Progress in Nutrition 2020; Vol. 22, N. 2: 596-602 DOI: 10.23751/pn.v22i2.8907

Original article

Investigation of Cronobacter sakazakii (Enterobacter sakazakii) Presence in Cereal Infant Foods

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Abstract. *C. sakazakii* is an opportunistic pathogen that may cause serious infections. Infant formulas are frequently reported as the source of infections caused by *C. sakazakii*. In spite of all of the taken precautions, there are recently published studies related to the isolation of *C. sakazakii* in formula and small children foods. In this study, we aimed to investigate the presence of *C. sakazakii* in cereal based infant formulas and complementary foods sold in the markets of Turkish Republic of Northern Cyprus (TRNC). We also aimed to determine the infection risk in these products. This research was carried out between May – December 2017 with the cereal-based continuing formulas and small child complementary foods of the brands offered for sale at T. R. N. C. In a total of 265 samples, including 36 varieties of cereal-based infant formula and 17 varieties of cereal-based infants and small children foods were analysed. Analysis of samples were carried out according to; ISO / TS 22964: 2006 method. *C. sakazakii* was not detected in any of the study samples. In conclusion, *C. sakazakii* was not detected in any of the cereal-based foods despite the reported detection of *C. sakazakii* in most of the microbiological analysis of baby foods in both the world and Turkey.

Key words: *Cronobacter sakazakii*, cereal infant foods, powdered infant formula

Introduction

Breastmilk is the most natural nutrient that can be given to baby alone for the first 6 months. However, from the 6th month onwards, since increased nutritional needs of the baby, complementary nutrients are given in addition to the breastmilk. Complementary foods started to be used with the development of swallowing reflex. There are two types of food, those are; specially formulated for babies and family like foods prepared in puree form by changing the consistency. Food forms specially prepared for infants can be found in various flavors and structures such as cereal, milk, fruit, vegetable or mixture and also they may be found in powder or ready-to-eat form (1).

Cronobacter sakazakii; is a gram-negative, facultative anaerobe which belongs to the *Enterobacteriaceae* family. *C. sakazakii*. It is a rod-shaped bacteria belonging to the coliform group of intestine origin which does not form spore (2-3). Until 1980, bacteria known as *Enterobacter cloacae* which produced yellow pigment, was reclassified as a distinct species by Farmer et al. (3) and the bacterium was renamed as *Enterobacter sakazakii*. As a result of taxonomic studies by Iversen et al. (4) with *E. sakazakii* strains, all of these bacterial strains were included in a new genus under the name *Cronobacter.* The researchers named *Cronobacter* as the bacteriologist inspired by the god named "Cranos" who took part in Greek mythology who is known as a God who used to swallow newborn babies.

C. sakazakii is an opportunistic pathogen that causes serious infectious. It may cause life-threatening, neurological diseases; especially, meningitis and infections such as septicemia and necrotizing enterocolitis in infants. Children which are under one year, newborns and premature babies are the most risky groups for this bacterium (5-7). The association of *C. sakazakii* with infants was reported in 1961 by a Urmenyi and Franklin case report, which was showing that two infant deaths caused by meningitis because of this bacteria. Thereafter, it is stated that this bacteria is the causative agent of meningitis (8). The International Commission on Microbiological Food Standards (ICMSF) classifies *C. sakazakii as* a life-threatening agent and classifies this effect in the same category as pathogens such as *Listeria monocytogenes* and *Clostridium botulinum* (3).

At the meeting organized by WHO and FAO in Switzerland in 2004, they stated that powder and ready-to-eat infant products may have some contamination with microorganism and those microorganisms can cause infections. At the same meeting, according to the risks that they may cause infectious microorganisms were categorized, as class A, B and C. *Salmonella enterica* and *E. sakazakii* which are isolated from infant foods are known as microorganisms that cause foodborne diseases and are categorized in class A (6).

Infant formulas have been reported as the source of *C. sakazakii*-mediated infections in infants, it is estimated that these factors are infected with the additives added to infant foods after raw material used and pasteurization has been made. *C. sakazakii*'s ability to survive even in low water activity allows it to develop in infant formula. It has been reported that lower stomach acidity in newborn babies, especially in premature babies, plays an important role in the development of *C. sakazakii* infections (9 – 11).

C. sakazakii, which can be found in infant foods and has a serious disease effect on infants, has been tried to be controlled by the criteria stated in the regulations about the microbiological criteria of foodstuffs. It is stated that *Cronobacter spp*. should not be present in the powdered formulas given to the 6 month-old infant which is stated in the regulation 2073/2005 / EC and in the revised regulation 362/2010 / EU prepared by the European Union Commission (12).

In Turkey and Turkish Republic of Northern Cyprus (T.R.N.C.), evaluation of *C. sakazakii* analysis results in foods are carried out by 2073/2005/EC on Microbiological Criteria for Foodstuffs prepared in parallel with the European Union Commission Regulation and according to Turkish Food Codex Microbiological Criteria Regulation revised in 2011. According to this regulation, *C. sakazakii* should not be present in infant formulas and continuing formulas. In the same regulation, *C. sakazakii* is not searched directly for infant and small children complementary food. It is stated that this bacterium is accepted within *Enterobacteriaceae* family and its limits are evaluated as $< 10^1$ (13) (Table 1).

Despite precautionary measures, infections rarely continue to be reported in the world, especially in newborns because of the *C. sakazakii* isolated from the infant formula and complementary food (14 – 18). Various studies conducted in Turkey in recent years have reported the presence of *C. sakazakii* (19 – 21).

In this study, it was aimed to investigate the presence of C. sakazakii in cereal based infant formulas and complementary food sold in T.R.N.C. Also, this study was designed to determine the risk of infection in these products.

Material and Methods

This research was carried out between May – December 2017 with the cereal-based infant continuing formulas and small child complementary foods of the brands offered for sale at Turkish Republic of Northern Cyprus (T. R. N. C.). Study has approved by Eastern Mediterranean University Scientific Research and Publication Board dated 14/04/17 with the line of Ethics Committee decision number 2017/41-15.

Sample Collection

In this study, by taking five samples from each item, a total of 265 cereal based infant formulas or complementary foods which are sold in different cities in T. R. N. C. were included. 36 different varieties of cereal-based infant formula and 17 varieties of cereal-based infant complementary foods from 10 brands on

Table 1. Infant- continuing formulas and recommended microbiological criteria for infant and small children supplementary foods (13).

Food	Microorganisms	Sampling plan		Limits	
		n	c	m	M
Baby formulas and continuing formulas (including diet foods for special medical purposes)	***B. cereus***	5	2	5×10^1	5×10^2
	Cronobacter sakazakii	10	0	0/25 g-mL	
	Salmonella spp.	10	0	0/25 g-mL	
	L. monocytogenes	10	0	0/25 g-mL	
Complemantary foods for infants and small children (including dietary foods for special medical purposes)	***B. cereus***	5	2	5×10^1	5×10^2
	Enterobacteriaceae	5	0	$< 10^1$	
	Salmonella spp.	5	0	0/25 g-mL	
	L. monocytıgenes	5	0	0/25 g-mL	

n: sampling number**, c:** number of samples that is allowed to be between m and M limits

T. R. N. C. marketed for sale were provided in a closed packed form. The products supplied are kept under proper conditions until the study is carried out. In the current study, total of 265 cereal based infant formulas or complementary foods which are sold in different cities in T. R. N. C. were included. 36 different varieties of cereal-based infant formula and 17 varieties of cereal-based infant complementary foods from 10 brands on T. R. N. C. marketed products were randomly selected. The samples were analysed in accordance with ISO / TS 22964: 2006 method.

Analysis

After products supplied from the market, they were brought to the laboratory in appropriate conditions. Afterwards, each item was opened on aseptic conditions and has been analysed. Analysis of samples were carried out according to; ISO / TS 22964: 2006 method. Before the samples were analysed, ATCC 51329 *Cronobacter muytjensii* (*Enterobacter sakazakii)* strain was used to check cross-reactivity and microbial background effect of the developed method. This strain used in this study was cultured in *E. sakazakii* isolation agar for 24 ± 2 hours at 44 ± 1 ° C on incubator (22). Each of the 53 kinds of 265 cereal-based samples was weighed at 25 g in aseptic conditions. 225 mL of Buffered Peptone Water was added to each of the weighed samples for pre-enrichment and incubated for 18 ± 2 hours at 37 ± 1 ° C on incubator. After incubation, 0.1 mL of pre-enriched Buffered Peptone Water was inoculated with 10 mL of Modified Lauryl Sulfate Tryptose Vancomycin Broth (mLST / Vancomycin) and incubated for 24 ± 2 hours at 44 ± 0.5°C on incubator.

Isolation

After incubation, using a 10µl loop, streak onto the surface of the Enterobacter sakazakii Isolation Agar plate, invert and incubate the dishes at 44°C ± 1°C for 24h ± 2h on incubator. After incubation development of blue-green colonies were checked on the agar. However, since no reproduction could be observed in the study, final identification steps were not applied.

Results

A total of 265 specimens of 36 different varieties of cereal-based infant formula, 17 varieties of cereal-based infant and child complementary foods were selected from the market for the determination of the presence *C. sakazakii*. The infant formulas and the infant/ small child complementary foods were separately examined, as it defined differently in the Turkish Food Codex.

In this study, the percentage of *C. sakazakii*, which is determined in a total of 180 cereal–based infant formulas of 36 varieties belonging to 10 different brands analyzed, and the detection result was found as 0%.

Table 2. *C. sakazakii* analysis results in cereal-based infant formula which are sold in the market

Product	Types of Sample	Number of Sample	Number Positive Samples per 25 grams	Positive Sample Ratio (%)
Cereal-based infant formula	36	180	0	% 0

Table 3. *C. sakazakii* analysis results in cereal-based infant/child complementary foods which are sold in the market

Product	Types of Sample	Number of Sample	Number Positive Samples per 25 grams	Positive Sample Ratio (%)
Cereal-based infant/child complementary foods	17	85	0	% 0

The percentage of *C. sakazakii,* which is detected in 85 cereal–based infants and children's complementary foods of 17 varieties belonging to 10 different brands analyzed and the detection result was found as 0%.

Discussion

C. sakazakii is a food-borne pathogen, which has become increasingly important in recent years and especially infants who are < 1 month, premature babies and babies who had low birth weight are at more risk since this pathogen may cause various clinical problems such as meningitis, necrotizing enterocolitis and septicemia. *C. sakazakii*, which can reproduce in infant formula due to its ability to sustain its activity in low water activity, is able to contaminate infant formulas through the raw materials added after pasteurization (3, 11).

In this study; 36 different varieties of cereal-based infant formulas and 17 varieties of cereal-based continuing formulas, which were sold in the market in TRNC, were collected and analyzed according to ISO / TS22964 (2006) standards. *C.sakazakii* was not observed in any of sample.

Muytjens et al. (23) collected 141 powdered infant foods from 35 different countries; and according to their analysis; out of 141 samples, 20 of them (14%) were identified with *C. sakazakii*. In the research conducted in Canada with 120 infant formulas, *C. sakazakii* was determined in 6.7% of infant formula (24). Leuschner et al. (25) collected 58 infant formulas from 11 countries and they found positivity of *C. sakazakii* in 8 samples (13.8%). Iversen and Forsythe (26) identified *C. sakazakii* in 2 out of 82 infant formula milk (2.4%) and 5 (10.2%) out of 49 dried infant formula which were collected from Asia, Europe, Africa and America have been found that they are contaminated with *C. sakazakii*. They concluded that the bacteria were isolated from food that had never been opened before, resulting in possible contamination after pasteurization during production stage (26).

Another study which was conducted by Shaker et al. (15) in 2007 stated that *C. sakazakii* was isolated in 2 (25%) of 8 powdered baby foods. Chap et al. (27) conducted a study with 136 continuing formulas and 179 infant formulas from 7 countries. After the analysis, out of 136 continuing formula samples 1% and out of 179 infant formulas, 22 samples (%12) have been identified with *C. sakazakii*. On the other hand, Hara et al. (17) carried out a study in Japan and they determined that *C. sakazakii* was present in only 9 samples (6%) out of 149 infant formulas. Kandhai et al. (28) carried out a study over a period of four years in Netherlands; they identified C. *sakazakii* in 7 samples out of 175 milk powder samples. At the same study, in powdered formulas which are suitable for infants below one year of age (8 samples out of 395) and in formulations for infants over 1 year of age (1 sample out of 5) have been investigated that they were contaminated with *C.sakazakii.*

In a study conducted in Bangladesh in 2010 (29), 32 powdered baby food samples collected from market; out of 32 sample, they have been isolated *C.sakazakii.* only in one sample. Between 2009 and 2012, Fei et al. (30,31) collected 1228 powdered infant formula and they isolated *C. sakazakii* in 66 of the collected samples. Also, they have found *C. sakazakii* in 56 of the 2020 powdered baby foods collected between years

2015-2017. When the data of *C. sakazakii* in infant formulas which have been reported from abroad have been compared with the data of this study, it has been observed that the results of the research are lower than the world average.

According to Turkey Statistical Institute's data for the last 8 years; 1.291.055 babies are born each year. Although the consumption of infant foods in the rural areas is lower than in the cities, the annual consumption amounts of infant foods are too high. Since, *C. sakazakii* has a potential to be found in baby food wordwidely, reveals the risk for babies of consuming food contaminated with *C. sakazakii* in infants in Turkey and T.R.N.C.

A study which is carried out by Tokatlı (32) in 2009 in Tekirdağ, *C. sakazakii* identified only in 3 (4.8%) out of the 62 powder baby food samples. The samples were consisting of 6 brands and they used the random sampling method to choose those 6 brands. Gökmen et al. (33) carried out a study in order to investigate the presence of *C. sakazakii* in 60 milk powder samples, 50 whey powder samples and 50 cheese samples. Results of the study indicated that they have determined *C. sakazakii* in 5% (3/60) of milk powder samples and 4% (2/50) of white cheese samples. In 2011, Yemiş (19) carried out a study by collecting 100 infant and continuing food, 40 children complementary food and 60 infant food and additional food component. Results of the study showed that none of the infant and continuing food had any *C. sakazakii* presence. While, only 1 sample of cereal-based foods and 14 of the additional food components of cereal products had *C. sakazakii* contamination. Kaya (34) has done a study in Istanbul in 2011, with 40 infant formula and 15 complementary foods; *C. sakazakii* was detected in 1 (2.5%) out of 40 infant formula and in 1 (6.7%) out of 15 complementary foods.

In 2012, Çakmak (20) conducted a study with a total number of 350 infant formula, including; 120 infant formulas, 80 continuing formulas and 150 infant/children complementary foods and they also analyzed 50 pasteurized milk products. None of the samples had any contamination with *C. sakazakii*. However, in the same study, *C. sakazakii* contamination was detected in 4 (4%) out of 100 cereal-based infants and small children complementary food. In 2013, Çetingürbüz (21), conducted a study with 50 raw milk and 20 powder baby foods in Ankara, 8 of the raw milk (16%) and only 1 (5%) of the baby foods determined with the contamination of *C. sakazakii.* However, in the same study, *C. sakazakii* contamination was detected in 4 (4%) out of 100 cereal-based infants and small children complementary food.

In Koluman (35) study which was conducted in 2011, they examined 20 baby food samples and concluded that none of the sample was contaminated with *C. sakazakii* (35). Also in another study which was carried out with 52 powdered baby food samples, 82 ready-to-use foods (with a water content) and 6 newborn feeding tubes, *C. sakazakii* was not found in any of the samples. The results of this study about infant and continuing foods are supported by the studies of Yemiş (19), Çakmak (20), Koluman (35), and Manga (36).

In conclusion, most of the microbiological analysis was carried out on baby foods in the world and Turkey despite the reported presence of *C. sakazakii,* in the current study no bacteria were found in any of the cereal based continuing foods. This result shows that the possible contamination of *C. sakazakii* is prevented and microbiologically safe food is produced during the production process of the foods. To determine the risk of infection of *C. sakazakii* in both formula foods and continuing foods, there is a requirement for new and comprehensive studies.

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