Archives de Pédiatrie xxx (2018) xxx–xxx

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Research paper

Breastmilk donations: Bacteriological assessment, analysis of causes of non-compliance and suggestions for improvement

C. Mullié [a,b,*], O. Obin [a], G. Outurquin [a], S. Grognet [c], A. Léké [c], C. Adjidé [a]

[a] Unité d'hygiène et d'épidémiologie hospitalière, CHU d'Amiens-Picardie, 80054 Amiens cedex 1, France
[b] Laboratoire AGIR (AGents Infectieux, Résistances et Chimiothérapie), UFR de pharmacie, 3, rue des Louvels, 80037 Amiens cedex 1, France
[c] Lactarium–biberonnerie, unité des soins intensifs de néonatologie et de médecine néonatale, CHU d'Amiens-Picardie, 80054 Amiens cedex 1, France

ARTICLE INFO

Article history:
Received 6 June 2017
Accepted 18 February 2018
Available online xxx

Keywords:
Human milk
Human milk bank
Microbiological screening
Compliance
Pasteurization
Safety

ABSTRACT

A total of 1099 breastmilk donations received by the milk bank at the Amiens University Hospital from January to June 2016 were assessed for bacteriological contamination according to French regulations. This consisted in enumerating the total aerobic flora before and after heat treatment as well as the specific enumeration of coagulase-positive staphylococci. Results above the mandatory limits for at least one of these parameters were found in 25.9% of the donations, resulting in the destruction of approximately one-quarter of the volume of the donations (~195 L). This is a huge loss in both economic and health-related terms for neonates, especially for pre-terms. To identify ways to improve the bacteriological assessment results and reduce the percentage of discarded milk, an analysis of the causes was conducted. The two main causes of non-compliance were the detection of a cultivable aerobic flora after heat treatment and the presence of coagulase-positive staphylococci above the mandatory limit (11.7% and 11.2% of the tested donations, respectively). *Bacillus* spp. were the leading cause of post-heat-treatment non-compliance. Therefore, the implementation of better environmental control could help reduce this kind of contamination. As for samples harboring coagulase-positive staphylococci, a further detection of toxins using molecular biology techniques could help discriminate actual health-hazardous donations that have to be destroyed while enabling the use of toxin-negative donations. Nevertheless, the economic viability of this proposal needs to be further assessed because these techniques are costly. Finally, a change in breastmilk dilutions used to enumerate the total aerobic flora to better reflect the actual level of these bacteria in the milk was proposed. Indeed, the comparison of various combinations of milk dilutions led to the conclusion that the association of the 1/10 and 1/100 dilutions was the best compromise between technical ease of enumeration and ensuring the safety of the donations. Implementing these suggestions would help reduce the rate of non-compliance and give better access to safe breastmilk donations for neonates.

1. Introduction

The scientific and medical communities agree that breastmilk is the best food for neonates due to its capacity to fulfil nutritional requirements as well as its health-protective properties [1–3]. To enable the vast majority of neonates, especially pre-terms, to benefit from the advantages of breastmilk even when mothers are not able to directly breastfeed, breastmilk donations are allowed in France through a regulated protocol. These donations are managed through milk banks validated by the national health authority (*Agence nationale de sécurité du médicament et des produits de santé* [ANSM]) [4]. Breastmilk donations can be personalized (donation of a given mother to her child) or anonymous (donation of a given mother to a child/children other than her own). In each and every case, a bacteriological assessment of the donation is mandatory.

In France, this assessment is regulated by public health legislation [4,5]. It has been designed and is performed to protect neonates, especially pre-terms, from a possible source of infection and/or intoxication. Indeed, several papers have previously reported that breastmilk can be a potential source of infection for neonates. To name a few, a cluster of infectious cases caused by an *Escherichia coli* strain producing an extended spectrum

* Corresponding author.
E-mail address: C.Mullié[ab,*]catherine.mullie@u-picardie.fr (C. Mullié).

https://doi.org/10.1016/j.arcped.2018.02.006
0929-693X/

β-lactamase was described in Japan in 2012 [6], *B. streptococci* (with a special mention of *Streptococcus agalactiae*) [7,8] as well as *Bacillus cereus* transmissions [9] through breastmilk have also been reported.

Bacteriological analyses are therefore implemented before and after pasteurization of breastmilk donations. Pasteurization is used as a preservation method if the donated breastmilk is not expected to be used within 48 h of its collection and consists in heating the milk at 62.5°C for 30 min. The pasteurized breastmilk is then frozen until use. The process of bacterial assessment of raw and pasteurized milk according to the French regulations is described in Fig. 1. Each noncompliant batch must be destroyed according to the required procedure [5]. To accurately enumerate the total aerobic flora (TAF) and coagulase-positive staphylococci (CPS) in raw milk, technical details such as the plating method and the choice of raw milk dilutions to be plated are the responsibility of the biologist in charge of the analysis. It has to be kept in mind that, for raw milk, these dilutions also impact breastmilk molecules, which are known for their antibacterial properties, such as lysozyme and lactoferrin [10,11] or antibodies and interleukins [12,13]. In some dilutions, the concentrations of these antibacterial molecules could therefore be reduced to levels below the threshold at which they are active and thus enable an increase in the TAF enumeration. The biologist in charge must then choose a combination of milk dilutions that will enable an accurate enumeration of the TAF and reduce the number of valuable breastmilk sub-batches discarded on the grounds of an excessively high TAF but that are not actually a threat to neonates' health when administered undiluted.

Therefore, this study first focused on validating the plating technical conditions for the enumeration of TAF in raw breastmilk. It also compared Chapman and Baird-Parker media for the detection of CPS, given that normative texts NF EN ISO 6888-1 and NF EN ISO 6888-1/A1 [14,15] have suggested the use of Baird-Parker medium although Chapman medium was routinely used in the laboratory. The second part of this paper reports the results obtained over a 6-month period following the adoption of technical solutions validated in the first part of the study. Additionally, the discarded breastmilk volume was evaluated and the causes of non-compliance were analyzed. From a continuous improvement point of view, the aim of this analysis was to identify technical parameters and/or potential contamination sources that could be acted upon so as to maintain optimal safety for neonates while reducing the volume of discarded breastmilk.

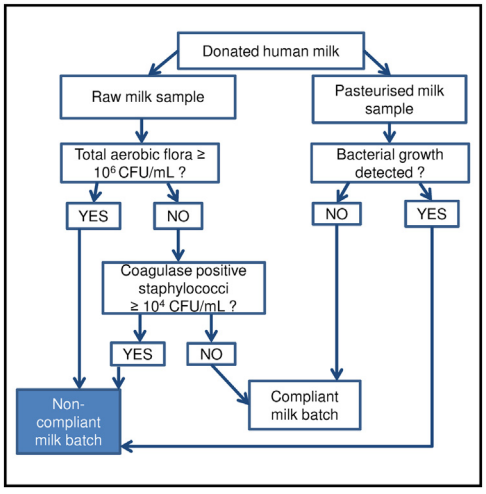

Fig. 1. Decision tree for compliance of donated human milk batches according to the French regulations. CFU: colony forming unit

2. Materials and methods

2.1. Comparison of media for CPS detection in raw breastmilk and selection of dilutions and seeding volumes for TAF enumeration

In July 2014, a series of 98 consecutive samples of raw milk from the Amiens milk bank were seeded on both Baird-Parker (Oxoid SA, Dardilly, France) and Chapman (bioMérieux, Marcy l'Étoile, France) ready-to-use agar media for CPS detection. A single batch of each cultivation medium was used for this assay. Additionally, the same samples were seeded on Columbia sheep blood agar (Becton-Dickinson, Le-Pont-de-Claix, France) for TAF enumeration. For each one of these media, a 10-μL volume of pure raw milk was seeded as well as a duplicate 10-μL seeding of the 1/10 and 1/100 dilutions of the pure sample in Tryptone-Salt broth (bioMérieux) to enumerate the TAF.

After a 48-h incubation at 36 ± 2°C, colonies were enumerated and the subsequent results were expressed as log10 CFU per 1 mL of the initial sample. Final counts for TAF were obtained using the following formulas [16]:

When three successive dilutions were taken into account: $N = (C/(V \times 1.1 \times d))$, where ΣC is the sum of colony counts from three successive dilutions, V is the inoculum volume (expressed in mL), and d is the first dilution retained for the calculation (d = 1 if it is the undiluted sample).

When two successive dilutions were taken into account: $N' = (C/(V \times 1.11 \times d))$, where ΣC is the sum of colony counts from two successive dilutions, V is the inoculum volume, and d is the first dilution retained for the calculation (d = 1 if it is the undiluted sample).

When a single dilution was taken into account: $N' = \frac{C}{V \times d}$, where C is the number of colonies, V is the inoculum volume, and d is the dilution retained for the calculation (d = 1 if it is the undiluted sample).

For each cultivation medium (Columbia sheep blood agar, Chapman, and Baird-Parker), colonies suspected of being CPS were submitted to a confirmatory agglutination test (Pastorex Staph plus®, Biorad, Marnes-la-Coquette, France). Final counts for CPS were obtained using the following formula:

$A = \frac{Bc}{Ac} \times Cc + \frac{Bnc}{Anc} \times Cnc$ where Ac is the number of phenotypically characteristic colonies submitted to the coagulase test, Anc is the number of uncharacteristic colonies submitted to the coagulase test, Bc is the number of characteristic colonies giving a positive result to the coagulase test, Bnc is the number of uncharacteristic colonies giving a positive result to the coagulase test, and Cc and Cnc are the total numbers of characteristic and uncharacteristic colonies enumerated on the Petri dish, respectively. The number of CPS per mL is then calculated with the following formula: $\frac{A}{V \times d}$, where A originates from the formula mentioned above, V is the inoculum volume (expressed in mL), and d the dilution retained for the calculation.

2.2. Study of results generated over a 6-month follow-up period

The validated new protocol was implemented routinely in January 2016. From then on, results from bacteriological analyses of samples collected from January to June 2016 were included in the statistical evaluation. During this period, 1099 sample pairs (raw and pasteurized milk) were processed. In addition to the analyses of the raw milk samples described above for TAF and CPS, 500 μL of undiluted pasteurized milk were seeded on Columbia horse blood agar to detect any remaining viable flora after pasteurization and incubated at 36 ± 2°C for 48 h. A systematic identification of bacteria isolated from pasteurized milk was carried out using MALDI-TOF mass spectrometry on a Bruker Biotyper (Bruker Daltonics, Bremen, Germany). Similarly, all Gram-negative

Please cite this article in press as: Mullié C, et al. Breastmilk donations: Bacteriological assessment, analysis of causes of non-compliance and suggestions for improvement. Archives de Pédiatrie (2018), https://doi.org/10.1016/j.arcped.2018.02.006