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Growth and survival of *Enterobacter sakazakii* in human breast milk with and without fortifiers as compared to powdered infant formula

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Abstract

Enterobacter sakazakii infections often involve debilitated neonates consuming contaminated reconstituted powdered infant formula. There is the possibility that expressed human breast milk can become contaminated with *E. sakazakii* in the hospital or home setting and through the use of contaminated breast milk fortifiers. In addition, although breast milk has been shown to have some antimicrobial effects, this has not been extensively researched in regards to *E. sakazakii.* Thus, we examined the survival and growth of 9 strains of *E. sakazakii* in breast milk, human breast milk with fortifiers and powdered infant formula at 10, 23 and 37 °C. The average generation times for clinical, food and environmental isolates in breast milk were 0.94±0.04, 0.75±0.04 and 0.84±0.04 h at 23 °C; and 0.51±0.03, 0.33±0.03 and 0.42±0.03 h at 37 °C, respectively. *E. sakazakii* was able to survive up to 12 days in breast milk with fortifiers at 10 °C. However, its average generation times among replicates and isolate sources ranged from 11.97±3.82 to 27.08±4.54 h in breast milk at 10 °C. Interestingly, average generation times in breast milk with fortifiers at 23 °C (0.83±0.05, 0.93±0.06 and 0.96±0.06 h) and 37 °C (0.41±0.04, 0.51±0.05 and 0.54±0.05 h) were longer than in powdered infant formula and breast milk at the same temperatures, indicating that human breast milk fortifiers may have an inhibitory effect on the growth of *E. sakazakii*. However, the intrinsically ascribed antimicrobial properties of breast milk do not appear to inhibit the growth of this foodborne pathogen *in-vitro*.

Keywords: *Enterobacter sakazakii*; Human breast milk; Milk fortifiers; Powdered infant formula; Growth; Survival

1. Introduction

Enterobacter sakazakii is an opportunistic Gram-negative, foodborne pathogen, which is a member of the family *Enterobacteriaceae*. Its association with outbreaks due to the consumption of powdered infant formula (Biering et al., 1989; Simmons et al., 1989; Clark et al., 1990; Muytjens and Kollee, 1990; Nazarowec-White and Farber, 1997a; Weir, 2002), have lead to the identification of high-risk populations that include newborns which have an immature immune system facilitating opportunistic infections (FAO/WHO, 2004). Further, premature infants have more permeable gastrointestinal tracts due to a delay in feeding and the absence of natural resident gut microbiota, which in full-term infants are known to protect against pathogenic invading microorganisms (Hammerman and Kaplan, 2006). Equally, it is possible that the lower acidity of the newborn stomach, especially those of premature infants, represents an additional factor contributing to survival and infection of neonates with this pathogen (FAO/WHO, 2004).

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The first complete infant formula was developed as a replacement for human breast milk almost 100 years ago. Its powder form constitutes over 80% of the infant formula used worldwide, and it has advantages over the liquid form in terms of cost and storage. However, while the latter is sterile, the former is not, and may contain low levels of microorganisms. Powdered infant formula is used as an alternative to human breast milk in providing newborns with nutritional needs either in addition to breast milk, or on its own when breast feeding is not possible. Although it has been previously demonstrated that *E. sakazakii* does not survive pasteurization temperatures (Nazarowec-White and Farber, 1997b), some dry powdered infant formula ingredients are added after the thermal treatment step. Moreover, the presence of microorganisms is probably due to post-heat-treatment contamination from the processing environment, equipment or product mishandling. Currently, there is a push to reduce the risk of bacterial contamination of this product (FAO/WHO, 2004), and the behavior of *E. sakazakii* cells in dry products may be a key element to be considered in the evaluation of potential treatments for inactivating *E. sakazakii* and possibly other pathogens (FAO/WHO, 2004) found in powdered infant formula. To-date, processing technology is unable to completely eliminate the potential for microbial contamination in powdered infant formula without affecting its organoleptic and nutritional requirements. A number of newer technologies, such as irradiation, ultra-high pressure and magnetic fields processes, combined with other potential hurdles, were suggested as potential candidates in the future (FAO/WHO, 2004). Two recent studies reported the effects of high-pressure processing and gamma radiation on *E. sakazakii* strains artificially inoculated in reconstituted and dehydrated powdered infant formula, respectively (Gonzalez et al., 2006; Lee et al., 2006). Both studies reported promising results regarding the reduction of *E. sakazakii* in powdered infant formula, however, nutritional and sensorial evaluation should be further investigated.

E. sakazakii has been isolated from unopened cans of powdered infant formula in concentrations ranging from 0.36 to 66 cfu/100 g (Muytjens et al., 1988; Iversen et al., 2004; Leuschner and Bew, 2004), and has been linked to infections in newborns. Identical molecular fingerprinting of clinical and food isolates from patients and unopened cans of powdered infant formula, respectively, suggest a causal link between consumption of powdered infant formula and infant infection (Biering et al., 1989; Simmons et al., 1989; Clark et al., 1990; Muytjens and Kollee, 1990; Weir, 2002).

Breast milk has been reported to contain compounds with immunological properties, for instance, soluble and cellular factors, such as immunoglobulins, lactoferrin, oligosaccharides, probiotics, fatty acids hormones and growth factors, as well as maternal leukocytes and cytokines (Isaacs, 2005; Newburg, 2005; Coppa et al., 2006; Paramasivam et al., 2006). These compounds provide protection to the neonate while its immune system is still immature, and also act in synergy with the gastrointestinal tract (GI), enhancing antimicrobial properties (Field, 2005; Isaacs, 2005). However, it has also been suggested that some of these breast milk components need specific conditions in order to be efficacious, thus often making it difficult for *in-vitro* evaluation of their antimicrobial effects (Newburg, 2005).

Human breast milk fortifiers are powdered or liquid calorie and/or nutrient supplements added to expressed breast milk when the nutritional requirements of infants are not satisfied by breast milk alone. Thickening agents, such as xanthan gum that increase the viscosity of the milk for feeding infants with gastro-oesophageal reflux, may also be added to breast milk. To our knowledge, *E. sakazakii* has never been isolated from human breast milk fortifiers. However, Jocson et al. (1997) demonstrated a larger increase (1 – log difference) in total bacterial colony counts in fortified human breast milk as compared to breast milk after 72 h storage at 4 or 26 °C. They hypothesized that nutrient fortification and storage duration may change some of the host defense properties of human milk, allowing bacterial proliferation to occur.

Based on the current guidelines for preparation of powdered infant formula, breast milk and breast milk with fortifiers in the hospital settings (Berseth et al., 2004), handling and fortification of breast milk may take place in the same environment where powdered infant formula is rehydrated. In addition, given that in outbreak situations *E. sakazakii* has been isolated from utensils and environmental samples of hospital milk kitchens (Biering et al., 1989; Clark et al., 1990; Janicka et al., 1999; Bar-Oz et al., 2001; Block et al., 2002) we hypothesized that breast milk could become contaminated with *E. sakazakii* during manipulation in the hospital setting. Thus, we attempted to compare the survival and growth of *E. sakazakii* in breast milk and breast milk with fortifiers at 10, 23 and 37 °C, as compared to growth in powdered infant formula. Growth rates, generation and lag times were calculated. The temperatures used for this study were chosen to represent 1) the optimum temperature of growth for *E. sakazakii* (37 °C), as well as temperatures close to which premature newborns are kept (30–35 °C), 2) room temperature in North America (23 °C), at which bottles of reconstituted powdered infant formula and of expressed breast milk are kept during infant feeding, and 3) an abusive refrigeration temperature of 10 °C, higher than the recommended refrigeration temperature (4 °C), but may more closely represent reality. The growth of 9 different strains of *E. sakazakii* from three geographically diverse isolation sources, i.e., environmental, food and clinical settings, were assessed to determine if differences, if any, existed.

2. Materials and methods

2.1. Inoculum preparation

Nine *E. sakazakii* isolates were used in this study (Table 1). All strains were stored at −80 °C in trypticase soy broth (TSB, Oxoid) containing 40% glycerol until time of use. Strains were grown in TSB with incubation at 37 °C, prior to inoculation of each media being tested.