International Journal of Micro Biology, Genetics and Monocular Biology Research
Vol.2, No.3, pp.12-26, September 2016
Published by European Centre for Research Training and Development UK (www.eajournals.org)

MICROBIAL COMPOSITION, ANTIBIOTIC SENSITIVITY AND PROXIMATE COMPOSITION OF POPULAR IMPORTED POWDERED INFANT MILK FORMULAS SOLD IN ADO EKITI, NIGERIA.

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ABSTRACT: *The study was carried out to determine the microbial and proximate composition of popular imported infant milk formulas in retail market at Ado-Ekiti. A total of six popular commercial infant milk poducts were purchased from the main market in Ado-Ekiti, Nigeria, and evaluated for microbial composition: total bacterial, coliform and fungal counts. Their proximate components were also determined. The total bacterial count ranged from $0.6x10^3$ to $0.9x10^4$ CFU/ml, with mean value of $3.4x10^3$CFU/g, the total coliform ranged from $0.2x10^3$ to $0.6x10^4$ CFU/ml, with the mean value of $1.7 x 10^3$CFU/g and the total fungal count ranged from $0.2x10^3$ to $0.5x10^4$ CFU/ml, with the mean value of $0.9 x10^3$CFU/g. Enteric bacteria and my cotoxigenic fungi were isolated from the formulas. Having subjected all the bacteria isolates to antibiotic susceptibility test, they all showed multiple antibiotic resistance index. The fat content ranged from 10mg/100g to 27.7mg/100g with mean value of 17.8mg/100mg; protein content, 1.5mg/100mg with mean value of 10.68mg/100mg and carbohydrate content ranged from 7.2mg/100mg with the mean value of 50.76 mg/100g. However ash, moisture and fibre contents of all the samples were insignificant.*
This study depicted the possibility of microbial contamination in infant milk formula supposedly sterile.

KEYWORDS: Antibiotic resistance, Microbial count, Proximate, Infant milk formula.

INTRODUCTION

Exclusive breast milk feeding is highly recommended as the perfect food in the first six months of humanlives (WHO, 2001; Joan, 2012). It is a sterile, complex nutritional fluid containing antibodies, enzymes, long chain fatty acids and hormones and other necessary nutrients required for infants'healthy growth and development (UNICEF, 2011). Breast milk provides protection against infection early in life by passing immune factors from the mother to infant. It protects against sudden infant death syndrome, diabetes and chronic digestive diseases (Arifeen *et al*., 2001).Babies breast-fed have fewer allergies, olfactory infections, respiratory infections, urinary tract infections and fewer problems of digestion, constipation and diarrhoea (Ekpete *et al.*, 2013). Hence, it is important to support breast feeding and promote its benefits to infants.

However, there are instances where expressed breast milk is not available at all and where quantity is insufficient and infant formulas serve as substitute. Also, at instance of maternal death before the baby is one year, infant formulas serve as best alternatives. Some working mothers who are employed outside their homes find it difficult to practise exclusive breast-feedng and have adopted infant formulas as supplement for convenience. Fashion crave to maintain breast shape restrain some modern females from breastfeeding (Rajput *et al*., 2009; Khaskheli, M., 1998). Nevertheless, there are few situations whereby breast feeding is not

advisable such as, a baby diagnosed with galactosemia, a mother receiving chemotherapy and mother with HIV or uncontrolled tuberculosis (Judith *et al*., 2013);infant formulas hence are totally given to babies as alternatives. Though they are advantageous for the proper growth and development of infants, they are still rated second to the natural breast milk because they are not always sterile.

Most infant formulas are manufactured from cow milk; its unprocessed form however is injurious to infants because of its high casein content which often leads to diarrhea and intestinal bleeding. Infant formulas are therefore designed based on the composition of matured breast milk providing an average of 68kcal/100 ml energy and 1.5g/100 ml protein (Fewtrell and Lucas, 1999). They are often supplemented with cysteine and taurine, as cows' milk contain lower quantities of these amino acids. Taurine is particularly involved in the development of the cardiovascular and nervous system; playing a role in modulatory neurotransmission (Jennie, 2007). Other components added to infant formulas making them resemble breast milk more closely are: probiotics, prebiotics and long chain polyunsaturated fatty acids.

Infant formulas exist in two forms; sterile ready-to-eat liquid and non-sterile powder (Marion, 2011). Powdered infant formulas and feed products have been associated with some pathogenic bacteria, the etiology of some serious infants' microbial infections, illness and death (Shadlia-Matug *et al.*, 2008). These formulas are made of dairy products, cereals, fruits and nuts which may contain some microorganisms or become contaminated during manufacturing processes. David *et al*., 2013 have investigated the occurrence of *Cronobacter sakazakii* (formerly known as *Eneterobacter sakazakii*) in infant formulas in Ado-Ekiti, which is responsible for fatal infection of the circulatory and central nervous system (Mayo Clinic, 2010); but the total count of fungi and bacteria in popular imported infant foods sold in Ado-Ekiti and the bacteria susceptibility to commercial antibiotics have not been reported. Since most of the ingredients used in the infant milk production may serve as nutrients for microbial growth, they are prone to microbial infestation. Also, they may be contaminated by potentially dangerous by heavy metals pesticides and polychlorinated biphenyls which infants are more susceptible to.

The aim of this study was to determine the microbial composition of a range of commercial baby formulas, ascertain the susceptibility of the bacterial isolates to antibiotics and determine the proximate and mineral composition of the imported infant formulas retailed in Ado-Ekiti, Nigeria.

MATERIALS AND METHODS

Source of Infant Milk Powdered Samples: A total of six powdered infant formulas of different brands for 1-6 months babies, were purchased from a supermarket at Ado-Ekiti namely: Cerelac, SMA Gold, Easy Scoop, Cow and Gate, FrisoCream and Eldorin. All the samples were sealed, not expired and verified by National Agency for Food and Drug Administration and Control (NAFDAC). The milk brands were brought to the laboratory, Department of Microbiology, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria for microbial and nutritional analyses.

Preparation of Test Samples

Each infant milkpowder (10g) was diluted in warm (50^{o}C) sterile diluents-buffered peptone water (90ml) to make primary dilution of 10^{-1}, which was incubated at ambient temperature for 2 -3 hrs. Then a series of dilution up to 10^{-5} dilution factor was prepared by transferring the primary dilution (1ml) into test tube containing the sterile diluents (9 ml), to obtain 10^{-2} dilution and the operation was repeated with sterile diluents (9 ml) using the 10^{-2} and further diluted to obtain 10^{-3}, 10^{-4} and 10^{-5}.

Enumeration of Total Microbial Count

Nutrient agar, MacConkey agar and malt extract were used to determine total bacteria count, total coliform count and total fungi count respectively.1 ml of 10^{-2} and 10^{-3} dilutions of each pre-prepared test samples were transferred into well labelled sterile petri- plates in duplicates and then overlaid with sterile warm (45^{o}C) 15ml of respective nutrient media. The plates were carefully swirled for uniform mixing and inoculums distribution. The mixture was allowed to solidify, then inverted and incubated aerobically at 37^{o}C for 72 hr. The plates having more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The total bacteria count (TBC), total coliform count (TCC) and total fungi count (TFC) were obtained by multiplying the number of colonies by the dilution factor and were recorded in Colony Forming Unit per milliliter (CFU/ml) (Olutiola *et al*., 2000).

Isolation and Identification of Distinct Microbial Colonies

Standard methods were used for isolation and identification of bacteria and fungi. Distinguished colonies of bacteria and fungi were sub-cultured from plates having appropriate numbers of colonies using the streaking method. The sub-cultured plates were incubated aerobically at 35^{o}C for 24 hours. The operation was done several times to obtain distinct microbial colony, which were transferred into nutrient agar slant. Microorganisms formed colonies with distinctive morphologies and were identified based on their morphology, physiological and biochemical features according to microbiological standard methods.

Antibiotic Sensitivity Tests

Each bacterial isolates was subjected to antibiotics sensitivity tests using the disc diffusion method as described by Cheesbrough (2006). Mueller-Hinton agar, normal saline, Gram-positive and negative antibiotics discs were used for this test. The commercial gram-positive and gram-negative discs used include: Ampicillin (AMP, 10mg), Streptomycin (STR, 10mg), Tetracycline (TET, 25mg), Cephalothin (CLT, 5mg), Colistin (COL, 5mg), Gentamycin (GEN, 10mg), Cotrimazole (COT, 25mg).

A sterile wire loop was used to pick 3-5 well isolated colonies of the organisms and emulsified in a 3-5ml of sterile physiological saline; the turbidity of the suspension was compared with McFarland Turbidity Standard of 0.5. Using a sterile swab-stick, each inoculum was swabbed in three directions over the surface of Mueller Hinton agar and, rotating the plate to ensure even distribution. With the petri-plates lid in place, the plates were left for 3-5minutes for the surface of the agars to dry. After, with a sterile forceps, an appropriate antibiotic disc was placed on the inoculated plates and left for about 30 mins; then the plates were inverted and incubated aerobically at 35^{o}C for 16-18hours and then

examined. The diameters of zone of inhibition of the discs were measured and the results were interpreted with reference to CLSI (2007).

Proximate and Mineral Analysis

The mineral composition: Na, K, Ca, Fe, and Zinc of the samples were determined using the Atomic Absorption Spectrophotometer (AAS). About 20 ml of each sample was obtained and kept in freezing state before taking for the analysis.The nutrient analysis of each milk sample was done using the standard methods of Association of Analytical Chemists (AOAC, 1997), for the determination of moisture content, ash content, crude protein, fibre and fat composition as described below.

Crude Protein Analysis

Crude protein contents of the infant samples were determined by weighing one gram of sample into digestion tubes. Two Kjeltabs Cu 3.5Fg (catalyst salts) were added into each tube. About 20 mL of concentrated sulphuric acid (H_2SO_4) was carefully added into the tube and then shaken gently to allow digestion. Digested samples were cooled for 10-20 min. Distillation procedure was then performed using distillation unit and the distillate was titrated with 0.025 N H_2SO_4 until the end point changes from green to pink. Volume of acid required in the titration was recorded. Blank was prepared with the exclusion of sample.

Carbohydrate Content

The Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

Moisture Content

The samples were dried in moisture dish in an oven at 105^oC until constant weight was obtained.

Ash Content Analysis

The samples obtained from moisture content analysis were turned to ash in furnace at 550^oC overnight to determine the ash content.

Mineral analysis

Each sample (1.0g) was measured and transferred to 200 mL beaker, 7.5 mL of nitric oxide HNO_3plus 2.5 mL of hydrochloric acid were added (Hack, 2000). The sample was digested by heating on a hot plate, about 20 mL of sterile distilled water was added and allowed to cool. The sample was filtered using Whatman No.1 filter paper and the filtrate was made up to 100 mL with distilled water. The minerals (calcium, magnesium, manganese, potassium, sodium, and iron) were determined using spectrometry method of Atomic Absorption Spectrophotometer(AAS) (ModelPhillip Pu9100x), with a hollow cathode lamp and a fuel rich flame(air-acetylene). Each sample was aspirated and the mean signal response recorded at each of the element respective wavelength.

RESULTS AND DISCUSSION

The total microbial count of samples (A to F) was investigated and the result is presented in Table 1. The total bacteria counts of the six formula samples ranged from 0.6 x 10^3 to 0.9 x 10^4 CFU/g, with the mean of 3.4x10^3CFU/g while the total coliform counts ranged from 0.2 x 10^3 to 0.6 x 10^4 CFU/g, with the mean of 1.7 x 10^3CFU/g and the total fungal counts ranged

from 0.2 x 10^3 to 0.5 x 10^4 CFU/g, with the mean of 0.9x10^3CFU/g.The mean value of total bacteria count, total coliform count and total fungal count are summarized in table 2. Total bacteria count and total coliform count are highest in Sample A. The following order of decrease in total bacteria count was observed in the infant milk formula: A>D>E>F>B>C. Also, the order of decrease in total coliform was observed in the infant samples: A>B>C>D>E>F. There was no significant difference in the total bacteria and total coliform count in all the samples; the counts exceed the standard and acceptable microbial plate count. The total fungal count is however highest in sample B and lowest in sample D and E. A total number of 29 Bacteria and 12 Fungi contaminants were isolated from the samples. The number of microbial contaminants is represented in Fig 1. The highest number of contaminating microorganisms; bacteria (7) and fungi (4), were isolated from A. While the least number; bacteria (3) and fungi (1) were isolated from F. The occurrence of each specific bacterial and fungal species within each formula is presented in Fig 2. It was observed that both *Staphylococcus aureus* and *Escherichia coli* seen in samples E and F respectively had the highest occurrence, each with value 3. In addition, the species of *Proteus*, *Aspergillus ochraceus* and *Aspergillus flavus* occurrence were found to be least; each had a value of 1. However, only *Enterococcus* and *Proteus* species were obtained respectively from A and C. The occurrences of bacterial isolates in samples are as follows: *Staphylococcus* spp (A, B, D and E), *Salmonella* ssp (A, B, C and D), *Shigella* (A, B, D, E), *Escherichia coli* (A, B, F), *Klebsiella* ssp (C, D), *Enterococcus* ssp (A),and *Proteus* ssp (C). Also, the occurrences of fungal isolates in the samples are stated: *Penicillum* spp (A, B, C, D, F), *Aspergillus niger* (A, D), *Aspergillus flavus* (A, B), *Aspergillus ochraceus* (C, E).

Table 1: Microbial Count of Six Infant Milk Formulas

	TBC (CFU/g**)**		**TCC(**CFU/g**)**		**TFC (**CFU/g**)**			
Sample	10^3	10^4	10^3	10^4	10^3	10^4	**No of Bacteria**	**No of Fungi**
A	3.3	0.9	3.0	0.6	0.4	0.1	7	4
B	0.6	0.4	1.0	0.3	0.9	0.5	5	3
C	1.4	0.2	0.3	0.2	0.3	0.1	3	1
D	3.0	0.6	0.2	0.2	0.2	ND	4	1
E	1.7	0.5	0.4	0.1	0.2	ND	4	1
F	1.3	0.4	0.3	0.1	0.4	0.1	3	1
Mean	3.4x 10^3		1.7x10^3		0.9 x10^3		**29**	**12**

Keys: Cerelac(**A**), SMA Gold **(B),** Easy Scoop **(C),** Cow and Gate (**D**),Friso Cream (**E),** Eldorin (**F**)

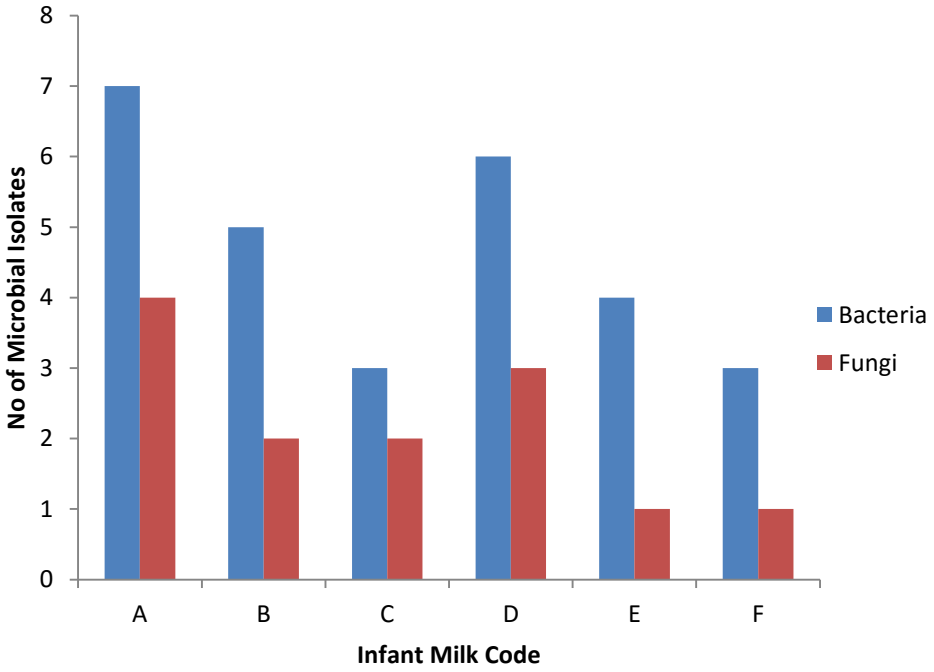
Table 2: Mean Value of Microbial Count in Infant Formulas

Keys: Cerelac(**A**), SMA Gold **(B),** Easy Scoop **(C),** Cow and Gate (**D**),Friso Cream (**E),**

Infant Milk Code	Total Mean (TBC)	Total Mean (TCC)	Total Mean (TFC)
A	6.2 X 10^3	4.5X10^3	7.0x10^2
B	2.3 X 10^3	2.0X10^3	3.0x10^3
C	1.7 X 10^3	1.2X10^3	6.5x10^2
D	4.5X10^3	1.1X10^3	2.0x10^2
E	3.4X10^3	7.0X10^2	2.0x10^2
F	2.7X10^3	6.5X10^2	7.0x10^2

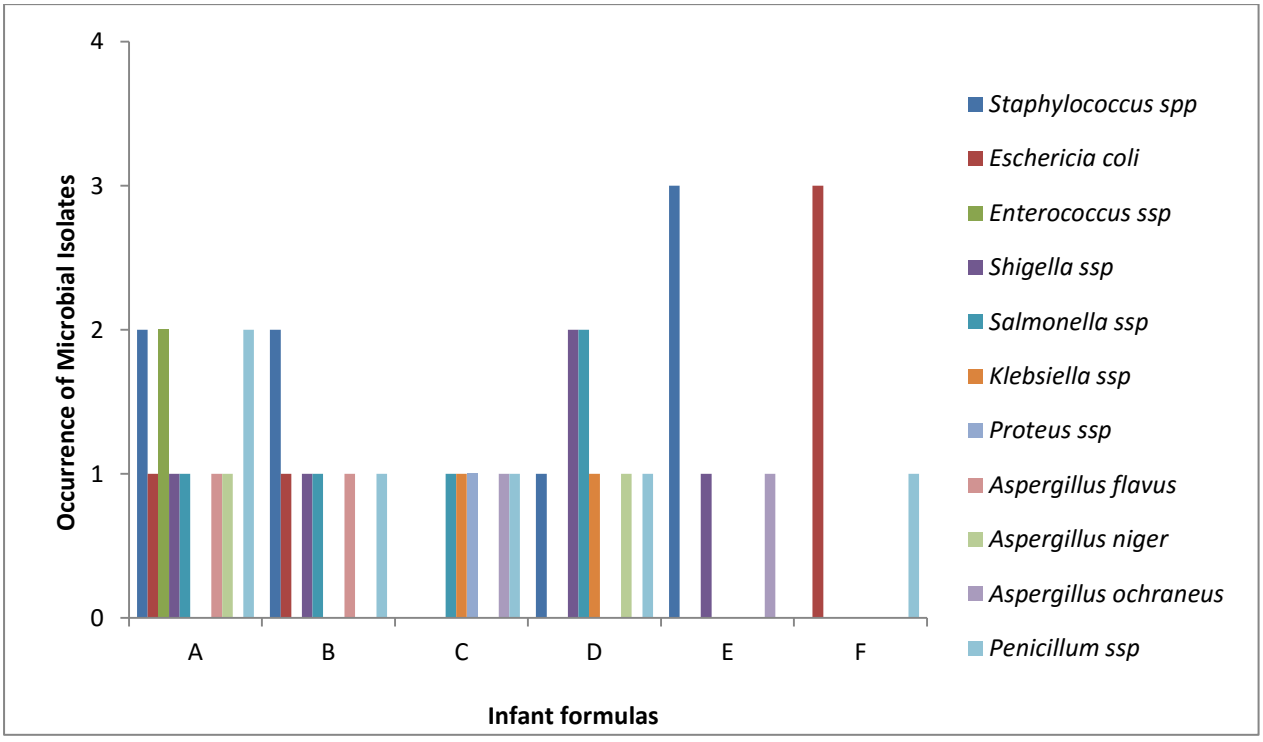
Eldorin (**F**)

Fig 1: Rate of Microbial Contamination of Six Infant Formulas



Keys: Cerelac(**A**), SMA Gold **(B),** Easy Scoop **(C),** Cow and Gate (**D)**,Friso Cream (**E),** Eldorin (**F**)

Fig 2: Occurrence of Microbial Isolates in All Infant Formulas



Keys: Cerelac(**A**), SMA Gold **(B),** Easy Scoop **(C),** Cow and Gate (**D)**,Friso Cream (**E),** Eldorin (**F**)

The percentage distribution of isolated organisms from the infant formulas is summarized in Table 3. *Staphylococcus* spp *(*20%) had the highest frequency, followed by *Penicillium* spp. (15%); then *Salmonella* spp. and *Escherichia coli* and *Shigella* spp (12.5%); *Klebsiella* spp (5%); all species of *Aspergillus*, *Enterococcus* spp and *Klebsiella* spp (5%), while *Proteus* spp. showed 2.5% distribution. The varying distribution of enteric bacteria; *Salmonella* spp. *Escherichia coli*, *Shigella* spp. (12.5%); *Klebsiella* spp. (5%); *Enterococcus* spp. (5%) and *Proteus* spp (2.5%), isolated from the infant formulas suggests that the raw materials of the infant products may have been contaminated with various intestinal bacteria due to the poor hygiene of the producers. Inadequate cleaning of the processing equipment and utensils used for production may also be a contributing factor Sarah *et al*., (2013). The presence of *Staphylococcus* spp. as the highest occurring organism may indicate a cross-contamination probably by personnel during handling and packaging as it was reported by (Chen *et al*., 2001).

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Table 3: **Percentage Distribution of Isolated Organisms from Infant Formulas.**

Isolates	**Number**	**Distribution**(%)
Staphylococcus spp.	8	20
Enterococcus spp.	2	5
Escherichia coli	5	12.5
Salmonella spp.	5	12.5
Klebsiella spp.	2	5
Shigella spp.	5	12.5
Proteus spp	1	2.5
Aspergillus ochraceus	2	5
Aspergillus flavus	2	5
Aspergillus niger	2	5
Penicillium spp	6	15
Total	40	100%

Table 4: **Zone of Inhibition in Diameter (mm) of antibiotic susceptibility pattern of isolated bacteria from infant formulas**

Code	Isolates	Antibiotics and Zone of Inhibition											
Sample A		GEN	STR	STD	TET	COT	AMP	CLT	COL	TRY	PEN	CHL	CXC
A_1	*Staphylococcus* spp.	15	16	NT	-	NT	-	NT	NT	11	-	11	-
A_2	*Staphylococcus* spp.	8	11	NT	8	NT	-	NT	NT	13	-	14	-
A_3	*Escherichia coli*	10	11	5	2	-	-	-	-	NT	NT	NT	NT
A_4	*Enterococcus* spp.	16	12	2	3	-	-	-	-	NT	NT	NT	NT
A_5	*Enterococcus* spp.	11	10	2	1	1	-	-	-	NT	NT	NT	NT
A_6	*Shigella* spp.	18	6	6	5	-	-	5	5	NT	NT	NT	NT
A_7	*Salmonella* spp.	19	18	17	-	-	-	-	-	NT	NT	NT	NT
Sample B													
B_1	*Salmonella* spp.	4	3	2	2	-	-	-	-	NT	NT	NT	NT
B_2	*Shigella* spp.	10	2	6	2	-	-	-	1	NT	NT	NT	NT
B_3	*Staphylococcus* spp.	11	11	NT	8	NT	-	NT	NT	13	-	14	-
B_4	*Escherichia coli*	14	10	12	2	6	-	-	-	NT	NT	NT	NT
B_5	*Staphylococcus* spp.	15	16	NT	-	NT	-	NT	NT	11	-	11	-
Sample C													
C_1	*Salmonella* spp.	18	8	-	-	-	-	-	-	NT	NT	NT	NT
C_2	*Klebsiella* spp.	12	11	13	-	-	2	6	5	NT	NT	NT	NT
C_3	*Proteus* spp.	12	13	9	-	-	-	7	8	NT	NT	NT	NT
Sample D													
D_1	*Shigella* spp.	10	2	1	-	2	1	1	-	NT	NT	NT	NT
D_2	*Salmonella* spp.	15	-	-	-	-	-	2	3	NT	NT	NT	NT
D_3	*Shigella* spp.	12	9	2	-	5	2	-	-	NT	NT	NT	NT
D_4	*Klebsiella* spp.	14	10	13	9	-	-	2	9	NT	NT	NT	NT
D_5	*Staphylococcus* spp.	8	11	NT	8	NT	-	NT	NT	13	-	14	-
D_6	*Salmonella* spp.	16	5	-	-	-	7	7	9	NT	NT	NT	NT
Sample E													
E_1	*Staphylococcus* spp.	11	11	NT	8	NT	-	NT	NT	13	-	14	-
E_2	*Staphylococcus* spp.	15	15	NT	3	NT	-	NT	NT	3	2	-	-
E_3	*Staphylococcus* spp.	14	11	NT	3	NT	-	NT	NT	11	-	-	-
E_4	*Shigella* spp.	8	7	3	-	1	-	-	-	NT	NT	NT	NT
Sample F													
F_1	*Escherichia coli*	12	8	-	1	1	1	1	2	NT	NT	NT	NT
F_2	*Escherichia coli*	8	4	-	1	1	1	1	-	NT	NT	NT	NT
F_3	*Escherichia coli*	17	14	-	-	-	-	-	8	NT	NT	NT	NT

Table 5: **Antibiotics Resistance and Susceptible Pattern of Isolated Bacteria from Infant Formulas.**

	Antibiotics								
Isolate	**GET**	**STR**	**STD**	**TET**	**COT**	**AMP**	**CLT**	**COL**	**Phenotype of resistance Pattern**
Salmonella spp.									
A_7	S	S	S	R	R	R	R	R	TET,COT,AMP,CLT,COL
B_1	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
C_1	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
D_2	S	R	R	R	R	R	R	R	STR,STD,TET,COT,AMP,CLT,COL
D_6	S	R	R	R	R	R	R	R	STR,TET,STD,COT,AMP,CLT,COL
Shigella spp.									
A_6	S	R	R	R	R	R	R	R	STR,STD,TET,COT,AMP,CLT,COL
B_2	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
D_1	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
D_3	R	I	R	R	R	R	R	R	GEN,STD,TET,COT,AMP,CLT,COL
E_4	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
Escherichia coli									
A_3	R	I	R	R	R	R	R	R	GEN,STD,TET,COT,AMP,CLT,COL
B_4	I	R	R	R	R	R	R	R	STR,TET,STD,COT,AMP,CLT,COL
F_1	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
F_2	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
F_3	S	S	R	R	R	R	R	R	STR,TET,COT,AMP,CLT,COL
Enterococcus spp.									
A_4	S	I	R	R	R	R	R	R	STD,TET,COT,AMP,CLT,COL
A_5	R	I	R	R	R	R	R	R	GEN,STD,TET,COT,AMP,CLT,COL
Klebsiella spp.									
C_2	R	I	S	R	R	R	R	R	GEN,TET,COT,AMP,CLT,COL
D_4	S	R	S	R	R	R	R	R	STR,TET,COT,AMP,CLT,COL
Proteus spp.									
C_3	R	I	I	R	R	R	R	R	GEN,TET,COT,AMP,CLT,COL
	GEN	STR	STD	TET	COT	AMP	CLT	COL	
Staphylococcus spp.									
A_1	I	S	R	R	R	R	R	R	STD,TET,COT,AMP,CLT,COL
A_3	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
B_3	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
B_5	S	S	R	R	R	R	R	R	STD,TET,COT,AMP,CLT,COL
D_5	R	R	I	R	R	R	R	R	GEN,STR,TET,COT,AMP,CLT,COL
E_1	R	R	R	R	R	R	R	R	GEN,STR,STD,TET,COT,AMP,CLT,COL
E_2	I	R	R	R	R	R	R	R	STR,STD,TET,COT,AMP,CLT,COL
E_3	S	I	R	R	R	R	R	R	STD,TET,COT,AMP,CLT,COL

Key: TET Tetracycline; **GEN** Gentamycin; **COL** Colistin; **AMP** Ampicillin; **COT** Cotrimoxazole,

CXC Cloxacillin; **CHL** Chloramphenicol; **STR** Streptomycin; **PEN** Penicillin.

R -Resistance,**S** -Sensitive, **I** -Intermediate.

Table 6: Multiple Antibiotics Resistance (MAR) Indices of Bacteria Isolates

Frequency of MAR index(%)							
MAR Index	*Salmonella* n=5	*Proteus* n=1	*Shigella* n=5	*Staphylococcus* ssp n=8	*E.coli* n=5	*Enterococcus* n=2	*Klebsiella* n=2
0.0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	0
0.2	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0
0.4	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0
0.6	1(20)	0	0	0	0	0	0
0.7	0	0	0	0	0	0	0
0.8	0	1(100)	0	3(37.5)	1(20)	1(50)	2(100)
0.9	2(40)	0	2(40)	2(25)	2(40)	0	0
1.0	2(40)	0	3(60)	3(37.5)	2(40)	1(50)	0

Key: (n) no of isolates

Table 7 and 8 below represents a profile of the Proximate Composition and mineral constituent of each of the six infant feeds respectively according to respective manufacturers.

Table 7: Proximate Composition of Six Infant Milk Formulas

Compositions	**Samples**					
(mg/100g)	**A**	**B**	**C**	**D**	**E**	**F**
Fat	10	3.6	27.7	25.5	12.9	27
Protein	15	1.5	9.6	9.6	16.7	10.6
Carbohydrate	65	7.2	57.83	53.3	64.2	57
Fibre	2.0	Nil	Nil	5.8	23.4	Nil
Ash	3.0	Nil	2.87	Nil	Nil	Nil
Moisture	Nil	Nil	2.0	Nil	1.1	3.0

Table 8: **Mineral Constituent of Infant Milk Formulas**

Compositions	**Samples**					
(mg/100g)	**A**	**B**	**C**	**D**	**E**	**F**
Sodium (Na)	145	16	133	127	150	155
Potassium (K)	635	70	525	474	420	500
Calcium (Ca)	600	46	330	364	480	385
Phosphorus (P)	400	33	183	202	420	230
Magnesium (Mg)	Nil	6.4	44	36	50	46
Manganese (Mn)	Nil	Nil	114	Nil	160	130
Iron (Fe)	7.5	0.80	5.2	3.9	9.1	6.0
Zinc (Zn)	5.0	0.60	5.4	3.6	1.7	4.6

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The total bacterial counts of samples are not significantly higher or lower than one another in this study. The total coliform count (Enterobacteriaceae count) ($0.6x10^{-4}\pm3.0x10^{-3}$cfu/g), ($0.3x10^{4}\pm1.0x10^{3}$cfu/g), ($0.2x10^{4}\pm0.3x10^{3}$cfu/g), ($0.2x10^{4}\pm0.2x10^{3}$cfu/g), ($0.1\ x10^{4}\pm0.4x10^{3}$cfu/g) ($0.1\ x10^{4}\pm0.3x10^{3}$cfu/g) recorded for all the samples showed a close

ranges which is lower than$13x10^6$as reported by Taha*et al*. (1972). However the bacterial counts are lower than$5.6x10^4 \pm 4.3x10^3$reported by Rueckert*et al*. (2005).

The presence of Enterobacteriaceae in some of the formula may be a result of postprocessed handling. It is a general concept and proven by some researches that Enterobacteriaceae (coliform bacteria in particular) is absent in commercial and sealed infant formulas. This is because infant formulas are produced and sealed in sterilized containers under a good hygiene during packaging (Rajput *et al*; 2009). However, the milk containers can be damaged when being transported for commercial purpose, and may lead to microbial contamination of milk contents especially when the containers are bored.

Despite the unfavourable temperature for vegetative cells growth during milk processing, they may still be found in final products. This is as a result of their ability to attach to stainless steels and folded surfaces by forming biofilms that are not easily removed by Clean-In-Place (CIP) system (Flint *et al*, 2006). The presence of yeasts and moulds in milk or milk products may create hazard to consumers' health, produce food allergen and irritants to human health (Parihar and Parihar, 2008). Fungi such as *Penicillum* and *Aspergillus* may be present in many raw and processed food commodities including cereals and milk used as ingredients in infant food products (Aidoo *et al*., 2011). *Aspergillusflavus and Aspergillus parasiticus* are main producers of aflatoxins; the presence of aflatoxins-producers in the human diets, and especially in the diets of infants, is a major health concern (Polychronaki *et al*., 2003; Mykkänen *et al*., 2005). Aflatoxin exposure has been associated with growth faltering and immune suppression in young children. Early life exposures could be a contributing factor towards the early onset of hepatocellular carcinoma (Gong *et al*., 2003).
The tables 4 and 5 state the summary of the diameter of zone of inhibition (mm) and the antibiotics resistance pattern of each isolated bacteria against different antibiotics namely: Ampicillin (AMP, 10mg), Streptomycin (STR, 10mg), Tetracycline (TET, 25mg), Cephalothin (CLT, 5mg), Colistin (COL, 5mg), Gentamycin (GEN, 10mg), Cotrimazole(COT, 25mg). The study revealed that all the bacterial isolates were highly resistant to all the test antibiotics. Table 6 showed the bacterial isolates multiple antibiotics resistance index; all of them showed multiple antibiotics resistance pattern, with *Klebsiella* having the highest index. All the organisms showed multiple resistances to all the antibiotics. The observed resistance to these antibiotics indicates the possibility of prior exposure of bacterial isolates to the drugs which may have enhanced them to develop resistance. Multiple antibiotics resistance (MAR) index is a tool that reveals the spread of resistance in a given microbial population. A MAR index greater than 0.2, implies that the strains of such bacteria originate from an environment where several antibiotics are used. The MAR indices observed in this study is a possible indication that a great proportion of the bacteria isolates have been exposed to several antibiotics. Resistance to antimicrobial agents is a public health threat and increasingly becoming a global problem (NNIS, 2004). It limits therapeutic options and leads to increased mortality and morbidity (Cosgrove and Carmell, 2003). Infections with antibiotic resistant bacteria make the therapeutic treatment against infection, extremely difficult or virtually impossible in some instances (El-Astal, 2004).

The proximate composition of the infant formulas is showed in table 7. The protein content of the formulas ranges from 1.5mg to 16.7mg, with the average value of 10.0mg. The highest protein content was obtained from Frisocream while the least was obtained from SMA Gold. The same value of 9.6mg was obtained from both Cow Gate and Easy Scoop.

The fat contents of the formulae varied widely and ranged between 3.6mg and 27.7mg, with the average of 17.7mg. The highest fat content was obtained from Cow and Gate formula (D), while the least was obtained from SMA Gold (B). Fat, an essential component of cell membranes, is needed for proper cell reaction and intra-cellular transportation of material in human. However, high levels of fat in human have been associated with several arteries, arterosclerosis and cardiac diseases. Unexpectedly, crude fibre was obtained from three of the infant formulas: Cerelac, Cow and Gate, and Friso Cream.However, it is relatively high in Friso Cream (23.4mg). Though fibres content in foods aid in digestion processes in animals and man, the digestive systems of infants are not yet developed enough for such function. Crude fibre content is hence not necessary in formula for infants below six months; fibre is not usually added to infant formula. Carbohydrate is the major source of energy in human body. The carbohydrate contents of infant formulae varied widely from 7.2mg to 65mg and mean value of 50.7mg. The composition of carbohydrate in the samples is in the following descending order A>E>F>C>D>B. It is observed that SMA Gold (B) contains the least of the three major essentials nutrients protein, carbohydrate and fat; it is least recommended for infant formula.

Microorganisms have low growth rate in infant formula milk owing to the low moisture nature of the powdered milk; hence they do not play any direct role in the milk spoilage. Their rate of occurrence in these products however has great significance as they serve as an index of hygienic standards maintained during formulae production, processing and handling (Yadav *et al*., 1993).The moisture contents of the infant formulae varied from nil to 3.0mg, with an average of 1mg. Moisture was not observed in Cerelac (A), SMA Gold (B) and Cow and Gate (D). The moisture contents of all the formulae are generally within the maximum codex standard for infant formula value of 5% (Codex, 1982).It is important to monitor the moisture content in foods as high moisture content increases microbial degradation activity of food resulting in reduction in food shelf life span and organoleptic. Moisture content of any food product is an index of water activity and storage stability (Uavy, 2003).More so consumption of such microbial degraded foods may have harmful effect on the health of infants. It is crucial to determine ash content in food when evaluating authenticity of food products. Ash contents are 2.87mg and 3.0mg in the infant formulas, Cerelac and Easy Scoop, respectively, while the remaining formulae did not give any value.

Protein is essential for normal growth, body development and general repair of body tissues. Protein deficiency in children leads to poor growth, kwashiorkor, liver and brain damage (Lawal and Adedeji, 2013). Hence, a relatively high amount of protein is required during infancy, as it is the period of rapid growth in human.

The values of analysed mineral contents in the samples are summarized in table 8.Ca and P are essential minerals for proper development of bone, teeth and connective tissues and deficiency of Ca as well as P may cause rickets diseases in infants. Moreso, the ratio of Ca to P may also affect bone metabolism (Olu-Owolabi *et al*., 2007). In this study, Potassium content is highest in all the samples while iron content is least in virtually all the samples and Ca to P ratio in all the formulae were generally within the recommended Codex standard for infant formula.Magnesium is also useful in the formation of bone structure in the body. Calcium ions have also been implicated with clotting process (Gaman and Sherrington, 1998). Iron has been found to be involved in the formation of haemoglobin which is necessary for respiratory and blood circulatory processes (Kathleen *et al*., 1996). Though zinc

is an essential mineral, when absorbed into human tissues in large quantities they become toxic; lead to alteration of the stereochemistry of enzymes and impairment of its catalytic activity. The presence of Manganese plays an essential role in skeletal formation and cholesterol metabolism.

CONCLUSION

This study confirmed the occurrence of pathogenic bacteria and fungal isolates in the infant formulas. The microbial composition suggests possible contamination of commercial powdered infant foods may be due to contaminated raw materials, cross-contamination by personnel or through contact with the equipment during production and packaging.

All the bacterial isolates were highly resistant to antibiotics. The microbial compositions of the formulas as well as the antibiotic resistance pattern of the composite bacteria are a major treat to infant lives; when the milk are consumed they also develop resistance when drugs are administered to them. Hence, childhood infections may be prolonged during illness and may even lead to increase in child mortality.

REFERENCES

Aidoo, E. A., Mohamed, S. M., Candlish, A.A., Tester, R. F. and Elgerbi, A. M. (2011). Occurrence of fungi and mycotoxins in some commercial baby foods in North Africa. *Food and Nutrition Sciences* 2: 751-758.

Arifeen, S., Black, R.E., Antelman, G., Baqui, A., Caulfield, L. and Becker, S. (2001) Exclusive breastfeeding reduces acute respiratoty infection and diarrhea deaths among infants in Dhaka slums. *Pediatrics* 108(4): E67.

Codex Standard 71-1982. Codex standard for infant formula. www.Euro.who.int.

Clinical and Laboratory Standards Institute **(**2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement, Wayne Pennsylvania.

Chen, Y.H. Jackson, K.M., Chea, F.P. and Schaffner, D. W. (2001). Quantification and variability analysis of bacterial cross contamination rates in common food service tasks *Journal of Food Protection* 64, 72-80.

Cheesbrough, M. (2006). Distinct Laboratory Practice in tropical countries. Cambridge University Press.London.

David, O.M., Falegan, C.R. and Oluyege, O.A. (2013).Pattern of breastfeeding and occurrence of *Cronobacter sakazakii* ininfant formula sold in Ekiti State, Nigeria. *International Journal of Current Microbiology Applied Science* (7): 1-11

Ekpete, O.A. and Festus, C.(2013).The nutrient value of breastmilk and some infant formulae. *The experiment, International Journal of Science and* Technology 8(2): 456-460

Fewtrell, M., and Lucas, A. (1999). Nutritional physiology: dietary requirements of term and preterm infants. In J. M. Rennie and N. R. C. Roberton (Eds.), Textbook of Neonatology, 3rd edition Edinburgh, Scotland: Churchill Livingstone. (pp. 305-325).

Flint, S., Drocourt, J.L., Walker, K., Stevenson, B., Dwyer, M., Clarke, I.and McGill, D. (2006). A rapid, two-hour method for the enumeration of total viable bacteria in samples from commercial milk powder and whey protein concentrate powder manufacturing plants. *International Dairy Journal*, 16: 379-384.

Gaman P.M. and Sherrington, K.B. (1998). The Science of Food: An introduction to Food Science Nutrition and Microbiology. Pergamon Press Oxford. 196-215.
Gong, Y.Y.Egal, S.Hounsa, S. Hall, A. J., Card-well, K. F and Wild, C. P. (2003) "Determinants of Aflatoxin Expo-sure in Young Children from Benin and Togo, West Africa: The Critical Role of Weaning," *International Journal of Epidemiology*, 32(4), pp. 556-662.
Hack, B. (2000). Analytical method of determination of mineral nutrients. In: *Text on Analytical in Practic*e, 1st edn. Dolphin and John S. Incorp. Press Ltd, NY, pp. 26-33.
Kathleen J, Wilson W, Anne W (1996). Anatomy and Physiology in Health and Illness. 8 th Edition. Longman, Asia Limited; Hong Kong. 274
Khaskheli, M., (1998).Some aspects of the production and quality improvements of fermented milk/cereal mixture (Kishk).Ph.D thesis, University of Glasgow, UK
Jennie, Y. (2007). The effects of breast milk versus infant formulae oncognitive development. *Journal on Developmental Disabilities* 1(13):1-3.
Joan, C.C.(2012). Breastfeeding pattern.In Reproductive Justice: A Global Concern, ABC-CLIO, LLC, Santa Barbara, California, USA. (pp 273-274).
Judith B., Janet I. and Bea, K. (2013). Medical Contradictions to breastfeeding. In Nutrition through the life cycle.5th edition Cengage Learning Stanford, USA.(pp 220).
Lawal and Adedeji (2013).Nutritional and elemental analysis of warankasi(fermented milk product) sold in Lagos metropolis. *International Research Journal of Biotechnology Vol. 4(6) pp. 112-116.*
Marion, N.(2011) Food Politics retrieved from http://www.foodpolitics.com
Mykkänen,H. Zhu, H., Salminen, E., Juvonen, R. O., Ling, W., Ma, J., Polychronaki, N. Kemiläinen, H., Myk-känen, O., Salminen, S. and El-Nezami, H. (2005). "Faecal and Uri-nary Excretion of Aflatoxin B1 Metabolites (AFQ1, AFM1 and AFB1-N7-Guanine) in Young Chinese Males," *International Journal of Cancer*, 115(6) 2005, pp. 879-884.
Olu-owolabi, B.I., Fakayode, S.O., Adebowale, K.O. and Onianwa, P.C. (2007). Proximate and elemental composition and their estimated daily intake in infant formulae from developed and developing countries: A comparative analysis. *Journal of Food, Agriculture and Environment 5 (2):* 40-44.
Olutiola, P.O. Famurewa, O. and Sonntag, H.G. (2000).An Introduction to Microbiology.
A practical approach.Tertiary Textbook Series.
Parihar, P. and L. Parihar, (2008). Dairy microbiology Chapter, 3.Microbes.Agrobios India, pp: 46-50.
Polychronaki, N. West, R. M., Turner, P. C. ,Amra, H., Abdel-Wahhab, M. , Mykkänen, H. , and El-Nezami, H. (2007) "A longitudinal assessment of Aflatoxin M1 excretion in Breast Milk of selected Egyptian mothers," *Food Chemistry and Toxicology*,. 45(7), pp. 1210- 1215.
Rajput, I.R., Khaskheli, M., Rao, S.,Fazlani, S.A. Shah, Q.A. and Khaskheli, G.B. (2009). Microbial quality of formulated infant milk powders.*Pakistan Journal of Nutrition* 8 (10): 1665-1670.
Rueckert, A., Ronimus, R.S. and Morgan, H.W.(2005). Rapid differentiation and enumeration of the total, viable vegetative cell and spore content of thermophilic bacilli in milk powders with reference to *Anoxybacillusflavithermus*. Journal of Applied Microbiology 99: 1246-1255.
Shadlia-Matug, M., Aidoo, K.E., Candlish, A.A. and Elgerbi, A.M. (2008). Evaluation of Some Antibiotics Against Pathogenic Bacteria Isolated from Infant Foods in North Africa. *The Open Food Science Journal* 2: 95-101

Taha, S., Naguib, M. and Ghani, A. (1972). Dried milk Microbiology. *Journal of Milk and Food Technology, 36: 559.*
Uavy, R. (2003). Securing the right complementary foods in early childhood: we should not delay taking effective action SCN News 27: 34-35.
United Nations Children's Fund (2011). Exclusive Breastfeeding rates have increased in most regions. Retrieved from http://www.childinfo.org/breastfeeding-progress.html
Who Health Organization (2001). The optimal duration of exclusive breastfeeding report of the expert consultation WHO/NHD/01.09
Yadav, J.S., Grover S. and Batish, V.K. (1993). Microbiology of dried milks. A Comprehensive Dairy Microbiology. B.V. Gupta, metropolitan book Co Pvt. Ltd. pp: 315-349.