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Isolation of *Enterobacter sakazakii* and other *Enterobacter* sp. from food and food production environments

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Abstract

Enterobacter sakazakii and other *Enterobacter* species have caused foodborne illnesses through consumption of a variety of foods, including infant foods. The prevalence of *E. sakazakii* and other *Enterobacter* sp. in infant food and milk formula, milk powder, cereal products, spices, sugar and food production environments were studied. A total of 106 samples were tested for the presence of *E. sakazakii* and other *Enterobacter* sp. The presence of *E. sakazakii* and *Enterobacter* sp. was detected using the FDA enrichment procedure and a chromogenic medium. *E. sakazakii* was isolated from 2/15 infant food formula, 2/8 infant milk formula, 1/18 cereal products. However none of the powder milk, spices, sugar and environmental samples were positive for *E. sakazakii*. *E. agglomerans* was isolated from infant food formula, infant milk formula, milk powder, cereal products, spices and environmental samples. *E. cloacae* was isolated from infant milk formula.

Keywords: *Enterobacter sakazakii*; Infant formula; Production environments

1. Introduction

Enterobacter sakazakii is a Gram negative, facultative, rod-shaped bacterium. It belongs to the family *Enterobacteriaceae* and genus *Enterobacter* that contains a number of species including *E. agglomerans*, *E. cloacae*, *E. aerogenes* and *E. gergoviae*. The differentiation among these species is based on biochemical reactions, and serological and molecular techniques (Farmer, Asbury, Hickman, & Brenner, 1980; Farmer & Kelly, 1992; Hoffmann & Roggenkamp, 2003; Iversen, Waddington, On, & Forsythe, 2004d). *E. sakazakii*, *E. agglomerans*, and *E. cloacae* are considered the main species of this genus that are frequently isolated from clinical samples and food products (Farmer et al., 1980).

E. sakazakii is considered an opportunistic pathogen that has been implicated in severe forms of necrotizing colitis (Van Acker et al., 2001) and meningitis (Bar-Oz, Preminger, Peleg, Block, & Arad, 2001) especially in neonates with a mortality rate varying from 40% to 80% (Muytjens, Roelfos, & Jaspar, 1988). The International Commission for Microbiological Specification for Foods (ICMSF, 2002) has ranked *E. sakazakii* as "Severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration".

E. sakazakii and *Enterobacter* species have been reported as frequently isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households (Kandhai, Reij, Gorris, Guillaume-Gentil, & Van Schothorst, 2004a; Neelam, Nawaz, & Riazuddin, 1987). *E. sakazakii* has been also isolated from a wide range of foods including ultra high-temperature treated milk (UHT milk), cheese, meat, vegetables, grains,

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sorghum seeds, rice seeds, herbs, spices, fermented bread, fermented beverage, tofu, and sour tea (Gassem, 1999, 2002; Iversen & Forsythe, 2003, 2004a; Leclercq, Wanequ, & Baylac, 2002; Muytjens et al., 1988; Skaldal, Mascini, Sal Vadori, & Zannoni, 1993), Despite this, studies have confirmed the connection between neonatal *E. sakazakii* infection and infant milk formulas (Biering, Karlsson, Clark, Jonsdottir, & Ludvigsson, 1989; Muytjens et al., 1988; Nazarowec-White & Farber, 1997b; Simmons, Gelfand, Haas, Metts, & Feruson, 1989; Van Acker et al., 2001).

The US Food and Drug Administration (FDA, 2002) has issued an alert to health care professionals about the risk associated with *E. sakazakii* infections among neonates fed with milk-based infant formula. The alert stated that a major contribution to the avoidance of *E. sakazakii* infections in premature babies and neonates is the prevention of contamination of infant milk formula during production and bottle preparation. However, knowledge of the etiological and ecological characteristics of *E. sakazakii* is sparse and its occurrence in factories that produce infant formulas and in hospital kitchens has not been studied in depth.

The objective of this study was to investigate the prevalence of *E. sakazakii* and *Enterobacter* sp. in commercial food products including infant food formula, infant milk formula, milk powder, cereal products, spices, and sugar and in two food factories producing infant formula and cereals products.

2. Materials and methods

All media materials used in the study were obtained from Oxoid, UK.

2.1. Food samples

A total of 59 different commercial food samples from different manufacturers were purchased from retail stores across Jordan. The samples composed of 15 infant food formula (recommended for above 6 months old infants), 8 infant milk formula (Recommended for from birth to 1 year old infants), 10 full cream milk powder, 18 cereal products, spices 5 and sugar 3. Food samples were manufactured or packaged in 13 different countries.

2.2. Samples from food production environments

A total of 47 dry swab samples were obtained from infant food formula and cereal factories using dry cotton swabs. These samples were collected from production areas, including floors, walls, equipment and spilled dry products.

2.3. Detection, isolation and identification of Enterobacter sp.

The procedure of FDA (2002) for detection, isolation, and identification of *E. sakazakii* and other *Enterobacter* sp. in food and environmental samples was followed.

An aliquot (10 ml) of homogenate sample (10 g of powder/90 ml sterile Peptone water) or swab sample was added to 90 ml Enrichment Broth (EE broth) which contains bile salt and brilliant green to suppress the growth of non-*Enterobacteriaceae*. The bottles were then incubated for 14–16 h at 36 °C.

Two plates of Violet Red Bile Glucose Agar (VRBGA) were inoculated (0.1 ml) by streak method form the EE broth culture. Another loopful of the suspension was streaked on VRBGA. The plates were incubated for 14–16 h at 36 °C. Five colonies of the red or purple colonies surrounded by purple halo were examined morphologically. *E. sakazakii* and other *Enterobacter* sp. grown on VRBGA appeared under microscope as short-rods in shape and was Gram negative. Typical colonies were streaked on Tryptic Soya Agar (TSA), plates were incubated for 24–72 h at 25 °C.

The isolated colonies that produce yellow pigment were identified using API 24E test. To avoid the lack of specificity of the FDA procedure for the isolation of *E. sakazakii* (Iversen et al., 2004), *E. sakazakii* isolates were confirmed by growing them on Druggan-Forsythe-Iversen (DFI) medium.

E. sakazakii ATCC 51329 was used as the positive control organism. The non *E. sakazakii* isolates were also identified.

3. Results and discussion

3.1. Isolation of Enterobacter sp. from commercial food samples

3.1.1. Infant formula

Table 1 summarizes the confirmed identification of *E. sakazakii* and other *Enterobacter* sp. in infant food and milk formula samples. 17.4%, 20% and 17.4% of the samples were found to be positive for *E. sakazakii*, *E. cloacae* and *E. agglomerans*, respectively. The positive strains of *E. sakazakii* formed yellow colonies on TSA after 24–72 h of incubation at 25 °C and blue-green colonies after 24 h of incubation at 37 °C on DFI medium. These results were expected and consistent with the others who have found a direct relationship between infant formula and *E. sakazakii* (Biering et al., 1989; Iversen & Forsythe, 2003; Muytjens et al., 1983; Nazarowec-White & Farber, 1997b; Noriega, Kotloft, Martin, & Schwalb, 1990; Simmons et al., 1989). Muytjens et al. (1988) tested 141 samples of powdered infant milk formula manufactured in different countries. They found that the *E. sakazakii* and other *Enterobacteriaceae* were isolated from 14.1% and 52.2% of the total samples, respectively. Nazarowec-White and Farber (1997b) surveyed the presence of *E. sakazakii* in 120 dried infant milk formula samples (five manufacturers) obtained from Canadian retail market and reported that the prevalence of this bacterium ranged between 0% and 12% of the samples/manufacturer. Iversen and Forsythe (2004a) isolated *E. sakazakii* from 2.4% of 82 powdered infant milk formulas.