

A Hidden Danger in Infant Formulas and Baby Foods: *Enterobacter sakazakii* Contamination

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Summary

Several contaminants, which can come from different stages of production and storage, may be present in infant formulas and baby foods. Dry infant formula has been considered as a good medium for bacterial growth of *Enterobacter sakazakii*. The presence of *Enterobacter sakazakii* in infant formulas is a significant public health problem as the bacterium has putative virulence factors and can cause fatal infections like enterocolitis and meningitis. Serious measures should be taken to minimize bacterial contamination of powdered products especially by the manufacturers and all caregivers should continuously be educated to reduce the risk of contaminated infant food. This review will focus on the biological contaminant "*Enterobacter sakazakii*", the toxicity caused by its virulence factors and solutions for the reduction of contamination by this particular bacterium.

Key Words: *Enterobacter sakazakii*, infant formula, baby food, virulence factor, toxicity.

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Bebek Formüllerinde ve Bebek Mamalarında Gizli Tehlike: *Enterobacter sakazakii* Kontaminasyonu

Özet

Bebek formüllerinde ve bebek mamalarında üretim ve depolamanın farklı aşamalarından gelen çeşitli kontaminasyonlar bulunabilir. Kuru bebek formülleri *Enterobacter sakazakii* bakteriyel gelişimi için iyi bir vasat olarak kabul edilir. Virulans etkenlerden kabul edilen *Enterobacter sakazakii*, enterokolit ve menenjit gibi fetal enfeksiyonlara neden olduğundan bebek formüllerinde bu bakterinin bulunuşu önemli bir halk sağlığı sorunudur. Toz haline getirilmiş ürünlerin bakteriyel kontaminasyonun en aza indirilmesi için üreticilerin ciddi tedbirler alması gerekmektedir ve bebek mamalarının kontaminasyon riskini azaltmak için bebeklerle ilgilenen kişilerin tümü sürekli olarak eğitilmelidir. Bu derleme biyolojik kontaminant "*Enterobacter sakazakii*" ile virulans etkenlerinin neden olduğu toksisite ve bu bakteriye özgü kontaminasyonun azaltılması için çözümlere odaklanacaktır.

Anahtar Kelimeler: *Enterobacter sakazakii*, bebek formülü, bebek maması, virulans etken, toksisite

INTRODUCTION

The Food and Drug Administration (FDA) regulations define infants as persons not more than 12 months old (1). The United Nations Convention on the Rights of the Child defines a child as "every human being below the age of 18 years unless under the law applicable to the child, majority is attained earlier". Proper, safe and adequate nutrition is a

crucial and universally recognized component of a child's right as stated in the Convention on Rights of the Child (2). Other than breast milk, infant formulas and baby foods are the most important part of a baby's diet in the first year of life. Baby foods are also consumed for three years as a part of the child's diet (3).

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The Food, Drug and Cosmetic Act (FFDCA) defines infant formula as “a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk” (4). Infant formulas, available in powder, liquid-concentrate and ready-to-feed formulas, are artificial substitutes mimicking human breast milk. They are based on cow milk or soy milk, designed for the consumption of infants. Medical community considers infant formulas as nutritionally acceptable for infants under the age of one year when breastfeeding is not possible. Infant formulas can be modified through the scientific evidence about the need of nutrients of the infants (5). Baby food is any food given to infants, with soft, liquidly paste or chewed, between ages from six months to two years (6, 7). Baby purees are also considered as baby foods and they are composed of several fruits/vegetables or vegetables plus meat, chicken or fish.

Formulation, handling and storage of baby foods are important for their quality of nutrition and physicochemical properties (6). There are several nutrients that an infant formula must include according to FDA through the advices of American Academy of Pediatrics and it is necessary to set upper limits for a nutrient in the formula (6, 8, 9).

Though human milk is the appropriate choice for the vast majority of full term, pre-term and low birth-weight infants, it may also pose the same risks as many toxic substances taken by the mother can be excreted into milk. On the other hand, infant formulas/baby foods can contain several contaminants that carry potential risk during their first years of life. A contaminant is an impurity; any material of an extraneous nature associated with a chemical, a pharmaceutical preparation, a physiologic principle, or an infectious agent. Several contaminants, which can come from different stages of production and storage, may be present in infant formulas and baby food. Food consumption is an important route of human exposure to contaminants such as pesticides and industrial pollutants (10).

Infant formula, like no other food, is regulated by its

own law, the Infant Formula Act of 1980 as amended in 1986 (11). EU Commission has set regulations (Commission Regulation 466/2001 and Commission Regulation 466/2005 Amendment) on the maximum levels of nitrates, mycotoxins (aflatoxins, ochratoxins, patulin, fusarium toxins), heavy metals and polycyclic aromatic hydrocarbons in infant food (12-14). In USA, The Center for Food Safety and Applied Nutrition, a center in FDA, is responsible for regulation of infant formulas. Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) and The Office of Food Additive Safety (OFAS) have program responsibility for ingredients and packaging of infant formulas (1).

Contaminants are a vast subject area of food safety and quality and can be present in our food chain from raw materials to finished products. These contaminants can be classified as follows (15):

1. Biological contaminants (*Enterobacter sakazakii*)
2. Toxins of biological contaminants (mycotoxins)
3. Chemical contaminants (acrylamide, xenoestrogens, heavy metals, pesticides, nitrates/nitrites, polyaromatic hydrocarbons [PAHs] and polycyclic biphenyls [PCBs])

This review will focus on the biological contaminant “*Enterobacter sakazakii*”, its contamination in baby foods with the toxicological outcomes and solutions for the reduction of contamination by this particular bacterium.

Enterobacter sakazakii

Contamination of baby food with biological contaminants can raise very important problems in a neonate’s or an infant’s health and may lead to severe problems or even death as the immune functions as well as other defense mechanisms of neonates/infants are not as well-functioned as adults. *Enterobacter sakazakii* (*E. sakazakii*) is a well-known *Enterobacter* species which can contaminate dry baby foods and infant formulas and cause severe intoxications and infections. During many years the several studies on the isolation of *E. sakazakii* suggested cases of neonatal meningitis or necrotizing enterocolitis were related to ingestion of powdered infant formulae. This led to the establishment of a

causal link. In some later cases the organism was isolated on utensils such as mixers used in bottle kitchens. Even though in some cases *E. sakazakii* could not be isolated from the infant formulae, a causal relationship was assumed. Recent publications have demonstrated that this microorganism can be found in a wide variety of foods, water and environments including homes and hospitals.

Several outbreaks in different parts of the world were recorded at through different decades. The high mortality rate (40-80%), the severity of the infection in infants, plus the scarcity of information on the ecology and pathogenicity of this organism warranted this review of the clinical and microbiological features of this putative food-borne pathogen.

E. sakazakii is a facultative, motile, peritrichous, gram-negative, rod-shaped bacterium belonging to the family *Enterobacteriaceae*, member of genus *Cronobacter*, which comprises of five species and its normal habitat is unclear. Spore formation has never been observed (16). It was originally designated as yellow-pigmented variant of *E. cloacae* until 1980 when it was introduced as a new species based on differences between *E. sakazakii* and *E. cloacae* in DNA-DNA hybridization, biochemical reactions, the production of yellow-pigmented colonies, and antibiotic susceptibility. The name *E. sakazakii* is here proposed in honor of the Japanese bacteriologist 'Riichi Sakazaki' for his many contributions to our current understanding of *Enterobacteriaceae*, *Vibrionaceae*, and enteric bacteriology (17). Most of the *E. sakazakii* strains described in the literature have been isolated from clinical sources. Besides, the bacterium was isolated from plant food and food ingredients like cereal, fruit and vegetables, legume products, herbs and spices as well as from animal food sources like milk, meat and fish and products made from these foods (18).

The spectrum of *E. sakazakii* contaminated food covers both raw and processed food. The kind of processing of *E. sakazakii* contaminated food was not restricted to dry products. Fresh, frozen, ready-to-eat, fermented and cooked food products as well as beverages and water suitable for the preparation of food, were

found to be contaminated by *E. sakazakii*. However, contamination with *E. sakazakii* can occur during the process of adding dry ingredients, including vitamins and minerals, to powdered milk infant formula and baby foods or during post-pasteurization packaging (18). On the other hand, contamination of powdered infant formula with *E. sakazakii* can occur extrinsically. Extrinsic contamination may result from the use of contaminated utensils, such as blenders and spoons, as well as the water used during preparation of the powdered infant formula (19).

Dry infant formula has been considered as a good medium for bacterial growth and reason of fatal infection with *E. sakazakii* (20). The presence of *E. sakazakii* infant formula is a significant public health problem (21). Commercially sterile, ready to-use infant feeds are recommended for use instead of powdered infant feeds (22, 23).

The presence of *Enterobacter* species is often used as an indicator of product quality and hygienic manufacturing practice. Verification of the presence of *Enterobacteriaceae* can be confirmed by using several techniques for detecting in both the product and the manufacturing environment (24-28).

Virulence Factors of *E. sakazakii*

E. sakazakii has putative virulence factors and some of these were characterized by different study groups:

a. Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria. This is composed of three parts, a complex lipid, called lipid A (consisting of sugars and fatty acids), that anchors the structure to the outer membrane, a conserved core oligosaccharide, and variable polysaccharide side chains (O antigen) that extend from the latter core. The O antigen is a major surface antigen present in gram-negative bacteria, and it is responsible for serological diversity. In these bacteria, LPS contains many oligosaccharide units (individual O units consisting of between 3 and 6 sugars) with typically between 10 and 30 repeats (29-31). Genes involved in O-antigen synthesis and downstream assembly map to the *rfb* locus located between the *galF* and *gnd* genes in

many *Enterobacteriaceae*. The O-antigen locus varies in size for each serotype depending on the sugar composition and complexity of the antigen structure (32). O-antigen serotypes emerge as a consequence of the gene content rather than sequence variation at this locus (33). Usually three gene types map to the O-antigen gene cluster, and these include the following: (i) genes that code for enzymes involved in the synthesis of sugars forming the O subunit, (ii) genes that encode glycosyltransferases, involved in the assembly of sugar substituents in the O subunit, and (iii) those genes that code for the transporter (*wzx*) and polymerase (*wzy*) proteins necessary for processing and assembly of the O antigen from the O subunit. The O-antigen gene cluster of *E. sakazakii* was first characterized by Mullane et al (2008). The group described the first characterization of two *rfb*-encoding loci defining two serotypes, O:1 and O:2, in *E. sakazakii*. These data served to further our understanding of this emerging neonatal pathogen. Serotype-specific genes were identified and were used to develop a PCR assay. Based on these primer pairs, these gene targets could be used to identify *E. sakazakii* serotype O:1 and O:2 strains from environmental, food, and clinical sources, contributing to the protection of neonates (34).

b. The first report describing another virulence factor of *E. sakazakii* was by Pagotto et al (2003). The group reported an enterotoxin production by the bacterium. The clinical and food-borne isolates of *E. sakazakii* were evaluated for enterotoxin production by the suckling mouse assay. In addition, suckling mice were challenged both orally and by i.p. injection. Of 18 *E. sakazakii* strains evaluated, four were found to test positive for enterotoxin production. All strains of *E. sakazakii* were lethal to suckling mice at 10(8) CFU per mouse by i.p. injection, while two strains caused death by the oral route. In in vitro assays, CHO, Vero, and Y-1 cells demonstrated both cell lysis and rounding when exposed to *E. sakazakii* strain LA filtrates (35). However, there were no reports until 2007 for the purification of this toxin. In 2007, Raghav and Aggarwal reported that they have purified and characterized the biochemical properties of the toxin. The toxin was purified by ammonium sulfate precipitation, followed by DEAE cellulose ion

exchange and desalting by Sephadex G-100. The 66 kDa toxin was most active at pH 6 and was stable at 90°C for 30 min. This stability combined with the potent activity of the toxin ($LD_{50} = 56$ pg) emphasizes the potential risk to neonates fed infant milk formula contaminated with *E. sakazakii* (36).

Enterobacter infections

Enterobacter infections are most common in neonates and in elderly individuals, reflecting the increased prevalence of severe underlying diseases at these age extremes. In the pediatric ICU setting, an age younger than 2.5 years is a risk factor for colonization. There have also been few reports of *E. sakazakii* infection in adults and it is not usually life-threatening. Most adults with reported *E. sakazakii* infections have also had serious underlying disease, such as malignancy (37).

Many different species comprise the genus *Enterobacter*. Some have never been associated with human infections. *Enterobacter* infections do not produce a unique enough clinical presentation to differentiate them clinically from other acute bacterial infections. The most commonly isolated species include *E. cloacae* and *E. aerogenes*, followed by *E. sakazakii*. Other species rarely encountered in the clinic include *E. asburiae*, *E. gergoviae*, *E. taylorae*, and *E. hormaechei*. The infections seen in clinic by *Enterobacter* species are as follows (38):

- **Lower respiratory tract infections** (asymptomatic colonization, tracheobronchitis, pneumonia, lung abscess, and emphysema)
- **Skin and soft-tissue infections** (cellulitis, fasciitis, myositis, abscesses, and wound infections)
- **Endocarditis**
- **Urinary tract infections**
- **Intra-abdominal infections** (emphysematous cholecystitis, suppurative cholangitis, and hepatic gas gangrene, hemorrhagic necrotizing pancreatitis)
- **Ophthalmic infections**
- **Bone and joint infections** (septic arthritis, on both native and prosthetic joints, and can result in osteomyelitis and discitis)
- **Bacteraemia, including sepsis:** *Enterobacter* bacteremia is the bacteremia caused by

Enterobacter species and the male-to-female ratio of this bacteremia is 1.3-2.5:1. This male predominance is also reported in the pediatric population. The portal of entry into the bloodstream is frequently unknown, but any infected organ may be the primary source of bacteremia. Symptoms of *Enterobacter* bacteremia are similar to those of bacteremia due to other gram-negative bacilli. Physical examination findings consistent with systemic inflammatory response syndrome (SIRS) include heart rate that exceeds 90 bpm, a respiratory rate of greater than 20, and temperature of greater than 38°C or less than 36°C. More than 80% of children and adults with *Enterobacter* bacteremia develop fever. Hypotension and shock occur in as many as one third of cases. Disseminated intravascular coagulation, jaundice, acute respiratory distress syndrome, and other organ failures reflect the severity of septic shock. Purpura fulminans and hemorrhagic bullae usually observed with meningococci or viruses causing hemorrhagic fever may be part of the clinical presentation of *Enterobacter* bacteremia. *Ecthyma gangrenosum*, usually associated with *Pseudomonas* or *Aeromonas* bacteremia, may also be observed. Cyanosis and mottling is frequently reported in children with *Enterobacter* bacteremia (38).

- **Meningitis:** Meningitis can be characterized by complicated by ventriculitis, brain abscess, cerebral infarction and cyst formation. Mortality rates of 33 – 80% have been reported in preterm infants affected by invasive *E. sakazakii* infection, with a high rate of neurological impairment among the survivors. Indeed, 94% of children who survive *Enterobacter*-associated meningitis develop irreversible neurological sequela, resulting in quadriplegia, developmental impedance, and impaired sight and hearing. These sequels are often attributed to secondary cerebral infarcts (37, 39).
- **Necrotizing enterocolitis** (40, 41, 42-44): Initial symptoms include feeding intolerance, increased gastric residuals, abdominal distension and bloody stools. Symptoms may progress rapidly to abdominal discoloration with intestinal

perforation and peritonitis and systemic hypotension requiring intensive medical support.

E. sakazakii Cases in Infants and Children

Conventional wisdom on the risks of infant formula has held that the major risk—apart from a lesser nutritional quality compared with human milk and the absence of immune factors—is unclean water used to reconstitute the powdered infant formula. But it is not the whole story, as several outbreaks of *E. sakazakii* over the last decade have shown. Although its epidemiology and reservoir are unknown, intrinsically and extrinsically contaminated dried infant formula has been implicated as the source of outbreaks and sporadic cases of *E. sakazakii* infections and colonization among neonates (45).

The first two known cases of neonatal meningitis caused by *E. sakazakii*, classified initially as *E. cloacae*, were reported in 1961 (46). The first reported case of *E. sakazakii* infection linked definitively to formula was in a male infant born at 33.5 weeks of gestation who was admitted to the neonatal intensive care unit (NICU) because of prematurity and respiratory distress. Subsequently, the patient was noted to be febrile and tachycardic and to exhibit systemic shock. At 11 days of life, he developed seizure activity, and a culture of his cerebrospinal fluid grew *E. sakazakii*. Intravenous antibiotics were administered, but the patient died on day of life 20. Close surveillance of the 49 patients in the NICU at that institution revealed an additional nine cases of *E. sakazakii* infection. All nine positive cultures were obtained from non-sterile sites (such as tracheal aspirates, stool, and urine), and therefore could not be confirmed as signs of infection. Putative risk factors, including age, birth weight, medications, incubators used, and need for mechanical ventilation were investigated. A significant association was found between a specific powdered infant formula and the patients with *E. sakazakii*-positive cultures. Cultures of the formula found a single batch to be contaminated with *E. sakazakii*. Cultures from the NICU environment and water supplies were negative. The manufacturer recalled the batch of formula voluntarily (47).

In 2000 a Belgian team reported on a 1998 outbreak of necrotizing enterocolitis a total of 12 neonates developed NEC in June-July 1998, and two of whom, a set of twins, died: 10 of the infants were being fed on the same powdered infant formula. *E. sakazakii* was isolated from both samples of the prepared formula and the unopened cans. *E. sakazakii* was isolated from a stomach aspirate, anal swab, and/or blood sample for 6 of the 12 neonates. A review of feeding procedures revealed that 10 of the 12 patients were fed orally with the same brand of powdered milk formula. *E. sakazakii* was isolated from the implicated prepared formula milk as well as from several unopened cans of a single batch. Molecular typing by arbitrarily primed polymerase chain reaction confirmed, although partially, strain similarity between milk and patient isolates. No further cases of necrotizing enterocolitis were observed after the use of the contaminated milk formula was stopped. With this outbreak, it was shown that intrinsic microbiological contamination of powdered milk formula can be a possible contributive factor in the development of necrotizing enterocolitis and this condition encountered almost exclusively in formula-fed premature infants (48).

In April 2001, the death from neonatal meningitis of a baby boy born 6 weeks prematurely triggered an investigation by the Tennessee Department of Public Health and the U.S. Centers for Disease Control and Prevention (49). The results were unsettling for families whose vulnerable infants were entrusted to the care of the neonatal intensive care unit. Of 49 infants screened, 10 (over 20%) were colonized by, or infected with, *E. sakazakii*. In 2004, powdered infant formula was linked to two other *E. sakazakii* outbreaks, in New Zealand and in France. The French outbreak involved nine cases, and resulted in the deaths of two infants. While eight of the cases were in premature infants of low birth weight (<2 kg), one case was in an infant born at 37 weeks and weighing 3.25 kg. The outbreak involved five hospitals, and a review of practices in the hospitals revealed that one hospital was not following recommended procedures for the preparation, handling and storage of feeding bottles, and four were storing reconstituted formula for >24 hours in domestic-type refrigerators, with no

temperature control or traceability (50). New Zealand outbreak, however, affected a total of 387 adults and these subjects were from one of four hospitality areas who consumed food or beverage at Eden Park on the evening of 17 June 2006 and subsequently suffered from diarrhea or vomiting; or, stomach cramps and nausea (51).

In New Mexico in 2008; one infant developed severe brain injury and hydrocephalus, and the other infant died. The first was a female infant, born in September 2008 at full term. The infant had been fed with prepared infant formula since birth and she was well until approximately age 3.5 weeks, when a family member noted that she had an axillary temperature of 38.3°C and took her to a hospital emergency department. Records for this visit noted a normal rectal temperature, normal fontanel, and overall healthy appearance. The infant was discharged without further testing or treatment. However, during the following week, the infant became notably fussier and began vomiting. Her illness progressed during early November, when at age 6.5 weeks she exhibited seizure-like activity. She was admitted to a hospital and on physical examination the infant was found to have a bulging anterior fontanelle, horizontal nystagmus, a positive Brudzinski sign with neck rigidity, and dehydration. Cerebrospinal fluid cultures collected during this admission yielded a *Cronobacter* organism; blood cultures were negative for *Cronobacter*. The infant was placed on intravenous antibiotic therapy and was transferred to a another hospital and there a computed tomography scan of the head revealed thick-walled hypodense lesions that were identified as abscesses and later at the third hospital, serial magnetic resonance imaging conducted and revealed multiple brain abscesses, diffuse brain injury, and hydrocephalus with lateral third and fourth ventricle dilation. Fluid obtained through percutaneous aspiration of an abscess in the right frontal region of the brain yielded a *Cronobacter* organism. And after 11.5 weeks on antibiotic therapy in this hospital, the infant was lost because severe brain injury, hypertonicity resulting from central nervous system damage, and hydrocephalus (52). The other infant was male, born in April 2008 at 40

weeks' gestation. The infant was breastfed exclusively until age 6 months and then began transitioning to prepared infant formula and age-appropriate foods. Other than a history of mild-moderate eczema, the infant was healthy, as confirmed by medical records and a visit by a trained new-parent support worker on November, 2008. The following day, at age 7 months, he died unexpectedly while taking his usual nap at home. A *Cronobacter* organism was found in the blood culture obtained during autopsy. However, aside from mild eczema, no congenital or histopathologic abnormalities were noted during postmortem examination. Therefore, the *Cronobacter* isolate was attributed to postmortem bacterial overgrowth, and the autopsy report listed sudden infant death syndrome as the cause of death (52).

In July 2009, the National Veterinary Research and Quarantine Service (NVRQS) in South Korea reported finding *E. sakazakii* in 695 kg of powdered infant formula made by Maeil Dairies. This amounted to over 53,000 individual packages, which, by good fortune, had not yet been delivered to retail outlets (53). Commenting on the scope of the problem in 2005, the International Food Safety Authorities Network (INFOSAN) indicated that: "Based on available information in 50-80% of cases, powdered infant formula is both the source and the vehicle of *E. sakazakii* induced illness" (48), and noted that mortality rates between 20 and 50% had been reported with lasting damage, including neurological disorders, among survivors. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) report recent reductions in these figures, "Surveys have identified *E. sakazakii* in 3-14% of powdered infant formula samples, but the levels of contamination reported have been low: 0.36-66.0 cfu/100 g" (54). However, an FAO/WHO Expert Meeting report concluded that "using current mix technology, it does not seem possible to produce commercially sterile powders or to completely eliminate the potential of contamination" (55).

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Looking for Solutions

The World Health Organization (WHO) 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 powdered infant formula, the WHO have made recommendations for its reconstitution and storage during the first 2 months of life, when the risk of milk-borne infection appears to be at its highest. For these at-risk groups in particular, the production, preparation and handling conditions of infant formulas must be such that the content of potentially pathogenic bacteria is kept to a minimum (57).

Alarmed by the cases in developed countries, in 2004, two United Nations agencies, the FAO and WHO, held an expert meeting. The World Health Assembly (WHA) in its 2005 Resolution, WHA 58.32, asked "the Organization [WHO] to develop such guidelines on the safe preparation, handling and storage of powdered infant formula in order to minimize the risk to infants" (57). A second meeting of the FAO/WHO expert group in January 2006 drafted guidelines on reducing the risk of *E. sakazakii* and *Salmonella enterica* contamination in powdered infant formula focusing on three major routes of that contamination:

- 1. Reducing intrinsic contamination during production:** Product contamination by *E. sakazakii* at manufacture and during preparation can be minimized by appropriate control measures such as storing at a specific temperature without detrimental impact on health (56, 57). The 2007 WHO guidelines urged the infant food industry to develop a greater range of commercially sterile alternative formula products for high-risk groups and to reduce the concentration and prevalence of *E. sakazakii* in both the manufacturing environment and powdered infant formulas by implementing

an effective environmental monitoring program and changing to more sensitive testing for hygienic control (58). The Codex Alimentarius Commission (a United Nations body tasked by FAO and WHO with determining international standards for food production and food safety) was asked to better address the microbiological risks of powdered infant formulas and to include the establishment of appropriate microbiological specifications for *E. sakazakii* in powdered infant formula (59), while FAO and WHO were asked to establish effective measures to minimize risk where breast-milk substitutes may be used in exceptionally difficult circumstances, e.g., feeding infants of HIV-positive mothers or low-birth-weight infants (58).

2. Reducing intrinsic contamination and minimizing cross-contamination during reconstitution: Using clean, sterilized equipment; and increasing the temperature of the water used for reconstitution of the baby food are choices for reducing the risk of intrinsic contamination. *E. sakazakii* is able to grow at refrigeration temperatures and attach to infant-feeding equipment (60). Therefore, there is an urgent need to minimize the dangers and insure the safety of formulas given to infants. Temperature control in the infant formula preparation and storage areas inhibit attachment and biofilm formation by *E. sakazakii* (61). On the last issue, the guidelines noted that the risk was dramatically reduced when powdered infant formula is reconstituted with water that is no less than 70°C, as this temperature would kill any *E. sakazakii* in the powder adding that a small number of cells may cause illness, therefore it is important that cells present in the powdered infant formulas are destroyed because there was still the potential for surviving cells to multiply in the reconstituted formula (62).

3. Minimizing *E. sakazakii* growth between reconstitution and consumption: This route addresses the third potential source of contamination—bacterial growth following reconstitution but before the infant is fed. Minimizing the time from preparation to consumption also reduces the risk, as does storage of prepared feed at temperatures no higher than 4°C (62).

Suggestions to Manufacturers, Institutions and Caregivers

Infants are particularly vulnerable to infections transmitted *via* food; therefore the microbiological safety of infant and follow-up formula is of utmost importance. Caregivers in hospital neonatal units should be continuously alert to the fact that powdered infant formula is not a sterile product and that the use of hygienic measures during preparation and reconstitution are essential (63, 64).

WHO in collaboration with the Food and Agriculture Organization (FAO) made the following recommendations to avoid *E. sakazakii* contamination (65):

- a. Inform infant caregivers about powdered formula which is not a sterile product and can be contaminated with pathogens
- b. Consider feeding high-risk infants commercially sterile liquid formula if they cannot breast-feed; encourage industry partners to develop a range of affordable sterile formula options
- c. Consider a guideline regarding the safe handling, preparation and delivery of powdered infant formula to minimize the risk
- d. Consider setting an industry standard for *Enterobacteriaceae* and *E. sakazakii* in infant formula

In maternity units, liquid formulas can be a suitable alternative to powder ones in order to reduce the risk of milk-borne infections, however, even they may pose infectious risks if handling after opening is not appropriate. Moreover, the use of liquid formula in small preterm infants is controversial and the nutritional and safety aspects of current formulations should be evaluated in detail (66-68). Powdered infant formula should be prepared fresh for each feed. If formula needs to be prepared in advance, it should be prepared on a daily basis and kept at 4°C or below, for not more than 24 h. Formula should only be warmed immediately before feeding. Formula must not be kept at room temperature for more than 4 hours if r continuous tube feeding is the matter. In home settings, if powdered infant formula is used, it should be prepared fresh for each meal. Remnants should be discarded and should not used as part of the following feed. Infant formula should never be kept warm in

bottle heaters or thermos flasks. For convenience, feeds given during the night and when away from home can be prepared using warm water kept in a thermos flask at feeding temperature and mixed with powdered formula immediately before feeding. (69). Formula should be reconstituted with water >70°C if the nutritional aspects will be maintained (66). The importance of thoroughly cleaning bottles and teats between feeds should be emphasized to parents. In addition, formula manufacturers must implement strategies aimed at reducing the risks of product contamination. Controlling the initial populations of *E. sakazakii* during the production of powdered formula and avoiding post-processing contamination, using suitable microbiological approaches, will have a positive effect. Data from surveys have shown that *E. sakazakii* can be cultured with various contamination frequencies in samples of powdered formulas, from manufacturing facilities and from environmental sources (70-73). 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. Increasing the awareness of *E. sakazakii* infection among medical personnel and the continuous education of all caregivers to the potential threats posed by this organism is essential to protect infants at high risk.

Considering the points mentioned above, these suggestions can be made:

a. Formula manufacturers

- Serious measures should be taken to minimize bacterial contamination of powdered products.

b. Clinics/Hospitals

- The manufacturers' instructions for preparation should be followed.
- Trained personnel should use aseptic techniques to prepare powdered products.
- All equipments (blenders, boiling spoons, bottles, teats) used in the preparation of the formula should be disinfected before use.

- The product should be refrigerated at 4°C if not used immediately and discarded if not used within 24 h.
- The maximum time formula should be kept at room temperature for continuous feeding is 4 h.
- A hospital action protocol should be implemented in the event of a product recall, including notification of hospital care providers, a reporting system, product batch follow-up and careful documentation.

c. Mothers and Other Caregivers

- Hands should be washed using soap, preferable antiseptic soap and the preparation area thoroughly cleaned with care.
- All equipments (bottles, spoons, teats) should be sterilized with sterilizer (or at least with boiling water) before formula preparation.
- Powdered infant formula should be freshly prepared for each feed; any remaining milk should be discarded.
- Infant formula should never be kept warm in bottle heaters or thermos flasks. As an alternative, warm water may be kept in a thermos and mixed with powdered formula just before feeding.
- In conclusion, we can state that increasing the awareness of *E. sakazakii* infection among medical personnel and the continuous education of all caregivers to the potential threats posed by this organism is essential to protect infants at high risk.

Footnotes:

- * An infant or a baby is the very young offspring of humans and can be defined infants as persons not more than 12 months old. Neonate or newborn (from Latin, neonatus, newborn) refers to an infant in the first 28 days after birth. These terms include premature infants, postmature infants and full term newborns.

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