Imported food risk advice

Cronobacter spp. in human milk and human milk products

Context of this risk advice

- Human milk means expressed milk collected from lactating women to be fed to infants that are not the biological infants of the women supplying the milk.
- Human milk products means products derived from human milk that have been specially formulated to meet the specific nutritional needs of infants such as fortifiers and formula.
- The level of risk for this hazard in human milk and human milk products was determined assuming that the most vulnerable category of infants (preterm infants in hospital neonatal intensive care units) would be receiving the products.

Nature of the hazard

Cronobacter spp. (formerly known as *Enterobacter sakazakii*, and includes *Cronobacter sakazakii*) are Gram-negative, non-spore forming rod-shaped bacteria belonging to the family *Enterobacteriaceae. Cronobacter* spp. are ubiquitous organisms as they have been isolated from a variety of foods, environmental samples and food processing facilities (FDA 2012; Healy et al. 2010; Pagotto and Abdesselam 2013). *Cronobacter* spp. are resistant to osmotic stress and drying but the bacterial cells are susceptible to pasteurisation (Breeuwer et al. 2003; Pagotto and Abdesselam 2013). In new-born infants *Cronobacter* spp. is a severe hazard and can cause life threatening illness.

Cronobacter spp. can form biofilms on surfaces such as silicon, latex, polycarbonate, polyvinyl chloride (PVC e.g. enteral feeding tubes) and stainless steel. Biofilms protect the bacterial cells from various environmental stresses and provide the bacteria protection against sanitizers (Iversen et al. 2004; Kim et al. 2006).

Transmission

Cronobacter spp. can be transmitted to infants due to poor hygienic practices. Studies have described infants with *Cronobacter* infection attributed to contaminated expressed human milk. McMullan et al. (2018) reported a case in which no *Cronobacter sakazakii* was detected in expressed mother's milk collected aseptically in the hospital, however after discharge *C. sakazakii* was detected in the expressed mother's milk. The breast pump used by the mother after discharge from the hospital was not sterilised, suggesting colonisation of the breast pump with *C. sakazakii* and subsequent contamination of the expressed breast milk. In a different case described by Bowen et al (2017), *Cronobacter* spp. were detected in the valves of the breast pump kit and in the expressed mother's milk collected using the pump.

Cronobacter spp. have not been reported to be transmitted directly through human milk via bacterial shedding in the milk.

Disease severity

Cronobacter spp. are a severe hazard in infants as they cause potentially life threatening illness with chronic sequelae, especially in preterm infants. The case fatality rate ranges from 10-80%, and the survivors often develop irreversible neurological sequelae (FDA 2012; Giovannini et al. 2008). Symptoms in infants include meningitis (leading to ventriculitis[1], brain abscess, cerebral infarction[2], hydrocephalus[3] and cyst formation), necrotizing entercolitis[4] and septicaemia (Chenu and Cox 2009; Feeney et al. 2014; Gurtler et al. 2005).

[1] Inflammation of the ventricules of the brain
[2] Severe damage to some of the brain tissue due to inadequate blood supply
[3] Abnormal increase in the amount of cerebrospinal fluid in the ventricles of the brain, often leading to skull enlargement and impaired brain function
[4] Inflammation and death of intestinal tissue

Some *Cronobacter* spp. produce enterotoxin. The role of the enterotoxin is unclear, however it has been postulated that it may increase bacterial virulence and disrupt the intestinal barrier causing necrotizing entercolitis[4] (Hunter and Bean 2013; Pagotto et al. 2003; Raghav and Aggarwal 2007).

Infectivity

The infectious dose of *Cronobacter* spp. in human milk is unknown. It has been estimated that the dose may be similar to other very infectious pathogenic bacteria such as *Escherichia coli* O157 and in the order of between 10-100 organisms (FDA 2012). The FAO/WHO has estimated the probability of one ingested organism causing illness as 8.9×10^{-6}, assuming that the ingestion of one *Cronobacter* cell has the ability, albeit slight, to cause illness in infants at risk (FAO/WHO 2007; FSANZ 2006).

Risk mitigation

Controls are needed to minimise contamination of human milk with *Cronobacter* spp., including pasteurisation of the milk. A study by Ueda (2017) showed that heating *Cronobacter* spp. suspended in nutrient broth (1×10^7 cfu/ml) at 60^{o}C for five minutes inactivated the bacteria. In food matrices the thermal resistance can differ, for example in rehydrated infant formulae it has been estimated that treatment at 60^{o}C for 15-17.5 minutes would be required to obtain a 6 or 7 log reduction in *Cronobacter* spp. (Gurtler et al. 2005; Nazarowec-White and Farber 1997). Also, the *D*-value of *Cronobacter* spp. in whole reconstituted milk was significantly higher than in low fat or skim milk, and was slightly higher than the *D*-value for various reconstituted infant formulae (Osaili et al. 2009).

Holder pasteurisation (62.5^{o}C, 30 minutes) kills most bacterial contaminants found in human milk (Baumer 2004; Picaud and Buffin 2017) and should inactivate *Cronobacter* spp. International human milk banks, including those in Australia, routinely perform Holder pasteurisation on human milk to ensure the microbiological safety of donor human milk (Bharadva et al. 2014; Hartmann et al. 2007; HMBANA 2015; UKAMB 2003). However, *Cronobacter* spp. can produce an enterotoxin that is stable at 90^{o}C which would be resistant to pasteurisation (Pagotto et al. 2003; Raghav and Aggarwal 2007).

The safe production of human milk and milk products is dependent on maintaining a high level of hygiene control during collection, handling, processing, storage and transport to minimise the contamination of milk with *C. sakazakii*. This is achieved by obtaining and treating donor human milk according to best practice guidelines followed by international donor milk banks, including those in Australia. Milk must be collected hygienically from the donors, with donors instructed about the importance of hand washing, cleaning and sterilising pumps, and the use of appropriate containers. Donor milk should be refrigerated (4^{o}C) immediately after collection and then stored frozen at -20^{o}C (Hartmann et al. 2007; HMBANA 2015; UKAMB 2003).

Human milk products should be produced from milk that has been subjected to Holder pasteurisation or an equivalent thermal treatment during processing to eliminate microbiological contamination. However, if human milk is heavily contaminated with microorganisms or if heat stable bacterial toxins are present, Holder pasteurisation used by international human milk banks may be ineffective. Therefore, pre- and post-pasteurisation microbiological criteria are used for human milk as described in international best practice guidelines to ensure the effectiveness of Holder pasteurisation (Bharadva et al. 2014; Hartmann et al. 2007; HMBANA 2015; UKAMB 2003). Process hygiene criteria are useful to verify that the hygiene measures in place in the manufacturing facility are working as intended (FSANZ 2018).

Milk banks and manufacturers of human milk products should utilise Good Manufacturing Practices, Good Hygienic Practices and an internationally recognised hazard management tool, such as the hazard analysis and critical control points (HACCP) process to identify, evaluate and control hazards (Codex 2008; Hartmann et al. 2007; HMBANA 2015; PATH 2013). Specifically, facilities and equipment used to process human milk and human milk products should be designed, constructed and laid out to prevent the entry of pathogens into high hygiene areas and to minimise their establishment or growth in harbourage sites, including the prevention of biofilm formation. Equipment should be designed, and appropriate procedures implemented, to facilitate effective cleaning and sanitising (Codex 2008; Marchand et al. 2012).

Pasteurised human milk is stored and transported frozen. Once thawed, human milk should be kept refrigerated (4^{o}C) until use and should be used within 24 hours. The human milk should be discarded after completion of the initial feed. If fortifiers are added to the human milk, the fortified human milk should be kept refrigerated and used

within 24 hours. Thawed pasteurised human milk and fortified human milk should not be refrozen (Hartmann et al. 2007; Jones 2011; UKAMB 2003).

Evaluation of uncertainty

There is uncertainty around the infectivity of *Cronobacter* spp. in human milk as the number of infectious particles required to cause infection is unknown. If, as has been estimated, the infectious dose is similar to *E. coli* O157, only small quantities of bacteria would be required for illness.

Pooling of human milk from multiple donors is common practice amongst many human milk banks and would dilute the bacterial load from a single donor, however some milk banks only pool milk from individual donors (Haiden and Ziegler 2016). The Australian Red Cross milk bank pasteurises human milk in single donor batches (Australian Red Cross 2018). However, potential environmental contamination of the human milk during collection, processing and/or post-processing may increase the bacterial load of the milk.

Risk characterisation

There is evidence that *Cronobacter* spp. can be present in human milk due to poor hygiene procedures and can be transmitted to infants and cause disease with a small number of bacteria. There is a high likelihood of exposure as *Cronobacter* spp. have caused illness in infants due to consumption of contaminated human milk. Also, inadequate hygiene practices during collection, handling and storage, and/or inadequate processing or post-processing practices could facilitate the contamination of human milk with this bacterium.

Cronobacter spp. causes severe illness in preterm infants with a high mortality rate. *Cronobacter* spp. in imported human milk and human milk products presents a potential medium or high risk to public health and safety.

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