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Detection of *Chronobacter sakazakii* and other Enteropathogenic Bacteria from Selected Brands of Commercial Powdered Foods in Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author MOB designed the study, wrote the protocol, performed the analyses, provided the literature searches, wrote the first draft of the manuscript, managed the analyses of the study, read and revised the drafts of the manuscript. Author OA managed the fieldwork, performed the analyses, provided literature searches and wrote the first drafts of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Food borne infections continue to debilitate human populations. Powdered Infant foods, cereal based, and malt based foods have been less investigated for the presence of *Chronobacter sakazakii* and other enteropathogenic bacteria as agents of diarrhea and Necrotizing enterocolitis in neonates and infants. This study was aimed at detecting the presence of multidrug resistant strains of *Chronobacter sakazakii* and other enteropathogenic bacteria from three brands of commercial powdered foods in Nigeria.
Methods: A total number of 45 samples comprising 15 units each, of NAN 2, Horlicks and Custard powder were purchased randomly from different retail outlets in Lagos, Ekiti and Ondo states Nigeria. The method of the US Food and Drug Administration was adopted in the bacteriological analysis of the samples, and susceptibility of the detected bacteria to 16 antibiotics was determined using the standard methods of CLSI on Mueller-Hinton Agar.
Results: A total number of 57 bacteria species were isolated from all the 45 powdered food

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samples. Twenty (20) isolates from the PIF were predominantly *C. sakazakii* 9/20 (45%), *S. enterica* 4/20 (20%), *E. aerogenes* 1/20 (5%), *S. rubidae* 1/20 (5%), *K. oxytoca* 2/20 (10%), *K. pneumoniae* 1/20 (5%) and *Erw. ananas* 2/20 (10%). Eight (8) isolates were obtained from malt-based Horlicks, comprising *C. sakazakii* 3/8 (37.5%), *B. licheniformis* 2/8 (25%) and *Erw. ananas* 3/8 (37.5%).Twenty nine (29) isolates were detected in the cereal-based custard, comprising *C. sakazakii* 11/29 (38%), *Erw. ananas* 8/29 (27.6%), *B. licheniformis* 5/29 (17.2%), and *S. enterica* 2/29 (6.9%), while *Erw. persicinus, E. aerogenes* and *B. cereus* were 1/29 (3.4%) respectively. Of all the 57 bacteria isolates, 8 (14%) were Gram positives while 49 (86%) were Gram negatives. All the Gram negative bacteria from the PIF were resistant to Ampicillin but susceptible to Gentamycin and Nalidixic acid, while all the isolates from Horlicks and custard powder were resistant to Ampicillin, Nalidixic acid, Nitrofurantoin and Streptomycin.

Conclusion: Multidrug resistant *Chronobacter sakazakii,* and other enteropathogenic bacteria are prevalent in commercial cereal based food (custard powder), malt based foods (Horlicks) as well as Powdered Infant Milk Formula (NAN2) in Nigeria.

Keywords: Chronobacter sakazakii; powdered infant formula; custard powder; horlicks; Erwinia ananas; multidrug resistant.

1. INTRODUCTION

Chronobacter sakazakii, formerly *Enterobacter sakazakii* [1] is a facultatively anaerobic, gram negative, non sporulating, peritrichously motile bacillus, belonging to the Enterobacter genus of the family Enterobacteriaceae. *Enterobacter sakazakii* was first discovered in 1958 within 78 cases of infants with meningitis infection. The bacterium cell approximately measures 3 by 1 micrometers in size. It produces a protective biofilm which has allowed this species to establish itself as food pathogen in most food industries and handling facilities. The international commission for microbiological specification for foods [2] has ranked *Chronobacter sakazakii* as severe hazard, life threatening or substantial chronic sequelae of long duration in premature, low-birth weight infants, neonates and children, with the rate of invasive infections by this bacterium in infants under 1 year of age at 1/100,000 [3] and mortality rate between 40-80%. *C. sakazakii* can cause various kinds of infection ranging from bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infection, gastroenteritis resulting in diarrhea and vomiting, jaundice, heart inflammation, arthritis, osteomyelitis, and eye infection. There were also few reports of *C. sakazakii* infection in adults, the elderly and people with compromised immune system. Necrotizing Enterocolitis (NEC), associated with *C. sakazakii* infection, is currently the most common gastrointestinal emergency in neonates, and it is characterized by necrosis of the gastrointestinal lumen. Once infected, neonates have been found to have a mortality rate of 40% to 80%, and a 20% chance of survival accompanied with serious neurological complications such as hydrocephalus, quadriplegia, brain abscess and retarded neural development [4]. Powdered infant food (PIF) products have been epidemiologically linked to several clinical cases and recalls of infants' formula that were contaminated with *C. sakazakii* have occurred in the United States and Europe. Several outbreaks have occurred in neonatal intensive care unit as a result of infection by this bacterium [5]. In France, three (3) neonates died of 13 infected cases in June 1994, in Belgium, two neonates died of necrotizing enterocolitis of 12 infected neonates, while five babies reportedly died in New Mexico due to *C. sakazakii* infection [6]. *Chronobacter sakazakii* and other Enterobacter species has been isolated from different cereal-based powdered food including semolina (crushed wheat), cheese, grounded rice, sesame seeds, lentils, tofu, fermented bread, and almond, as part of the associated microbial flora of the seeds [7]. The spectrum of *C. sakazakii* contaminated food include raw and processed foods, dry products, fresh, frozen, ready-to-eat, fermented and cooked food products, beverages, and water, were found to be contaminated by *C. sakazakii* [8]. In addition, *C. sakazakii* is not the only bacteria contaminants of Infant foods, other notable enteropathogenic bacteria had been incriminated in the contamination of powdered food products. The US FDA categorized the microorganisms or microbial toxins of concern in powdered infant formula and other food products (malt-based and cereal-based foods), and their strength of the evidence of a causal association between their presence in the food and the illness they caused, as follows:

1.1 Category "A" Organisms

Clear evidence of causality**.**The presence of *C. sakazakii* in powdered infant formula, malt-based, and cereal-based foods, and its association with illness in infants and adults.

1.2 Category "B" Organisms

Causality plausible, but not yet demonstrated. Other Enterobacteriaceae in this category are well-established causes of illness in infants e.g. systemic infection, NEC and severe diarrhea, and have been found in other food apart from powdered infant formula. These organisms include; *Pantoea agglomerans* or *Escherichia vulneris*, *Hafnia alvei*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *C. freundii*, *Klebsiella oxytoca* and *Enterobacter cloacae*.

1.3 Category "C" Organisms

Causality less plausible or not yet demonstrated. This category of organisms despite causing illness in infants e.g. systemic infection, NEC and severe diarrhea, have not been identified in powdered infant formula, cereal-based food, and malt-based foods, and they have not been implicated as causing such illnesses in infants. These organisms include *Bacillus cereus, Clostridium difficile, C. perfringens, C. botulinum, Staphylococcus aureus* and *Listeria monocytogenes* [9].

Powdered Infant Milk formula is manufactured powdered food designed and marketed for feeding babies and infants under 12 months of age, usually prepared for bottle-feeding or cup-feeding from reconstituted powder, mixed with water or other liquid, with or without additional water. Only about one quarter of all mothers were breastfeeding babies as majority of parents believed that these foods had more nutritional value. However, lack of sufficient breast milk (in cases of lactation failure, or incorrect believe of a mother or her family that her breast milk is of low quality or low in supply), health problems, inadequate sucking reflex, painful nursing, lengthy mother-infant separation and work demands, all of these contributed to the evolution of considerations for using infant formula.

Horlicks is a malt based food drink formulated with combinations of wheat flour, malt extract, malted barley, milk solids, sugar, minerals, Iron, salt, vitamins, protein isolate and acidity stabilized with potassium bicarbonate for family nourishment and vitality.

Custard powder is a cereal, corn flour-based powdered food which thickens to form a custard-like sauce when mixed with milk and heated to a sufficient temperature. Custard is a variety of culinary preparations based on a cooked mixture of milk or cream and egg yolk popularized by Alfred Bird (1837). Custard may vary in consistency from a thin pouring sauce to a thick pastry cream depending on how much egg or thickener used. The most common custards are used as desserts or dessert sauces and typically include sugar and vanilla. The powdered form is now packaged into sachet or plastic for human consumption.

1.4 Objectives of the Study

In response to the worldwide reports of multiple brain abscess, critical infections, the scarce information about the ecology of *Chronobacter sakazakii*, and the uncertainty concerning the source of Necrotizing Enterocolitis infection in the Nigerian children, this basic study investigated into the prevalence and antibiotic susceptibility of *C. sakazakii* and other *Enteropathogenic* bacteria in selected commercial powdered food products in Nigeria.

2. MATERIALS AND METHODS

2.1 Samples

A total number of 45 samples comprising 15 units each, of NAN2 (Powdered Infant Milk formula), Horlicks (Malt- based powdered food) and Custard powder (Cereal- based powdered food) were purchased randomly from different retail outlets in Lagos, Ekiti and Ondo states Nigeria. The products borne comparable batch numbers, production dates, expiry dates and none were expired prior to analysis at the Microbiology Laboratory of Adekunle Ajasin University.

2.2 Bacteriological Analysis of Samples

The method of the US FDA [10] was adopted in the analysis of the samples, following the four stepwise essential stages of Pre-enrichment, Enrichment, Selective, and Identification. In the pre-enrichment step, each sample was aseptically opened, from which 10 grams of the powder was measured and dispensed in each culture bottle containing 90ml sterile distilled water and incubated at 36ºC for 24 hours. In the Enrichment stage, 10 mls of the pre-enrichment culture was inoculated into 90mls of sterile

Enterobacter Enrichment Broth (EEB) in culture bottles and incubated at 36℃ for 24 hours. Uninoculated bottle was included as growth control. In the selection step, from the Enrichment step, 1 ml of inoculum was taken from the incubated Enterobacter Enrichment Broth to seed sterile plates of Violet Red Bile Glucose Agar (VRBGA) using the pour plate method and gently swirled to ensure optimum dispersion of the aliquot with the agar. The media was allowed to gel, inverted, labeled and incubated at 36℃ for 24 hours. The plates were replicated with additional uninoculated plate as growth control. In the Identification stage, presumptive pink and yellow colonies of the bacteria isolates were picked with the aid of sterile inoculating loop from the VRBGA cultures, and streak inoculated onto sterile Tryptic Soy Agar (TSA) plate in duplicates. The plates were then incubated at 27℃ for 72 hours. The cellular morphology and cultural characteristics of the isolates were studied and recorded. The isolates were further sub cultured onto freshly prepared sterile TSA plates for another 72 hours from which pure colonies were obtained and preserved in TSA slants for further analysis. The stock cultures were further subcultured at 2-weeks interval to ensure viability and purity. In addition various biochemical tests including Catalase, Motility, Indole, Citrate Utilization, Methyl Red, Hydrogen sulphide production and fermentation of sugars were done for conventional identification of the isolates [11,12].

2.3 Antibiotic Characterization of the Bacteria Isolates

The antibiotic susceptibility of the detected bacteria was determined using the standard method of [13]. The following antibiotics and their disk concentrations were tested against the isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Oxoid). The antibiotics for Gram positive were Gentamicin (10 µg.disk^{-1}), Amoxicillin (25 µg.disk^{-1}), Cotrimoxazole (25 µg.disk^{-1}), Augmentin (30 µg.disk^{-1}), Tetracycline (10 µg.disk^{-1}), Chloramphenicol (30 µg.disk^{-1}), Cloxacillin (5 µg.disk^{-1}) and Erythromycin (5 µg.disk^{-1}), while the antibiotics for Gram negative were Tetracycline (25 µg.disk^{-1}), Ampicillin (25 µg.disk^{-1}), Colistin (25 µg.disk^{-1}), Nalidixic (30 µg), Nitrofuratoin (200 µg), Gentamicin (10 µg), Streptomycin (25 µg) and Cotrimoxazole (25 µg). Inoculum of each pure isolate was prepared in a direct broth suspension of Nutrient broth, incubated at 35℃ for 18 hrs and standardized by adjusting its density to match the 0.5 McFarland turbidity standard using Barium sulphate ($BaSO_4$). Using a sterile cotton swab, the inoculum suspension of each isolate was used to carefully inoculate the entire surface of sterile Mueller- Hinton agar plate in replicates. The inoculated plates were allowed to stabilize for 5 minutes before the antibiotic impregnated discs were aseptically laid on the surface, inverted, and incubated at 35℃ for 18 hrs, examined for zones of inhibition, measured and recorded.

3. RESULTS

3.1 Detection rate of *Chronobacter sakazakii* and other Enteropathogens

Physical assessment of the tested commercial food products showed that manufacture dates, batch numbers, expiry dates and NAFDAC number were present on the NAN2 powdered Infant Milk Formula, some of the Horlicks product and all of the custard powders lack batch numbers and NAFDAC numbers. On analysis, all isolates on the VRBGA that appeared whitish, pink and light yellow were presumptively identified as belonging to the family Enterobacteriaceae. The isolates in culture appeared as smooth colonies ranging between 2-3.5 mm in diameter. The biochemical characterization (Table 2) showed that the isolates were Motile, catalase (+), oxidase (-), fermented glucose with production of acid and gas, Methyl Red (+), Voges Proskauer (-), Indole (-), citrate (+), H_2S (-) and were identified according to [12].

A total number of 57 bacteria species were isolated from all the 45 powdered food samples analyzed. Twenty (20) isolates from the PIF (NAN2) were predominantly *C. sakazakii* 9/20 (45%), *S. enterica* 4/20 (20%), *E. aerogenes* 1/20 (5%), *S. rubidae* 1/20 (5%), *K. oxytoca* 2/20 (10%), *K. pneumoniae* 1/20 (5%), and *Erw. ananas* 2/20 (10%). Eight (8) isolates were obtained from the malt-based Horlicks, comprising *C. sakazakii* 3/8 (37.5%), *B. licheniformis* 2/8 (25%) and *Erw. ananas* 3/8 (37.5%).Twenty nine isolates were detected in the cereal-based custard, comprising *C. sakazakii* 11/29 (38%), *Erw. ananas* 8/29 (27.6%), *B. licheniformis* 5/29 (17.2%), and *S. enterica* 2/29 (6.9%), while *Erw. persicinus, E. aerogenes* and *B. cereus* were 1/29 (3.4%) respectively. Of all the 57 isolates, 8 (14%) were Gram positives while 49 (86%) were Gram negatives. The isolates were able to ferment both

glucose and lactose to produce acid, while some produced both acid and gas (Table 2).

3.2 Antibiogram of the Bacteria Isolates

Table 4 shows the antibiogram of Gram negative bacteria isolates from the Powdered Infant Milk Formula, Horlicks and custard powder. From the NAN2 brand of powdered infant Formula, the isolates were all Resistant to Ampicillin and all were sensitive to Gentamycin and Nalidixic acid. *Serratia rubideae* (NN2C) was most susceptible to all the antibiotics except Ampicillin.

While all the isolates from the Horlicks brand were resistant to Ampicillin, Nalidixic acid, Nitrofurantoin and Streptomycin, *Chronobacter sakazakii* isolates HL 2-a and HL 2-b were susceptible to Tetracycline, Cotrimoxazole, Colistin and Gentamicin. *Erwinia ananas* isolates HL 4-aii and HL 4-b were moderately susceptibile to Tetracycline, Cotrimoxazole, Colistin and Gentamicin.

Table 5 shows the antibiotic susceptibility of Gram Positive bacteria isolates from Horlicks and custard powder. *Bacillus licheniformis* isolate HL 3-bii was susceptible to Gentamicin, Augmentin and Cloxacillin but resistant to the other drugs. All the isolates from custard powder were resistant to all the antibiotics.

4. DISCUSSION

The global impact of *Chronobacter sakazakii,* as an emerging food-borne pathogen transmissible through the consumption of contaminated powdered infant foods and other milk products is becoming a serious concern for public health. Pediatric infections associated with the consumption of *E. sakazakii* contaminated powdered food products have been less reported in Nigeria. This research investigated the presence of *Chronobacter sakazakii,* and other Enterobacteriaceae in some commercially available powdered foods which are widely consumed by infants and young children in Nigeria.

Table 1. Physical attributes of the tested commercial powdered infant formula, horlicks and custard powder

Samples	Man. Date	Batch N^{o}	Expiry date	NAFDAC N^{o}	Visible defects	Date of analysis
Powdered infant formula						
NN1 & NN2	06/11	+	08/13	+	Nil	08/11
NN3 &NN4	07/11	+	09/13	+	Nil	08 &09/11
NN5 &NN6	01/10	+	01/12	+	Nil	09/11
NN7 &NN8	06/10	+	05/12	+	Nil	09/11
NN9	08/10	+	07/12	+	Nil	09/11
NN10 –NN12	08/09	+	08/11	+	Nil	08/11
NN13	11/09	+	10/11	+	Nil	09/11
NN14&NN15	10/09	+	09/11	+	Nil	09/11
Horlicks						
HL1 &HL2	05/11	-	10/12	-	Nil	08/11
HL3	05/10	-	10/11	A1-7651	Nil	08/11
HL4	06/10	2302H	12/11	A1-7651	Nil	08/11
HL6 –HL8	07/11	-	12/12	-	Nil	09/11
HL9 & HL10	08/11	-	01/13	-	Nil	10/11
HL5 & HL11	06/10	2392H	12/11	A1-7651	Nil	08 &10/11
HL12	06/11	2394H	12/12	A1-7651	Nil	10/11
HL13	06/11	2494H	12/12	A1-7651	Nil	10/11
HL14	06/11	2245A	12/12	A1-7651	Nil	10/11
HL15	06/11	2390H	12/12	A1-7651	Nil	10/11
Custard						
CS1- CS4	05/11	-	12/14	-	Nil	09/11
CS5 –CS7	06/11	-	12/14	-	Nil	08/11
CS8 –CS11	04/11	-	10/14	-	Nil	09/11
CS12 –CS15	07/11	-	09/14	-	Nil	10/11

All the Custard powder samples had neither a Batch number nor NAFDAC number. No visible defects were observed on all the tested samples

Table 2. Cellular morphology, sugar fermentation, biochemical characteristics, and probable identities of bacteria isolates from powdered infant milk formula, horlicks and custard powder

Isolates	Gram	Mot	Catalase	Indole	MR	VP	Citrate	H_2S	NR	Gluc	Lac	Probable organism
PIF												
NN1 A	-v e Rod	+	+	_	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
NN1 B	-ve Rod	+	+	_	_	+	+	_	+	AG	A	*Chronobacter sakazakii*
NN1 C	-ve Rod	+	+	_	_	+	+	_	+	AG	AG	*Chronobacter sakazakii*
NN2 D	-ve Rod	+	+	_	_	+	+	_	+	AG	A	*Chronobacter sakazakii*
NN2 E	-ve Rod	+	+	_	_	+	+	+	Nd	AG	A	*Salmonella enterica*
NN3 A	-ve Rod	+	+	_	_	+	+	+	Nd	AG	AG	*Salmonella enterica*
NN3 B	-ve Rod	+	+	_	_	+	+	+	+	-	AG	*Enterobacter aerogenes*
NN4 C	-ve Rod	+	+	_	_	+	+	-	Nd	-	A	*Serratia rubidaea*
NN4 D	-ve Rod	+	+	_	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
NN5 E	-ve Rod	_	+	+	_	+	+	+	Nd	AG	AG	*Klebsiella oxytoca*
NN6 A	-ve Rod	+	+	-	_	+	+	-	+	AG	AG	*Chronobacter sakazakii*
NN6 B	-ve Rod	+	+	+	_	+	+	+	Nd	AG	AG	*Samonella enteric*
NN7 C	-ve Rod	_	+	+	_	+	+	+	Nd	AG	AG	*Klebsiella oxytoca*
NN7 D	-ve Rod	+	+	-	_	+	+	-	+	AG	AG	*Chronobacter sakazakii*
NN8 E	-ve Rod	+	+	+	_	+	+	+	Nd	AG	AG	*Salmonella enteric*
NN9A	-ve Rod	_	+	-	_	+	+	+	Nd	AG	A	*Klebsiella pneumoniae*
NN12 B	-ve Rod	+	+	-	_	+	+	+	Nd	_	A	*Erwinia persicinus*
NN12 C	-ve Rod	+	+	-	_	+	+	+	Nd	_	A	*Erwinia persicinus*
NN14 D	-ve Rod	+	+	-	_	+	+	-	+	_	A	*Chronobacter sakazakii*
NN14 E	-ve Rod	+	+	-	_	+	+	-	+	AG	A	*Chronobacter sakazakii*
Horlicks												
HL 1A	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
HL 2B	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
HL 2C	+ve Rod	+	+	-	-	+	+	+	Nd	AG	A	*Bacillus licheniformis*
HL 3B	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
HL 3C	+ve Rod	+	+	-	-	+	+	+	Nd	AG	AG	*Bacillus licheniformis*
HL 4A	-ve Rod	+	+	+	-	+	+	+	Nd	A	AG	*Erwinia ananas*
HL 6B	-ve Rod	+	+	+	-	+	+	+	Nd	A	AG	*Erwinia ananas*
HL 8C	-ve Rod	+	+	+	-	+	+	+	Nd	A	AG	*Erwinia ananas*

Isolates	Gram	Mot	Catalase	Indole	MR	VP	Citrate	H_2S	NR	Gluc	Lac	Probable organism
Custard												
CS 1A	-ve Rod	+	+	-	-	+	+	-	+	AG	A	*Chronobacter sakazakii*
CS 1B	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*
CS 1C	+ve Rod	+	+	-	-	+	+	+	Nd	AG	AG	*Bacillus licheniformis*
CS 2A	+ve Rod	+	+	-	-	+	+	+	Nd	A	AG	*Bacillus licheniformis*
CS 2B	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
CS 3A	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
CS 3B	+ve Rod	+	+	-	-	+	+	+	Nd	AG	AG	*Bacillus licheniformis*
CS 3C	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*
CS 4A	-ve Rod	+	+	+	-	+	+	+	Nd	A	AG	*Erwinia ananas*
CS 4B	+ve Rod	+	+	-	-	+	+	+	Nd	AG	A	*Bacillus licheniformis*
CS 4C	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
CS 5B	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
CS 6A	-ve Rod	+	+	-	-	+	+	-	+	A	AG	*Chronobacter sakazakii*
CS 7A	-ve Rod	+	+	-	-	+	+	-	+	AG	AG	*Chronobacter sakazakii*
CS 8A	-ve Rod	+	+	-	-	+	+	-	Nd	AG	AG	*Chronobacter sakazakii*
CS 8B	-ve Rod	+	+	+	-	+	+	+	Nd	A	AG	*Erwinia ananas*
CS 9A	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*
CS 10A	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*
CS 11A	-ve Rod	+	+	-	-	+	+	-	+	A	AG	*Chronobacter sakazakii*
CS12 A	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Salmonella enterica*
CS 12B	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*
CS13 A	-ve Rod	+	+	-	-	+	+	+	Nd	-	A	*Erwinia persicinus*
CS 13B	-ve Rod	+	+	-	-	+	+	-	+	A	AG	*Chronobacter sakazakii*
CS14 A	-ve Rod	+	+	-	-	+	+	+	+	-	AG	*Enterobacter aerogenes*
CS14B	+ve Rod	+	+	-	-	+	+	+	Nd	AG	A	*Bacillus licheniformis*
CS14C	+ve Rod	+	+	-	-	-	+	-	Nd	A	A	*Bacillus cereus*
CS 15A	-ve Rod	+	+	-	-	+	+	-	+	A	AG	*Chronobacter sakazakii*
CS15 A	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Salmonella enterica*
CS 15B	-ve Rod	+	+	+	-	+	+	+	Nd	AG	AG	*Erwinia ananas*

Legends: Mot: Motility; MR: Methyl Red; VP; Voges Proskauer; H_2S: Hydrogen sulphide production; NR: Nitrate reduction; Gluc: Glucose; Lac: Lactose; AG: Acid and gas; A: Acid; -ve Rod: Gram negative Rod; +: positive; -: Negative; Nd; Not done

In this study, 11 units (73.3%) of the powdered infant food formula, 6 units (40%) of the Horlicks, and 15 units (100%) of the custard powders were contaminated (Table 3). The occurrence, characteristics and probable identities of the bacteria isolates are presented in Table 2. Of the contaminated samples, *C. sakazakii* was detected at a rate of 54.5% in the Powdered Infant Milk formula, 50% in the Horlicks and 73.3% in the custard powders. Other Enterobacteriaceae were detected at a rate of 45.5% in the PIF, 50% in the Horlicks, and 80% in the custard powders. Other bacteria species were not detected in the PIF, but 33.3% in the Horlicks and 40% in the custard powders (Tables 2 and 3). These results were consistent with the reports of [14-16] who have found a direct relationship between *C*. *sakazakii* infections and consumption of infant formula. From the eight isolates that were detected in the Horlicks samples, 6 (75%) were Gram negatives while 2 (25%) were Gram positive bacteria. The predominant bacteria were *Chronobacter sakazakii*, *Erwinia ananas* and *Bacillus licheniformis*. Previous studies have focused on Powdered Infant Milk Formula as the main source of *Chronobacter sakazakii*. The highest rate of detection of the organism in the custard samples indicated that the organism was more prevalent in the food products than the PIMFs. However, the presence of *Samonella*, *Klebsiella* and *C*. *sakazakii* in the tested brands of the Powdered Infant Milk Formula (NAN2) indicated that the products are therefore not actually sterile as presumed. These bacteria species are considered critical by the FDA and WHO as etiological agents of septicaemia, meningitis, enterocolitis and gastroenteritis in infants. These outcomes are engendered by the exceptional ability of *C*. *sakazakii* to invade the brain capillary endothelial cells, persistence in human mcrophages and distruption of intestinal enterocytes' tight junctions [17,18]. When babies are repeatedly exposed to the *C. sakazakii* intrinsically contaminated food formula, there is the tendency of developing the ailments vis-a-vis the rudimentary development of immune response of infants, as the pathogen had been earlier incriminated in several cases of neonatal meningitis, brain damage and death associated with consumption of powdered infant milk Formula in Iceland [19], in the USA [20], in Israel [21], in France [22], in India [23], and in Japan [24] among others. This study showed that these commercial PIF (NAN2) in Nigeria were contaminated to varying levels with Enterobacteriaceae and other bacteria species, making them risky for human consumption especially infants. These findings corroborate the reports of Shaker et al. [25] as well as Aigbekaen and Oshoma [26] who reported in separate studies, a direct relationship between detection in powdered infant formula and *Chronobacter sakazakii* infection. The detected category A bacterium as established causes of illness in infants according to FAO/WHO [27] were *Chronobacter sakazakii,* in all the food samples analysed, while category B included *Klebsiella oxytoca* and *Klebsiella pneumoniae*, which were detected in 50% of the samples. This research therefore is consistent with the submission of the FAO / WHO [27] on the basis of their detection in Powdered Infant Formula, and the detection of Salmonella is consistent with the report of the CDC [28].

The demand for powdered infant formula was spurred by the recommendation for infants of HIV-positive mothers, that where replacement feeding is acceptable, feasible, affordable, sustainable and safe, all breastfeeding should be avoided [29]. In addition, human milk fortifiers are required to compensate the nutritional needs of very low-birth-weight infants. In circumstances when the mother cannot breastfeed or chooses not to breastfeed for any reason, special powdered infant formula and other powdered foods were often required for feeding of low-birth-weight infants, and weaning. However, in this study, the microbiological analysis of PIF revealed the presence of Enterobacteriaceae and *Klebsiella* in concentrations that make the product unfit for human consumption going by the FDA recommendations and guidelines [10]. Therefore there is a potential health risk in the use of the products, for weaning, feeding low birth weight babies, a baby born to HIV positive mothers, a baby with an underlying illness and so on.

The antibiogram showed that the isolates in this study were resistant to 70% of the antibiotics contrary to what was obtained by Aigbekan and Oshoma [26] In the Powdered Infant Milk formula, *Salmonella enterica* was resistant to Streptomycin, Tetracycline Cotrimoxazole, Gentamycin and Nalidixic but were susceptible to Nitrofurantoin, Colistin and Ampicillin. *Enterobacter aerogenes, Serratia rubidaea, Klebsiella oxytoca*, and C. *sakazakii* were susceptible to Nitrofuratoin, Streptomycin, Tetracycline, Cotrimoxazole, Gentamycin and Nalidixic acid but resistant to Ampicillin and Colistin (Table 4). These findings, in consistence

with the reports of Oonaka et al. [30] may be attributable to the increased ability of *C. sakazakii* to deactivate β-lactam antibiotics as earlier reported by Block et al. [21]. Biofilm formation by the bacterium may also be a factor associated with altered susceptibility to antimicrobials. *Erwinia persicinus,* and *Klebsiella pneumoniae* were susceptible to Streptomycin, Tetracycline, Cotrimoxazole, Nitrofurantoin, Gentamycin and Nalidixic acid but were resistant to Colistin and Ampicillin. Therefore in the event of infection resulting from consumption of the contaminated infant food formula, the stated antibiotics will prove useful in the treatment. The detected Gram positive bacteria comprising *Bacillus licheniformis* and *Bacillus cereus* were susceptible to Augmentin and resistant to Amoxicillin, Cotrimoxazole, Erythromycin and Tetrcycline. All the Gram positive isolates from custard powder were resistant to all the antibiotics, while only *Bacillus licheniformis* isolate HL 3-bii was susceptible to Gentamicin, Augmentin and Cloxacillin (Table 5). The current detection of *B. cereus* in this study is consistent with the report of Kim et al. [31] on cereal-based follow-up infant formulas. These findings raises serious concern as *B. cereus* had been implicated by Rowan and Anderson [32] in cases of diarrhea enterotoxin associated with infants' reconstituted milk.

Considering the survival and transmission of the pathogens, the presence of *C. sakazakii* in powdered infant milk formula depends on the process conditions and nature of the product [14]. *C. sakazakii* can gain access to the powder from the environment or from the addition of the ingredient at the powder processing stage [33]. Despite the fact that powdered formulas are exposed to heat treatment during drying process, *Chronobacter sakazakii*, was still detected in the final products, possibly due to the inherent ability of the bacterium to resist desssication and osmotic stress [34]. In custard powders the main vehicle (source) of this enteropathogenic bacteria especially, *Chronobacter sakazakii* could be from the milk and egg yolk additives in the production, and the contaminated product may transmit the pathogen when adopted in weaning babies.

The ability of *C. sakazakii* to attach to stainless steel, plastic, enteral feeding tubes and silicon rubber surfaces and grow in a biofilm has been established by Kim et al. [35] especially when the reconstituted formula are held warm for subsequent feeding of infants. The external feeding tubes and teats of feeding bottles can harbor the bacterium, and biofilm formation may generate infectious doses enough to initiate infection. There has been no determination of infectious dose of *C. sakazakii*, but 3cfu/100 g can be used as an initial estimation of dose of infection. The minimum lethal dose in neonates requires high number of viable cells (an event likely to occur if reconstituted formula is held at inappropriate temperature). *C. sakazakii* appears more thermotolerant than other Enterobacteriaceae from dairy products and well adapted to growth at temperature around 37℃ to 44℃. In addition, certain strains show an increased tolerance and resistance to temperatures around 50℃ to 60℃. It was observed during the benchwork of this research that the *Chronobacter sakazakii*, isolates were almost lost as they were dying off when preserved at temperature below 25℃. This observation showed that the bacteria is thermophilic and could be responsible for their survival in the food product during manufacturing, distribution, storage and when reconstituted. This is consistent with the findings of Nazarowec-White and Farber [33] who reported that the minimum growth temperatures for *E. sakazakii* in Brain Heart Infusion (BHI) broth varied from 5.5℃ to 8℃; and that strains actually began to die off slowly at 4℃.

Table 3. Prevalence of *Chronobacter sakazakii,* enterobacteriaceae, and other bacteria species from commercial powdered infant formula, horlicks and custard powders

Food Products	N^{o} of sample tested	N^{o} of contaminated sample (%)	Prevalence of *C. sakazakii* (%)	Prevalence of other Enterobacteriaceae (%)	Prevalence of other bacteria (%)
PIF (NAN2)	15	11 (73.3)	06 (54.5)	05 (45.5)	00
Horlicks	15	06 (40)	03 (50)	03 (50)	02 (33.3)
Custard Powder	15	15 (100)	11 (73.3)	12 (80)	06 (40)

Table 4. Antibiotic susceptibility patterns of gram negative bacteria isolates from powdered infant formula, horlicks and custard powder

Tested samples	Isolates / Identities	Zone of inhibition by the antibiotic (mm)							
		Tet	Amp	Cot	Nal	Nit	Col	Str	Gen
Infant formula	NN1-A / *C. sakazakii*	19	09	19	18	00	14	19	20
	NN1-B / *C. sakazakii*	17	00	20	20	20	00	16	20
	NN1-C / *C. sakazakii*	18	00	18	20	19	10	15	20
	NN1-D /*C. sakazakii*	15	00	05	19	04	10	15	20
	NN1-E / *Salm. enterica*	05	00	00	20	19	19	19	17
	NN2-A / *Salm. enterica*	20	00	20	20	12	00	15	20
	NN2-B / *C. aerogenes*	19	00	19	19	19	19	19	19
	NN2-C / *Serratia rubidaea*	11	00	18	19	17	09	13	19
	NN2-D / *C. sakazakii*	02	00	05	17	02	00	10	11
	NN2-E / *Klebsiella oxytoca*	13	00	00	19	15	14	03	15
	NN3-A / *C. sakazakii*	12	00	20	20	20	00	20	20
	NN3-B / *Salm. enterica*	05	00	20	15	05	00	20	18
	NN3-C / *Klebsiella oxytoca*	15	00	19	19	14	11	14	17
	NN3-D / *C. sakazakii*	16	10	03	19	18	15	07	19
	NN3-E / *Salm. enterica*	14	00	14	19	19	14	19	16
	NN4-A / *Kleb. pneumoniae*	10	00	20	20	20	00	19	20
	NN4-B / *Erwinia persicinus*	15	00	15	15	10	00	20	20
	NN4-C / *Erwinia persicinus*	11	00	18	20	17	09	13	19
	NN4-D / *C. sakazakii*	10	00	19	19	00	12	20	17
	NN4-E / *C. sakazakii*	08	00	20	15	01	00	20	15
Horlicks	HL2-a /*C. sakazakii*	16	00	24	00	00	29	00	16
	HL 2-b /*C. sakazakii*	18	00	12	00	00	23	19	19
	HL3bi /*C. sakazakii*	00	00	00	00	00	00	00	16
	HL 4-ai / *Erwinia ananas*	00	00	00	00	00	00	00	20
	HL 4-aii /*Erwinia ananas*	15	00	00	00	00	19	00	21
	HL 4-b /*Erwinia ananas*	16	00	20	00	00	18	00	19
Custard powder	CS1-ai /*C. sakazakii*	00	00	00	00	00	00	00	00
	CS1-aiii/*C.sakazakii*	00	00	00	00	00	00	00	00
	CS1-b /*C. sakazakii*	00	00	00	00	00	00	00	00
	CS2-b /*C. sakazakii*	00	00	00	00	00	00	00	15
	CS2-c /*C. sakazakii*	10	00	00	00	00	00	00	18
	CS3-ai /*C. sakazakii*	00	00	00	00	00	00	00	00
	CS3-aii /*C. sakazakii*	00	00	00	00	00	00	00	15
	CS3-bi / *C. sakazakii*	00	00	00	00	00	00	00	00
	CS3-ci /*C. sakazakii*	00	00	00	00	00	00	00	00
	CS3-cii /*Erwinia ananas*	00	00	00	00	00	00	00	00
	CS3-ciii /*Erwinia ananas*	00	00	00	00	00	00	00	00
	CS4-ai /*Erwinia ananas*	15	00	00	00	00	30	00	18
	CS4-aii /*Erwinia ananas*	00	00	00	00	00	00	00	25
	CS4-ci /*Erwinia ananas*	00	00	00	00	00	00	00	00
	CS4-cii /*C. sakazakii*	14	00	00	00	00	10	00	18

Chronobacter sakazakii is an emerging food-borne pathogen that causes sepsis, meningitis or necrotising enterocolitis and gastrointestinal emergency and death in newborn infants, particularly premature infants with rudimentary immune systems [4,36] and this present report corroborates the earlier submission of Drury et al*.* [37] that signified *C. sakazakii* as emerging pathogen in powdered infant formula.

Table 5. Antibiotic susceptibility patterns of gram positive bacteria isolates from horlicks and custard powdered foods

Tested samples	Isolates	Zone of inhibition by the antibiotic (mm)							
		Amx	Cot	Gen	Aug	Chl	Cxc	Ery	Tet
Horlicks	HL2-c / *B. licheniformis*	00	00	00	00	00	00	00	00
	HL 3-bii /*B. licheniformis*	00	00	18	25	12	18	00	00
Custard	CS1-c/*Bacillus licheniformis*	00	00	00	00	00	00	00	00
	CS2-a / *Bacillus licheniformis*	00	00	00	00	00	00	00	00
	CS3-bii / *Bacillus licheniformis*	00	00	00	00	00	00	00	00
	CS4-b / *Bacillus licheniformis*	00	00	00	00	00	00	00	00
	CS14-c / *Bacillus cereus*	00	00	00	00	00	00	00	00

The US Food and Drug Administration [10] has issued an alert to health care professionals about the risk associated with *Chronobacter sakazakii* infections among neonates fed with milk-based infant formula. A possible important factor contributing to infection with *Chronobacter sakazakii* in infants is the less acidic stomach of newborns, especially of premature babies [38] which may permit establishment of infection and colonization by pathogenic organism.

The detection of *C. sakazakii* is an established enigma whose impact must be mitigated as a matter of research urgency. In the US, monoglycerides are currently added to infant formula as emulsifiers. Therefore, the incorporation of monocaprylin as an antimicrobial ingredient in PIF is potentially feasible as Carprylic acid is a natural eight-carbon chain fatty acid, present in human breast milk and has a generally recognized as safe (GRAS) status. At concentrations of 50mM, monocaprylin rapidly inactivates *E. sakazakii* at 37℃, reducing its population to undetectable levels [39].

5. CONCLUSION

Chronobacter sakazakii and other enteropathogenic bacteria were found in commercial powdered infant Milk formula, custard powders and Horlicks. Commercial PIF should carry valid expiry dates. To achieve this goal, the manufacturers, the government and the end users should come together and evolve acceptable standard that will elicit consumers' confidence in the products with a view to eliminate *E. sakazakii* induced illness attributable to consumption of these powdered infant foods. Considering the risks associated with artificial infant feeding formula, breasfeeding should be strictly encouraged except in exceptional cases. Healthcare professionals should also consider the possibility of powdered infant food associated *C. sakazakii* in the diagnosis of neonatal necrotizing enterocolitis, meningitis and gastroenteritis that may be encountered in the hospitals. It is imperative to develop public health education that target healthcare workers, parents and other caregivers, in both the hospital and the community, on the safe handling, health hazards of inappropriate preparation, storage and use of powdered infant formula, and other powdered foods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Iversen C, Lehner A, Mullane N, Bidlas E, Cleenwerck I, Marugg J, Fanning S, Stephan R, Joosten H. The taxonomy of *Enterobacter sakazakii*: Proposal of anew genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. Malonaticus subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies*. BMC Evolutionary Biology. 2007;7(64): 1471-2148.
2. International Commission on Microbiological Specification for Foods (ICMSF). Microbiological testing in food safety management. Academic/Plenum Publisher, New York; 2002;7.
3. Li-chun I, Beuchat LR. Survival of *Enterobacter sakazakii* in infant cereal. Food Microbiology. 2007;24(7-8):767-777.
4. Friedemann M. *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). Int J Food Microbiol. 2007;116:1-10.

5. Lai KK. *Enterobacter sakazakii* infections among neonates, infants, children and adults. Medicine. 2001;80:113-122.
6. CDC. Chronobacter species isolation in two infants- New Mexico, 2008. Morbid. Mortal. Weekly Report. 2009;58: 1179-1183.
7. Gassem MA. Study of the microorganisms associated with the fermented bread (Khamir) product produced from sorghum in Gizan region, Saudi Arabia. Journal of Applied Microbiology. 1999;86:221-225.
8. Coulin P, Farah Z, Assavno J, Spillmann H, Puhan Z. Characterisation of themicroflora of attieke, a fermented cassava product, during traditional small-scale preparation. International Journal of Food Microbiology. 2006;106:131-136.
9. Gericke B, Thurm V. Identification of infant food as a vehicle in a nosocomial outbreak of *Citrobacter freundii*: Epidemiological subtyping by allozyme, whole-cell protein and antibiotic resistance. Journal of Applied Bacteriology. 1994;553-558
10. FDA. Isolation and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula; 2002.
    Available:http://www.cfsan.fola.gov/~comm/mmes akaz.html
11. Cappuccino JG, Sherman N. Laboratory Manual of Microbiology 4th edition. Benjamin Cummings Pub. Inc. 1996; 59-150.
12. Abbott SL. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Plesiomonas* and other Enterobacteriaceae. In; Manual of Clinical Microbiology, 8th ed. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken, RH, eds. ASM Press, Washington, DC. 2003;1:684-700.
13. Clinical Laboratory Standards Institute. Performance for antimicrobial susceptibility testing, 15th informational supplement 3. 2005;m100-515.
14. Iversen C, Forsythe SJ. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends Food Sci. Technol. 2003; 14:443-454.
15. Simmons BP, Gelfand MS, Haas M, Melts I, Ferguson J. *Enterobacter sakazakii* infection in neonates associated with intrinsic contamination of a powdered infant formula. Infect. Control Hosp. Epidemiol. 1989;10:398-402.
16. Willis J, Robinson JE. *Enterobacter sakazakii* meningitis in neonates. Pediatr. Infect. Dis Journal. 1998;7:19-199.
17. Townsend SM, Hurrell E, Gonzalez-gomez I, Lowe J, Frye JG, Forsythe S, Badger JL. *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. Microbiology. 2007;153:3538-3547.
18. Kim KP, Loessner MJ. *Enterobacter sakazakii* invasion in human intestinal caco-2 cells requires the host cell cytoskeleton and is enhanced by disruption of tight junction. Infect. Immun. 2008;76: 562-570.
19. Biering G, Karlsson S, Clark NC, Jónsdóttir KE, Lúdvígsson P, Steingrímsson O. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. J Clin Microbiol. 1989;27(9):2054-2056.
20. Burdette JH, Santos C. E*nterobacter sakazakii* brain abscess in the neonate: the importance of neuroradiologic imaging. Pediatrics Radiology. 2000;30:33-34.
21. Block C, Peleg O, Minster N, Bar-oz B, Simhon A, Arad I, Shapiro M. Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of E*nterobacter sakazakii*. European Journal of Clinical Microbiology Infectious Disease. 2002;21:613-616.
22. Acoignard B, Vaillant V, Vincent JP, Leflèche A, Mariani-kurkdjian P, Bernet C, L'hériteau F, Sénéchal, H, Grimont P, Bingen E, Desenclos JC. Infections severes a *Enterobacter sakazakii* chez des nouveaunes ayant consomme une preparation en poudre pour nourrissons, france. Bull. Epidemiol. Hebd. 2006;2(3): 10-13.
23. Ray P, Das A, Gautam V, Narang A, Sharma M. *Enterobacter sakazakii* in infants: Novel phenomenon in india. Indian Journal of Medical Microbiology. 2007; 25(4):408-410.
24. Ibuki K, Hashida Y, Shimura S, et al. A scase of multiple brain abscess in a very low birth weight infant caused by *Enterobacter sakazakii.* Journal of Japanese Society for Perinatal and Neonatal Medicine. 2009;4:129-133. (In Japanese).
25. Shaker R, Osaila T, Al-omary W, Jaradat Z, Alzuby M. Isolation of *Enterobacter sakazakii* and other Enterobacter sp. from

food and food production environments. Food Control. 2007;18:1241-1245.
26. Aigbekaen BO, Oshoma CE. Isolation of E*nterobacter sakazakii* from powdered foods locally consumed in Nigeria. Pakistan J. of Nutrition. 2010;9(7):659-663.
27. FAO/WHO. E*nterobacter sakazakii* and other microorganisms in powdered infant formula; 2004. Available:ftp://ftp.fao.org/codex/ccfh36/fh04_12e. pdf
28. CDC. *Salmonella* serotype Tennessee in powdered milk products and infant formula - Canada and the United states. Morbidity and Mortality Weekly Report. 1993;42: 516-517.
29. WHO. New data on the prevention of mother-to-child transmission of HIV and their policy implications: Conclusions and recommendations. Who technical consultation on behalf of the UNFPA /UNICEF/ WHO/UNAIDS inter-agency task team on mother-to-child transmission of HIV. Report no. WHO/rhr/01.28. 2001. Geneva: WHO.
30. Oonaka K, Katsunori F, Montonobu H, Masafumi F. Powder infant formula milk contaminate with *Enterobacter sakazakii.* Japanese Journal of Infectious Diseases. 2010;63:103-107.
31. Kim SA, Oh SW, Lee YM, Hwang IG, Kang DH, Rhee MS. Microbial contamination of food products consumed by infants and babies in Korea. Letters in Applied Microbiology. 2011;53(5):532-538.
32. Rowan NJ, Anderson JG. Diarrhoeal enterotoxin production by psychrotrophic *Bacillus cereus* present in reconstituted milk-based infant formulae (MIF). Letters in Applied Microbiology. 1998;26(2):161-165.
33. Nazarowec-white M, Farber JM. Isolation, survival and growth of *Enterobacter sakazakii* in infant formula. J Food Protection. 1997;60:226-230.
34. Arku B, Mullane N, Fox E, Fanning S, Jordan K. *Enterobacter sakazakii* survives spray drying. Int. J. Dairy Technol. 2008; 61:102-108.
35. Kim H, Ryu JH, Beuchat LR. Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. Appl. Environ. Microbiol. 2006;72:5846-5856
36. Van Acker J, De Smet F, Muyldermans G, Bougatef A, Naessens A, Lauwers A. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J. Clin. Micribiol. 2001;39:293-297
37. Drudy D, Mullane NR, Quinn T, Wall PG, Fanning S. *Enterobacter sakazakii*: An emerging pathogen in powdered infant formula. Clinical Infectious Diseases. 2006;42:996-1002.
38. CDC. *Enterobacter sakazakii* infections associated with the use of powdered infant formula-Tennessee, 2001. Morbid. Mortal. Weekly Reports. 2002;51:297-300.
39. Nair MK, Joy J, Venkitanarayanan KS. In activation of *Enterobacter sakazakii* in reconstituted infant formula by monocaprylin. Journal of Food Protection. 2004;67:2815-2819.

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