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REVIEW

Cronobacter sakazakii (Enterobacter sakazakii): Only An Infant Problem?

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Summary

Cronobacter sakazakii has emerged as a rare cause of neonatal meningitis, septicemia and enterocolitis. Contaminated infant milk formula (IMF) has been identified as one infection route. Currently no agreed standardized typing protocol has been developed to trace *Cronobacter sakazakii.* This review article aims to inform the readers about the agent's taxonomy, isolation and typing, epidemiology, incidence in foods, and behavior in powdered infant formula.

Keywords: *Cronobacter sakazakii, Enterobacter sakazakii, Powdered infant formula, Neonatal meningitis*

Cronobacter sakazakii (Enterobacter sakazakii): Sadece Bebeklerin Problemi mi?

Özet

Cronobacter sakazakii, neonatal meningitis, septisemi ve enterokolitis hastalıklarının ana etkenlerinden biri olarak bildirilmektedir. Kontamine toz formül bebek mamaları ise önemli bir kontaminasyon kaynağıdır. *Cronobacter sakazakii*'nin izini sürmek için kullanılan standardize edilmiş bir izolasyon ve identifikasyon prosedürü bulunmamaktadır. Bu derlemede, etkenin taksonomisi, izolasyon ve tiplendirilmesi, epidemiyolojisi, gıdalardaki insidensi ve formül bebek mamalarındaki davranışları hakkında okuyuculara bilgi verilmesi amaçlanmışır.

Anahtar sözcükler: *Cronobacter sakazakii, Enterobacter sakazakii, Formül bebek mamaları, Neonatal meningitis*

INTRODUCTION

Cronobacter sakazakii (C. sakazakii) is an opportunistic pathogen causing meningitis, septicaemia and enterocolitis in neonates [1,2]. Preterm, low-birth-weight or immuno-compromised infants exposed to *C. sakazakii* are at particular risk [3]. Mortality rates of 10-80% have been described and survivors often suffer from neurological sequel [3-5]. Clinical outbreaks of infection in neonatal intensive care units associated with contaminated infant milk formula (IMF) have been reported [6,7] .

C. sakazakii is a ubiquitous organism [8]. The source of *C. sakazakii* and vehicle of transmission is not always clear however infant formula has been epidemiologically implicated as the source of *C. sakazakii* in several clinical cases [7,9]. The source of contamination of IMF is thought to include a broad range of dry blended raw material, together with possible environmental sources associated with the production environment. To minimize possible contamination of IMF both the raw materials and the production environment must be constantly monitored.

Molecular subtyping has been applied as a useful tool to facilitate surveillance, tracing routes from a source to an infected individual. Importantly, this approach makes it possible to distinguish a persistent environmental strain that could intrinsically contaminate IFM from extrinsic isolates introduced post-manufacturing. Generally methods based on phenotype analysis are acknowledged to be unreliable due to the unstable expression of the corresponding marker(s). For this

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reason DNA-based protocols offer an attractive alternative. Furthermore, DNA fingerprinting allows for a direct comparison of isolates in outbreaks. Previously, non-standardized DNA fingerprinting protocols have been applied to *C. sakazakii* [10]. Reported methods used include ribotyping, pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD) [10]. These molecular tools facilitate the trace back of outbreak isolates from clinical sources to the contaminated batch of powdered infant milk formula and/or the manufacturing environment. In addition, they are useful tools to target control strategies and reduce the risk of transmission. The only comparison between various molecular subtyping protocols in the literature was reported by Nazorowec - White and Farber [10].

In this paper it is aimed to inform the readers about *C. sakazakii*, which is a very important food borne pathogen that causes serious infections at almost all different age groups but especially neonates.

TAXONOMY and CHARACTERIZATION

Taxonomy, classification and nomenclature of genera in the family *Enterobacteriaceae* have evolved over the years based on various distinctions in serology, morphology, biochemical traits and genetic characteristics. There are 14 species or biogroups in the genus *Enterobacter* [11], however, for the recent years, the agent has been included in *Cronobacter* genus and this genus currently consists of 6 different species; *C. sakazakii, C. malonaticus, C. dublinensis, C. muytjensii,C. turicensis and C. genomospecies* [12]. This recently defined nomenclature is the result of a polyphasic taxonomic investigation aimed at re-defining this group of organisms. *Cronobacter spp.* are described as opportunistic pathogens, causing bacteremia, necrotizing enterocolitis (NEC), and meningitis in immunocompromised neonates [13]. More recently it has also emerged that *Cronobacter spp.* may cause infections among immunocompromised adults, in particular, elderly. Due to their ubiquitous nature, *Cronobacter spp.* have been isolated from a wide variety of foods. While primary reservoir of *Cronobacter* has yet to be defined, plant material is believed to be the likely source [2,12,13]. Major differences between *C. sakazakii* and other Enterobacter species have been traditionally thought to be its inability to ferment d-sorbitol and its ability to produce an extracellular deoxyribonuclease [14] . However, some strains of *C. sakazakii* more recently have been shown to ferment d-sorbitol [15].

Based on DNA-DNA hybridization showing yellow-pigmented strains to have less than 50% homology with non-pigmented strains [16], suggested that yellow-pigmented *E. cloacae* should comprise a new species. Phenotypic characterization and differentiation based on biochemical traits, serotyping, bacteriophage typing and antibiotic resistance are frequently among the first steps used to distinguish characteristics of isolates [10,17,18]. Some have suggested using phenotype tests (eg, biotyping, bacteriocin typing, serotyping and phage typing) to differentiate *Enterobacter* species; however, none of these tests has proven effective in distinguishing strains within the species, nor can they be used for all species of *Enterobacter* [10,19]. Iversen et al.[20], investigated the phylogenetic relationships of *C. sakazakii* using 16S ribosomal DNA and hsp60 sequencing. They found that strains were distributed among four clusters, indicating taxonomic heterogeneity. The type strain 16S rDNA sequence was 97.8% similar to that of *Citrobacter roseri* and 97.0% similar to that of *E. cloacae*. Studies have shown that both *Enterobacter* and *Cronobacter* genus are polyphyletic [21]. Strains currently classified as *C. sakazakii* fall into two distinct groups which can be further subdivided based on hsp60 sequences. Both genotypes include clinical strains and do not correspond to biochemical profiles.

Farmer et al.[14], extended the work of Brenner [16] and Brenner et al.[22] by further distinguishing 57 strains of yellow-pigmented *C. sakazakii* based on DNA hybridization, antibiotic susceptibility and biochemical reactions. Other distinguishing characteristics of the bacterium include greater pigment production at temperatures less than 36.8°C, with optimum pigment production at 25.8°C, survival of cells in stock cultures stored at 17-30.8°C without transfer for up to 8 years, utilization of citrate as a sole carbon source, 31-49% DNA-DNA homology with *E. cloacae*, and 57% guanine + cytosine ratio [14]. Production of the diffusible yellow pigment is unstable with repeated subculturing.

In addition to phenotypic characterization of *C. sakazakii*, advances have been made in fingerprinting DNA and RNA by several techniques, eg, PCR, randomly amplified polymorphic DNA (RAPD) PCR, pulsed-field gel electrophoresis (PFGE), chromosomal DNA restriction analysis, ribotyping and plasmid typing [23]. Nazarowec-White and Farber [10] ribotyped *C. sakazakii* with the *EcoR1* restriction endonuclease and found that 18 isolates were represented by 10 ribotypes. This analysis has been determined to be more discriminatory than that of restriction endonuclease analysis (REA) [24]. In another study [30], *C. sakazakii* isolates from an infant formula factory comprised only 8 ribotypes [25]. Kornacki [26] isolated 17 *EcoR1* ribotypes from a factory environment. Nazarowec-White and Farber [10] analyzed 18 isolates by

PFGE using the restriction endonuclease *Xba1* and found each to have a distinct pattern. Characterization was superior to ribogrouping in that two sets of three isolates, comprising only two ribogroups, were distinguishable as six distinct pulsovars.

C. sakazakii has been shown to exhibit substantial resistance to acid pH. Edelson-Mammel and Buchanan [27] examined survival characteristics of 12 strains of *C. sakazakii* in tryptic soy broth adjusted to pH 3.0 and 3.5 with HCl. Ten of twelve strains showed less than a l-log decline over a 5-h period at 37.8°C; reductions in TSB at pH 3.0 were 4.9 to 6.3 log CFU/ml. There was no correlation in acid resistance based on 1-h/pH 3.0 results and previously determined heat resistance of test strains [27]. Skladal et al.[28] examined the fermentation of milk inoculated with 10-15 CFU of *C. sakazakii* per 500 ml and incubated at 30.8°C. Changes in pH and the production of L-lactate and D-lactate were monitored. *C. sakazakii* fermented milk rapidly, reducing the pH from 6.6 to 5.6 in less than 20 h. Concentrations of L-lactate and D-lactate reached 0.40 mM and 10.7 mM, respectively.

ISOLATION, IDENTIFICATION and TYPING

FDA [29,30] developed a method to isolate and enumerate *C. sakazakii* in dehydrated powdered infant formula. *Table 1* indicates the enumeration procedure described above.

The method of ISO31 to isolate and enumerate *C. sakazakii* in dehydrated powdered infant formula is indicated in *Table 2* below.

Further characterization of *C. sakazakii* isolated from food and environmental samples can be accomplished by using pulsed PFGE, RFLP, multilocus enzyme electro-phoresis tests, or ribotyping. Other potential methods of analyses include testing for antibiotic resistance patterns (antibiograms), toxin assays, hemagglutination, serotyping and phage typing.

INCIDENCE in FOODS

C. sakazakii has been isolated from a wide spectrum of environmental sources [32] including water waste [33] and thermal spring water [34], soil, dust from households and food production-lines [8]. *C. sakazakii* has an unusual surviving ability under dry conditions [35], but the thermal tolerance of *C. sakazakii* strains may differ [36]. Pasteurization is effective in destroying *C. sakazakii* [37]. Acidification reduced the concentration of *C. sakazakii* in different types of infant formula and vegetable based food products [38]. In juices of vegetables, the reduction of pH after 48 h was correlated with a reduction of the numbers of *C. sakazakii*, but with increasing numbers of *C. sakazakii* in juices of different fruits [39].

Table 1. *Enumeration procedure of C. sakazakii for infant formula according to FDA*

Tablo 1. *C. sakazakii için, FDA'ya gore formül mamalardaki sayım prosedürü*

Step	Time	Temperature
Dilute 100 g, 10 g, 1 g of powdered infant formula with pre-warmed sterile distilled water at 1:10 ratio. Mix and incubate	Overnight	36ºC
Add 10 ml of each suspension to 90 ml of *Enterobacteriaceae* enrichment broth and incubate	Overnight	36ºC
Mix suspensions and surface plate 0.1 ml on VRBG agar, streak on VRBG agar with a 10 Al inoculating loop onto three quadrants for isolation and incubate	Overnight	36ºC
Pick five presumptive-positive *C. sakazakii* colonies from both sets of VRBG plates and subculture by streaking onto TSA and incubate	48-72 h	25ºC
Select yellow-pigmented colonies only and confirm per manufacturer's instructions for the API 20E biochemical confirmation system	-	-
Calculate the most probable number (MPN) after determining the number of positive tubes at each dilution	-	-

Table 2. *Enumeration procedure of C. sakazakii for infant formula according to ISO*

Tablo2. *C. sakazakii için, ISO'ya gore formül mamalardaki sayım prosedürü*

Step Number	Name of the Procedure	Description	Time	Temperature
1	Pre - enrichment in non selective liquid medium	The pre - enrichment medium is inoculated with the test portion	16 h to 20 h	37ºC±1ºC
2	Enrichment in selective liquid medium	The selective enrichment medium is inoculated with culture obtained in step 1	22 h to 26 h	44ºC±0.5ºC
3	Plating and identification	A chromogenic agar is inoculated with the enrichment culture obtained in step 2	22 h to 26 h	44ºC±1ºC
4	Confirmation	Typical colonies are selected from the chromogenic agar and isolates producing yellow pigment on tryptone soya agar are biochemically characterized	-	-

There is no essential need for special microbiological criteria of *C. sakazakii* in food other than infant formula, because *C. sakazakii* is a ubiquitous opportunistic micro-organism. *C. sakazakii* will be detected only in studies with the aim of differentiating genus *Cronobacter* or specially of tracking *C. sakazakii*. Iversen and Forsythe [40] referred in their risk-profile to *C. sakazakii* contamination in food. In 2004, they published a survey about the isolation of *C. sakazakii* from a variety of powdered infant formulas, milk powders and related food products [36]. Drudy et al.[32] compared biochemical and molecular-genetic methods in the investigation of 57 European and Australasian *C. sakazakii* isolates. The researchers indicated that 51 isolates of 57 were food originated.

C. sakazakii was isolated from wheat [41] and as endophytic bacteria from the leaves of rice plants [42]. Kanivets and Pishchur [43] detected *C. sakazakii* in the bacterial colonization flora of disinfected sugar beet seeds. As *C. sakazakii* belongs to the cultivable endophytic and epiphytic flora of rice [42] and soy bean plants [44], it could be isolated from related food products. Some traditional cereal, herb and legume-based food and beverages were found to be contaminated with *C. sakazakii* [45]. *C. sakazakii* may be part of starter cultures for fermentation of traditional vegetarian food products. Osterblad et al.[46] detected *C. sakazakii* in mixed salad vegetables and imported fresh and deep-frozen vegetables at retail level.

C. sakazakii contaminated food of animal origin comprise a variety of meat and meat products from camel, pig, beef and poultry, and, additionally, eggs, raw milk and different dairy products and, less frequently, fish. *C. sakazakii* was isolated from a variety of raw and ready-to-eat meat and its products. Watanabe and Esaki [47] isolated *C. sakazakii* during a complicated curing process of meat products. *C. sakazakii* is a histamine forming microorganism in the ripening process of cheese [48]. *C. sakazakii* has been isolated from a cheese whey substrate [49]. Lipolytic activity of a *C. sakazakii* strain was demonstrated by Chaves-Lopez et al.[50]. *C. sakazakii* has been detected in fresh and prepared fish. Miranda et al.[51] isolated a tetracycline-resistant *C. sakazakii* strain from a Chilean freshwater salmon farm with no history of recent antibiotic use. *C. sakazakii* has been isolated from smoked sardines after 12 weeks of storage after irradiation [52].

Schindler and Metz [53] found *C. sakazakii* in total frequencies of 1.8% (10/564 strains) and 0.4% (1/256 strains) investigating central and local drinking water supplies. Lee and Kim [54] identified *C. sakazakii* as bacteria indigenous to the water distribution system during their investigations for biofilm formation. Even bottled beverages should not be considered as free of micro-organisms, as shown by the results of Schindler [53] for *C. sakazakii* contaminated bottled mineral water.

EPIDEMIOLOGY

Many reservoirs exist for these bacteria, including water, soil, food, and the intestines of humans and animals. There are several modes of transmission for these organisms, including exogenous, such as fecal-oral, person-person, mother-child, food, hospital equipment, and personnel, and endogenous, from the patient's own intestinal flora. Passive carriage on the hands of medical personnel constitutes the major mode of transmission. *C. sakazakii* can also be isolated from tap and bottled water and can survive and multiply on or in hospital equipment such as hemodialysis and respiratory instruments [11].

Respiratory tract infections are often caused by gram-negative bacteria. Indeed, sputum is the first or second most common clinical specimen to yield *Enterobacter* isolates and, although these bacteria do not represent the most predominant pathogens causing respiratory infections, they are significant because of their antibiotic resistance. *E. cloacae, E. amalonaticus, E. agglomerans, E. amnigenus, E. asburiae, E. cancerogenus, E. gergoviae, E. normaechei,* and C*. sakazakii (Enterobacter sakazakii)* are species that have been isolated from respiratory tract infections [11]. These bacteria can be transmitted exogenously through hospital procedures, such as surgery or with intubation, inhalation / aspiration, or hematogenous spread to the lungs. Prior antibiotic treatment may predispose patients to *Enterobacter pneumonia,* and *Cronobacter* are a major cause of pneumonia in early post-lung transplant patients, with the bacteria originating from the donor [55].

Meningitis and brain abscesses resulting from *Citrobacter* or *Cronobacter* infections occur most often in neonates, but can also appear in immunocompromised patients and following neurosurgery [56]. The causative agents are primarily *Citrobacter koseri (diversus)* and *Cronobacter sakazakii* and, occasionally, *Citrobacter freundii* [57]. Transmission of the bacteria to the infant can occur horizontally during nosocomial outbreaks in neonatal hospital wards or from contaminated infant formula/powdered milk [58]. They can also be transmitted vertically from a colonized mother [59]. While premature or low birth-weight babies are more susceptible to such infections, any neonate can be affected. Among neonates, the meningitis often results in vasculitis, cerebritis and/or ventriculitis, the development of hydrocephalus, and a surprising rate of brain abscesses and cyst

formation [59]. The brain abscess can persist with little response to antimicrobial therapy.

CLINICAL ETIOLOGY and PATHOGENICITY

Cronobacter species can create community infections, are responsible for approximately half of all nosocomially acquired infections and are often implicated in co-infections. Infections reported in infants include meningitis leading to ventriculitis, brain abscess, and infarction and cyst formation [13]. *C. sakazakii* can cause also systemic respiration, cardiovascular and neurologic symptoms such as destruction of the frontal lobes of the brain, seizures, spastic quadriplegia, hypothermia, fever Cheyne-Stokes respirations, bradycardia, poor feeding, irritability, jaundice, grunting respirations, instability of body temperature, hemorrhagic cerebral necrosis, meningo encephalitis, necrotic softened brain, cyst formation, liquefaction of cerebral white matter and severe neurologic complications at infants, adults and also at elderly patients, too [60].

The symptoms of respiratory tract infections are similar to those seen with *Streptococcus pneumoniae*. Symptoms, which generally occur gradually, include malaise, slowly increasing fever, and/or chills and a cough. The cough will eventually produce sputum, which may be discolored and foul smelling, and the patient may experience shortness of breath. In cases of chronic pneumonia or lower respiratory tract infection, the individual may also experience appetite and weight loss [61]. A chest radiograph and culture of sputum samples are useful in identifying the etiological agent.

Gastroenteritis infections produce symptoms similar to those that occur with other enteropathogenic bacteria such as *E. coli* or *Shigella spp*. The symptoms generally appear suddenly, with loss of appetite, nausea, vomiting, intestinal/abdominal cramps, gas, and watery diarrhea. A fever and mylagia may also be present. Also drop in blood pressure may come out from loss of electrolytes due to dehydration at the infected cases. Patients with hemorrhagic colitis may experience little or no fever and bloody and/or watery diarrhea. Some may develop hemolytic-uremic syndrome that can lead to kidney failure, anemia, seizures, strokes, and nerve or brain damage [62].

Sepsis occurs when bacterial numbers in the blood are too high for efficient removal by white blood cells leading to septic shock. Bacteria normally enter the bloodstream and cause sepsis when there is an infection elsewhere in the body. Symptoms include fever, chills, shaking, nausea, vomiting, diarrhea, and general malaise. The patient will normally have a high white blood cell count. Sepsis can also lead to infections in other parts of the body, such as the brain (meningitis), heart (endocarditis), bone (osteomyelitis), or soft tissue [63].

Meningitis and brain abscesses most commonly occur in neonates and present with fever, vomiting, lack of appetite, irritability, high-pitched crying, and seizures. The forehead may bulge and the head may swell. In those older than one year of age, fever, irritability, drowsiness, confusion, and a painful stiff neck are common. The symptoms can progress to coma and death very rapidly. A lumbar puncture is required to determine the cause of infection if meningitis is suspected [63].

TREATMENT

Cronobacter and Enterobacter infections are treated with antibiotics. Care must be taken when choosing an antibiotic because of the intrinsic resistance to beta-lactams and cephalosporins, and the emergence of plasmid-mediated resistance to aminoglycosides, quinolones, and third-generation cephalosporins [64].

For *Cronobacter* and *Enterobacter* infections, carbapenems or antipseudomonal penicillins (ie, mezlocillin, piperacillin, piperacillin/tazobactam, ticarcillin, and ticarcillin/clavulanate) are recommended as drugs of choice, with ciprofloxacin as an alternative [64]. It is recommended that the drug resistance pattern of the organism be established early on so the infection can be treated properly form the beginning [65]. In the case of brain abscesses, surgery may be necessary to drain the abscess, as they do not always respond to antibiotic therapy [66]. Patients with gastrointestinal illness must have fluid and electrolytes replaced.

RISK MANAGEMENTS, CRITICAL CONTROL POINTS and HAZARD ANALYSIS

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly convened a workshop on *C. sakazakii* in early 200467 in response to a request for scientific advice from the Codex Committee on Food Hygiene to provide input for the revision of the Recommended International Code of Hygienic Practice for Goods and Infants and Children. An extensive list of recommendations to FAO, WHO, Codex Committee on Food Hygiene, their member countries, and Non-Governmental Organizations (NGOs) was issued [67] *(Table 3)*.

Table 3. *Joint FAO/WHO recommendations to the powdered infant formula industry and infant caregivers concerning processing, preparing and handling powdered and reconstituted products*

Tablo 3. *FAO/WHO kurumlarının formül mama endüstrisi ve sektörle ilgili kuruluşlar için tavsiye ettikleri hazırlama, üretim, proses prosedürleri için tavsiyesi*

Recommendations to the Powdered Infant Formula Industry By FAO/WHO
In situations where infants are not breast-fed, caregivers, particularly of infants at high risk, should be regularly alerted that powdered infant formula is not a sterile product and can be contaminated with pathogens that can cause serious illness and provided with information that can reduce the risk
In situations where infants are not breast-fed, caregivers of high-risk infants should be encouraged to use, whenever possible and feasible, commercially sterile liquid formula or formula which has undergone an effective point of use decontamination procedure (e.g., use of boiling water to reconstitute or by heating reconstituted formula)
Guidelines should be developed for the preparation, use and handling of infant formula to minimize risk
Research should be promoted to gain a better understanding of the ecology, taxonomy, virulence and other characteristics of *C. sakazakii* and on ways to reduce its levels in reconstituted powdered infant formula
Investigation and reporting of sources and vehicles, including powdered infant formulae, of infection by *C. sakazakii* and other Enterobacteriaceae and *Cronobacter* genus should be encouraged. This could include the establishment of a laboratory-based network
The use of internationally validated detected and molecular typing methods for *C. sakazakii* and other relevant microorganisms should be promoted
FAO/WHO should address the particular needs of some developing countries in establishing effective measures to minimize risk in situations where breast-milk substitutes may be used in exceptionally difficult circumstances, e.g., feeding infants of HIV-positive mothers or low-birth-weight infants
In revising its Code of Practice, Codex should better address the microbiological risks of powdered infants formula and, if deemed necessary, include the establishment of appropriate microbiological specifications for *C. sakazakii* in powdered infant formula
The infant food industry should be encouraged to reduce the concentration of prevalence of *C. sakazakii* in both the manufacturing environment and powdered infant formula. To this end, the infant food industry should consider implementing an effective environmental monitoring program
The infant food industry should be encouraged to develop a greater range of commercially sterile alternative formula products for high-risk groups

Summarized from FAO/WHO (2004) [67]

CONCLUSION

C. sakazakii can be found in a wide range of foods and beverages, many of which are not subjected to treatments or processes that inactivate the pathogen. Its ability to survive and grow in these products raises concern about safety risks not only to neonates and infants but also to older immunocompromised consumers. The suitability of infant cereals and some types of fresh fruits and vegetables to support luxuriant growth of *C. sakazakii* is of particular concern. The ability of the pathogen to produce biofilms, coupled with its resistance to sanitizers and disinfectants when present in organic matrices, emphasizes the importance of properly cleaning and sanitizing food preparation areas and utensils and containers used to prepare and serve foods to neonates and others in hospital, daycare center, and home settings.

Studies involving *C. sakazakii* have focused on methods to eliminate the coliform from powdered infant formula, to determine thermal resistance, environmental reservoirs, pathogenicity, antibiotic resistance, exopolysaccharide production and to develop rapid methods detection, enumeration and identification, subtyping and predictive modeling, but additional researches in these and other areas are needed. One study using a suckling mouse model to determine virulence mechanisms and minimum infectious dose has suggested the possibility of enterotoxin production by *C. sakazakii*.

Studies to determine conditions that influence survival and growth or cause death of *C. sakazakii* in dry and reconstituted infant formulae are needed, given the likelihood that post-process contamination is the principle route of contamination. Other areas in need of research attention include studies of conditions affecting biofilm formation by *C. sakazakii* in processing plants and hospital settings (eg, in tubes used for enteral feeding), competitive exclusion to control or prevent growth, efficacy of sanitizers, methods to recover and and evaluation of practices associated with preparing and feeding infant formulae in hospitals and in the home. Surveys of neonatal wards, Neonatal Care Units and food processing environments for the presence of *C. Sakazakii* and an evaluation of hygienic practices in hospitals and the home that may contribute to neonatal infections would also provide information of value when developing intervention strategies to eliminate *C. sakazakii* infection.

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