Early Human Development 105 (2017) 1–6

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

A retrospective audit of bacterial culture results of donated human milk in Perth, Western Australia☆

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ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 22 December 2016
Accepted 22 December 2016
Available online xxxx

Keywords:
Donor
Human milk
Human milk bank
Bacteria
Pasteurization
Pathogenic bacteria
Enterotoxin

ABSTRACT

Introduction: The bacterial content of donated human milk is either endogenous or introduced via contamination. Defining milk bank bacterial content will allow researchers to devise appropriate tests for significant and commonly encountered organisms.
Objective: A retrospective audit was conducted on data recorded from the Perron Rotary Express Milk Bank, King Edward Memorial Hospital, Subiaco, Western Australia. This aimed to describe the incidence of bacterial species detected in donated human milk and to identify potentially pathogenic bacteria.
Material and methods: The data comprised of 2890 batches donated by 448 women between 2007 and 2011.
Results: Coagulase negative *Staphylococcus* (CoNS) represented the highest prevalence of bacteria in donated milk, isolated from 85.5% of batches (range: 20 to 650,000 CFU/mL) followed by *Acinetobacter* species in 8.1% of batches (range: 100 to 180,000 CFU/mL). *Staphylococcus aureus* was the most prevalent potentially pathogenic bacteria in 5% of batches (range: 40 to 100,000 CFU/mL).
Conclusion: Further investigation is warranted to better define the risks posed by the presence of toxin-producing *S. aureus* in raw and pasteurized human milk which may allow minimization of risk to the preterm infants.

1. Introduction

The advantages of feeding preterm infants human milk instead of formula are well established. However, maternal milk cannot always be provided to these high-risk infants. This can be due to a mother's inability to establish her milk supply after delivering prematurely or the infant's inability to feed directly from the mother. For this reason, donor milk banks have been established in some hospitals to avoid risks associated with early formula feeds in vulnerable preterm infants [1]. For the purpose of this paper we define a donor human milk (DHM) bank as a service that receives donated human milk (that is in excess of their infant's needs) from eligible breastfeeding women. This donated milk is then pasteurized to ensure it does not contain any potentially pathogenic bacteria. It may then be dispensed to premature infants whose own mothers have insufficient milk for their infant's needs.

Human milk often contains normal microbiota associated with the human gut and/or skin such as *Bifidobacteria* spp., *Lactobacillus* spp., coagulase-negative *Staphylococcus* (CoNS), diphtheroids, *Acinetobacter* spp. and viridans group streptococci [2–5]. Other commensals, which can be potentially pathogenic such as *Staphylococcus aureus*, Group B *Streptococcus* (GBS), *Escherichia coli, Pseudomonas* spp., *Klebsiella* spp., *Enterococcus* spp., *Enterobacter* spp., *Bacillus* spp. and *Moraxella* spp., have also been detected in expressed milk [3,4,6,7]. Potentially pathogenic bacteria can be found endogenously in the mothers' milk; for example by expressing milk while suffering from mastitis [8]. Bacteria can also be introduced into donated milk through contamination during

Abbreviations: (DHM), donor human milk; (CoNS), Coagulase negative *Staphylococcus aureus*; (NEC), Necrotizing enterocolitis; (CFU/mL), Colony forming unit per milliliter; (LFGNB), Lactose fermenting Gram-negative bacilli; (NLFGNB), Non lactose fermenting Gram-negative bacilli.

☆ **Funding:** This research was funded by an unrestricted grant from Medela AG, Switzerland. Medela AG provides an unrestricted research grant to Donna Geddes. Taibah University, Saudi Arabia provides a PhD scholarship for Yahya Almutawif. Medela AG and Taibah University had no input into the design, analysis, interpretation and publication of the study.

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http://dx.doi.org/10.1016/j.earlhumdev.2016.12.011
0378-3782/

collection (using contaminated or improperly cleaned equipment), storage (delaying refrigeration) and processing (handling) of donated milk. Case reports suggest that the presence of bacteria or bacterial toxins in expressed milk that is fed to preterm infants may be associated with complications such as late-onset sepsis and necrotizing enterocolitis (NEC) [9–12].

In order to address this issue, milk banks have established safety standards that define the acceptance or rejection criteria for donated milk. In Perth, Australia, at the Perron Rotary Express Milk Bank (PREM Bank, King Edward Memorial Hospital), each milk batch is tested microbiologically. Milk batches that contain confluent growth of organisms indicating a total count of $>10^5$ colony forming units per mL (CFU/mL) and/or potential pathogens that are capable of producing heat-stable enterotoxins, endotoxins and spores pre-pasteurization are rejected, while any bacterial growth in the post-pasteurized batches is unacceptable [13]. Other milk banks accept some potential pathogens e.g. $\leq 10^3$ CFU/mL *S. aureus*, *Pseudomonas* spp., *Streptococcus pyogenes* or *Enterococcus faecalis* [14], and others do not assess bacteria in donated batches pre-pasteurization [15]. Post-pasteurization microbial testing is also variable, in some milk banks it is performed every month or every ten pasteurization cycles [16]. Differences in practice may impact the efficiency and availability of donor milk and expose preterm infants to different and currently undefined risks. Defining bacterial content will allow milk banks to devise technologies and practices to appropriately manage these hazards. This retrospective audit of the PREM Milk Bank from 2007 to 2011 was carried out to describe the bacterial content of donor breast milk. We hypothesized that CoNS would be the predominant bacteria in DHM and, *S. aureus* the predominant potential pathogen.

2. Materials and methods

2.1. Study data and donation selection

These data were collected during the normal operation of the PREM Milk Bank, King Edward Memorial Hospital, Subiaco, WA, Australia from 2007 to 2011. All donations were from registered donors. All donors completed a written health questionnaire about their medical history and lifestyle and submitted to serological screenings that are consistent with blood and tissue donation screenings in Australia. Written informed consent was given by all donors for the use of their milk for clinical and research purposes (Human research ethics committee; 2014127EW). Donors were either mothers who had an infant in the NICU or mothers of healthy term infants with excess milk supply. Serological screenings were repeated every 90 days for long-term donors.

2.2. Milk collection and processing

Before donation, donors were given instructions on breast milk collection techniques, including washing hands for 30 s, paying attention to under finger nails, use of a paper towel to turn off the tap, to dry the hands with a clean paper towel and the application of alcohol gel when handling the pump kit, attachment pieces and the breast. Mothers were also instructed to ensure that clean pump parts and milk collection bottles are kept away from clothing. All mothers collected milk, either at home or in the hospital, using a hospital grade breast pump and were advised to clean their expressing kit according to the manufacturers recommendations by rinsing items coming into contact with the milk in cold water, then washing equipment in hot soapy water using a bottle brush and detergent, followed by a final rinse with hot water. All breast shields, bottles, valves and membranes were put in sterilizing bags (Medela AG) with 60 mL water and microwaved for 3 min.

Expressed milk was placed in thermally disinfected plastic (High-density polyethylene or polypropylene) bottles and either frozen in the donor's home freezer until transportation to the milk bank, or frozen in the milk bank freezer. Following PREM Milk Bank guidelines [13], milk donations were stored frozen (−20 °C) for up to 90 days from the date of expression. Prior to pasteurization, a batch of milk (pooled donations from a single donor) was thawed rapidly in an orbital incubator (37 °C) and pooled in a sterile flask under laminar flow. The pooled batches ranged in volume from 80 to 3000 mL and contained milk obtained from multiple expressions collected over an extended time period (up to 90 days) the expression dates were not available and the analysis was based on the date samples were processed. Using an aseptic technique, 1 mL from each batch was collected for pre-pasteurization microbiological testing. All batches were pasteurized at 63.5 ± 1.0 °C for 30 min. Three types of pasteurizers were used between 2007 and 2011. An independently calibrated temperature probe that logged the time and temperature of the product during pasteurization was used to ensure accurate pasteurization. Once batches were pasteurized, 1 mL was collected for post-pasteurization microbiological testing. Both pre and post-pasteurization samples were immediately frozen (−20 °C) before transfer to the microbiology laboratory.

During 2007–2011 the batch volumes varied between 600 and 3100 mL due to the capacity of the different Holder pasteurizers used by the milk bank. The maximum capacity of the pasteurizer was 800 mL (Saurin Industries, Australia) in early 2007, and the purchase of a new pasteurizer allowed a maximum batch volume of 3000 mL (Sterifeed T30, Medicare Colgate Ltd., United Kingdom) from September 2007. In July 2009 the pasteurizer capacity increased again to 9000 mL (Sterifeed S90, Medicare Colgate Ltd., United Kingdom). However, each 9000 mL cycle was made up of 4 batches and as such the highest batch volume processed during this period was 3100 mL.

2.3. Milk bacteriological screening, acceptance criteria and reporting

A single 100 μL (pre-pasteurization) and 200 μL (post-pasteurization) sample from each batch was cultured on blood agar (5% horse blood) and CLED (Cystine-lactose-electrolyte deficient) agar and incubated at 35 °C in 5% CO_2 overnight (18–24 h). Any bacterial growth was quantified and identified through Gram staining, morphological structure, and conducting biochemical tests [13]. The methods were those used in human milk bank guidelines that are followed by the external laboratory responsible for processing the samples (PathWest, KEMH, Subiaco, Western Australia).

In order to meet the pre-pasteurization acceptance criteria, all batches containing any bacteria capable of producing harmful factors, such as heat-stable enterotoxins, endotoxins and spores, were considered potential pathogens and rejected even if no bacterial growth was detected post-pasteurization. Batches that contained other bacteria with a total colony count $\leq 10^5$ (CFU/mL) of others such as CoNS, viridans group streptococci, diphtheroids and *Acinetobacter* spp. were considered probable commensals and accepted for pasteurization. Any bacterial growth in the post-pasteurized batches was unacceptable and these batches were discarded.

The primary aim of the microbiological reporting was to allow the PREM Milk Bank to assess the result in the context of the acceptance and rejection criteria. Consequently there was variability in the level of identification reported. Where possible reports identified bacteria to the genus or species level. However, where broader identification was sufficient to interpret the results, the genus and species where not always reported. For example lactose and non-lactose fermenting Gram-negative bacilli (LFGNB and NLFGNB) and coliforms where often reported as such. Positive cultures were expressed in quantitative colony counts from 1 to >100,000 CFU/mL.

2.4. Statistical analysis

Descriptive analyses were performed using R3.0.3 for Mac OSX [17]. Lattice package [18] was used to create time series graphs. Monthly changes in numbers of batches, number of rejected batches, and proportion of batches rejected are presented graphically with LOESS (local