Revista de Saúde Pública
ISSN 0034-8910 versão impressa

Rev Saúde Pública 2003; 37(6)

Microbiological quality of human milk from a Brazilian milk bank

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ABSTRACT

Objective

The objectives of the present study were to determine the prevalence of potentially pathogenic microorganisms that indicate the hygienic and sanitary conditions of human milk samples collected at a Human Milk Bank.

Methods

Three hundred and thirty eight (338) samples of human milk collected from a milk bank in a maternity in the municipality of Goiânia, in the state of Goias, Brazil were submitted to microbiological analysis. The latter were plated on McConkey agar according to the type of bacteria. Among the total number of samples collected, 194 consisted of raw milk and the remaining 144 were pasteurized milk.

Results

The presence of Staphylococcus spp., Streptococcus spp., yeasts and molds, and Enterobacteriaceae was verified in the raw milk samples. Staphylococcus aureus were isolated in 10 (5.2%) samples, Staphylococcus epidermidis in 28 (14.4%) samples, Streptococcus spp. in three (1.6%) samples, yeasts and molds in 43 (22.2%) and Enterobacteriaceae in 49 (25.3%) samples. In a hundred and forty four (144) samples which underwent thermal treatment Staphylococcus aureus was detected in five (3.5%) samples, Staphylococcus epidermidis in 15 (10.4%), Staphylococcus lugdenensis in two (1.4%), Streptococcus spp. in four (2.8%), yeasts and molds in 37 (25.7%), and Enterobacteriaceae in nine (6.3%).

Conclusions

Analysis indicated a high degree of contamination in raw human milk, and as for the pasteurized milk, despite elimination of the great majority of potentially pathogenic microorganisms, the percentage of yeasts and molds was higher than in raw milk, demonstrating that a lower degree of initial contamination would be necessary for pasteurization to be an efficient means of microbiological control.

Keywords

Milk banks. Milk, human. Quality control.

INTRODUCTION

Exogenous infections, originating from the hospital environment, deserve greater attention from public health professionals than endogenous ones, for human beings are the sources of the agents that provoke them. The former include hospital employees, patients and visitors as well as hospital instruments and equipment.[5] Since it is very difficult to implement measures to control hospital infections in all sectors of the hospital simultaneously, efforts should be directed primarily towards higher risk sectors, such as nurseries, nutritional services, surgical wards, intensive care units and centers of sterilization.

Among these sectors, particular attention should be given to the nursery, due to cross-contamination or to the transmission of pathogenic or potentially pathogenic microorganisms by means of milk stored in the human milk bank, which may be considered a potential microbiological risk factor. It is widely acknowledged that for breastfed infants, the first six months can be the healthiest period in their lifetime. Human milk fulfills all of the baby's nutritional and hygienic requirements.[25] However, premature newborns do not have the strength to suckle their mother's breasts and must be fed by other means. Furthermore, due to physiological or emotional problems, some mothers are unable to produce milk. On the other hand, milk derived from other animals may cause allergies. For these as well as for other reasons, many infants are fed milk from human milk banks (HMB), supplied by voluntary donors who produce a surplus.

The microbiological quality of expressed milk distributed by these milk banks is a public health issue, for the children who will consume this product have low resistance to neonatal infections. [1,2,13,21] The most important issue concerning HMBs is the bacteriological control of donated milk, [11] for consumption of contaminated human milk may be the cause of neonatal diseases.[23]

It is important to obtain more epidemiological data on bacterial contamination of human milk and the development of educational work with the mothers, nurses, nurses' aides, nutritionists, pediatricians, and intensive care physicians, must be carried out so as to make them aware of the risks involved in the preparation and consumption of human milk.[21] The purpose of this study is to investigate the prevalence of microorganisms in milk being offered by a human milk bank so as to subsidize the competent authorities conducting programs for the prevention of neonate infections.

METHODS

Three hundred and thirty eight (338) samples of human milk were collected from a milk bank in a maternity in the municipality of Goiânia, in the state of Goias. Among the total number of samples collected, 194 consisted of raw milk and the remaining 144 were pasteurized milk. These samples were packaged and sent immediately to the Laboratório de Microbiologia de Alimentos do Departamento de Microbiologia do Instituto de Patologia Tropical e Saúde Pública de Goiânia (Laboratory of Food Microbiology of the Department of Microbiology of Tropical Pathology and Public Health of Goiania), where they were submitted to microbiological analysis.

Due to the quantity of samples and the fact that the material in question was produced by secretion, procedures adopted for microbiological analysis were those indicated by Koneman et al[14] (1997) with samples being plated initially on blood agar and McConkey's agar and, according to the type of bacteria isolated, posterior identification was undergone in appropriate mediums. Bacteria pertaining to the *Stapphylococci* gender were tested for their capacity to produce coagulase and resistance to novobiocin. As to the *Streptococci*, based on hemolysis, tests of resistance to bacitracin, optochin and other biochemical tests were conducted. In order to identify gram-negatives, screening was undertaken in triple sugar iron (TSI) agar and identification was obtained by means of biochemical tests. Molds and yeast were identified by means of a technique indicated by the American Public Health Association[25], which consisted in plating the samples on a 10% potato dextrose agar, acidified by tartaric acid until the pH was 3.5, and incubating it at 25° C± 1 for 5 to 7 days.

RESULTS

Potentially pathogenic and/or commensal organisms were identified in 70.4% (136 /194) of the raw milk samples and 50.7% (73/144) of the samples of pasteurized milk (see Table 1).

Table 1 - Percentage of positive and negative samples found in raw and pasteurized expressed human milk (EHM) from a milk bank in Goiania, GO.

Microorganisms	Not pasteurized EHM		Pasteurized EHM	
	N	%	N	%
Positive	136	70.1%	73	50.7%
Negative	58	29.9%	71	49.3%
Total	194	100%	144	100%

EHM– Expressed Human Milk

Among the samples of raw milk, *Streptococci* of the viridans group were isolated in three (2.2%); 10 samples (7.35%) presented colonies of *Staphylococcus aureus*; *S. epidermidis* were isolated in 28 (20.59%) samples; and 49 (36.0%) samples were contaminated by *Enterobacteriaceae*. Furthermore, 43 (31.6%) samples of raw milk were contaminated by molds and yeast (see Table 2).

Table 2 - Distribution of the results of microbiological analysis of the samples of expressed raw and pasteurized human milk, collected in milk banks from 1999 to 2001, in Goiania, GO.

Microorganismo	Not pasteurized EHM			Pasteurized EHM		
		analyzed	positives		analyzed	positives
	N	%	%	N	%	%
Staphylococcus aureus	10	5.2	7.4	5	3.5	6.9
S. epidermidis	28	14.4	20.6	15	10.4	20.6
S. lugdenensis	-	-	-	2	1,4	2,7
Streptococcus viridans group	3	1.6	2.2	2	1.4	2.7
S. pyogenes	-	-	-	2	1.4	2.7
Klebsiella sp	3	1.6	2.2	1	0.7	1.4
K. oxitoca	3	1.6	2.2	1	0.7	1.4
K. rhinoscleromatis	6	3.1	4.4	-	-	-
K. pneumoniae	4	2.1	2.9	2	1.4	2.7
Enterobacter sp	1	0.5	0.7	-	-	-
E. aerogenes	2	1.0	1.5	-	-	-
E. agglomerans	3	1.6	2.2	-	-	-
E. cloacae	12	6.2	8.8	3	2.1	4.1
E. hormaechei	2	1.0	1.5	-	-	-
Escherichia coli	3	1.6	2.2	-	-	-
Citrobacter freundii	1	0.5	0.7	-	-	-
C. diversus	1	0.5	0.7	-	-	-
Morganella morganii	1	0.5	0.7	-	-	-
Serratia marcescens	3	1.6	2.2	-	-	-
S. liquefaciens	-	-	-	1	0.7	1.4
Proteus mirabilis	1	0.5	0.7	1	0.7	1.4
Hafnia alvei	2	1.0	1.5	-	-	-
Pantoea sp.	1	0.5	0.7	-	-	-
Pseudomonas sp.	3	1.6	2.2	1	0.7	1.4
Molds and yeast	43	22.2	31.6	37	25.7	50.7
Total: analyzed samples	194	100	-	144	100	-
Total: positive samples	136	70.4	-	73	50.7	-

Microorganisms were identified in seventy-three of the 144 samples of pasteurized milk from the HMB. Among these, *Staphylococcus lugdenensis, Streptococcus pyogenes*, and *Streptococcus* of the *viridans* group were isolated in two (2.7%) samples; *Staphylococcus aureus* in five (6.9%) samples; *S. epidermidis* in 15 (20.6%) and *Enterobacteriaceae* were detected in 12.3% of the samples. Furthermore, 37 (50.7%) samples tested positive for molds and yeasts (see Table 2).

DISCUSSION

The presence of high levels of contaminants in raw human milk entails in a reduction of its biological benefits because its nutrients are being utilized by the microflora present in the milk, thus diminishing its protective immunological qualities.[2] These situations, in the majority of cases, lead to the classification of this product as improper for consumption, taking into account the vulnerability of the recipient infants. Furthermore, the greater the quantity and diversity of microorganisms present in the milk, the less efficient will the outcome of the process of pasteurization be. It may be noted that there is a great gap in the criteria for selection of milk for pasteurization.[3]

The prevalence of *Staphylococcus aureus* in the oropharynx of human beings varies from 35to 40% and in the mouth and saliva its prevalence varies from 10 to 35%. However, it's presence in human milk may be interpreted as being due to contamination from a secondary source, such as the skin and nasal fossa, or unsatisfactory hygienic or sanitary conditions of the utensils employed in its manipulation. The greatest concern with respect to its presence is the occurrence of strains which produce toxins that are resistant to pasteurization.[3]

Pereira et al (1995) reported the presence of *Staphylococcus* in al the samples of maternal milk collected from 19 women with symptoms of mastitis. Among the 19 samples, 8 synthesized detectable quantities of enterotoxins, being that in some cases, it was observed that they also produced the toxin of the toxic chock syndrome.

As to the raw milk, the results presented in this study are similar to those reported by Caroll et al[7] (1979) who observed *S. aureus* in 13 (6.2%) of the 207 samples of expressed human milk. Other researchers found coagulase positive *Staphylococcus* in different proportions of human milk samples submitted to analysis: 28.1% (Nikodemuz[16]), 29% (Almeida et al[3]), 51.7% (Almeida et al[2]) and furthermore, among 7,570 samples, bacterial growth was observed in 230, being that 40% of these were *S. aureus*.[8]

Wyatt and Mata[26] (1969), isolated enterobacterias in 18% of the 51 samples of colostrum they submitted to analysis, revealing the low level of personal hygiene and sanitary conditions of the study population, living in the small village of Santa Maria Cauqui in Guatemala.

Other authors found *E. coli* in 8.5% of the 59 samples of maternal milk[22], in 2% of 44 samples[9], indicating faecal contamination of the milk, and enterobacterias in 15 (7%) samples. The present results reveal the presence of enterobacterias in 25.3% of the samples of raw milk analyzed and in 6.2% of the samples of pasteurized milk. Therefore, they presented inferior hygienic conditions than the studies cited above.

Among the enterobacterias, coliforms have been singled out as particularly important in bacteriological control of HMBs, since their presence may indicate faecal contamination, even if it originates from an indirect source and doesn't necessarily imply in the isolation of *E. coli*.[2] Research has indicated that contamination by coliform microorganisms may originate from the environment, as observed in a study in which this group of bacteria were isolated in 38.4% of a total of 472 samples[17], and among a total 5,710 samples of human milk, coliforms were detected in 1,139 (19.9%)[17] and in a total of 837 samples, 71 (8.48%) were contaminated with coliform microorganisms, whereas only three of these were *E. coli*.[18] In this study, coliforms were present in 21.1% of the samples of raw milk and in 5.6% of the pasteurized milk samples, indicating that the efficiency of pasteurization may be compromised by an initially high count of microbes.

The presence of molds and yeast in foods may also indicate contamination originating from the environment or resulting from handling in inadequate hygienic and sanitary conditions.[15] Almeida[4] (1986) found a 69.4% prevalence of fungus and yeast in samples obtained after employing the usual methods of hygiene (soap and water) on the mammary gland. Other researchers, however, observed

that 230 (6.5%) of a total of 7,570 samples analyzed contained molds and yeast.[8] The results of this study, in which molds and yeast were detected in 22% of the samples of raw milk, are inferior to those observed by other authors. However, the percentages of molds and yeast detected in the pasteurized milk (25.7%) are a matter of concern, for they indicate a possible environmental contamination occurring after the process of pasteurization or that this process may be ineffective in itself. The presence of pathogenic yeast in pasteurized human expressed milk suggests that this could be a source of infection to neonates during breastfeeding. Quality control by means of yeast counts in expressed human milk may be a good indicator of problems related to hygiene, storage or transportation.[21]

Inadequate pasteurization not only presumably endangers the beneficial properties of expressed human milk, but may also increase the susceptibility to subsequent contamination.[6]

The RDC (Resolução da Diretoria Coletiva) [Resolution of the Collective Directory] Resolution nº 12 , instituted by the Ministry of Health[14] in January, 2001, establishes criteria for the microbiological control of human milk for the first time in Brazil. According to this resolution, the quantity of viable mesophilic aerobic microorganisms permitted is up to 100 CFU/mL, coliforms at 35ºC and *Staphylococcus* coagulase-positive absent in 1 mL and *Salmonella sp* absent in 25 mL. According to this resolution, 49% of the samples analyzed in the present investigation were improper for human consumption, for they did not present the analytical parameters established in Annex 1 of the aforementioned Resolution. Taking into consideration the results obtained, the authors believe that efforts should be made to improve the microbiological control of expressed human milk, including the milk which is going to be pasteurized. In this sense, more rigorous measures for monitoring the quality of human milk are indispensable so as to guarantee safe feeding for neonates.

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Financiado pela Fundação de Apoio à Pesquisa/FUNAPE/UFG (Processo n 66.357).
Received on 23/1/2002. Reviewed on 27/6/2003. Approved on 3/7/2003.