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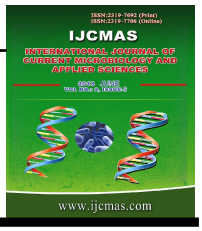

Original Research Article

Pattern of breastfeeding and occurrence of *Cronobacter sakazakii* in infant formula sold in Ekiti State, Nigeria

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A B S T R A C T

The presence of *Cronobacter sakazakii* in infant formula has been commonly reported in the recent time and has been implicated in infection of infants with high mortality rates. There is paucity of information the occurrence of *C. sakazakii* in infant formula in the study area hence this study. This work aimed at the determination of aerobic bacterial load and the susceptibility pattern of *C. sakazakii* isolated from the infant formula and to determine the factors that encourages bottle feeding. Standard microbiological methods were used to isolate and identify *C. sakazakii* and disc diffusion method was used to determine the susceptibility of the isolates to the tested antibiotics. Standard pre-test questionnaire was used to collect information on the pattern of infant formula feeding and breastfeeding among nursing mothers in the study area. The total aerobic bacterial counts of ten different infant formula screened ranged from $1.1x10^2$ to 8.0×10^3 cfu/g. The least counts occurred in brand CWG whiles the highest was recorded in brand DNN. Out of ten brands of infant formula examined only four were positive for *C. sakazakii*. Eight antibiotics were tested against the isolated *C. sakazakii*. The isolates showed the highest resistance to tetracycline (99.93%) followed by nitrofurantoin (96.97%). Out of the total isolates tested 42.43%, 51.51% and 52.51% were resistant to amoxicillin/clavulanate, gentamicin and ofloxacin respectively. Based on the level of education, 83.33% of the participated nursing mothers had minimum of secondary education. A greater part of the respondents (82.35%) fed infant formula to their babies while about 17.65% does not. Mother gave different reasons for choosing infant formula for their babies. A total of 39 mothers reported their babies showed different signs of infections after been fed with infant formula. Most of the infant formula screened did not meet the international acceptable standards and the rate of bottle feeding is still high in the study area.

K e y w o r d s

Cronobacter sakazakii; breast feeding; infant; Infant formula; antibiotic resistance.

Introduction

Milk substitutes play an indispensable role in infant nutrition when breast-feeding is not possible, desirable and sufficient (Greiner, 1977; Muytjens et *al.*, 1988; Howie, 2002). Infant formula is manufactured to support adequate growth of infants under six months of age when fed as a sole source of nutrition (Cohen *et*

al., 1984; Becker *et al.,* 1994). Their composition is roughly based on a mother's milk in most cases they are not sterile due to contamination (Alles *et al.,* 2004). They have been designed to provide infants with the required nutrients for optimal growth and development. Infant formulas, like other food items contain microorganisms of different types and in different amounts and microorganism that dominate depend on several factors (Fleming and McFeeters, 1981; Isolauri *et al.,* 2002; Breeuwer *et al.,* 2003).

Introduction of complementary foods and the use of artificial baby milk have been associated with a high degree of infection among infants. Isolates from most of which are major bacteria have been reported to be pathogenic (Erku and Ashenafi, 1998*;* Morais *et al.,* 1997). Bacterial contamination of supplementary milk feeds to infants and children up to 2 years of age is common. Higher degree of contamination could significantly be associated with lower income, lower caste, illiteracy among the mothers, unclean utensils, and lack of hand hygiene in the mother (Iroegbu *et al.,* 2000).

Most studies report that the stool flora of breast-fed infants differs from that of formula-fed infants (Satokari *et al.,* 2002; Rubaltelli *et al.,* 1998). Breastfeeding contribute largely to child health by protecting the infant and child against infections and death (Habicht *et al.,* 1988). In 1988, a Malaysian study found a relative risk of 5 deaths after 1 week of age children not breast fed compared with breast fed infants (MMWR, 2002). Providing the mother is given the right information and support from the family, community and from the health care system, exclusive breastfeeding from birth is possible for most mothers except in a few medical conditions (Pursall *et al.,* 1978; Iroegbu *et al.,* 2000).

Cronobacter sakazakii (formerly known as *Eneterobacter sakazakii*) belongs to the family *Enterobacteriaceae.* It is closely related to the genera *Enterobacter* and *Citrobacter* (Sani and Yi, 2011; Himelright *et al.,* 2002). *C. sakazakii* is a normal flora of milk powder, wheat, rice, vegetables, cheese, sausage meat, teas, and various spices (Joseph and Forsythe, 2011). It is associated with various food products like powdered infant formula. Is an opportunistic pathogen that has been implicated in food-borne outbreaks or sporadic cases worldwide (Leclercq *et al.,* 2007). It is the causative agent of meningitis, septicemia, and necrotizing colitis in infants, particularly neonates.

Muytjens *et al*., (1988) reported a study examining powdered infant formulae from 13 European countries and found *Cronobacter* species in 53% of 141 samples. *Cronobacter sakazakii* is a gram-negative, rod-shaped bacterium that has a wide spread and relatively resistant to heat (Nazarowec-White and Farber, 1997a; Edelson-Mammel and Buchanan, 2004) hence survive in infant formula and poses serious risks on infants (Kandhai, 2004). Multiplication of *C. sakazakii* causes severe invasive infections in preterm and term infants, particularly during the first weeks of life. The pathogen causes high rates of meningitis, brain abscesses and necrotizing enterocolitis (Lai, 2001) with mortality of 33% to 80% (Lai, 2001; Burdette and Santos, 2002; Pagotto *et al.,* 2003).

C. sakazakii is a pathogen of emerging public health concern. It has been detected in a range of foods especially infant

formula. Infants requiring formula feeding are at high risk for developing life-threatening *C. sakazakii* infections, which are associated with significantly high morbidity and mortality rates. In southern Nigeria, there is no report on the occurrence of the pathogen in the powder infant formula hence the aim of this study. The objectives of this study were to determine the level of bacterial contamination and antibiotic resistance of the associated *C. sakazakii* in common infant formula products in Ekiti Sate, Nigeria.

Materials and Methods

Source of samples

Five samples each for the ten brands of powdered infant formula were purchased from different supermarkets in Ado-Ekiti, Nigeria. None of the products was seal-pampered nor expired. And all the products were certified by the National Agency for Food and Drug Administration and Control (NAFDAC).

Determination of total aerobic bacterial count and isolation of *C. sakazakii*

Each of the powdered infant formula samples was opened in a sterile environment and the methods of Fawole and Oso (2001) and Banwart (1994) were used to determine the aerobic bacterial loads in the samples. The FDA (2002) method was used for the isolation of *C. sakazakii*. The sample was first pre-enrichment by dispensing 10 g of each powder was aseptically into a 50 ml of sterile distilled water in a conical flask. Ten-fold serial dilution of the solution was made This was incubated at 36^{o}C for 24 h. Ten milliliter of the diluted solution was added into 90 ml Enrichment Broth (EEB) and incubated at 36^{o}C for 24 h. After incubation a loop full of the culture was aseptically inoculated onto violet red bile glucose (VRBG) agar (Oxoid). The plates were incubated for 24 h at 36^{o}C. Colonies with purple colour were picked and sub-cultured onto tripticase soy Agar (TSA). The plates were incubated at 25^{o}C for 72 h in an incubator. The tests were conducted in triplicates. For each of the brands positive for *C. sakazakii* we selected an average of 10 isolates for the possibility of detecting clones. The cultural and biochemical test were performed on the isolates and the results interpreted according to Holt *et al*., (1994).

Determination of Antibiotic Susceptibility of *C. sakazakii*

The isolates were standardized by growing at 37°C in Mueller-Hinton broth (Oxoid) for 16-18 h and adjusted to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625 nm. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (CLSI) (2008). The isolates were tested against eight commercial antibiotics. The commercially prepared antibiotic disks (Abtek Biologicals Limited) and their concentrations are as follows: amoxicillin (25μg), gentamicin (10μg), cotrimoxazole (25μg), nitrofurantoin (30μg), nalidixic acid (30μg), ofloxacin (30μg), amoxicillin/clavulanate (30μg) and tetracycline (30μg).

Subjects and Questionnaire
Study population

This study was conducted between May and July 2009. At the onset of the study the intent of the study was disclosed to the subjects (the nursing mothers). A

structured questionnaire interview was used to collect data from consented mothers who gave a verbal consent and signified their intention to participate in the study. The questionnaire used in the interview was evaluated, reviewed carefully and then pre-tested on 20 respondents. The questionnaire consisted of two parts. The first focused on socio-demographic characteristics of respondents in terms of academic qualifications and occupation. The second involved issues with infant formula and breast feeding pattern.

Two hundred and fifteen questionnaires were distributed to the consented nursing mothers at Ado-Ekiti and Ilawe-Ekiti, representing urban and rural areas respectively. The questionnaires were administered and data were collated. Only two hundred and four questionnaires were returned out of the total distributed.

Result and Discussion

Table 1 shows the result of the total aerobic bacterial counts in the 10 brands of infant formula screened. None of the powders were sterile. The total aerobic bacterial counts ranged from $1.1x10^2$ to $8.0 X 10^3$ cfu/g. The least counts occurred in brand CWG whiles the highest was recorded in DNN. Out of ten brands of infant formula examined only four [ISM (4/5), JGO (3/5), PKK (2/5), and CWB (3/5)] were positive for the *C. sakazakii*.

As shown in Table 2, the susceptibility pattern of *C. sakazakii* shows that the isolates had highest resistance to tetracycline (99.93%) followed by nitrofurantoin (96.97%). Out of the total isolates tested 42.43%, 51.51% and 52.51% were resistant to amoxicillin/ clavulanate, gentamicin and ofloxacin respectively. Amoxicillin/ clavulanate was most effective against the isolates while nitrofurantoin recorded the least effectiveness.

Table.1 Total bacterial count of screened infant formula (Log_{10}cfu/g)

Samples*	Total bacterial load
ISM	3.301±0.128
DNN	3.903±0.913
JGO	3.806±0.108
PKK	2.079±0.317
NNN	3.556±0.428
NTD	3.716±0.253
CWB	2.041±0.025
FRG	3.301±0.136
SML	3.556±0.083
CWB	3.602±0.461

Data are the mean±SEM of triplicate determinations; These are represented and not the real names of the products

Table.2 Antibiotic susceptibility pattern of *C. sakazakii* isolated from infant formula

Antibiotics	Susceptible	Intermediate	Resistance
AMX	143	12	174
TET	5	7	317
GEN	160	0	169
NIT	7	7	315
OFL	155	0	174
NAL	13	9	307
AMOX/CLAV	169	16	144
COT	31	4	294

Table 3 shows the distribution of respondents based on their level of education and occupation. It is clearly shown that 83.33% of the respondents have at least secondary education while

only 16.67% of the respondents had primary school education only. It also shows the distribution of respondents by occupation. Most of the respondents (65.69%) were either self employed or unemployed while 34.31% were government workers.

Table 4 shows the level of infant formula feeding among nursing mothers participated in the study. A greater part of the respondents (82.35%) fed infant formula to their babies while about 17.65% does not. Out of the 168 (82.35%) respondents that feed infant formula to their babies a total of 41(24.40) were public servants while 71(42.26%) were trader. Respondent gave different reasons for bottle feeding.

Table 5 shows the type of infant formula, factors affecting their choices and effect on babies' health. Brand NNN enjoys widest acceptance among mothers followed by FRG with 45.10% and 14.71% respectively. Mother gave different reasons for choosing infant formula for their babies. A total of 26 mothers reported their babies showed different signs of infections after been fed with infant formula. A total of 176 (86.27%) mothers responded that they use to feed their babies with chocolate drink or adult milk before the age of 12 months.

Ten different types of infant formulas were examined for their microbial quality and the presence of *C. sakazakii*. The total bacterial counts ranged from $1.1x10^2$ to $8.0 X 10^3$ cfu/g in brands CWG and DNN respectively as shown in Table 1. Thus, this suggests that infant formula could support the growth of bacterial pathogen despite their low water activity[11,13,19,34] hence could be a good vehicle for the transmission of pathogens. The results of this study also indicated that the products were not sterile. This supports the report of Chap *et al*., (2009) which detected different species of family *Enterobacteriaceae* in infant formula.

However, the aerobic bacterial load was much higher than the microbiological specification for powdered infant formula (NZFSA, 2009; Oonaka *et al.,* 2010). The contamination of infant formula with *C. sakazakii* in this study was higher than the previous studies by Muytjens *et al*., (1988) that found *C. sakazakii* in 14.8% of 141 samples analysed. Nazarowec-White and Farber (1997a), reported 6.7% of 120 samples, Leuschner *et al.,* (2004), in 13.8% of 58 samples, while Seo and Brackett (2005), did not find any positive samples in 50 infant formulas evaluated. The result of this study was lower than that of Aigbekaen and Oshoma (2010) that detected the pathogen in all the brands screened. This contamination could be due to the dry mixing or other methods of processing for the current samples (Gutler *et al.,* 2005). Nazarowec-White and Farber (1997b), indicated that the absence of pasteurization after mixing ingredient may result in a high level of contamination in dry mixed infant formulas. Four out of ten infant formulas screened for *C. sakazakii* were culture-positive.

As shown in Table 2; the highest resistance of *C. sakazakii* to tested antibiotics was notice against tetracycline (93.93%), followed by nitrofurantoin (96.97%). The resistant pattern of the pathogen was 42.42%, 51.51% and 51.51% to amoxicillin/clavulanate, gentamicin and ofloxacin respectively. Amoxicillin/clavulanate was most effective against the isolates while

Table.3 Level of education and occupation of the respondents (nursing mothers)

Items		**Response n (%)**	**EBFn (%)**	**Infant formula feeding (with or without breastfeeding) n (%)**
Level of education				
	Primary school	34 (16.67)	14 (3889)	20(11.90)
	Secondary school	95(46.57)	13(36.11)	82(48.81)
	Polytechnic/ university	30(14.71)	3(8.33)	27 (16.07)
	College of education	125(61.27)	4(11.11)	121(72.02)
	Others	20(9.80)	2(5.56)	18(10.71)
	Total	**204**	**36 (17.65)**	**168 (82.35)**
Occupation				
	Public servant	43(21.08)	2(5.56)	41(24.40)
	Trader	90(44.12)	19(52.78)	71(42.26)
	Farmer	5(2.45)	4(11.11)	1(0.6)
	Full housewife	20(9.80)	6(16.67)	14(8.33)
	Food seller	6(2.94)	2(5.56)	4(2.38)
	Teacher	25(12.25)	1(2.78)	24(14.29)
	Unemployed	15(7.35)	2(5.56)	13(7.74)
	Total	**204**	**36 (17.65)**	**168 (82.35)**

EBF: Exclusive breast feeding

Table.4 Level of infant formula feeding among nursing mothers participated in the study

Items		**Response**	**Percentage**
Do you feed infant formula to your child?			
	Yes	168	82.35
	No	36	17.65
	Total	**204**	
If yes, why?			
	As supplements to breast milk	86	51.19
	Good for baby	18	10.71
	Due to the nature of my job	32	19.04
	Weaning	24	14.28
	On advice	2	1.19
	Baby refusal of breast milk	2	1.19
	Medical advice	2	1.19
	Sickness of mother	0	0
	Total	**168**	

Table.5 Type of infant formula, factors affecting their choices and effect on babies health

Items		**Response**	**Percentage**
What type/brand of infant formula do you give to your child(ren)?			
	NNN	74	44.04
	NTD	15	8.93
	SML	16	9.52
	FRG	32	19.05
	ISM	2	1.19
	JGO	-	0
	CWB	11	6.55
	DNN	1	0.6
	PKK	13	7.74
	CWG	0	0
	Others	6	3.57
	Total	**168**	
What is the basis for infant formula selection?			
	Composition	11	6.55
	Price	8	4.76
	Advice from friends	15	8.93
	Medical advice	25	14.88
	Previous experience	21	12.50
	No answer	88	52.38
	Total	**168**	
Do you notice any negative effect on your baby's health after using infant formula?			
	Yes	39	23.21
	No	129	76.79
	Total	**168**	
If yes, what effect?			
	Vomiting	4	10.26
	Passing of stool	29	74.36
	Sickness	4	10.26
	Others	2	5.13
	Total	**39**	
Do you feed your babies with chocolate drink or adult milk before the age of 12 months?			
	Yes	172	84.31
	No	32	15.67
	Total	**204**	
If yes, which type?			
	Adult milk	28	16.28
	BRVT	118	68.60
	MILL	12	6.98
	VITL	8	4.65
	Other	10	5.81
	No response	4	2.33
	Total	**172**	

The names of the products given here are not their real names but representatives

Table.6 Knowledge of nursing mothers about duration of breastfeeding and commencement of infant formula

Items		**Response**	**Percentage**
At what age do you think breast feeding should stop?			
	Below 9 months	14	6.86
	9-10 months	4	1.96
	11-12months	106	51.96
	13-14 months	44	21.57
	15-16 months	8	3.92
	17-18 months	2	0.98
	19-20 months	0	0
	21-22 months	6	2.94
	23-24 months	12	5.88
	25 months and above	0	0
	No response	8	3.92
	Total	**204**	
What is the basis for infant formula selection?			
	Composition	12	5.88
	Price	8	3.92
	Advice from friends	16	7.84
	Medical advice	28	13.73
	Previous experience	24	11.76
	No answer	116	56.86
	Total	**204**	
At what age do you think infant formula should begin if at all?			
	1 months	6	2.94
	2 months	16	7.84
	3 months	24	11.76
	4 months	78	38.22
	5 months	12	5.88
	6 months	44	21.57
	7 months	12	5.88
	8 months	0	0
	9 months	4	1.96
	I do not know	2	0.98
	No response	12	2.94
	Total	**204**	
Factors influencing type of supplementary food fed			
	Price	28	13.72
	Availability	18	8.82
	Cultural	20	9.80
	Quality	10	5.10
	Suitability for baby	72	45.08
	Others	16	7.84
	No response	8	3.92
	Total	**204**	

nitrofurantoin recorded the least anti-cronobacter. The resistance of the *C. sakazakii* to first line antibiotics was higher than other antibiotics. The isolates were moderately susceptible to gentamicin and amoxicillin/clavulanate. This supports the finding of Lai (2001) who reported increasing resistance to *Cronobacter* species to common antibiotics.

Table 3 shows the educational status of the respondents. It could be suggested from these results that education contributes to the use of infant formula among nursing mothers. Educated mothers fed their babies with formula than the less educated ones. In a similar study in a Nigeria Ojofeitimi (1981) reported that the highest frequency of mothers who introduced infant formula at less than one month was among the mothers with formal education. However, this finding is against the report of Pursall *et al.,* (1978) who noted that better education encourages breastfeeding. Educated women were quick to introduce their infants to infant formula compared to the less educated ones. The distribution of respondents by occupation shows that most of the respondents (65.69%) were either self employed or unemployed while 34.31% were government workers.

Table.5 shows that majority of the respondents feed their babies with NNN brand of infant formula (44%) while 14% of these respondents fed their babies with FRG brand. The choice of the infant formula could be due to their price and/or nutritionally values, age in the circulation and advertisement. The respondents (86.27%) feed their babies with other beverages (chocolate based) before the ages of 12 months. This may be due to the cheap prices and availability of the chocolate drinks.

Some of the babies fed with infant formula manifested different signs of infections. This may be as a result of infections acquired from the food as noted by Cameron and Hofvander (1983) and Brown (1989). Poor hygiene commonly associated with bottle feeding could also be the factor responsible. This has been identified to lead to increased mortality rate among infants (Cameron and Hofvande, 1983). Infant infections may not be directly as a result of formula feeding but due to the contamination of the utensils and water used for the preparation. More than half of the respondents, 51.96%, as shown in Table 6 planned to wean their babies between the age of 10 and 12 months. This may be due to some cultural and personal reasons.

In conclusion, the result of this research work underscore the fact that most of the infant formula screened did not meet the international acceptable standards and there is high contamination level of *C. sakazakii* in powdered infant formulas which could be due to methods of processing, preparation, handling and storage of formulas. The rate of bottle feeding is still high especially among educated mothers.

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