CRONOBACTER SAKAZAKII: AN EMERGING CONTAMINANT IN PEDIATRIC INFANT MILK FORMULA
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ABSTRACT
Cronobacter sakazakii (C. sakazakii) previously known as Enterobacter sakazakii is a motile, Gram-negative, non-spore-forming bacterium belonging to the Enterobacteriaceae family. C. sakazakii is ubiquitously found in soil, air, floor drains, and dry product processing environment. It has been isolated from hospitals, clinical materials, and cutting fluids and is also present in cerebrospinal fluid (CSF), blood, sputum, throat, nose, stool, gut, skin, wounds, bone marrow, eye, ear and breast abscesses. C. sakazakii is a virulent pathogen and can adhere to silicon, latex, polycarbonate, and stainless steel. Therefore, Feeding-bottles or utensils used to prepare pediatric infant formula (PIF) should be thoroughly cleaned to diminish the development of biofilms, which could be the source of infections. Due to its virulence, C. sakazakii causes life threatening infections such as septicaemia, necrotizing enterocolitis, bacteremia and meningitis. Hence, the regulatory authorities such as Food and Drug Administration (FDA), Food and Agriculture Organization/World Health Organization (FAO/WHO), Centers for Disease Control and Prevention (CDC) and Health Canada strongly recommend breast-feeding over the bottle-feed to minimize the risk of infections caused by C. sakazakii.

Keywords: Cronobacter sakazakii; Pediatric infant formula; Contamination; Necrotizing enterocolitis; Meningitis

INTRODUCTION
Cronobacter sakazakii (C. sakazakii) is an emerging foodborne, motile, peritrichous, Gram-negative rods, non-spore-forming bacterium belonging to the Enterobacteriaceae family. It is an opportunistic human and food-borne pathogen previously known as yellow pigmented Enterobacter sakazakii. Farmer et al. mentioned the first used of the name E. sakazakii 1 after the Japanese bacteriologist Riichi Sakazakii. Several genera and species of Enterobacteriaceae have been isolated from the reconstituted pediatric infant formula (PIF). C. sakazakii’s physiological traits aid in its environmental survival, and give the ability to produce a yellow pigment that protects the cell against UV rays in sunlight. It also provides capsular and film barrier formation to assist in adherence to surfaces including other cell types, and its ability to resist desiccation during long dry periods 2. C. sakazakii has been of much concern in life-threatening bacterial infections especially in low birth-weight neonates and infants. The clinical isolates of the bacterium produce only slightly yellow pigmentation when cultured on nutrient agar at 37 °C, but produce a non-diffusible yellow-gold pigment when incubated at room temperature. Pangalos in 1929 was the first to report that septicaemia in an infant was caused by a yellow-pigmented coliform in tryptone soy agar TSA 3. Willis and Robinson reported 40-80% mortality rates among infected infants 4. Neonatal infections have been associated with C. sakazakii colonization of the food preparation equipment such as brushes, blenders, and spoons 5,6. Its contamination from the samples of commercially available dry PIF has been reported 7,8. C. sakazakii is an opportunistic pathogen most commonly affecting immunocompromised patients and neonates 4,8,9. Neonatal infections have been reported to be on rise via contact with C. sakazakii in the birth canal or through post-birth environmental sources 4,9-13. Detection of Cronobacter sakazakii
Steigerwalt and his co-workers have observed that the presence or absence of yellow pigmentation can distinguish between the two strains of Enterobacteriaceae family (Enterobacter cloacae and C. sakazakii) when these strains are cultured on tryptone soy agar (TSA) 14. It was further reported that fermentation of D-sorbitol helps in the differentiation among different strains 15. C. sakazakii can be identified by some traditional biochemical methods, but there is a need of isolation in pure culture from mixed contamination in PIF before identification can be carried out 16. Biochemical tests for the identification of C. sakazakii are shown in Table 1. Currently, molecular detection methods such as polymerase chain reaction (PCR), real-time PCR, and immunoassays are commonly employed for its identification 17. Sometimes even more advanced methods such as DNA microarray-based assays have been used for the detection of C. sakazakii 18. In the investigation of PIF contamination, a combination of methods such as antibigrams, ribotyping, plasmid analysis, multilocus enzyme electrophoresis, and chromosomal restriction fragment analysis have been used 19.

Sources of contamination
C. sakazakii has been isolated from floor drains, air, vacuum canister, broom bristles, room heater, electrical control box, transition socks, a clean-in-place valve, floor, and condensate in a dry product processing plant 4. Van Os et al. have isolated C. sakazakii from grass silage in The Netherlands 20. It has also been isolated from hospital air 21, clinical materials 22,3,6, rats 24, soil 25, rhizosphere 26, sediment and wetlands 27, crude oil 28 and cutting fluids 29.

Clinical Sources
Farmer et al. have reported that most of the C. sakazakii isolates from infected patients originate from CSF, blood, sputum, throat, nose, stool, gut, skin, wounds, bone marrow, eye, ear and breast abscess 4. Although C. sakazakii infections in vaginally born newborns have been suspected to originate from mother’s birth canal, the presence of its infection in neonates born through caesarian section has questioned this hypothesis 27,30,31. Moreover, often the fecal, vaginal, and
cervical swabs of the mothers who delivered newborn with C. sakazakii were negative on colonization. Samples from the anus, skin, nose, umbilical cord, and outer ear of two infants on their day of the birth were the also negative as were the samples from outer ear, nose, umbilicus, and gastric aspirate of another newborn tested for C. sakazakii.

Contamination in milk and milk-products
It has been reported the chances of microbial contamination are reduced by the use of sterile liquid formula instead of PIF. C. sakazakii has been found in dry powdered foods including unopened non-fat dried milk. In one survey of the powdered foods, about 27% of the total 140 foods tested positive for C. sakazakii and the powdered infant foods had the highest frequency of contamination (34%) followed by PIF (about 33%) and milk-based products (about 31%) in addition. It has been isolated from various non-PIF sources including dried baby food, cheese, dry food ingredients, rice, herbs, and spices. It was isolated from the three food type of powdered infant food, PIF and milk-based products in 27.1% of samples. Krieg and Holt stated that C. sakazakii was more prevalent in foods and the environment than in the clinical settings.

Contamination in PIF
Contaminated PIF is a recognized source of C. sakazakii, which poses a serious risk of infection to newborn infants. Two main routes by which C. sakazakii can enter the reconstituted PIF are known as intrinsic contamination either through contaminated ingredients added after drying or from the processing environment following drying and before packing; and/or through external contamination of the formula during reconstitution and handling (e.g. through poorly cleaned utensils). The level of C. sakazakii is ranged from 0.36 to 66 cfu/100 g. The Food and Agriculture Organization of the United Nations (FAO/ WHO) has recommended bacterial counts for coliforms in PIF of less than 3 cfu/g. The production of PIF is achieved by either wet or dry processing. The wet process involves combining all the essential ingredients with liquid skimmed milk and fat components and heating the mixture at 81°C. In the dry process, pasteurized evaporated skimmed milk is dry blended with the balance of essential ingredients (essential fatty acids, vitamins, whey, stabilizers and emulsifiers), pasteurized at 110°C and spray dried. FDA has published bulletins highlighting the dangers of bacterial contamination of enteral formula products, most of which contain PIF as the major ingredient. A number of studies have not only found the contamination of C. sakazakii in PIF but also in the other infant or baby foods as listed in Table 2.

Recalls of C. sakazakii contaminated PIF
It is recommended by FDA that written guidelines should be available in the event of PIF recall. There must be the notification of health care providers, a system for reporting and following up on specific formula products used, and retention of recall records. There have been a number of recalls of PIF due to the contamination of C. sakazakii as listed in Table 3.

Outbreak of C. sakazakii in PIF
Since 1958, more than 70 cases have been reported with a mortality of 24 in the outbreak of C. sakazakii, out of these 23 cases have been reported in USA with a causality of only 3. In the last decade, twenty major outbreaks of E. sakazakii have been reported in France (2004), NewZealand (2004), Belgium (2002), and USA (2001) with a death tool of 2, 1, 1 and 1 respectively. In the era of 1990s, major outbreak in Belgium in 1998 with 12 cases was reported with 2 causalities. But in the period of 1980s, two main outbreaks occurred in Denmark in 1983 and Greece in 1984, 8 and 11 cases were reported with mortality of 6 and 4 respectively.

Storage of reconstituted PIF
According to the FAO/WHO, risk assessment for C. sakazakii in reconstituted PIF has increased many fold as compare to the powdered formula. The risk is increased in warmer ambient temperatures (30-35 °C). Reconstituted PIF in bottle for a period of time probably maximize the growth of C. sakazakii. have found that six clinical and food strains of C. sakazakii have grown between 6-45 °C, with maximum growth between 37-43 °C. Have reported that C. sakazakii has flourished in reconstituted PIF at 8-47 °C. It has been indicated that up to 20% of household refrigerator temperatures are kept at approximate 10 °C, thus providing the temperature that is suitable for the growth of C. sakazakii.

Clinical etiology and pathogenicity
In 1992, Enterobacter species were reported as being the third most common among those recovered from the urinary and respiratory tracts, of patients in intensive care units. C. sakazakii may have some relationship associated with several bacterial pathogens and is the most common cause of gastrointestinal disease in newborns. The illness affects around 2-5% of premature neonates, leads to death in 10-55% and is characterized by ischaemia, bacterial colonization of the intestinal tract and increased levels of protein in the gastrointestinal lumen, the latter is often attributable to the consumption of PIF. C. sakazakii produces viscus capsular material and therefore the organism could form a biofilm on feeding equipment and contact surfaces (i.e. stainless steel). A capsulated strain formed denser biofilms compared to a noncapsulated type strain. It has been suggested that bottles and utensils used to prepare PIF should be cleaned thoroughly as soon as possible after use to eliminate or minimize the formation of biofilms, which could be the sources of infection.

In addition, the stomach of newborns, especially of premature babies, is less acidic than that of adults, a possible important factor contributing to the survival of an infection with C. sakazakii in newborns. The frequency of intrinsic C. sakazakii contamination in PIF is of concern, even though intrinsic concentration levels of the organism appear to be typically very low. Other reports have confirmed or implicated PIF as a source of bacteria responsible for meningitis and associated with neonatal necrotizing enterocolitis. Contaminated PIF is one of the sources of neonatal infection that subsequently leads to high morbidity and occasional mortality in the infants. Two unrelated cases involving meningitis and bacteraemia have been investigated and it was found that each victim could be linked to a particular PIF. Pagotto and his colleagues were the first to describe putative virulence factors for C. sakazakii. Using tissue cultures, some strains produced a cytotoxic effect. The International Commission for Foods, due to the seriousness of pathologies with C. sakazakii has severed as hazard for restricted
populations, life threatening or substantial chronic sequelae or long lasting.

Meningitis
Meningitis is the most frequently reported condition in neonatal *C. sakazakii* infections, resulting in 90% of the cases leading to brain abscesses. Meningital infection of *C. sakazakii* have been reported as arising between the fourth and fifth day after birth and can be fatal within a few hours to several days following the first clinical signs with death often occurring within hours of infection. Seizure activity has been reported in about one-third of the cases of neonatal *C. sakazakii* meningitis, with physiological responses including gruntling, bulging fontanelles, convulsions, twitching and an increase in cranial circumference. *C. sakazakii* was first implicated in a case of neonatal meningitis in 1958, and since then there have been around 70 reported cases of *C. sakazakii* infections.

Necrotizing Enterocolitis
A positive correlation between necrotizing enterocolitis and oral formula feeding has been suggested by various researchers. Babies fed only on PIF rather than breast milk are 10 times more likely to contract necrotizing enterocolitis. Another study confirmed that in 125 infants, prior to the administration of antibiotics, *Enterobacteriaceae* species were the most prevalent bacteria, present in 29% of neonates.

High risk individuals
Although *C. sakazakii* can cause illness in all age groups, infants (children <1 year) are at most risk with neonates and infants under two months at greatest risk. The groups of infants at greatest risk include, in particular pre-term infants, low-birth-weight (<2.5 kg) infants or immunocompromised infants. The prominent predisposing factors to the onset of meningitis in neonates are premature birth, low birth weight (<2500 g), or prolonged rupture of membranes prior to delivery. Infants of HIV-positive mothers are also at risk because they may be immunocompromised and may specifically require PIC. Two distinct infant risk groups for *C. sakazakii* infection include premature infants who develop bacteremia after one month of age; and term infants who develop meningitis during the neonatal period. Therefore, the FAO/WHO expert working group in 2006 concluded that while infants appear to be the group at particular risk, neonates and also those of less than two months of age are at greatest risk.

Risks and hazards control
FDA in 2002 issued an alert to healthcare professionals about the risk associated with *C. sakazakii* infections among neonates fed with milk-based PIF. The alert stated that a major contribution to the avoidance of *C. sakazakii* infections in premature babies and neonates is the prevention of contamination of PIF during production and bottle preparation. Ingredients such as raw milk might be contained with *Enterobacteriaceae*, and these organisms are eliminated following pasteurization, whereas dry ingredients, added post-pasteurization must be free of bacterial and other types of contamination. The manufacturing plant design should specially separate different processing areas (essentially dry and wet processing), and frequent cleaning regimes should be implemented to minimize the persistence of pathogens in the manufacturing process and environment.
survival of heat treatments, and subsequent presence in desiccated products. The lowest temperature of growth is 5 °C and therefore potentially the organism can grow during refrigerated storage. When PIF is reconstituted with ≥70 °C water, the risk is dramatically reduced, and this risk reduction remains valid for feeding times of two hours. This finding has practical implications for the reduction of risk of *C. sakazakii* infection for slow-feeding infants and for infants in warm climates where ambient room temperature may be around 35 °C. It has been recommended that the formula should not be held at room temperature for more than two hours, even if water at no less than 70 °C is used to reconstitute PIF. This is because the feed may have become contaminated during preparation, or harmful bacteria may have been introduced into the cup or feeding bottle from the infant's mouth. Also, hot water (70 °C) may have activated bacterial spores of harmful bacteria in the formula. Lihono et al. have examined the use of probiotic cultures to control the growth of *C. sakazakii* in rehydrated PIF at 30 and 35 °C. Holding prepared feeds above refrigeration temperature for extended periods provides the opportunity for the growth of *C. sakazakii*.

### Table 1: Biochemical Identification Test of *Cronobacter sakazakii*

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80 esterase</td>
<td>Positive</td>
</tr>
<tr>
<td>Phosphomucidase</td>
<td>Positive</td>
</tr>
<tr>
<td>α-glycosidase</td>
<td>Positive</td>
</tr>
<tr>
<td>Yellow pigmentation on TSA</td>
<td>Positive</td>
</tr>
<tr>
<td>D-sorbitol fermentation</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### Table 2: Contamination of *Cronobacter sakazakii* in Infant/Baby Food and Milk

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Size</th>
<th>Positive Sample</th>
<th>Percentage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric Infant Formula</td>
<td>141</td>
<td>21</td>
<td>14.9%</td>
<td>8</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>120</td>
<td>8</td>
<td>6.7%</td>
<td>9</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>82</td>
<td>2</td>
<td>2.4%</td>
<td>43</td>
</tr>
<tr>
<td>Milk Formula</td>
<td>72</td>
<td>3</td>
<td>4.2%</td>
<td>43</td>
</tr>
<tr>
<td>Infant Formula</td>
<td>49</td>
<td>5</td>
<td>10.2%</td>
<td>43</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>124</td>
<td>3</td>
<td>2.4%</td>
<td>44</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>35</td>
<td>2</td>
<td>5.7%</td>
<td>45</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>58</td>
<td>8</td>
<td>13.8%</td>
<td>46</td>
</tr>
<tr>
<td>Milk Formula</td>
<td>170</td>
<td>7</td>
<td>4.1%</td>
<td>47</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>40</td>
<td>1</td>
<td>2.5%</td>
<td>47</td>
</tr>
<tr>
<td>Pediatric Infant Formula</td>
<td>8</td>
<td>2</td>
<td>25%</td>
<td>48</td>
</tr>
<tr>
<td>Infant Formula</td>
<td>15</td>
<td>2</td>
<td>13.3%</td>
<td>48</td>
</tr>
</tbody>
</table>

### Table 3: Recalls of *Cronobacter sakazakii* contaminated Pediatric infant formula

<table>
<thead>
<tr>
<th>Year Of Recall</th>
<th>Region/ Country/ Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Nestle Beba 1 was manufactured by Nestle Germany Kapeln (Belgium and Switzerland, but is also being recalled from Luxembourg)</td>
<td>50</td>
</tr>
<tr>
<td>2001</td>
<td>Tennessee (USA)</td>
<td>51</td>
</tr>
<tr>
<td>2002</td>
<td>Mead Johnson Nutritional (batch BMC17 of Portagen)</td>
<td>52</td>
</tr>
<tr>
<td>2002</td>
<td>Store brand powdered formula Contaminated with <em>C. sakazakii</em></td>
<td>53</td>
</tr>
<tr>
<td>2002</td>
<td>Wyeth Nutritional Inc., Georgia, Vermont</td>
<td>54</td>
</tr>
<tr>
<td>2003</td>
<td>(Mead Johnson) EnfaCare Lipil</td>
<td>55</td>
</tr>
<tr>
<td>2004</td>
<td>Mead Johnson Nutritional (Pregestimil in December)</td>
<td>56</td>
</tr>
<tr>
<td>2011</td>
<td>Wall-Mart is pulling Enfamil infant formula (Missouri)</td>
<td>57</td>
</tr>
</tbody>
</table>

**CONCLUSION**

*Cronobacter sakazakii* is one of the most lethal contaminant found in pediatric food and/or milk formula. It is a motile, Gram-negative, non-sporing yellow pigmented rod belonging to the *Enterobacteriaceae* family. *C. sakazakii* is found everywhere in air, soil, floor drains, room heater, clean-in-place valve, and floor. It has also been isolated in hospitals, clinical materials, and cutting fluids. *C. sakazakii* has also been found in throat, blood, sputum, nose, stool, gut, skin, bone marrow, eye, ear, and breast abscess. Because of its pathogenicity and virulence, it mainly causes necrotizing enterocolitis, meningitis, bacteremia and septicemia. It may adhere to latex, silicon, polycarbonate, and stainless steel and form biofilm. Moreover, FDA, Health Canada, FAO/WHO, and CDC forcefully advocate mother-feeder over bottle-feeder to avoid the possible life threatening illness to neonates and infants caused by the contamination of *C. sakazakii*.

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