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REVIEW

Enterobacter sakazakii: an emerging foodborne pathogenic bacterium

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Abstract *Enterobacter sakazakii* (*Cronobacter* spp.) is an emerging pathogen associated with the ingestion of contaminated powdered infant formula (PIF) that causes necrotizing enterocolitis, sepsis, and meningitis in low-birth-weight preterm neonatal infants. The natural habitat of *E. sakazakii* is unknown, but PIF has been suggested as a possible mode of transmission in neonatal infections. The presence of *E. sakazakii* is not limited to powdered infant formula, it can also be found in a broad range of foods and in water. In recent years, the International Commission on Microbiological Specifications for Foods has ranked *E. sakazakii* a "severe hazard for restricted populations." The present review is mainly focused on environmental stress on *E. sakazakii* and its antibiotic resistance.

Keywords *Enterobacter sakazakii* · Powdered infant formula · Neonatal infections · Environmental stress · Antibiotic resistance

Introduction

Enterobacter sakazakii, a Gram-negative, rod-shaped bacterium, is a rare cause of invasive infection with high death rates in neonates. The organism was formerly referred to as "yellow-pigmented" *Enterobacter cloacae*, and was characterized as a unique species 30 years ago (Farmer et al. 1980). Initially, it was noted to be an opportunistic pathogen responsible for neonatal sepsis and meningitis (Simmons et al. 1989; Biering et al. 1989; Clark et al. 1990). The infections have been linked to ingestion of powdered infant formula (PIF) that has not been thermally sterilized (Hunter et al. 2008). In a recent study on the occurrence of *E. sakazakii* in production environments from food (milk powder, chocolate, cereal, potato, and pasta) factories and households, this organism was isolated with varying frequency from nearly all environments examined, strongly indicating that it is widespread (Lehner and Stephan 2004). It was reported that the *E. sakazakii* was able to adhere to and grow on latex, polycarbonate, silicon and to a lesser extent stainless steel (Iversen et al. 2004). Schindler and Metz (1991) found *E. sakazakii* with overall frequencies of 1.8% (10/564 strains) and 0.4% (1/256 strains) investigating central and local drinking water supplies.

Recently, most of the attention paid to *E. sakazakii*-related contamination of food products has focused on PIF. In 2002, the U.S. Food and Drug Administration (FDA) published a warning regarding the presence of *E. sakazakii* in baby formula (U.S.FDA. 2002). PIF is not manufactured as a sterile preparation, and some heat-resistant *E. sakazakii* isolates expressed a higher level of *infB* than did the heat-sensitive isolates. The *infB* gene encodes the prokaryotic translation initiation factor (IF2), which plays a very important functional role in stress-response mechanisms of this pathogen (Asakura et al. 2007). Moreover, producing capsular materials may provide protection for the organism, facilitating its survival in a desiccated environment (Drudy et al. 2006). *Enterobacter sakazakii* can adhere to plastics and silicon rubber surfaces and grow in a biofilm; biofilm formation may also be a factor associated with altered

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susceptibility to antimicrobials (Iversen et al. 2004; Lehner et al. 2005). *Enterobacter sakazakii* may exhibit long-term persistence in dried infant formula and has been reported to be the only organism isolated after a 2.5-year period of storage (Riedel and Lehner 2007).

This paper is aimed at giving an overview on *E. sakazakii*, a very important foodborne pathogenic bacterium that causes serious infections among infants, especially the preterm neonates.

Reservoirs of *Enterobacter sakazakii*

The natural reservoir of *E. sakazakii* has not yet been characterized, but the organism can be found in a variety of environments and food (FAO 2004; Iversen and Forsythe 2004). Kandhai et al. (2004) isolated *E. sakazakii* from milk powder manufacturing facilities and household vacuum cleaners, thus confirming its ubiquitous distribution. *Enterobacter sakazakii* has also been isolated from milk powders, baby foods, beef, sausage meat, cheese products, and vegetables (Leclercq et al. 2002, Muytjens and Kollee 1990). Nevertheless, *E. sakazakii* was not detected in other environmental settings, including surface water, soil, mud, rotting wood, grain, bird droppings, domestic animals, cattle, or cows' milk (Muytjens and Kollee 1990). In addition, *E. sakazakii* has been isolated from a wide range of clinical sources, including cerebrospinal fluid (CSF), blood, bone marrow, sputum, urine, inflamed appendix tissue, intestinal and respiratory tracts, and wounds (Gurtler and Beuchat 2005; Ray et al. 2007; Bhat et al. 2009). In addition, the hospital environment provides a befitting survival space for *E. sakazakii.*

Infections associated with *Enterobacter sakazakii*

Feeding with *E. sakazakii*-contaminated PIF has been epidemiologically implicated in several clinical cases. The first cases of neonatal meningitis probably caused by *E. sakazakii* were reported in 1961 (Urmenyi and Franklin 1961). Since then, a lot of infection cases associated with this organism have been reported in many countries (Table 1). Although most cases have been reported in developed countries, few *E. sakazakii* infections described in India have been reported (Ray et al. 2007; Bhat et al. 2009).

Bowen and Braden (2006) analyzed 46 cases of invasive infant *E. sakazakii* infection. Twelve infants had bacteremia, 33 had meningitis, and 1 had a urinary tract infection. The PIF samples associated with 15 (32.6%) of 46 cases yielded *E. sakazakii*; in 13 cases, clinical and formula strains were indistinguishable. *E. sakazakii* was obtained from the vaginal smear (PH 5.5) of a 26-year-old woman in Budapest. Importantly, she had bathed in the resort lake Balaton with water temperature 26–28°C (Ongrádi 2002).

The mortality rate of infections caused by *E. sakazakii* ranged from 40 to 80% (Nazarowec-White and Farber 1997; Sethi and Prakash 2010). Clearly, the severity of the infection in infants as well as the scarcity of information on the ecology and pathogenicity of this organism warrant further studies on clinical and microbiological features of this foodborne pathogen.

Environmental stress on *Enterobacter sakazakii*

Thermal treatment of foods prior to consumption has long been used as a primary means of reducing the risks associated with foodborne pathogens. *Enterobacter sakazakii* has been reported thermotolerant. Kim and Park (2007) studied thermal resistance and inactivation of *E. sakazakii* isolates during rehydration of PIF and found that the thermal resistance of *E. sakazakii* increased in rehydrated PIF compared with in saline. Moreover, the ability of *E. sakazakii* to form biofilms and survive desiccation conditions may contribute to its survival in infant formula factory environments and subsequent desiccated products (Iversen et al. 2004; Grimm et al. 2008). Beuchat et al. (2009) studied factors affecting the survival, growth, and inactivation of *E. sakazakii*, and their studies clearly indicated that its survival was influenced by α_w and temperature, and that survival was favored by low α_w and low storage temperature. Shaker et al. (2008) have already shown that desiccation and heat stresses caused significant reduction in *D*-values of *E. sakazakii* strains as used in the present study.

Nazarowec-White and Farber (1997) found that *E. sakazakii* strains was highly heat-tolerant and showed a wide range of growing temperatures. Shaker et al. (2008) studied thermal inactivation of stressed *E. sakazakii* in PIF with hot water at various temperatures, when the temperature of water was increased to 70°C, a significant reduction in stressed cells compared with the unstressed cells by approximately 1 $\log_{10}$. But there were no significant differences when PIF was reconstituted with water at 80, 90, and 100°C where the populations were <1 $\log_{10}$. So, to reduce the risk of *E. sakazakii* contamination from PIF, it is recommended that the water at the appropriate temperature for rehydration.

Environmental stresses such as acid and cold have been reported. Johler et al. (2010) found *E. sakazakii* isolates survived under conditions of low pH and were able to grow to approximately 10^9 CFU/ml within 24 h at pH 4.5. This is of particular interest in terms of the survival of *E. sakazakii* in the stomach after ingestion, since neonates tend to show higher gastric pH levels than adults (Maffei and Nobrega

Table 1 Cases of *E. sakazakii* infection in neonates and infants (1961–2009)

year of report	No. of cases	No. of deaths	Symptoms	Source	References
1961	2	2	Meningitis	Unknown	Urmenyi and Franklin (1961)
1965	1	Unknown	Meningitis	Unknown	Joker et al. (1965)
1979	1	0	Bacteremia	Unknown	Monroe and Tift (1979)
1981	1	Unknown	Meningitis, sepsis	Unknown	Adamson and Rogers (1981)
1981	1	0	Meningitis	Unknown	Kleiman et al. (1981)
1983	8	6	Meningitis	PIF	Muytjens et al. (1983)
1984	NS	NS	NS	NS	Postupa and Aldova (1984)
1985	1	0	Meningitis	Unknown	Naqvi et al. (1985)
1987	11	5	Meningitis and sepsis	Unknown	Arseni et al. (1987)
1988	2	0	Meningitis	Unknown	Willis and Robinson (1988)
1989	3	1	Meningitis	PIF	Biering et al. (1989)
1989	2	2	Appendicitis, conjunctivitis	NS	Reina et al. (1989)
1989	4	0	Meningitis	Unknown	Lecour et al. (1989)
1989	1	0	Sepsis, diarrhoea	PIF	Simmons et al. (1989)
1990	4	NS	Septicemia	PIF	Clark et al. (1990)
1990	1	0	Bacteremia	PIF	Noriega et al. (1990)
1991	NS	NS	Meningitis	NS	Gallaghar and Ball (1991)
1994	1	NS	Necrotising enterocolitis	Unknown	Chan et al. (1994)
1994	1	0	Meningitis	Unknown	Reis et al. (1994)
1996	1	0	wound infection	Unknown	Tekkok et al. (1996)
2000	1	0	Meningitis	NS	Burdette and Santos (2000)
2001	12	0	Enterocolitis	PIF	Van Acker et al. (2001)
2001	2	0	Bacteremia, meningitis	PIF	Bar-Oz et al. (2001)
2001	1	0	Bacteremia	NS	Lai (2001)
2002	NS	NS	NS	PIF	Block et al. (2002)
2002	11	1	Meningitis, enterocolitis	PIF	Himelright et al. (2002)
2004	2	NS	NS	NS	Coignard et al. (2004)
2005	2	-	-	-	Unpublished data
2007	2	1	Respiratory distress, sepsis	PIF	Ray et al. (2007)
2009	1	0	Urinary tract infection	NS	Bhat et al. (2009)

NS Not specified in papers, - not stated

1975). Iversen et al. (2004) reported that *E. sakazakii* were able to grow in PIF during storage at refrigeration temperatures and attach to infant-feeding equipment, which may become reservoirs of infection. Therefore, trying to reduce the storage time of PIF is obviously essential.

Antibiotic resistance

Although *E. sakazakii* has been detected in multiple food sources, a strong association has been found only with PIF. Its antibiotic susceptibility has also been conducted after some fatal infections among infants reported (Van Acker et al. 2001; Simmons et al. 1989; Arseni et al. 1987). Oonaka et al. (2010) found *E. sakazakii* isolates from PIF were resistant to ampicillin and lincomycin, while sensitive to gentamicin and cephalosporins. Shadlia-Matug et al. (2008) reported that *E. sakazakii* isolates were resistant to penicillin G, ampicillin and cephalothin, and also sensitive to gentamicin. *Enterobacter sakazakii* infections have been traditionally treated with ampicillin-gentamicin or ampicillin-chloramphenicol (Lai 2001). However, resistance to ampicillin has emerged due to the acquisition of transposable elements and the production of β-lactamases (Pitout et al. 1997; Girlich et al. 2001). Consequently, consideration should be given to the use of carbapenems or the newer cephalosporins in combination with a second agent, such as an aminoglycoside. The use of trimethoprim-sulfamethoxazole may also be useful (Lai 2001). It is recommended that the antibiotic-resistance pattern of the organism be established early on, so the infection can be treated properly from the beginning.

In conclusion, surveillance of emerging resistance of *E. sakazakii* is of paramount importance. And collection of updated data is mandatory for appropriate use of antibiotics. To select antibiotics conscientiously according to susceptibility tests is very important.

Prevention strategies

Enterobacter sakazakii is recognized as a foodborne pathogen associated with the contaminated PIF. In order to reduce the risk associated to consumption of contaminated formulas, correct information and education regarding good practices required during preparation and handling of this product are required.

A variety of strategies have been suggested to minimize the risk of *E. sakazakii* contamination of infant formula, including the use of gamma radiation and *E. sakazakii*-targeted bacteriophage therapy to reduce bacterial growth (Lee et al. 2006; Kim et al. 2007). Furthermore, in considering the thermal characteristics of *E. sakazakii*, Kim and Park (2007) recommended that rehydration of powdered infant formula for infant feeding with water at more than 60°C may be more helpful for the reduction of *E. sakazakii* with minimal nutrient reduction. For the high risk population (preterm neonates, infected infants), a sterilized rehydrated PIF to feed them is demanded. And physicians and other caregivers must advocate breast-feeding as the preferred means of feeding infants.

Conclusion

Enterobacter sakazakii is an emerging foodborne pathogen, often transmitted through PIF and responsible for a series of infections, some of which with potential fatal outcomes in a particular segment of the population. So, preventive measures by parents, infant formula manufacturers, and health care providers will be important in the prevention of *E. sakazakii*-related infections. We recommend more preventative strategies be applied in the process of PIF products to avoid being contaminated by this foodborne pathogen.

Given increasing reports of antibiotic resistance, we hope there will be antibiotic susceptibility test before using antibiotics. And better understanding of the pathogenesis *E. sakazakii*-related diseases will help in the development of new modes of prevention for this emerging pathogen.

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