

Bacillus species cultured repeatedly from the breastmilk of a donor milk mother

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A 33-year-old woman with no known comorbidities and a healthy baby donated breastmilk to a local milk bank. *Bacillus* species were cultured from recurrent batches of milk donated by this mother. All batches in which this skin commensal was detected subsequently had to be discarded. A contaminated breast pump apparatus was identified as the source of the contamination. The potential effect of the microbial contamination was explained to the mother to prevent emotional distress and allow her to continue breastfeeding. A good relationship between the microbiology department and donor milk bank ensured that the participant was not lost to the donor milk programme and that she did not unnecessarily switch to formula feeding.

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The National Health Laboratory Service at Groote Schuur Hospital performs culture tests on donor breastmilk post pasteurisation to verify sterility. No global consensus exists for obtaining cultures pre-pasteurisation in milk banks.^[1] Post-pasteurisation reports indicate only presence or absence of microorganism growth to detect growth of significant aerobic bacteria, in line with the recommendations of the Human Milk Banking Association of South Africa (SA). The laboratory received 1 732 pasteurised milk samples for testing during 2016 and 1 753 samples in 2017. Bacterial growth was found in 10.3% of the samples in 2016 and 7.4% in 2017. Normal skin flora were the main contaminants and included organisms such as *Bacillus*, *Micrococcus*, *Corynebacterium* and coagulase-negative *Staphylococcus* species (spp.).

Case presentation and investigation

A 33-year-old primigravida with a healthy term infant was breastfeeding and participated in a donor milk programme. She had no episodes of mastitis or foul-smelling nipple discharge. It was noted that *Bacillus* spp. were cultured from post-pasteurisation milk batches from this mother. Samples were processed according to the National Department of Health's Human Milk Banking Regulations and guidelines from a community-based milk bank (Milk Matters) in Cape Town, SA.

The donor mother was subsequently advised to follow good hand hygiene before expressing. The breast pump was disassembled and all parts were washed separately in hot, soapy water. The milk bank recommends boiling or steaming milk pumps and to avoid decontamination solutions where possible. The mother was encouraged to use clean, autoclaved containers for milk collection as provided by the milk bank and not to merge milk samples, even from the same day. This reduced the risk of contamination and maintained the cold chain by preventing hot milk mixing with cold milk from the freezer. Milk from the mother was then pooled into batches of up to 1.2 L at the sterile section of the milk bank and treated according to the Holder pasteurisation technique within 24 hours of defrosting. This reference technique incorporates the elements

of slow, low-temperature pasteurisation (62.5 °C for 30 min). This pasteurisation method, which is commonly used worldwide, offers a good compromise between the preservation of milk components and microbiological safety.

Pasteurised milk samples (100 µL) were aseptically plated onto 4% blood agar medium and incubated for 24 hours in 5% CO₂. *Bacillus* spp. were identified based on colony morphology. Beta haemolysis and lecithinase activity were excluded on sheep blood agar medium (Fig. 1) and lecithin agar medium (Fig. 2), respectively, to rule out *B. cereus*. Samples were not tested for anaerobes, the presence of toxin-producing organisms or preformed toxins. If bacterial growth was detected in a sample, the whole batch of pooled milk (1.2 L) was discarded and not dispensed to the relevant neonatal units. *Bacillus* spp. were cultured from 12 of the 16 samples received between March and May 2018, and the respective pooled batches had to be discarded. A faulty microwave oven used to decontaminate the collection equipment was subsequently identified as the reason for insufficient decontamination, thereby allowing the recurrent growth of *Bacillus* spp. from the donor milk. Bacterial growth was not detected in any subsequent samples from this donor mother after the decontamination practice was changed to boiling the pump apparatus in hot water for 5 minutes before use.

Discussion

Human breastmilk offers considerable nutritional, developmental and immunoprotective benefits, especially in preterm neonates. Suboptimal breastfeeding results in about 800 000 infant deaths annually, estimated at 11.6% of childhood deaths worldwide. A supplement of donor breastmilk is recommended for infants of very low birthweight (LBW) (<1 500 g) who do not receive sufficient mother's milk.^[2] An increase in the demand for donor milk banking to supplement infants in the neonatal intensive care unit has been reported.^[3]

Breastmilk is not sterile and may contain various bacteria.^[4] *Bacillus* spp., excluding *B. cereus* or *B. anthracis*, are defined as skin commensals or normal microflora when cultured from breastmilk.^[3] *Bacillus* spores

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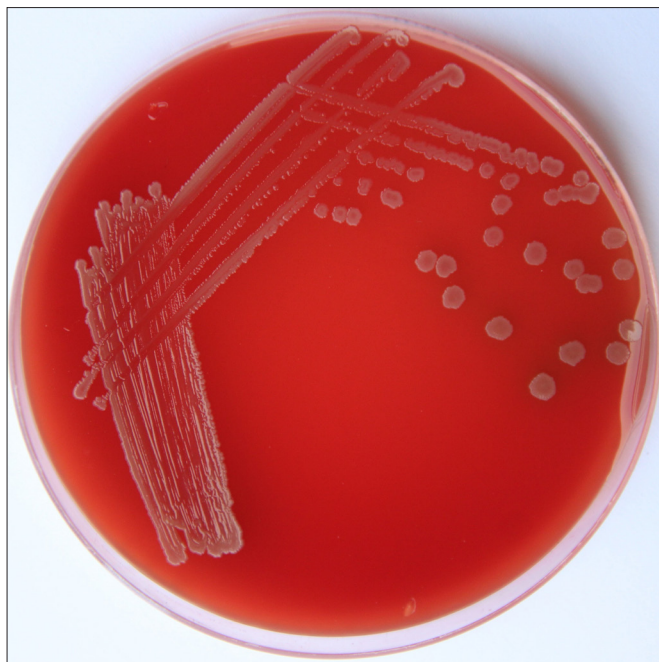


Fig. 1. *Bacillus* spp. cultured on sheep blood agar medium, incubated in aerobic conditions. No beta haemolysis activity was observed after 24 hours' incubation at 35 °C.

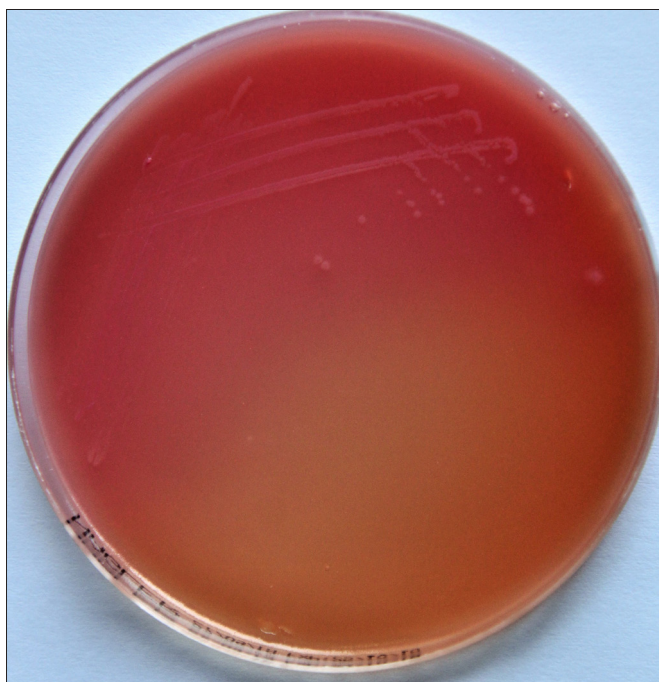


Fig. 2. *Bacillus* spp. cultured on lecithin agar medium, incubated in aerobic conditions. No lecithinase activity was observed after 24 hours' incubation at 35 °C.

are known to be resistant to pasteurisation. This microorganism can be introduced at the collection, storing or processing of human donor milk.^[1] Milk pumps and storage containers were the main source of bacterial contamination in a study that investigated expressed milk from mothers in a neonatal intensive care unit.^[5] Haiden *et al.*^[6] found that milk expressed in the hospital setting had a 10% lower rate of bacterial contamination than home-expressed milk. They suggested

the re-use of collection equipment as a major route of contamination.^[6] Contaminated breast pumps and storage containers may harbour potential pathogens if not decontaminated appropriately. It is important that doctors ask about these practices specifically when interviewing a mother of a sick baby who is bottle fed. The Centers for Disease Control and Prevention recommends the aforementioned boiling technique when a baby has a weakened immune system, was born prematurely or is younger than 3 months. In rural or low-resource settings, this is a feasible approach for decontaminating breastmilk equipment if manufacturer specifications allow.

Our findings are limited by not having tested the pre-pasteurised milk, collection equipment or milk expressed by hand, as such an approach would have allowed a more accurate identification of the bacterial source. Discarding donor milk has an emotional effect on the mother, as giving milk to a baby at home when it has been rejected by the milk bank can cause anxiety about the milk's safety with a mother. Terms such as 'normal skin flora' are not always understood or interpreted correctly by mothers, which may result in the donor mother not giving their own infant breastmilk and changing to formula feeds or discontinuing providing milk to a donor milk bank. In this case, there would probably not have been any adverse consequences to the mother's child receiving the breastmilk if the contamination had gone undetected.

The microbiologist has a key role in explaining the potential effects of microbial contamination in donor milk. By following a systematic approach to identify the source of contamination and pathogens during culture, the laboratory can review donations and ensure that safe donor milk is supplied to neonatal intensive care units that nurse infants of LBW.

Conclusion

Bacillus spp. may represent skin contamination in donor milk and can survive the pasteurisation process. Milk pumps and collection containers are common sources of contamination of breastmilk expressed at home. Healthcare workers should ask a lactating mother about decontamination processes of breast pump equipment and storage containers even if her baby is not sick. Thorough cleaning and boiling is a practical alternative for decontamination in rural or low-resource settings. Explaining the effect of the presence of normal microflora to breastfeeding mothers can prevent emotional distress, unnecessary formula feeding and the loss of participants to a donor milk programme. Finally, a good relationship is recommended between the microbiology laboratory, donor milk banks, general practitioner and neonatologist to address issues of milk sterility and safety.

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