Contamination of expressed human milk by mycelial fungi

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Abstract

Objective: to characterize the genera of mycelial fungi detected in expressed human milk received at the human milk bank of Instituto Fernandes Figueira after home collection.

Methods: we studied 821 expressed human milk samples randomly obtained from flasks filled by the donors at home. The possible presence of molds, yeasts and mesophilic microorganisms was investigated. A total of 48 strains of mycelial fungi were isolated from the human milk bank samples and identified through standard laboratory techniques.

Results: microbiological analysis revealed the occurrence of molds and yeasts in 43 samples (5.2%), with counts reaching 103 CFU/ml. The following microorganisms were identified: Aspergillus niger group (6.3%), Aspergillus sp. (4.2%), Paecilomyces sp. (12.6%), Penicillium sp. (60.4%), Rhizopus sp. (2.0%), and Syncephalastrum sp. (14.5%). Four samples showed the presence of more than one mycelial fungus type.

Conclusions: the presence of molds and yeasts in human milk manually expressed at home suggests that the hygiene conditions of the collection site may contaminate milk. Thus, when hospitalized priature babies receive the raw product, it is very important to observe the collection, storage and transport conditions in order to avoid the presence and consequences of contaminants increase.


Introduction

The human milk bank is a specialized center where the breastfeeding practice is encouraged and where collection, processing and quality control of colostrum, transition milk and mature milk are carried out for later use (according to the physician’s or nutritionist’s recommendations) by infants who depend on breastmilk for survival.1
At present, Brazil has the world’s largest network of human milk banks, totaling 151 units in 22 states. In the year 2000 alone, more than 90,000 liters of human milk were collected, benefiting more than 80,000 preterm and low-weight infants. At the time, approximately 60,000 donors were registered at the bank.2

In technological terms, human milk is an unstructured foodstuff, since it offers no physical protection against infectious microorganisms. Although human milk is the ideal food for infants, with all necessary nutrients (with appropriate quality and quantity), it can be an excellent culture medium for different kinds of microorganisms when its protection factors are no longer present.3 Mother’s milk obtained from healthy donors, submitted to a strict hygiene control, is free from pathogens. The presence of pathogens is associated with external contamination factors.3

There is scientific evidence of the efficacy and safety of human milk pasteurization in the elimination of pathogenic agents.4-6 However, the presence of mycelial fungi in expressed human milk can be a problem when the milk is to be used in natura, in case of mother’s milk given directly to the infant, as occurs at neonatal ICUs.

Fungi are a diversified group of organisms with remarkable ecological and economic importance. Approximately 70,000 fungal species have already been identified, but their total number is estimated at 1.5 million.7 These fungal species are of relevant importance since they are the primary decomposers in all terrestrial ecosystems, form important symbiotic associations with vascular plants (mycorrhiza), include most plant pathogens, provide genetic systems for molecular biologists and are essential to industrial biotechnology.7

However, these species of microorganisms are undesirable in foods, since they can produce a wide variety of enzymes that cause deterioration. On top of that, several fungi can produce toxic metabolites during their reproduction. These metabolites are generally called mycotoxins and, when eaten with foods, they cause harmful biological alterations, which can range from allergies to carcinogenesis.8

Since molds are largely present in nature, their occurrence in expressed human milk can be a sign of external contamination. In addition to deterioration, these microorganisms can produce mycotoxins.3

Part of the microbiological criterion used worldwide for screening milk-based foods consists in counting molds and yeasts.10 Since the available literature does not contain enough information on fungal microflora of technological interest for the preservation of expressed human milk, the present study aims at characterizing the genera of mycelial fungi found in samples of expressed human milk, thus contributing towards filling this gap in literature.

Methods

The study was carried out at the Human Milk Bank of Instituto Fernandes Figueira (BLH/IFF) with the aim of describing the occurrence of mycelial fungi in 821 individual samples of expressed human milk (EHM), obtained from 20% of the registered donors. The convenience samples were randomly obtained from the flasks received from each donor, in 262 different locations in the city of Rio de Janeiro, between October 1998 and January 2000.

The donors were asked to follow the health and hygiene protocol established by the Ministry of Health for the milking procedure(11). After milking, the milk was stored in flasks previously sterilized at 121°C for 15 minutes and kept at −6°C to −10°C, for at most 5 days, in the ice-box of the refrigerator or in the freezer at the donor’s house. The flasks were transported in isothermal boxes, which contained a thermometer for the measurement of minimal and maximum temperatures.

At the Human Milk Bank of Instituto Fernandes Figueira (BLH/IFF), the flasks were thawed and samples were taken for the screening of molds, yeasts and mesophilic microorganisms. Since collection was not closely followed, information such as time, lactation period, or whether the milk was obtained at the beginning or at the end of the milking procedure, was not taken into consideration.

The microbiological analyses were carried out at the laboratory of food control of Instituto Fernandes Figueira, and the identification of the strains of mycelial fungi was made at the Laboratory of Mycology of the Hospital Evandro Chagas Research Center (CPqHEC). Both laboratories belong to Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

The screening of molds and yeasts was carried out according to the method described by Marvin (1976). Samples of 1.0 ml of milk and of its decimal dilutions were plated in duplicate on potato dextrose agar (Merck) using the pour plate technique. After medium solidification, the plates were incubated at 25°C for five days. The colonies were counted and the results were expressed in colony-forming units per ml (CFU/ml).

Mesophilic bacteria were screened according to the method described in the Compendium of Methods for the Microbiological Examination of Foods.12 Samples of 1.0 ml of milk and of its decimal dilutions were selected and plated in duplicate on standard agar (Merck) using the pour plate technique. After medium solidification, the plates were incubated at 35°C for 48 hours. The colonies were counted and the results were expressed in CFU/ml. Mesophil counts were performed since mesophils presented a direct relationship with the total population of microorganisms and could be somewhat proportionate to the population of mycelial fungi, if both presented the same source of contamination.12

Mycelial fungi were initially isolated from the plates that presented mold and yeast growth. Typical colonies of
mycelial fungi were selected, plated on potato dextrose agar, and incubated at 25°C for 3-5 days. The pure cultures that were isolated were maintained in tubes with the same inclined agar up to identification.

The identification of mycelial fungi was made by taking the colonies from the primary isolation medium, inoculating them onto Sabouraud agar and keeping them at room temperature. After growth, the colonies with at least 10mm in diameter were used in the preparation of slides for direct microscopy. The colonies were stained with Aman’s lactophenol and/or cotton blue, and were bleached with NaOH at 10% so that the morphological structures of the fungi could be observed. The fragments were examined in a microscope and the morphologies were compared with those described in specialized literature.13

The statistical analysis involved two steps: frequency distribution analysis (for assessing the sociocultural profile of the donors) and the correlation test between the counts of mycelial fungi and mesophilic microorganisms, by using the System Analysis Statistical (SAS) software version 6.11, by SAS Institute Inc., Cary, NC, USA.

The study was based on the regulatory guidelines for human research (CNS 196/96 resolution).14 After approval by the Research Ethics Committee of Instituto Fernandes Figueira (IFF), the samples of expressed human milk were collected, with no change to the milking routine at the IFF milk bank. The collected samples were used only for microbial counts and for the isolation of strains of mycelial fungi.

The data on the sociocultural profile of the donors were directly obtained from the electronic file at the Human Milk Bank of Instituto Fernandes Figueira (BLH-IFF). There was no need to view the medical history of the donors in order to get additional information, or establish personal contact with them, thus preserving their identity. Therefore, we consider that donors were not exposed to any kind of risks.

**Results**

The sociocultural profile of the donors was as follows: nurturing mothers from different sociocultural background aged between 13 and 45 years, of which 2.1% were more than 40 years old, and 13.7% were aged between 13 and 19 years. With respect to the level of education, 334 (40.7%) donors had primary education, 254 (30.9%) had finished their secondary education, and 233 (28.4%) had a university degree.

Of the 821 analyzed samples of expressed human milk, 43 (5.2%) were contaminated with 48 strains of mycelial fungi. Three samples presented two strains and one sample, three strains, with different macroscopic aspects.

The results (Figure 1) show the presence of molds and yeasts, with counts up to $10^3$ CFU/ml, in addition to the presence of the following microorganisms: *Aspergillus niger* group (6.3%), *Aspergillus* sp. (4.2%), *Paecilomyces* sp. (12.6%), *Penicillium* sp. (60.4%), *Rhizopus* sp. (2.0%) and *Syncephalastrum* sp. (14.5%).

By studying the correlation between mold and yeast counts and the total populations represented by mesophil counts, we found a correlation coefficient ($r$) of 0.55, with a 95%CI: $0.32 < R < 0.72$, which does not show a strong correlation between these two groups.

**Discussion**

Expressed human milk, even when obtained from healthy women, following all hygiene procedures, may present normal-occurring contaminants. However, the presence of mycelial fungi of different species suggests that the health

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**Figure 1** - Count of molds and yeasts and mesophilic microorganisms found in the human milk samples
and hygiene care was not strictly followed during the milking procedure.

The results of the present study show the presence of molds and yeasts in only 5.2% of the samples, with counts up to 10^3 CFU/ml, suggesting that most donors follow the recommendations for collection and storage of expressed milk.

The fungal spores present on the foods handled by the donors are probably the source of the fungi detected in expressed human milk, since they are very similar to those found by other researchers in different foods.

A study on the presence of microorganisms in table butters sold in the city of São Paulo revealed that 94% of the samples contained the following fungi: Cladosporium (18%), Penicillium (12%), Geotrichum (8%), Aspergillus (6%), and Trichoderma (6%).

Alexandre et al. (1996) investigated 71 samples of dehydrated fruits sold in Santiago do Chile and observed that 45 (73.8%) presented fungal growth, which usually included Aspergillus, Rhizopus, Penicillium and Mucor.

Taniwaki et al. (1989) analyzed three apple cultivars produced in the state of São Paulo and isolated the following fungi: Cladosporium sp., Phoma sp., Fusarium sp., Trichoderma sp., Alternaria sp., Phomopsia sp. and Penicillium sp., in addition to other molds that could not be identified.

Of 30 samples of cassava flour bought from different stores in Niterói, state of Rio de Janeiro, Kraemer et al. (1998) isolated fungi of the following genera: Aspergillus (36.5%), Penicillium (18.2%), Rhizopus (10.5%), Paeclomyces (7.1%), Mucor (5.4%), Neurospora (3.1%), Cladosporium (2.3%), Aureobasidium (1.4%), Syncephalastrum (1.1%), Metarrhizium (0.8%), Trichoderma (0.3%), Trichosporon (0.3%) and Humicola (0.3%).

Costa et al. (1986) detected molds and yeasts in 11.7% of 2,533 samples of cow’s milk obtained from 32 properties in 18 towns in the state of São Paulo.

In the present study, the milking procedure was carried out at home by the donors themselves. We can infer that the hygiene of the donors or of the collection site also contributed to the presence of such contaminants. There is no doubt that the environment plays an essential role in the quality of expressed human milk, as widely discussed by Assis in the environment plays an essential role in the quality of expressed human milk. There is no doubt that hygiene of the donors or of the collection site also contributed to the presence of such contaminants. There is no doubt that hygiene of the donors or of the collection site also contributed to the presence of such contaminants.

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Almeida, in 1986, observed molds and yeasts in 69.4% of expressed human milk samples collected at Instituto de Viçosa; 1986.

References

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