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Enterobacter sakazakii: An Emerging Pathogen in Powdered Infant Formula

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Enterobacter sakazakii **represents a significant risk to the health of neonates. This bacterium is an emerging opportunistic pathogen that is associated with rare but life-threatening cases of meningitis, necrotizing enterocolitis, and sepsis in premature and full-term infants. Infants aged <28 days are considered to be most at risk. Feeding with powdered infant formula (PIF) has been epidemiologically implicated in several clinical cases. Infants should be exclusively breast-fed for the first 6 months of life, and those who are not should be provided with a suitable breast-milk substitute. PIF is not a sterile product; to reduce the risk of infection, the reconstitution of powdered formula should be undertaken by caregivers using good hygienic measures and in accordance with the product manufacturer's food safety guidelines.**

Enterobacter sakazakii is a member of the family Enterobacteriaceae, genus *Enterobacter,* and is a motile peritrichous, gram-negative bacillus [1]. The organism, which was initially referred to as "yellow-pigmented cloacae," was reclassified as *"E. sakazakii"* in 1980 on the basis of differences in DNA-DNA hybridization, biochemical reactions, pigment production, and antibiotic susceptibility, compared with *Enterobacter cloacae* [1]. Recent studies have demonstrated that *E. sakazakii* is a genomically heterogeneous and, therefore, poorly defined species [2, 3].

E. sakazakii is regarded as an emerging opportunistic human pathogen and the etiological agent of life-threatening bacterial infections in infants [4–7]. *E. sakazakii* was first implicated in a case of neonatal meningitis in 1958, when an outbreak in England resulted in the deaths of 2 infants [8]. Since that time, there have been ~70 reported cases of *E. sakazakii* infection (table 1). Although the incidence of *E. sakazakii* infection is low, the prognosis is poor, and infection is associated with significant morbidity and mortality. Powdered infant formula (PIF) products have been shown to contain *E. sakazakii* and have been epidemiologically linked to several clinical cases [19, 23, 24, 26]. Related coliforms, such as *Citrobacter diversus,* have also been isolated from PIF [29]. Like *E. sakazakii,* these organisms can cause invasive infections. There is also evidence from surveillance activities that low-level contamination of PIF with *Salmonella* species has led to cases of disease in infants [30]. Recalls of infant formula contaminated with *E. sakazakii* have occurred in the United States and Europe [19, 23, 26]. This has resulted in increased efforts to implement appropriate strategies to reduce the health risks associated with the use of PIF.

INFECTIONS CAUSED BY *E. SAKAZAKII*

E. sakazakii infections are an important cause of life-threatening meningitis, septicemia, and necrotizing enterocolitis in infants [31]. Premature and low-birth-weight infants and those aged <28 days are considered to be more at risk than are older infants [4–7]. Clinical presentation includes meningitis (complicated by ventriculitis, brain abscess, cerebral infarction, and cyst formation), bacteremia, and necrotizing entercolitis [20]. A CT scan of the skull should be considered early during the management of symptomatic infected infants, because it nearly always reveals abnormalities, including cystic changes, abscesses, fluid collection, dilated ventricles, and infarctions. *E. sakazakii* has also been identified in the stool or urine [4, 6, 18] of asymptomatic infants, and stool carriage has been demonstrated for up to 18 weeks.

There have been few reports of *E. sakazakii* infection in adults, and it is not usually life threatening [20, 31–33]. Indeed,

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Table 1. Sporadic cases and outbreaks of *Enterobacter sakazakii* infection for which powdered infant formula (PIF) was implicated as the source agent.

Location (year)	No. of cases	No. of deaths	Source	Reference(s)
England (1958)	2	2	Unknown	[8]
Denmark (1958)	1	1	Unknown	[9]
Georgia (1958)	1	0	Unknown	[10]
Oklahoma (1958)	1	1	Unknown	[11]
Indiana (1981)	1	0	Unknown	[12]
Denmark (1983)	8	6	Suspected PIF	[13]
Greece (1977–1981)	1	1	NS	[14]
Greece (1984)	11	4	Unknown	[15]
Missouri (1984)	1	0	Unknown	[16]
Massachusetts (1984)	2	1	Unknown	[17]
Iceland (1986–1987)	3	1	PIF[a]	[18]
Tennessee (1988)	4	0	PIF, blender	[19, 20]
Maryland (1990)	1	0	PIF, blender	[21]
Ohio (1990)	1	0	NS	[22]
Belgium (1998)	12	2	PIF	[23]
Israel (1999–2000)	2	0	PIF and blender	[5, 6]
Tennessee (2001)	10	1	PIF	[24]
Belgium (2002)	1	1	PIF	[25]
New Zealand (2004)	5	1	PIF	[25]
France (2004)	4	2	PIF	[26]

NOTE. Data are based on [20, 27, 28]. NS, not specified.

[a] One of the causal factors responsible may have been reconstituted formula was held at 35°C–37°C for lengthy periods.

most adults with reported *E. sakazakii* infections had serious underlying diseases, such as malignancies. *E. sakazakii* infections continue to be more common in neonates and infants, among whom they are usually associated with a poor prognosis. Mortality rates of 33%–80% have been reported [31]. *E. sakazakii* infections are also associated with significant morbidity. Most children who survive *Enterobacter*-associated meningitis (94%) develop irreversible neurological sequelae resulting in quadriplegia, developmental impedance, and impaired sight and hearing. These sequelae are frequently attributed to secondary cerebral infarcts.

RESERVOIRS OF *E. SAKAZAKII*

The natural habitat of *E. sakazakii* is currently unknown. This bacterium can be found in the environment and in food [34, 35]. The organism's natural habitat may be on plant material, and this may account for the organism's isolation from dry herbs and spices [35]. Kandhai et al. [36] isolated *E. sakazakii* from milk powder manufacturing facilities (8 of 9 samples) and household vacuum cleaners (5 of 16 samples), thus confirming its ubiquitous distribution. *E. sakazakii* has also been isolated from milk powders, cheese products, baby foods, minced beef, sausage meat, and vegetables [35, 37]. In addition, the isolation of *E. sakazakii* from animal sources—Mexican fruit flies and from the gut of stable fly larvae—has been documented [38–40]. The organism was not detected in other environmental settings, including surface water, soil, mud, rotting wood, grain, bird droppings, domestic animals, cattle, or cows' milk [41]. *E. sakazakii* has also been isolated from a wide range of clinical sources, including CSF, blood, bone marrow, sputum, urine, inflamed appendix tissue, intestinal and respiratory tracts, eye, ear, wounds, and feces [11, 22, 27]. This organism has also been isolated from the hospital environment [42].

MODE OF TRANSMISSION

The sources of *E. sakazakii* and its vehicles of transmission are not always clear. Although the organism has been detected in multiple food sources, a strong association has been found only with PIF. Intrinsic and extrinsic contamination of PIF with *E. sakazakii* can occur. Intrinsic contamination results from the introduction of the organism to the PIF at some stage during the manufacturing process. In contrast, extrinsic contamination may result from the use of contaminated utensils, such as blenders and spoons, in the preparation of PIF [21].

Several investigations into the presence of *E. sakazakii* in PIF have been performed. Muytjens et al. [43] examined 141 dif-

ferent powdered formulas from 35 countries and isolated *E. sakazakii* at levels ranging from 0.36 to 66 cfu per 100 g from 20 formula samples from 13 countries. Simmons et al. [19] isolated *E. sakazakii* (8 cfu per 100 g) from PIF in association with an outbreak in Memphis, Tennessee. Biering et al. [18] isolated *E. sakazakii* from 5 different lot numbers of unopened packages of PIF after an outbreak of neonatal meningitis in Iceland. A Canadian survey that investigated the incidence of *E. sakazakii* in PIF isolated the organism from 8 of 120 cans from 5 different manufacturers [44].

Van Acker et al. [23] reported an outbreak of *E. sakazakii* infection involving 12 infants who had necrotizing enterocolitis in 1998 in Belgium; *E. sakazakii* was isolated from liquid formula prepared from PIF. In Belgium in 2002, an infant died of *E. sakazakii*–associated meningitis after consuming a commercial PIF. The product was withdrawn after the detection of low levels of *E. sakazakii* in the implicated infant formula. In New Zealand in July 2004, a premature infant contracted *E. sakazakii* meningitis and died. The subsequent investigation found that 4 other babies in the neonatal intensive care unit were colonized with this organism, but none became unwell. The investigation attributed the source of the organism to PIF used in the nursery [45]. Most recently, another PIF was withdrawn after a possible link to 5 cases of presumed *E. sakazakii* infection in premature infants in France in 2004 that led to the death of 2 infants [26].

PATHOGENICITY AND VIRULENCE FACTORS

In mammalian tissue culture, the organism can attach to intestinal cells and survive internally in macrophages [46]. However, the specific bacterial adhesins and host cell receptors involved in these processes are unknown. Some strains of *E. sakazakii* produce capsular material, and how this material contributes to macrophage evasion remains to be determined [47]. Furthermore, this capsule may also provide protection for the organism, facilitating its survival in desiccated environments.

E. sakazakii can attach to plastics and silicon rubber surfaces and grow in a biofilm. Enteral feeding tubes and feeding-bottle teats can harbor the bacterium in large numbers [48]. Biofilm formation may also be a factor associated with altered susceptibility to antimicrobials [49, 50].

No reports have investigated the dose-response relationship in *E. sakazakii* infection. Using a suckling mouse model, Pagotto et al. [46] determined a minimum lethal dose for 18 *E. sakazakii* isolates (9 clinical, 8 food, and 1 type strain). They challenged newborn mice orally and intraperitoneally and showed that all isolates administered intraperitoneally were lethal at 1×10^8 cfu. For 2 strains, the minimum lethal dose was 1×10^5 cfu. Two of 18 isolates were lethal when administered orally. The authors concluded that the minimum lethal dose in neonates would most likely require an unusually high number of viable cells (an event likely to occur if reconstituted formula is held at an inappropriate temperature over time). This study also suggested the possibility of enterotoxin production. However, the importance of putative enterotoxin production remains unclear, because neither the genes encoding the putative toxin nor the protein itself have been identified as of yet. Furthermore, the possibility of differences in virulence among isolates cannot be ruled out. Future research should address these issues and provide a better understanding of the mechanism(s) of pathogenesis.

The thermotolerance of *E. sakazakii* has been determined by a number of researchers. Although there are significant discrepancies regarding the degree of heat resistance, *E. sakazakii* appears to be more thermotolerant than other Enterobacteriaceae cultured from dairy products [44, 49]. Whether this variation in thermoresistance correlates with the genetic diversity of *E. sakazakii* remains to be established.

ANTIMICROBIAL RESISTANCE

E. sakazakii is naturally resistant to all macrolides, lincomycin, clindamycin, streptogramins, rifampicin, fusidic acid, and fosfomycin [51]. It is susceptible to some antibiotics, including tetracyclines, aminoglycosides, numerous β-lactams, chloramphenicol, antifolates, and quinolones [51]. *E. sakazakii* infections have been traditionally treated with ampicillin-gentamicin or ampicillin-chloramphenicol [31]. However, resistance to ampicillin has emerged owing to the acquisition of transposable elements and the production of β-lactamases [52, 53]. *Enterobacter* species are known to be capable of inactivating broad-spectrum penicillins and cephalosporins through the production of β-lactamase enzymes. This situation also appears to be increasing among isolates of *E. sakazakii.* 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 [31].

LABORATORY DETECTION OF *E. SAKAZAKII*

Reliable detection of *E. sakazakii* poses a major challenge to PIF manufacturers; thus, the action to be taken on its identification is important. PIF is not a sterile product, and current Codex Alimentarius Commission specifications for PIF permit 1–10 coliform bacteria per gram of formula. It should be noted that *E. sakazakii* belongs to this group of organisms. Nevertheless, PIF manufacturers implement a policy of zero tolerance for both *Salmonella* and *Listeria* species in products. Current drafting of microbiological specifications for *E. sakazakii* is under consideration by the International Committee for the Microbiological Safety of Food and the Codex Alimentarius Commission.

CULTURE AND BIOCHEMICAL-BASED IDENTIFICATION

The US Food and Drug Administration–recommended procedure for the isolation of this organism uses standard isolation methods for Enterobacteriaceae, with additional selection for yellow-pigmented organisms and subsequent biochemical identification [54]. Furthermore, this protocol is only selective for Enterobacteriacae, is not specific for *E. sakazakii,* and requires 5 days to complete [54].

Muytjens et al. [55] first identified α-glucosidase enzyme activity in 129 isolates (100%) of *E. sakazakii.* This was not detected in 97 other *Enterobacter* species, including *E. cloacae, Pantoea agglomerans,* and *Enterobacter aerogenes.* Farmer et al. [56] confirmed this finding, identifying 53 of 57 *E. sakazakii* strains positive for α-glucosidase activity. To facilitate the detection of *E. sakazakii,* culture media—including Druggan-Forsythe-Iversen [57], Oh-Kang [58], and Leuscher-Baird-Donald-Cox [59] agar—have been recently formulated that exploit this key biochemical characteristic. However, α-glucosidase activity is not solely restricted to *E. sakazakii* [3].

MOLECULAR DETECTION

As a means of improving the detection of *E. sakazakii,* molecular-based methods are being developed. Seo and Brackett [60] described a quantitative real-time PCR technique in which primers and a TaqMan probe were developed to target the macromolecular synthesis operon of *E. sakazakii.* The specificity of the assay was established using 68 *Enterobacter* and 55 non-*Enterobacter* strains. The method could detect 100 cfu/mL in reconstituted PIF without an enrichment step. More recently, Liu et al. [61] developed 2 real-time PCR assays based on TaqMan and SYBR green technology. Both of these assays used primers that target the 16-23S rRNA spacer region and could detect 1.1 cfu of *E. sakazakii* per 100 g of infant formula after a 25-h enrichment.

SURVEILLANCE AND SUBTYPING

Bacterial subtyping systems are unrivalled in their ability to track pathogens along the food chain. DNA fingerprinting facilitates a direct comparison of bacterial isolates in outbreak investigations. These techniques are based on the fact that identical isolates share highly similar or indistinguishable DNA profiles that can differentiate these isolates from epidemiologically unrelated ones. Molecular tools enable the tracing back of outbreak isolates from clinical sources to the contaminated batch of PIF and/or the manufacturing environment, to facilitate corrective action and the proper focusing of control protocols. Reported methods applied to *E. sakazakii* include ribotyping, PFGE, and random amplification of polymorphic DNA [62]. A standardized subtyping scheme would be useful. This could be designed on the basis of PFGE protocols similar to those applied to other enteric organisms under surveillance by PulseNet (available at http://www.cdc.gov/pulsenet). This database would facilitate the global comparison and traceability of *E. sakazakii* isolates. Molecular subtyping methods are valuable analytical tools for the identification and tracing of sources of environmental contamination in PIF-processing facilities. Once standardized, these methods can provide important epidemiological data that would be useful in the control and elimination of *E. sakazakii* from the PIF food chain.

PUBLIC HEALTH SIGNIFICANCE OF *E. SAKAZAKII* AND FOOD SAFETY

Infants and young children are particularly vulnerable to foodborne infections. Therefore, the microbiological safety of infant and follow-up formula is of utmost importance. Caregivers in hospital neonatal units should be continuously alerted to the fact that PIF is not a sterile product and that, therefore, the use of hygienic measures during preparation and reconstitution are essential.

PIF has been fed to millions of infants for years, and it constitutes the majority of infant formula used worldwide. This product is formulated to mimic the nutritional profile of human breast milk [63]. Because PIF is not a sterile product, it is an excellent medium to support bacterial growth. Bovine milk is an essential ingredient of PIF and a potential source of bacteria that are pathogenic to humans. On occasion, bacterial pathogens have been cultured from PIF, including *Citrobacter, Enterobacter, Klebsiella, Staphylococcus, Streptococcus,* and *Yersinia* species. Should *E. sakazakii* multiply in PIF, it can result in infection [64]. A definitive link between the presence of *E. sakazakii* in an unopened can of PIF and an outbreak of infection has been reported [4]. Cases of invasive *E. sakazakii* infection have recently been added to the list of notifiable diseases in New Zealand, after the death of an infant due to *E. sakazakii* meningitis in July 2004 [45]. These actions highlight the importance of this opportunistic pathogen and the risk posed to vulnerable infants.

The World Health Organization recommends that infants should be exclusively breast-fed for the first 6 months of life. Infants who are not breast-fed should be provided with a suitable breast milk substitute, formulated in accordance with Codex Alimentarius Commission standards. To reduce the risk of infection in infants fed PIF, recommendations have been made for the preparation and storage of PIF. A summary of the current guidelines to care givers in hospitals and the home is shown in table 2.

Manufacturers of PIF are being encouraged to develop a greater range of commercially sterile alternative formula products for high-risk groups. In addition, formula manufacturers must implement strategies aimed at reducing the risks of prod-

Table 2. Summary of the general guidelines for the reconstitution of powdered infant formula (PIF) in hospitals and homes

Type of protocol, action
Hospital based
Formula should be selected on the basis of nutritional needs
Trained personnel should use aseptic techniques to prepare PIF
Disinfect all blenders, boiling spoons, bottles, and teats before preparation
Follow the manufacturers' instructions for preparation; product should be refrigerated at ≤4°C if not used immediately and discarded if not used within 24 h
The "hang" time for continuous enteral feeding should be <4 h
A hospital action protocol should be implemented in the event of a product recall, including the notification of hospital care providers, a reporting system, product batch follow-up, and careful documentation
Home based
Wash hands using soap and thoroughly clean the preparation area
Sterilize all bottles, spoons, and teats with boiling water or other suitable sanitizer before preparation
PIF should be freshly prepared for each feeding; any remaining milk should be discarded
To reconstitute PIF, water should be brought to a rolling boil, then cooled for a few minutes to a temperature of 70°C–90°C
Cool the reconstituted PIF to body temperature before feeding
Never keep bottles warm in heaters or thermoses

NOTE. Summarized from references [24, 25, 28, 64]. See also the World Health Organization's list of publications related to food safety (http://www.who.int/entity/foodsafety/publications).

uct contamination. Controlling the initial populations of *E. sakazakii* during the production of PIF and avoiding postprocessing contamination, using suitable microbiological approaches, will have a positive effect. Data from surveys showed that *E. sakazakii* can be cultured at various frequencies in samples of PIF, from the manufacturing facility, and from environmental sources [7, 23, 36, 43]. However, the true frequency of contamination is unknown, making it difficult to quantify the level of risk to vulnerable groups. The role of the broader infant food chain and of dairy animals and their environment as sources of contamination has not been investigated. Standardized analytical approaches are necessary to ensure product safety. The European Food Safety Authority has recommended the introduction of a performance objective for PIF and follow-up formula that is aimed specifically at low levels of *Salmonella* and *E. sakazakii* (e.g., absence in 1, 10, or 100 kg) [65].

FUTURE NEEDS

Current research on *E. sakazakii* has focused on the elimination of this coliform from PIF. Investigations into thermal resistance, osmotic tolerance, exopolysaccharide production, and pathogenicity, among others, have been performed, and attempts have been made to identify environmental reservoirs. Only 1 study has suggested the possible existence of an enterotoxin produced by *E. sakazakii* on the basis of an animal model [46]. Other virulence factors remain to be identified. Furthermore, why infection can occur in all age groups but is more frequent among full-term infants and neonates remains to be understood [18, 27].

Physicians and other care givers must advocate breast-feeding as the preferred means of feeding infants. Where this is not possible, hygienic practices for the preparation of PIF in both the home and hospitals should be carefully followed. These guidelines will contribute toward the minimization of risk. Increasing the awareness of *E. sakazakii* infection among medical personnel and the continuous education of all care givers to the potential threats posed by this organism will be essential to protect infants at high risk.

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