Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science and Science Education, Anchor University Lagos
URL: journal.aul.edu.ng
Vol. 1 No 1, June 2020, Pp. 35 - 40

Occurrence of *Cronobacter sakazakii* in Powdered Milks Obtained within Kaduna Township

Oloninefa S. D.*, Daniyan, S. Y. , Abalaka, M. E.

Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria

***Corresponding author:**
E-mail: : dami4nefa2013@gmail.com;
Phone: +2348038893341

Submitted 5 April, 2020
Accepted 24May, 2020

Competing Interests: The authors declare no competing interests.

ABSTRACT

Background: *Cronobacter sakazakii* is a foodborne pathogen, posing a high risk of disease to infants and immunocompromised individuals. Powdered milk (such as infant milk formula and opened-vended milk) are the main food sources that have been linked with epidemic diseases caused by *C. sakazakii*.
Objectives: The objective of this study is to determine the occurrence of *C. sakazakii* in powdered milk comprising powdered infant formulas (PIF) (used and retailed PIF) and opened-vended powdered milk (OVPM) obtained within Kaduna Township, Kaduna State, Nigeria.
Methods: The occurrence of *C. sakazakii* was investigated in three hundred and six (306) samples of powdered milk comprising powdered infant formulas (PIF) (used and retailed PIF) and opened-vended powdered milk (OVPM) obtained within Kaduna township were investigated using standard methods comprising *Enterobacteriaceae* Enrichment Broth and HardyCHROM sakazakii medium.
Results: The results showed that the occurrence of *C. sakazakii* was highest in OVPM with 8 (7.21%) followed by used PIF with 5 (5.05%) and the least occurred in retailed PIF with 2 (2.08%) out of three hundred and six (306) investigated.
Conclusion: The work suggests that OVPM was a major source of contamination of *C. sakazakii*. Hence, the consumption of contaminated OVPM by infants will make them susceptible to septicemia, meningitis, bacteremia caused by *C. sakazakii*.

Keywords: *C. sakazakii*, Powdered Infant Formulas, Opened-Vended Powdered Milk, HardyCHROM sakazakii medium

INTRODUCTION

The need for food safety and microbial contamination of powdered infant formulas (PIF) and opened-vended powdered milk (OVPM) is of great concern to public health all over the world. The health, growth and infants' behaviour can be affected by the consumption of powdered infant formulas and opened-vended milk contaminated by *Cronobacter sakazakii*. The publications on *Cronobacter sakazakii* infections are very rare in adults because the seriousness is less compared to the high mortality recorded in neonates and infants. Hence, adequate feeding is needed for a child of age zero to two years for optimum development and to achieve full potential (Lai, 2001; NAFDAC, 2013). In Nigeria, *C. sakazakii* was isolated from locally consumed powdered foods as reported by Aigbekaen and Oshoma (2010). Serious forms of neonates meningitis, necrotizing enterocolitis and septicemia with mortality rates that vary from 40-80% have been recorded from the consumption of contaminated milk-based powdered infant formula. As a result of this, alert on the risk of consuming contaminated powdered infant formula was issued to the healthcare professionals by US Food and Drug Administration (Muytjens *et al*., 1988; Lai, 2001; Van Acker *et al*., 2001; Bar-Oz *et al*., 2001). *C. sakazakii* was isolated from different foods which include sorghum, rice seeds, cheese, herbs, meat, vegetables, spices, grains, sorghum, fermented bread, sour tea, tofu, fermented beverage and ultra-high-temperature treated milk (UHT milk) (Gassem, 2002; Leclercq *et al*., 2002; Iversen and Forsythe, 2003). However, studies have revealed the connection between infections caused by *C. sakazakii* in neonates and

powdered infant milk formulas (Muytjens *et al*., 1988; Biering *et al* 1989; Simmons *et al*., 1989; Van Acker *et al*., 2001).

Furthermore, CAC (1979) reported the isolation of *C. sakazakii* from infections such as bacteremia and a variety of infections (found in older babies, children and adults) coupled with devastating meningitis in neonates. However, available literature reported that the majority of infections such as sepsis, meningitis and necrotizing enterocolitis caused by *C. sakazakii* occurred in neonates, premature infants, post-mature infants and full-term newborns (CAC, 1979).

The occurrence of *C. sakazakii* in powdered infant formulas (PIFs) has been made possible because of the current method of not subjecting the final packaged product to high temperature. This eventually made PIFs not to be commercially sterile and the safety of the products guarantee (United States Food and Drug Administration, 2002). This study however investigates the occurrence of *C. sakazakii* in powdered milk obtained within Kaduna Township, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

The study covered selected shops and day-care centres located in Sabon Tasha in Chikun Local Government Area, Kakuri in Kaduna South Local Government Area and Kawo in Kaduna North Local Government Area.

Sample Collection

Retailed infant formulas (RIF) and opened-vended powdered milk (OVPM) samples of different brands were bought from the markets while used infant formulas (UIF) were collected from day-care centres. Both the markets and the day-care centres were located in Sabon Tasha, Kakuri and Kawo within Kaduna Township. The samples were coded as SM, CE, NT, NA, FR, LC, PK, DA, ML, GM, JG, NU, NN, OL and CB. They were transported to the laboratory for microbiological analysis for the determination of the occurrence of *C. sakazakii.*

Experimental Design

The occurrence of *C. sakazakii* was investigated in PIF (comprising UIF and RIF) and OVPM obtained within Kaduna Township. The sample size was determined using equation 1.

$$n = \frac{z^2pq}{d^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where:
n = Sample size
z = Standard deviation set at 1.96 corresponding to 95% confident level
p = Prevalence of *C. sakazakii* from previous studies equal to 27.1% = 0.271 (Aigbekaen and Oshoma, 2010)
d = Degree of freedom at 5% = 0.05
q = 1 – p

Therefore, $$n = \frac{1.96^2 \times 0.271 \times 0.729}{0.05^2} = 303.57$$

Three hundred and six (306) samples comprising of branded UIF, RIF and OVPM were randomly selected and used for the research work. The samples were made up of 96 unopened RIF, 111 OVPM and 99 UIF obtained from infant day-care centres and shops within Kaduna Township. Thirty-two (32) unopened RIF, 37 OVPM and 33 UIF samples of different brands were collected per study areas respectively. The microbiological analysis was carried out in triplicates.

Occurrence of *C. sakazakii*

The occurrence of *C. sakazakii* in powdered milk was carried out according to the modified method outlined in Oloninefa *et al*. (2016). Two grams (2 g) from each sample was weighed aseptically and delivered into 18 ml of sterilized buffer peptone water and mix very well to give a dilution of 1:10. One millilitre (1 ml) from each of the food homogenate was then added to 9 ml of the prepared *Enterobacteriaceae* Enrichment Broth (EEB) and incubated at 37^{o}C for 24 h. The HardyCHROM Sakazakii medium plates were inoculated with a loopful from EEB and incubated at 37^{o}C for 24-48 h in a dark incubator (Hardy Diagnostics Manual, 2011). The enumeration of *C. sakazakii* was done by counting and multiplying the number of distinct, viable and separated colonies on the HardyCHROM Sakazakii medium plates by the reciprocal of the dilution factor and then expressed in cfu/g (Hardy Diagnostics Manual, 2011).

Identification of *C. sakazakii*

Gram Staining and biochemical tests were carried out on the isolated *C. sakazakii* isolated for further identification as outlined by Brooks *et al*. (2007) and Cheesbrough (2010).

RESULTS

Cultural Characteristics and Biochemical Tests

C. sakazakii isolated on HardyCHROM sakazakii medium plates appeared greenish and were all Gram negative. They were all motile, catalase-positive, methyl red negative, oxidase negative, positive for citrate utilisation and nitrate reduction (Table 1).

Occurrence of *C. sakazakii*

Table 2 shows the occurrence of *C. sakazakii* in retailed infant formulas, used infant formulas and opened-vended powdered milk in the three study areas.

Out of 306 samples screened for the occurrence of *C. sakazakii* in the three study areas, 15 (4.90%) were positive while 291 (95.10%) were negative. Ninety-six (96) of the retailed infant formulas (RIF) screened had 2 (2.08%) positive and 94 (97.92%) were negative. On the other hand, 5 (5.05%) and 94 (94.95%) were positive and negative respectively out of 99 used infant formulas (UIF) screened. One hundred and eleven (111) samples of opened-vended powdered milk (OVPM) screened had 8 (7.21%) positive and 103 (92.79%) negative (Table 2).

The results obtained from the study areas revealed that 32 (33.33%) were negative in Sabon Tasha; 2 (2.08%) were positive, 30 (31.25%) were negative in Kakuri while 32 (33.3%) were negative in Kawo for the occurrence of *C. sakazakii* when 96 samples of RIF were screened for the occurrence of *C. sakazakii* (Table 2). On the other hand, out of 99 samples of UIF screened, 2 (2.02%) were positive, 31 (31.31%) were negative in Sabon Tasha; 33 (33.33%) were negative in Kakuri; 3 (3.03%) were positive and 30 (30.30%) were negative in Kawo (Table 2).

However, 2 (1.80%) were positive, 35 (31.53%) were negative in Sabon Tasha; 3 (2.70%) were positive, 34 (30.63%) were negative in Kakuri; 3 (2.70%) were positive and 34 (30.63%) were negative out of 111 samples of OVPM screened for the occurrence of *C. sakazakii* (Table 2).

DISCUSSION

The presence of greenish isolates on HardyCHROM Sakazakii medium which distinguishes *C. sakazakii* from other species of *Cronobacter* agreed with the report stated in Hardy Diagnostics Manual (2011) while the cultural characteristics and biochemical tests for the

Table 1: Cultural Characteristics and Biochemical Tests

	Morphology		Gram Reaction	Biochemical tests						
ISOLATE	Colour	Shape	Reaction	Shape	Catalase	Citrate Utilisation	Methyl red	Oxidase	Nitrate Reduction	Motility	Probable Organism
n= 20	Red	Straight	-	Rods	+	+	-	-	+	+	*C. sakazakii*

n= Number of *C. sakazakii* tested

Table 2: Occurrence of *C. sakazakii* in Retailed Infant Formulas, Used Infant Formulas and Opened-vended Powdered Milk

		Study Areas					
		Sabon Tasha		Kakuri		Kawo	
Types	Number of Samples	Positive	Negative	Positive	Negative	Positive	Negative
RIF	96	0 (0%)	32(33.33%)	2(2.08%)	30(31.25%)	0 (0%)	32(33.33%)
UIF	99	2(2.02%)	31(31.31%)	0(0%)	33(33.33%)	3(3.03%)	30(30.30%)
OVPM	111	2(1.80%)	35(31.53%)	3(2.70%)	34(30.63%)	3(2.70%)	34(30.63%)
Total	306	4(1.31%)	98(32.03%)	5(1.63%)	97(31.70%)	6(1.96%)	96(31.37%)

RIF- Retail Infant Formulas; UIF- Used Infant Formula; OVPM- Opened- Vended Powdered Milk

identification of *C. sakazakii* were in agreement with the results reported by Iversen *et al*. (2008) and Buchana and Gibbons (2007).

Also, the results showed that 2 (2.08%) samples of RIF were positive for occurrence of *C. sakazakii* in Kakuri. The current method used for the manufacturing of infant formulas which does not guarantee complete sterility of powdered infant formulas might be responsible for the positive result (FDA/CDC, 2011). This result also agreed with FAO/WHO (2004) that reported that powdered infant formulas (PIF) are susceptible to contamination by *C. sakazakii*. However, 32 (33.33%), 30 (31.25%) and 32 (33.33%) samples of RIF were negative for the occurrence of *C. sakazakii* in Sabon Tasha, Kakuri and Kawo respectively. This result might be due to the intactness of the containers since they were in sealed condition before being screened for the occurrence of *C. sakazakii*. Good Manufacturing Practice (GMP) also must have contributed to this result (WHO, 2007).

Furthermore, 2 (2.02%) and 3 (3.03%) samples of UIF were positive for the occurrence of *C. sakazakii* in Sabon Tasha and Kawo respectively. The samples positive for *C. sakazakii* might be due to poor hygienic practices, poor handling practices and the use of unsterilized feeding equipment (FAO/WHO, 2006). Poor information dissemination to the day-care centres personnel on possible sources of contamination of UIF by *C. sakazakii* might also be responsible for the positive result. On the other hand, 31 (31.31%), 33 (33.33%) and 30 (30.30%) samples of UIF were negative for the occurrence of *C. sakazakii* in Sabon Tasha, Kakuri and Kawo respectively. This result might be due to good hygiene practices especially that of food handling and handwashing practices which prevent the contamination of prepared infant formulas in homes and at day-care centres by *C. sakazakii*, a member of *Enterobacteriaceae* as reported by Drudy *et al.* (2006) and FAO/WHO (2006). The absence of *C. sakazakii* might also be because the day-care centres maintain a temperature of not less than 70^{o}C when preparing infant formulas for babies (FAO/WHO, 2006; Gurtler *et al*., 2005).

The results further revealed that 2 (1.80%), 3 (2.70%) and 3 (2.70%) samples of OVPM were positive in Sabon Tasha, Kakuri and Kawo respectively. The occurrence of *C. sakazakii* in the samples might be due to poor hygienic practices especially that of food handling and handwashing practices. In the same vein, the positive result might be due to frequent openings of the OVPM samples to contaminated environments and poor hygienic practices in the course of dispensing opened-vended powdered milk samples. Frequent openings of the opened-vended powdered milk samples to a contaminated environment and poor hygienic practices in the course of dispensing coupled with improper handling of the opened-vended powdered milk (OVPM) samples might have contributed to the positive result as reported by Drudy *et al*. (2006); FAO/WHO (2006) and FDA/CDC (2011). On the other hand, 35 (31.53%), 34 (30.63%) and 34 (30.63%) samples of OVPM screened were negative for the occurrence of *C. sakazakii* in Sabon Tasha, Kakuri and Kawo respectively. Good hygiene practices and the carefulness in the openings of the OVPM bags in the course of selling to customers might be responsible for this result.

However, it was observed that 15 (4.90%) samples were positive for the occurrence of *C. sakazakii* while 291 (95.10%) were negative for the occurrence of *C. sakazakii* in the three study areas. The highest percentage of 8 (7.21%) for occurrence of *C. sakazakii* was recorded in OVPM followed by 5 (5.05%) in used infant formulas (UIF) while the least result of 2 (2.08%) was obtained from retail infant formulas (RIF) samples. The highest percentage of occurrence of *C. sakazakii* recorded in OVPM compared to others might be due to frequent openings, poor hygienic practices especially during handling and dispensing. The occurrence in OVPM, UIF and RIF implies they are all susceptible to *C. sakazakii* contamination. Hence, sellers and users must adhere to good hygienic practices in food handling and handwashing practices and maintaining a temperature of not less than 70^{o}C when preparing infant formulas for babies to prevent the contamination by *C. sakazakii*.

CONCLUSION

The findings from this study showed that *C. sakazakii* occurred in RIF possibly because of the current methods used for manufacturing of powdered infant formulas (PIF) which does not guarantee complete sterility. The study also revealed the presence of *C. sakazakii* in UIF and OVPM.

It is therefore recommended that current methods used for the manufacturing of PIF should be reviewed and in situations where infants are not breastfed, sterile liquid formula or formula that has undergone an effective

point-of-use decontamination procedure (e.g. use of boiling water to reconstitute or by heating reconstituted formula) should be used. Good handling practices, fewer openings of OVPM bags and good hygienic practices should be encouraged to prevent contaminations of UIF and OVPM by *C. sakazakii*.

ACKNOWLEDGMENTS

We would like to appreciate God for the success recorded in the study. We also wish to thank the following people for their immense contributions to the study: Prof. Evans Egwin (Department of Biochemistry, Federal University of Technology, Minna, Niger State); Dr. Abdulsalam Ihimma (Department of Applied Science, Kaduna Polytechnic, Kaduna, Kaduna State); Prof (Mrs) Abimbola Orukotan (Department of Microbiology, Kaduna State University, Kaduna, Kaduna State) and Mallam Sani Mohammed (Department of Microbiology, Kaduna State University, Kaduna, Kaduna State).

REFERENCES

Aigbekaen, B. O. and Oshoma, C. E. (2010). Occurrence of *Enterobacter sakazakii* from Powdered Foods Locally Consumed in Nigeria. *Pakistan Journal of Nutrition,* 9 (7): 659-663.

Bar-Oz, B., Preminger, A., Peleg, O., Block, C. and Arad, I. (2001). *Enterobacter sakazakii* infection in the newborn. *.Acta Pediatrics,* 90: 356–358.

Biering, G., Karlsson, S., Clark, N., Karlsson, N. C., Jonsdottir, K. E., Ludvigsson, P. and Steingrimsson, O. (1989). Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *Journal of Clinical Microbiology*, 27: 2054-2056.

Brooks, G. F., Carroll, K. C., Butel, J. S. and Morse, S. A. (2007). Microscopy and Stains. In: Jawetz, Melnick and Adelberg's *Medical Microbiology*. Twenty-fourth Edition (pp.700-701). U.S.A: The McGraw-Hill Companies, Inc.

Buchana, R. E. and Gibbons, N. E. (2007). *Cronobacter sakazakii*. In: *Bergey's Manual of Determinative Bacteriology*. Eight Edition (pp 1246). Bartimore, Maryland: The Williams and Wilkins Company.

Centre for Disease and Control (CDC) (2001). *Enterobacter sakazakii* infections associated with the use of powdered infant formula-Tennessee. *Morbidity* and *Mortality Weekly Report,* 51(14): 298-300.

Cheesbrough, M. (2010). Laboratory Manual. In: *District Laboratory Practice in Tropical Countries* (pp.146-157). United Kingdom: Cambridge University Press.

Codex Alimentarius Commission (CAC) (1979). Recommended international code of hygienic practice for foods for infants and children (CAC/RCP 21-1979).

Drudy, D., Mullane, N. R., Quinn, T., Wall, P. G. and Fanning, S. (2006). *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. *Clinical Infectious Diseases,* 42: 996–1002.

Food and Agriculture Organization of the United Nations/World Health Organization (2004).*Enterobacter sakazakii* and other microorganisms in powdered infant formula. Meeting report. Geneva, Switzerland. *Microbiological Risk Assessment Series*, No. 6.

Food and Agriculture Organization of the United Nations/World Health Organization (2006). *Enterobacter sakazakii* and *Salmonella* in powdered infant formula. Meeting Report. Joint FAO/WHO Technical Meeting on *Enterobacter sakazakii* and *Salmonella* in Powdered Infant Formula, Rome, Italy. *Microbiological Risk Assessment Series,* No.10.

Food and Drug Administration/Centre for Disease Control (2011). Investigation of Cronobacter bacteria illness in infants. CDC Media Relations. Retrieved from*http://www.cdc.gov/mediar releases /2011/s1230_ Cronobacter.html.*

Gassem, M. A. A. (2002). A microbiological study of sobria: a fermented beverage in the Western province of Saudi Arabia. *World Journal of Microbiology and Biotechnology,* 8: 173–177.

Gurtler, J. B., Kornacki, J. L. and Beuchat, L. R. (2005). *Enterobacter sakazakii*: a coliform of increased concern to infant health. *International Journal of Food Microbiology*, 104: 1-34.

Hardy Diagnostics Manual (2011). HardyCHROM sakazakii Medium-Microbiology Culture Media (pp.1-5). USA: 1430, West McCoy Lane, Santa Maria, CA93455.

Iversen, C. and Forsythe, S. (2003). Risk profile of *Enterobacter sakazakii* an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*, 14: 443-454.

Iversen, C., Mullane, F. W., McCardell, N. B., Tall, B. D., Lehner, A., Fanning, C., Stephen, R. R. and Joosten, H. (2008). *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter genomospecies* 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter blinensis* subsp. *lausannensis* subsp. nov. and*Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *International Journal of Systematic Evolution of Microbiol*ogy, 58: 1442-1447.

Lai, K. K. (2001). *Enterobacter sakazakii* infections among neonates, infants, children and adults. Case reports and a review of the literature. *Medicine* (Baltimore), 80: 113-122.

Leclercq, A., Wanegue, C. and Baylac, P. (2002). Comparison of faecal coliform agar and violet red bile lactose agar for faecal coliform enumeration in foods. *Applied and Environmental Microbiology,* 68: 1631-1638.

Muytjens, H. L., Roelofs-Willemse, H. and Jaspar, G. H. (1988). Quality of powdered substitutes for breast milk with regard to members of the family *Enterobacteriaceae. Journal of Clinical Microbiology,* 26: 743-6.

National Agency for Food and Drug Administration and Control (NAFDAC) (2013). Infant Nutrition Policy. In: *Consumer Safety Publication*. Issued 2 (pp.5).

Oloninefa, S. D., Abalaka, M. E. and Daniyan, S. Y. (2016). Preparation of food homogenate and occurrence of *C. sakazakii*. In: *Antibacterial Susceptibility of C. sakazakii to Known Antibiotics.* 1st edition (pp. 53-54), Saarbrucken, Deutschland, Germany: Lambert Academic Publishing Omniscriptum GmbH & Co. KG, BahnhofstraBe, 28, 66111.

Simmons, B. P., Gelfand, M. S., Haas, M., Metts, L. and Ferguson, J. (1989). *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infection Control Hospital Epidemiology*, 10: 398-401

United States Food and Drug Administration (2002). Occurrence and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula. Retrieved from *http://www. cfsan. fda.gov /~comm/mmesakaz.html*.

Van Acker, J., De Smet, F., Muyldermans, G., Bougatef, A., Naessens, A. and Lauwers, S. (2001). Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *Journal of Clinical Microbiology*, 39 (1): 292-297.

World Health Organisation (2007). Guidelines for safe preparation, storage and handling of powdered infant formula. Retrieved from *http://www.Whonintnfoodsafety/ publications/micro/pif2007/en/index.htl.*