AUCTORES
Globalize your Research

Journal of Clinical Pediatrics and Mother Health
André Léké *

Open Access

Research Article

Disposal of Donated milk is Unsuitable for Consumption: Effect on a Human Milk Bank's Ability to meet the needs of Hospitalized Preterm Infants

André Léké[1*], Sévérine Grognet[2], Guy Kongolo[3], Elion Dzon Bertin[4], Maurice Biendo[5]

[1]Unité de Soins Intensifs et Médecine Néonatale, CHU Amiens Picardie, France.

[2]Milk bank nurse and Technician, Biberonnerie, CHU Amiens Picardie, France.

[3]PhD, MD, Unité de Soins Intensifs et Service de Surveillance Continue, CHU Amiens Picardie, France.

[4]Student PhD, Service de Chirurgie Vasculaire et de Nutrition Parenterale, CHR de Lille, France.

[5]PhD, MD, Laboratoire Péritox, UMR 101 CURS, Université Jules Verne de Picardie, Amiens, France.

***Corresponding Author:** André Léké, Soins Intensifs de Néonatologie et Médecine Néonatale-Biberonnerie et Lactarium, CHU Amiens-Picardie Site Sud, F-80054 Amiens cedex 1 France.

Received Date: September 27, 2021; **Accepted Date:** October 05, 2021; **Published Date:** October 12, 2021

Citation: André Léké, Sévérine Grognet, Guy Kongolo, Elion Dzon Bertin, Maurice Biendo. (2021) Disposal of Donated milk is Unsuitable for Consumption: Effect on a Human Milk Bank's Ability to meet the needs of Hospitalized Preterm Infants. *J. Clinical Pediatrics and Mother Health*, 1(2); **Doi:** 10.31579/jcpmh.2021/014

Abstract

Background: Breast milk, produced after childbirth during the flow of milk, is food whose composition evolves to adapt to the newborn's needs. The purpose of this study was to evaluate the amount of non-compliant mother's milk donations during the study period.

Materials and Methods: We performed a prospective study of milk samples donated by home donors or mothers of hospitalized preterm newborns.

Results: The disposal milk was due to bacterial contamination in 91.6% of cases. The dominant bacteria detected in raw milk were E. coli in 4.2% of samples, S. epidermidis (4.2%), S. aureus (4.2%) and S. hominis (4.2%), and in pasteurized milk were Bacillus spp in 16.2% of samples followed by B. cereus (6.2%), Paenibacillus (6.2%), S. hominis (5%) and P. aeruginosa (5%).

Conclusion: The role of the milk bank is to provide adequate nutrition for preterm infants. Pasteurized breast milk intended for preterm infants must be bacteriologically consistent with consumption.

Keywords: human milk bank; breastfeeding; neonatal intensive care unit; donor milk; raw milk; pasteurized milk

Running title: Disposal of donated milk

Abbreviations

APUH: Amiens-Picardie University Hospital;

BM: breast milk;

CFU: colony-forming unit;

DM: donor milk;

FHPSA: French Health Product Safety Agency;

HMB: human milk bank;

HM: human milk;

MB: milk bank;

NDHPSA: National Drug and Health Product Safety Agency;

nICU: neonatal intensive care unit;

Yrs: years

Introduction

Healthy breastfeeding mothers and mothers of preterm babies should send their milk to human milk banks (HMB) [1]. The main missions of the HMB are to collect, screen, store, process, pasteurize, and distribute the woman's milk and promote breastfeeding [2]. Another role of HMB is to obtain enough milk to meet the needs of preterm infants or sick newborns admitted to the neonatal intensive care unit (nICU) [3]. Donor women are screened for Human immunodeficiency virus (HIV-1 and HIV-2); Human T-cell leukemia virus (HTLV-1 and HTLV-2); Hepatitis B virus (HBV), Hepatitis C virus (HCV), and syphilis [2].

The need for milk bank (MB) use is globally increasing with more preterm newborns [4]. Personalized milk donation gives the hospitalized newborn the possibility to benefit from their mother's milk, either directly in the form of raw milk (unpasteurized) that the mother expresses and keeps at home or in MB, or after pasteurization by an MB. This practice depends on the gestational age and the stage of lactation, ensuring an adequate composition of donated milk to preterm infants [5].

Human milk (HM), apart from its nutritional qualities, has immunocompetent functions particularly suitable preterm children. HM is known as the most complete nutrition for infants worldwide [6]. If a mother cannot breastfeed her child, either because she is not available or because of the milk produced is insufficient, the best alternative is breast milk (BM) from a healthy breastfeeding mother or milk of HMB [4, 7]. Therefore, in 2005, in the MB, the milk was processed according to the Ministry of Health guidelines [(French Health Product Safety Agency (FHPSA) and National Drug and Health Product Safety Agency (NDHPSA)] [8]. Moreover, the bacteriologic quality of raw milk given to a very preterm infant, even when provided by his/her mother, has to be checked carefully because the newborn's immature immune system may not be able to combat bacterial contamination. The milk's fat, protein, and carbohydrate contents are checked to ensure that the nutritional profile is appropriate for a very preterm baby. The purpose of this study was to evaluate the amount of the donated milk non-compliant for consumption during the study period, as well as the reasons for the disposal of these donated milk samples.

Materials and Methods

Ethical considerations

The study was approved by Ile de France3 Ethics Committee of Amiens-Picardie University Hospital (APUH) (Ref DSLILG/2018-039). Study participants were all breastfeeding mothers, who voluntarily decided to participate in the study. These mothers provided their written informed consent to participate before the study's initiation.

Study design and donor population

A prospective study was undertaken on milk samples of healthy breastfeeding mothers. These mothers gave their extra BM to other babies out of their free will. The donors also include mothers attending well-baby clinics, mothers whose babies are in nICUs, and those who have lost their babies. Lactating donors were home donors or mothers of preterm newborns hospitalized in the nICU at APUH from January 1st, 2019 to December 30th, 2020.

During this period, a total of 154 lactating mothers who have their newborns hospitalized at the nICU and produced an excess of milk for their infants were carefully selected and included in this study. Most of them delivered prematurely (84.4%, 130/154), but some had term babies (15.6%, 24/154). Out of 154 milk donors, 112 (72.7%) were personalized, and 42 (27.3%) were anonymous donors. Each mother had a questionnaire to check for absence of formal contraindications to donation (tabacco use, alcohol consumption, blood transfusion) or a positive serologic test for HIV1, 2; HTLV1, 2; HBV, HCV, and Treponema pallidum [1], and, if all requirements are met, the donor is eligible as a milk donor. These women donated a total of 2,000 ML of milk to HMB, including 1,800 ML of personalized milk (90%) and 200 ML of anonymous milk donation (10%). The total quantity of distributed milk was 1,400 ML (70%), and 600 ML (30%) were discarded (Table 1).

Human milk bank generally follows standardized procedures for the collection and handling of donated milk [1, 9]. Donors are instructed by the MB in breast cleaning and breast pumping procedures. The donor obtains milk by mechanical pump or manual expression and stores it in the freezer compartment of their home refrigerator before delivery to the MB. The milk is transported to the MB either by the mother herself or by a transport service provided by the MB. The milk from 4 donors is pooled in individual 100 ML bottles and stored at -20 °C until performed. Pooling serves the purpose of estimating macronutrients, such as fat, protein, carbohydrate, and energy, using infra-red spectroscopy technology [9].

Microbiological testing

Raw and pasteurized milk samples were estimated. They were plated onto 5% Columbia blood agar for facultative anaerobes and onto mannitol salt agar. Following the Holder pasteurization method [10], a final bacteriological check is carried out after pasteurization, by inoculation onto 5% Columbia blood agar incubated aerobically at 35±2 °C 48 times. The raw milk was unsatisfactory when the total viable facultative anaerobe count was $\geq 10^6$ CFU/ML, the S. aureus count $\geq 10^4$ CFU/ML, and the total viable facultative anaerobe count in the pasteurized milk $\geq 10^1$ CFU/ML [11].

Results

A total of 220 milk samples from 154 breastfeeding mothers were studied, including 140 raw and 80 pasteurized milk samples. Of those, 15.7% (22/140) of raw milk samples, and 46.3% (37/80) of pasteurized milk samples were culture-positive.

During the study period, a total of 750 newborns received BM, and the common indication of recipients was prematurity (100%). Among them, five had malabsorption syndrome (0.6%), three had an intolerance to BM (0.4%), and two had congenital defects (0.2%). The mean birth weight (BW) of the preterm infants was 1150.02±442.7 g, and the median 992.2 g (range: 590—2,600 g). An infant can be classified in extremely low birth weight (ELBW) (<1,000 g); very low birth weight (VLBW) (<1,500 g), and low birth weight (LBW) (<2,500 g) [12]. The mean gestational age was 28.01±4.75 weeks, and the median 28 weeks (range: 20—36 weeks). An infant can be classified as extremely preterm (<28 weeks), very preterm (28 to<32 weeks), and moderate preterm (32 to <37 weeks) [13]. The mean age of mothers' milk donors was 30±6.43 years (yrs), and the median 31 yrs (range: 16—44 yrs).

In our hospital, the proportion of discarded donated milk increased yearly, 18% in 2016, 25% in 2017, and 29.4% in 2018. The donated milk discarded for bacterial reasons during the same periods was 86.8%, 87.5%, and 86.4%, respectively (unpublished data). The current results have shown that the quantity of mother's milk between 2019 and 2020 was 2,000 ML, 1,400 ML (70%) of whom were distributed and 600 ML (30%) thrown, 550 ML (91.6%) for bacterial reasons and 50 ML (8.4%) for other causes (Table 1).

Number of milk donors	Personalized milk donors	Anonymous milk donors	Total of milk collected (ML)	Personalized milk donations distributed (ML)	Anonymous milk donations used(ML)	Total milk distributed (ML)	Total milk discarded (ML)	Milk discarded due to bacterial causes	Milk thrown due to other reasons
154	112 72.7%	42 27.3%	2000	1800 90.0%	200 10.0%	1400 70.0%	600 30.0%	550 91.6%	50 8.4%

During this period, there were 154 milk donors (112 personalized milk donors and 42 anonymous milk donors), this represented 2000 ML of milk of which (70.0%) were distributed and 600 ML (30.0%) were discarded of whom 550 ML (91.6%) due to bacterial causes and 50 ML (8.4%) due to other reasons

Table 1: *Volume of milk (in ML) collected, distributed, and discarded between 2019 and 2020*

The most common reason for discarding raw milk was bacterial contamination. All raw milk samples had bacterial load ranging from $1.5X10^6$ to $1.2X10^7$ CFU/ML. The types of bacteria isolated in raw milk cultures were the following: Escherichia coli (E. coli) (4.2%), Staphylococcus epidermidis (S. epidermidis) (4.2%), Staphylococcus. aureus (S. aureus) (4.2%), Staphylococcus hominis (S. hominis) (4.2%), and Enterobacter cloacae (E. cloacae) (3.5%). These bacteria were detected at low levels in pasteurized milk, suggesting possible post-pasteurization contamination (Table 2).

Bacterial count in pasteurized milk samples ranged from $1.0X10^2$ to $1.2X10^3$ CFU/ML. The most frequent bacteria strains were the following: Bacillus spp. (16.2%), Bacillus cereus (B. cereus) (6.2%), Paenibacillus spp. (6.2%), Staphylococcus hominis (S. hominis) (5.0%), and Pseudomonas aeruginosa (P. aeruginosa) (5.0%) (Table 2).

Most microbial types found both before and after pasteurization of milk samples have seen that the number of Gram-negative bacilli and Gram-positive cocci recovered decreased after pasteurization: E. coli, E. cloacae, S. epidermidis, S. aureus, and Staphylococcus lugdinensis (S. lugdinensis). Gram-positive bacilli include: Bacillus spp., B. cereus, Mycobacterium lacticum (M. lacticum), while Gram-negative bacilli include: Stenotrophomonas maltophilia (S. maltophilia), and P. aeruginosa. Gram-positive cocci include: S. hominis, and Streptococcus salivarius (Str. salivarius), whose amount has increased after this process (Table 2). Such results have been observed by Cherif-Antar et al. [14].

This study also showed that the non-spore-forming Gram-negative bacteria heat-sensitive in both raw and pasteurized milk were predominantly represented by P. aeruginosa (5.0%), S. maltophilia (3.7%), E. coli (4.2%), E. cloacae (3.5), and Klebsiella pneumoniae (K. pneumoniae) (1.4%). The non-spore-forming Gam-positive bacteria included M. lacticum (2.5%) and Lactobacillus plantarum (L. plantarum) (1.2%). The psychrotrophic spore-forming bacteria heat-resistant isolated included Bacillus spp. (16.2%), B. cereus (6.2), and Paenibacillus spp. (6.2%) (Table 2).

Type of bacterial isolated	**Raw milk samples: n=140 No of strains (%)**		**Pasteurized milk samples n=80 No of strains (%)**	
Bacillus spp	0	0	13	16.2
Escherichia coli	6	4.2	2	2.5
Staphylococcus epidermidis	6	4.2	2	2.5
Staphylococcus aureus	6	4.2	2	2.5
Staphylococcus hominis	6	4.2	4	5.0
Enterobacter cloacae	5	3.5	1	1.2
Bacillus cereus	0	0	5	6.2
Staphylococcus lugdunensis	3	3.2	0	0
Stenotrophomonas maltophilia	3	3.2	3	3.7
Corynebacterium tuberculostearicum	2	1.4	0	0
Campylobacter coli	2	1.4	0	0
Klebsiella pneumoniae	2	1.4	0	0
Paenibacillus spp	0	0	5	6.2
Staphylococcus pasteurii	2	1.4	0	0
Streptococcus salivarius	1	0.7	3	3.7
Pseudomonas aeruginosa	1	0.7	4	5.0
Mycobacterium lacticum	0	0	2	2.5
Staphylococcus capitis	0	0	1	1.2
Lactobacillus plantarum	0	0	1	1.2
Bacillus subtilis	0	0	1	1.2
Aeromonas caviae	0	0	1	1.2
Moraxella oslensis	0	0	1	1.2
Acinetobacter pittii	0	0	1	1.2
Acinetobacter johnsonnii	0	0	1	1.2

Six pasteurized milk samples were contaminated by more than one species: *Bacillus* spp+ *Acinetobacter pittii* (n=1), *Bacillus cereus* + *Paenibacillus* spp (n=1), *Klebsiella pneumoniae* + *Acinetobacter pittii* (n=1), *S.maltophilia* +*Acinetobacter pittii* (n=1), *Streptococcus salivarious* + *Moraxella osloensis* (n=1), and *Bacillus cereus, Pantoea agglomerans*+ *Acinetobacter ursinguii* (n=1).
Three raw milk samples were contaminated by more than one species: *Staphylococcus hominis* + *Escherichia coli* (n=1)*; Staphylococcus hominis* + *Stenotrophomonas maltophilia* (n=1)*; Staphylococcus aureus* + *Enterobacter cloacae* (n=1)

Table 2: *Results of bacterial strains isolated from raw and pasteurized milk samples by culture*

Discussion

The bacterial load detected in raw milk samples ranged from $1.5X10^6$ to $1.2X10^7$ CFU/ML and was slightly lower than that found by Banik et al. ($5.2x10^2$ to $1.3X10^7$ CFU/ML) [15]. The results of bacterial count observed in pasteurized milk agree with those by Banik et al. ($1.2X10^2$ to $1.8X10^1$ CFU/ML) [15].

The most frequent reason for discarding both raw and pasteurized milk was the non-compliance of quality (collection procedures not complying with guidelines, the lack of information on the label concerning the donor's identity, the date of collection, and any medications that the donor may have been taking) [16].

The presence of the aforementioned bacteria suggested that the milk samples had been contaminated by people and environmental sources. High bacterial counts indicated that the bacteria had not only contaminated the milk but had grown and multiplied in it [17].

The big problem in ensuring a suitable shelf life of pasteurized milk is to destroy, by pasteurization, a sufficient proportion of the heat-resistant flora, such as Streptococcus, Lactobacillus spp., Paenibacillus spp., and Bacillus spp. and to minimize the contaminating flora, such as coagulase-negative Staphylococcus, S. aureus, and Enterobacteriaceae [15, 18]. Different bacterial species can enter the udder through the teat canal and are excreted with the milk. By developing in the udder, some germs, particularly Staphylococci, Streptococci, and Enterobacteriaceae, cause milk contamination.

There are several reasons for the occurrence of bacterial contamination in the pasteurized milk samples, such as a defect in pasteurization machinery, or contamination in the post-pasteurization due to a lack of respect for the hygienic conditions defined in the pasteurization protocols by the personnel.

Some bacteria, such as E. coli, S. aureus, and Bacillus spp. produce enterotoxins, extracellular enzymes, heat-resistant spores, and can form biofilm [19]. They remain active after pasteurization, even though bacteria have been eliminated. Bacteria within biofilms may attach to tools and equipment, thus persisting in the dairy environment. Biofilms are considered a source of microbial contamination leading to food spoilage and shelf-life reduction of foods, which can be a way of pathogen transmission.

According to literature data, a variety of pathogenic bacteria have been isolated from raw milk, including Mycobacterium spp., Salmonella spp., Listeria monocytogenes, B. cereus, Campylobacter jejunii, Yersinia enterocolitica, E. coli, S. aureus, Brucella abortus, Klebsiella spp., Proteus spp., and P. aeruginosa [20-22]. These results are different from those found in the present work. Depending on the country of origin, species, climate, and sanitary conditions, raw milk can contain one or more pathogens listed above.

Gram-negative bacteria detected during the processing of donated BM are generally heat-sensitive protease and lipase producers and can cause product deterioration. P. aeruginosa is a psychrotrophic bacterium that lives in cold water, refrigerated milk, and the soil. It is also found as a commensal in the digestive tract. The effective adaptation to cold is probably due to the presence of many unsaturated lipids in the cell membranes [23-25]. The presence of indicator Gram-negative bacteria and some other bacteria in lesser numbers determines the safety and quality of milk and its products [26-28].

In this study, we paid more attention to the quality of BM, including infection control and nutrition density. Some HMB provides fresh-frozen raw milk to infants, and only samples containing S. aureus, Klebsiella spp., Enterobacter spp., Serratia spp., E. coli, or a bacteria count of $>10^4$ CFU/ML) were pasteurized and supplied when germ-free [29]. At present, however, we still use the protocols for milk pasteurization that meet the FHPSA and NDHPSA criteria [8]. All DM was provided after pasteurization and was germ-free. The bacteriological screen of DM pre- and post-pasteurization resulted in approximately 30% of DM discarded in our MB between 2019 and 2020. We then applied rigorous screening standards because safety should always be paramount. Following the FHPSA and NDHPSA criteria for bacterial screening, only samples $\geq 10^6$ CFU/ML of microorganisms or $\geq 10^4$ CFU/ML of S. aureus or Enterobacteriaceae were discarded before pasteurization. That would allow taking corrective measures to increase the volume of collected milk available for consumption and reduce the unnecessary waste of valuable milk.

Most post-pasteurization psychrotrophic spore-formers bacteria found in this study have been reported in the literature [24, 25]. In this category, Bacillus spp. is predominant and can be introduced into milk from water used for rinsed milking machines and equipment. It is an environmental germ, ubiquitous, spore-former, and heat-resistant. These spores remain viable and give rise to vegetative forms (germination) and can develop toxins. Bacillus spp. produces degradation enzymes such as protease, lipases, and phospholipases, which can deteriorate dairy products.

The presence of pathogens in pasteurized milk may be due to the dysfunction of the pasteurization equipment or insufficient pasteurization temperature and time, untimely handling of the milk after pasteurization [17]. Raw milk may be a prime source of psychrotrophic spore-formers during milk transport, storage, and handling. Additionally, poorly cleaned milk-processing equipment, both raw and post-pasteurized types of milk are a potential source of these organisms, as the latter is more likely to withstand the rigor programs of cleaning [30].

Conclusion

This study shows that the microbiological quality of most of the raw and post-pasteurized milk samples studied were not satisfactory for consumption as indicated by their high loads. As corrective measures, following the results of this work, we decided to strictly and rigorously apply the criteria defined in the FHPSA or NDHPSA guidelines. Further work will allow us to assess the effectiveness of the measures recommended regarding the reduction in the amount of milk bank discarded.

Conflict of interest

The authors declare that they have no conflict of interest

References

1. Bharadva K, Tiwari S, Mishra S, Mukhopadhyay K, Yadav B, Agarwal RK, et al. Human milk banking guidelines. Indian Pediatr 2014; 51: 469-474.
2. Halden N, Ziegler EE. Human milk banking. Ann Nutr Metab 2016; 69 (suppl 2): 8-15.
3. Eidelmen AI, Schanler RJ. Breastfeeding and the use of human milk. Pediatr 2012; 129: e827-e841.
4. Underwood MA. Human milk for the premature infant. Pediatr Clin N Am 2013; 60: 189-207.
5. Sanchez Luna M, Caballero Martin S, Sanchez Gomez-de-Orgaz C. Human milk bank and personalized nutrition in the NICU: a narrative review. Eur J Pediatr 2021; 180: 1327-1333.
6. Keim SA, Hogan JS, McNamara KA, Gudimetla V, Dillon CE, Kwiek JJ, Geraghty SR. Microbial contamination of human milk purchased via the internet. Pediatr 2013; 132: 1227-1235.

7. Arnold LDW. Human milk in the NICU policy into practice. Ontario Jones and Bartlett Publishers. 2010.
8. French Health product Safety Agency (FHPSA) or National Drug and Health Product Safety Agency (NDHPSA). Hygiene recommendation for preparation and storage of baby bottles. July 2005. P. 116 (NosoBase n° 16092).
9. Léké A, Grognet S, Deforceville M, Goudjil S, Chazal C, Kongolo G, et al. Macronutrient composition in human milk from mothers of preterm and term neonates is highly variable during the lactation period. Clin Nutr Exp 2019; 26: 59-72.
10. Guerra AS, Mellinger-Silva C, Rosenthal A, Luchese RH. Hot topic: holder pasteurization of human milk affects some bioactive proteins. J Dairy Sci 2018; 101: 2814-2818.
11. Léké A, Goudjil S, Mullie C, Grognet S, Biendo M. PCR detection of Staphylococcal enterotoxin genes and exfoliative toxin genes in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains from raw human breast milk. Clin Nutr Exp 2017; 14: 26-35.
12. Quinn J-A, Munoz FM, Gonik B, Frau L, Cutland C, Mallet-Moore T, et al. Preterm birth: case definition & guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine 2016; 34: 6047-6056.
13. Howson CP, Kinney MV, Lawn J. Born too soon: the global action report on preterm birth. March of Dimes. PMNCH, save the Children. WHO 2012.
14. Cherif-Antar A, Moussa-Boudjemâa B, Didouh N, Medjahdi K, Belén Florez A. Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant. Dairy Sci Technol 2016; 96: 27-38.
15. Banik SK, Das KK, Uddin MA. Microbiological quality from different locations in Bangladesh. Stanford J Microbiol 2014; 4: 5-8.
16. Biendo M, Elion Dzon B, Goudjil S, Chazal C, Léké A. Influence of breast milk bacteria on breast milk macronutrient levels during lactation. Acta Pediatr 2019; 00: 1-2.
17. Mubarack HM, Doss A, Rangasamy D, Balachander Sl Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu. Indian J Sci Technol 2010; 3: 61-63.
18. Chandan RC, Patel DA, Almeida RA, Oliver SP. Mammary gland and milk biosynthesis nature's virtual bioprocessing factory. In: Chandan RC, Kilara A, Sha NP, (2015).
19. Centola Vidal AM, Rossi Junior OD, de Abreu IL, Bürger KP, Cardoso MV, Siqueira Gonçalves AC, et al. Detection of Bacillus cereus isolated during ultrahigh temperature milk production flowchart through random amplified polymorphic DNA polymerase chain reaction. Ciência Rural, Santa Maria 2016; 46: 286-292.
20. te Giffel AMC, Wells-Bennik MHJ. Good hygienic practice in milk production and processing. In Improving the safety and quality of milk. Milk production and Processing Woodhead Publishing Series in Food Science, Technology and Nutrition 2010, p179-193.
21. Msalya G. Contamination levels and identification of bacteria in milk samples from three regions of Tanzania: evidence from literature and laboratory analyses. Vet Med Intern 2017. ID 9096149.
22. Sudhasaravanan R, Binukumari S. Microbial quality of raw and pasteurized milk samples collected from different regions of Madural Distric, (TN) India. J Environ Sci Toxicol Food Technol 2015; 9: 71-73.
23. Alves MP, Salgado RL, Eller MR, Vidigal PMP, de Carvalho AF. Characterization of a heat-resistant extracellular protease from Pseudomonas fluorescens 07A shows that low temperature treatments are more effective in deactivation its proteolytic activity. J Dairy Sci 2016; 99: 7842-7851.
24. de Oliveira GB, Favarin L, Luchese RH, Mclntosh D. Psychrotrophic bacteria in milk:how much do we really know? Brazilian J Microbiol 2015; 46:313-321.
25. Ivy RA, Raniery ML, Martin NH, den Bakker HC, Xavier BM et al. Identification and characterization of Psychrotolerant sporeformers associated with fluid milk production and processing. Appl Environ Microbiol 2012; 78: 1853-1864.
26. Uddin MA, Haque HMMU, Noor R. Isolation and identification of pathogenic Escherichia coli, Klebsiella spp and Staphylococcus spp in raw milk samples collected from different areas of Dhaka city. Bangladesh Stamford J Microbiol 2011; I: 19-23.
27. Armesto MRG, Sutherland AD. Temperature characterization of psychrotrophic and mesophilic Bacillus species from milk. J Dairy 1997; 64: 261-270.
28. Seraug T, Stepaniak L. Psychrotrophs and their enzymes in milk and dairy products: quality aspects. Trends in Food Sci Technol 1997; 8: 35-40.
29. Lindemann PC, Foshaugen I, Lindeman R. Characteristics of breast milk and serology of women donating breast milk to a milk bank. Arch Dis Child Neonatal Ed 2004; 89: F440-F441.
30. Bogo M, Cruz KL, Revello AG, Correa APF, Brandelli A, et al. Thermal resistance of proteolytic enzymes produced by psychrotrophic bacteria isolated from Buffalo milk. J Dairy Sci 2017; 12: 339-347.

DOI: 10.31579/jcpmh.2021/014