Preterm milk differs in composition to term milk and may have less effective antibacterial properties, thus reducing the length of time for which it can be safely stored¹².

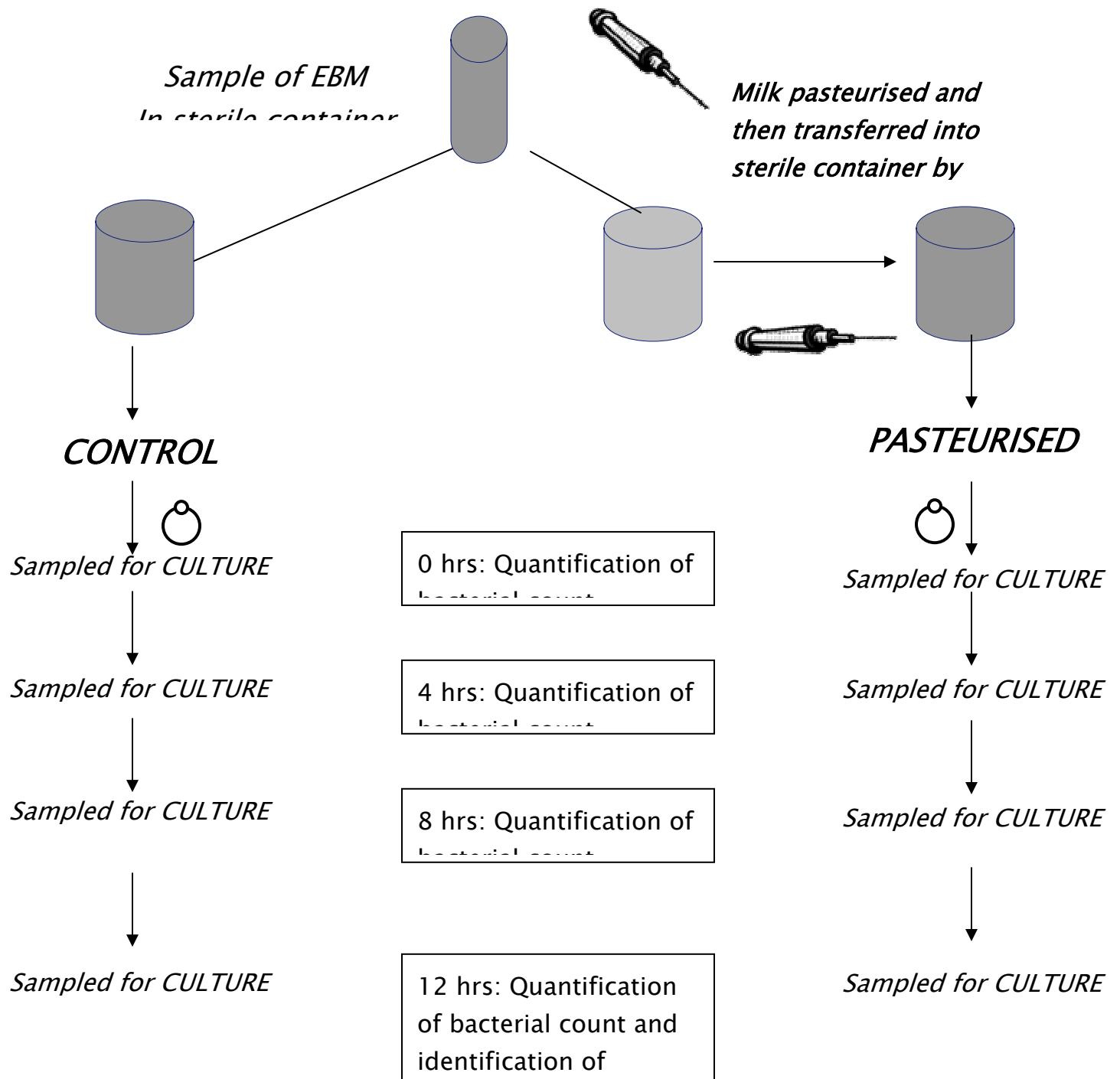
Heat treatment of EBM has been shown to kill common pathogens contaminating the milk. It is hypothesized that this may prolong the time for which the milk can be safely stored, however, heat treatment will kill macrophages and other cells in the milk thus reducing some of the intrinsic antibacterial properties and may thus allow for rapid regrowth of pathogens. This study sought to determine whether Pretoria Pasteurisation of EBM effectively eliminates commensal and pathogenic bacteria contaminating EBM and to determine the length of time for which EBM which has undergone Pretoria Pasteurisation can safely be kept at room temperature before the development of unacceptable bacterial contamination.

Methods

Women in the postnatal ward at a secondary hospital in Pretoria, South Africa were approached and invited to participate in the study by donating a sample of 30-50 ml of expressed breast milk. HIV serostatus was not used as a selection criterion and most women approached were of unknown serostatus. Women with clinical symptoms or signs of mastitis or other breast pathology were excluded from the study. Women were not reminded about handwashing or other hygienic measures before collecting the samples. All samples were hand expressed into sterile specimen containers.

Each sample of EBM was split into two portions. One portion of each sample acted as the control and was kept in the sterile specimen container with the lid closed. The other was transferred via sterile syringe to a glass jar in which it underwent Pretoria Pasteurisation. After pasteurization the sample was then transferred under sterile conditions into another sterile specimen container and the lid closed. The milk handler wore sterile latex gloves at all stages of milk transfer. The processing of the samples is shown in figure 1.

figure 1. Processing of specimens



The control and Pasteurised samples underwent baseline sampling for culture (immediately after expression for the control and immediately after pasteurization for the pasteurized specimen) and then stood at room temperature and were sampled again at 4, 8 and 12 hours. Semi-quantitative Colony Forming Unit (CFU/ml) counts were obtained on all cultures and all bacterial isolates were identified using standard microbiological techniques¹³.

End points

A bacterial culture was considered to be clinically significant if it contained a commensal with a colony count of 100 000 CFU/ml or greater or a known pathogen with a colony count of 1000 CFU or greater. Any growth of a gram negative organism was considered clinically significant. Numbers of significant cultures in the pasteurized samples were compared to their controls. Simple descriptive statistics were used.

The study was approved by the University of Pretoria Ethics committee.

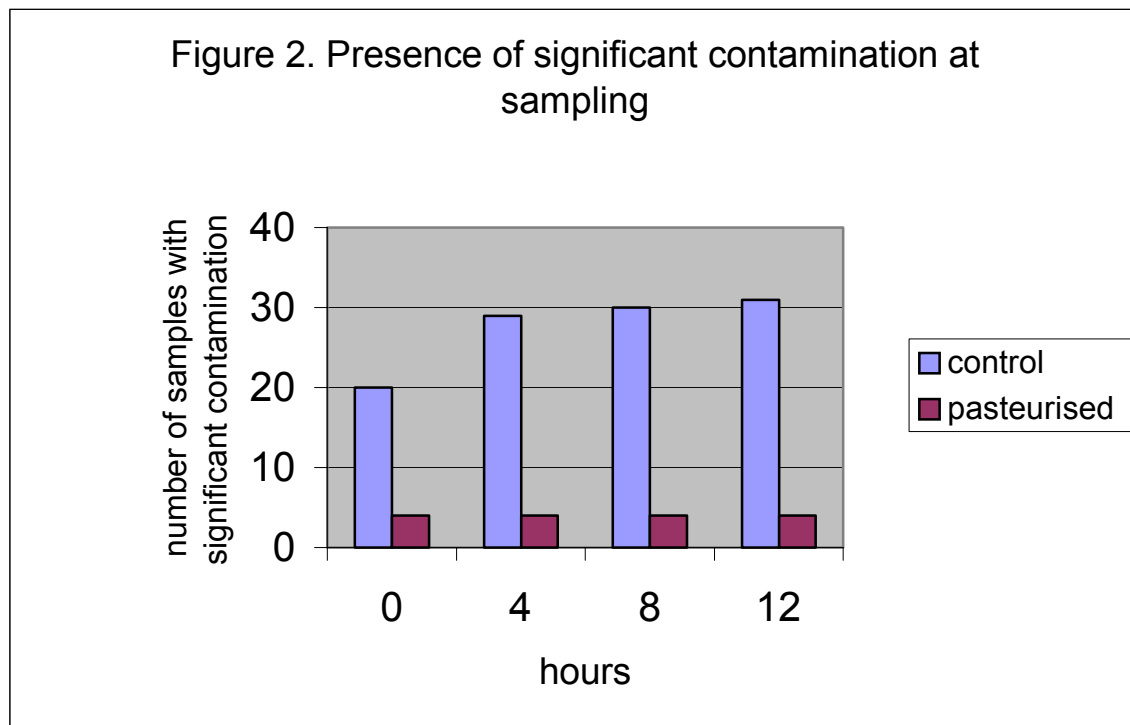
Results

A total of 58 samples were obtained, each of which was split into a control and pasteurized sample. The results are shown on table 1 and figure 2. 6.8% of the portions which underwent Pretoria Pasteurisation had bacterial growth considered to be clinically significant compared to 59% of the matching control portions ($P= 0.0000$, OR 0.0523, CI 0.01389- 0.178523). Up to and including the 12 hour sampling, 53 of the pasteurized samples remained sterile compared to only 5 of the controls ($p = 0.0000$ OR 0.0089, CI 0.001861 - 0.0373).

Table 1. Clinically significant growth of organisms from stored samples, comparison of Pasteurised compared to control portions.

	Pasteurized	Control
Significant growth	4	34
Nonsignificant growth or sterile	54	24
Total	58	58

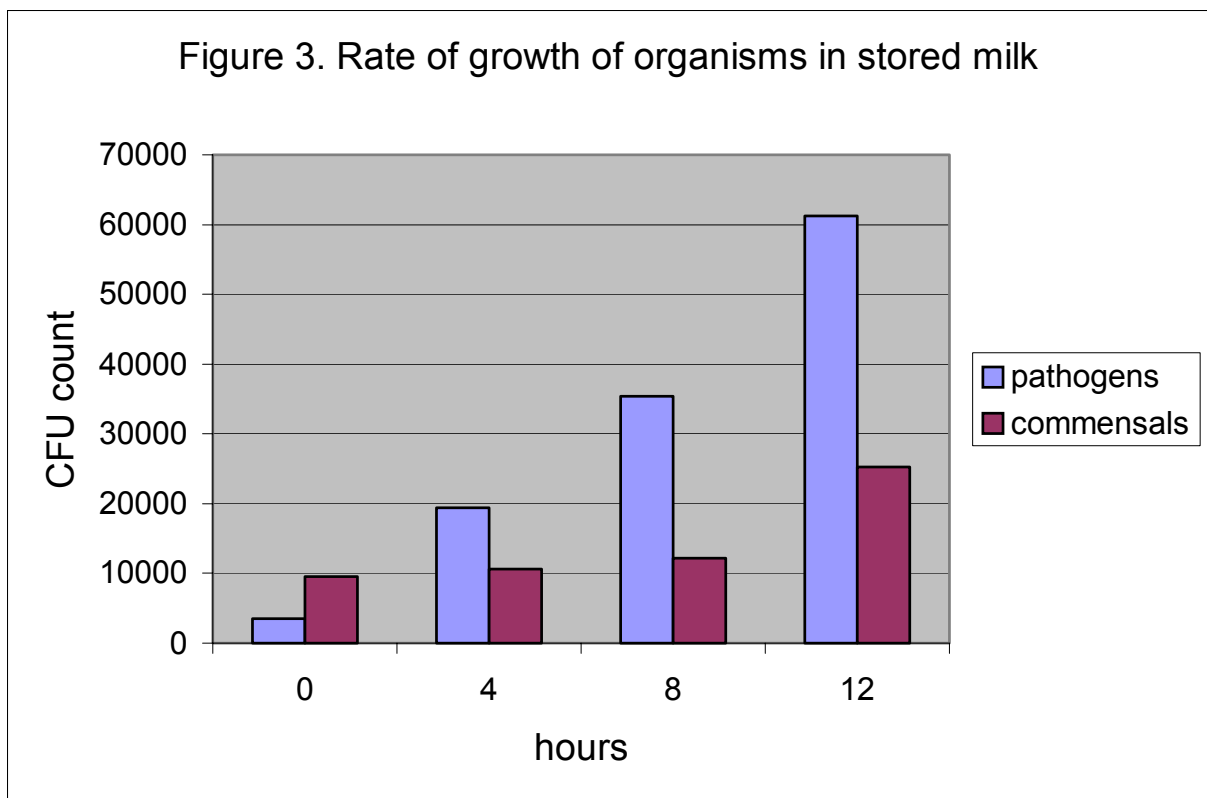
p=0.0000, OR 0.0523, CI 0.01389- 0.178523



Of the 34 control samples with significant growth, 21 contained more than one organism. 32 of the 34 samples with clinically significant growth had at least one pathogen, and two samples were considered clinically significant on the sole basis of high colony count for commensals. Of the four pasteurized samples which had clinically significant growth, all contained more than one pathogen. All of these samples were processed on the same day and all contained a

combination of the same three pathogens (which differed from the organisms grown in the corresponding control portions of these samples). One pasteurized sample showed growth of a commensal organism, but this did not reach clinically significant levels. Of the 24 control samples without clinically significant growth, 19 had growth but which was non-significant and 5 were sterile at all samplings up to 12 hours.

Of the 38 samples in both groups which had clinically significant growth, 28 (73%) already had unacceptable levels of contamination at the 4 hour sampling. The average CFU count at each sampling in the control samples is shown in figure 3. The rate of growth of pathogens was faster than that of commensal organisms and in 10 cases the CFU for the commensal dropped after the baseline sampling, in nine of them to zero at the 12 hour sampling. A drop in CFU count was found in only two pathogens.



Discussion

Pretoria Pasteurisation successfully eliminated contaminant bacteria in the expressed breast milk in all samples. This is shown by the fact that the organisms identified at baseline in the control portions were not identified at any sampling in the corresponding pasteurized portions. The four pasteurized portions which displayed pathogen growth all contained different organisms from the unpasteurised portions of those samples. The organisms grown from these four samples before and after pasteurization are shown on table 3.

Table 3. Organisms grown from contaminated pasteurized specimens and corresponding control portions

	Control portion	Pasteurised portion
Specimen 1	A1: Staphylococcus epidermidis	B1: Pseudomonas aeruginosa Klebsiella spp.
Specimen 3	A3: Staphylococcus epidermidis Streptococcus milleri	B3: Pseudomonas spp. Enterobacter cloacae
Specimen 4	A4: Staphylococcus epidermidis	B4: Pseudomonas aeruginosa Enterobacter cloacae
Specimen 6	A6: Klebsiella spp. Streptococcus viridans	B6: Enterobacter cloacae

This indicates that the pathogens grown from the pasteurized samples had not survived the pasteurization process, but must have been introduced after pasteurization. This could have happened if the apparatus used to sample the milk was contaminated and the organisms were introduced into all specimens sampled at that time. This is supported by the fact that growth in all four samples consisted of combinations of the same three pathogens, as well as the fact that these samples were all processed on the same day. They were also the first group of samples to be processed for this study, thus making a lapse in technique more likely. These samples displayed rapid bacterial growth with

CFU counts reaching >100 000 at the four hour sampling in three of them and at 8 hours in the fourth. Although no conclusion can be drawn from 4 samples, it would appear that there is the potential for rapid bacterial growth in pasteurized milk if contaminants are introduced after pasteurization. Hernandez et al have shown that pasteurization of expressed breast milk reduces the bacteriostatic activity of human milk against pathogenic bacteria when compared to unprocessed EBM. It is hypothesised that this is because macrophages and other protective factors would have been destroyed or reduced in quantity during pasteurization¹⁴. Because of the potential for bacterial contamination after pasteurization, milk that is to be stored after undergoing Pretoria Pasteurisation should not be handled after pasteurization. If too much milk is expressed for the infant to consume at one feed, the portion which is intended for storage should be pasteurized in a separate jar and kept sealed in that jar until use, thereby avoiding the possibility for introduction of pathogens after pasteurization.

Several investigators have recommended that raw expressed breastmilk can be safely stored without refrigeration for up to 8 hours⁷. In this study, 41% of samples already had unacceptable levels of bacterial contamination at 4 hours. This underlines the importance of hygienic measures before expressing. In this study women were not reminded to wash their hands before donating a sample of EBM.

Conclusion

Hand expression of breast milk without strict hygiene and hand washing can result in unacceptable levels of bacterial contamination in the milk. Pretoria Pasteurisation effectively kills commensal and pathogenic bacteria in expressed breast milk. Contamination of EBM after pasteurization can result in rapid colonization, and pasteurized milk should be stored sealed in the container in which it was pasteurized in order to avoid the introduction of bacterial contaminants. Milk which has undergone Pretoria Pasteurisation and is stored

in this way can be safely kept for up to 12 hours without refrigeration, provided it remains sealed in the pasteurizing container and is not handled after pasteurization.

References

1. Mc Coy D, Besser M, Visser R, Doherty T. Interim findings on the national PMTCT pilot sites. Lessons and Recommendations. Health Systems Trust. Durban. 2002
2. Dunn DT, Newell ML, Ades AE, Peckham CS. Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet* . 1992;340:585-588
3. Jeffery BS, Mercer KG Pretoria Pasteurisation: a potential method for the reduction of postnatal mother to child transmission of the Human Immunodeficiency Virus. *J Trop Ped*. 2000;46:219-223
4. Jeffery BS, Webber L, Mokhondo R, Erasmus D. Determination of the effectiveness of Pretoria Pasteurisation to inactivate Human Immunodeficiency Virus in human milk. *J Trop Ped*. 2001;47:345-349.
5. Olowe SA, Ahmed I, Lawal SF, Ransome-Kuti S. Bacteriological quality of raw human milk: effect of storage in a refrigerator. *Ann Trop Paediatr*, 1987;7(4):233-37
6. Garg AK, Rejaver RK, Al Hifzi I. Safety of expressed breast milk. *J Inf*. 1995;31:249-54
7. Ajusi JD, Onyango FE, Mutanda LN, Wamola A. Bacteriology of unheated expressed breast milk stored at room temperature. *East Afr Med J*. 1989;66(6):381-7
8. Delport SM, Videvs I. Bacterial contamination of expressed breast milk. Unpublished
9. Low BJ, Urias BA, Lertzman J, Robson D, Romance L. Is ingestion of milk-associated bacteria by premature infants fed raw human milk controlled by routine bacteriologic screening. *J clin microbiology*. 1989; 1560-66
10. Arnold L. contamination of expressed breast milk: a non-issue. *J human Lact*. 1997;13(4):273-4

11. Donowitz LG, Marsik FJ, Fisher KA, Wenzel RP. Contaminated breast milk: A source of Klebsiella bacteremia in a newborn intensive care Unit. *Reviews of Infectious diseases*. 1981;3(4):716-20.
12. Nwankwo MU, Offor E, Okolo AA, Omene JA. Bacterial growth in expressed breast-milk. *Ann Trop Paediatr*. 1988;8(2):92-5.
13. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology, Fifth edition, Lipincott.
14. Hernandez J, Lemons P, Lemons J, Todd J. Effect of storage processes on the bacterial growth-inhibiting activity of human breast milk. *Pediatrics* 1979;63(4):597-601.