Detection and Enumeration of Gram-Positive Pathogens in Some Powdered Infant Foods in Ikot Ekpene Local Government Area, Akwa Ibom State, Nigeria.

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Abstract

The study was carried out to detect and enumerate Gram positive bacteria in powdered infant foods sold within Ikot Ekpene Metropolis. Ten different samples of commercial (10) powdered infant foods were analyzed using the conventional culture based method of enumerating bacteria. The bacterial count ranged between 0.3 x10^3 to 3.5 x10^3 CFU/ml. The highest bacterial count was 3.5 x 10^3 CFU/ml which was recorded in sample three, followed by 2.8 x 10^3 CFU/ml which was recorded in sample two, while the least of 0.3 x 10^3 CFU/ml was recorded in sample five. The bacterial isolates and their percentage frequency of occurrence of bacterial isolates were, Clostridium spp (20 %), Listeria monocytogenes, Staphylococcus spp and Lactobacillus spp had (16 %)each, Bacillus spp (12 %), while Enterococcus spp had the lowest percentage frequency of occurrence of 8%. The antimicrobial susceptibility profile of bacterial isolates showed different susceptibility pattern of some commonly used antibiotics. 85 % of the bacterial isolates were susceptible to some antibiotics. The results of this study revealed contamination of powdered infant food from many sources. The predominant bacteria could pose a significant health risk to consumers. Therefore, pasteurization, proper hygiene and sanitary practices should apply for controlling of pathogens to prevent food poisoning. Furthermore, Government should implement food safety policy with the view of reducing food-borne illness among the consumers especially infants.

Keywords: *Susceptible, contamination, predominant, bacteria, Gram positive, pathogens.*

1. INTRODUCTION

Powdered infant foods come in powdered form, liquid concentrate and ready to feed forms. They are designed to be prepared by the parent or caregiver in small batches and fed to infants, usually with either cup or a baby feeding bottle and with or without additional water (WHO, 2011).

Powdered infant food is a food which intent to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk (FAO, 2006).
Manufacturers state that the composition of powdered infant milk is designed to be roughly based on a human mother's milk at appropriately one to three months post-partum, however, there are significant differences in the nutrient content of these products (Wells, 1996).
The most commonly used infant milks contain purified cow's milk whey and casein as a protein source, a blend of vegetable oils as a fat source, lactose as a carbohydrate source, a vitamin-mineral mix and other in gradients depending on the manufacturer. Foodborne diseases have become a major public health problem worldwide due to the significantly increased incidence of foodborne diseases over the last 20 years.
Powdered infant foods (PIF) has been associated with serious illness, and even death, due to infection with *Cronobacter sakazakii* and other microorganisms that can be introduced to PIF during its production. Although, *C. sakazakii* can cause illness in all age groups, infant are believed to be at greatest risk of infection (Geneva, 2007).
Dairy powders can be used to boost other dairy products as well as an ingredient in a wide variety of foods including soups and sauces, confectionary, infant formula, sports dietary supplements and in foods for health recovery (Gill *et al*., 2001; Lagrange *et al.*, 2015).

Powdered infant foods can be used for infants who are healthy and full term and also for high – risk infants in situations where sterile liquid infant milk is not available. Unlike liquid infant milk, which is heat treated to sterilize the product, powdered infant milk is not manufactured to be sterile (Forsythe, 2009). One of the methods of preserving various foodstuffs is by drying, and thus depriving microorganisms of the water necessary for their growth. The drying of food products has been used for a very long time as a food preservation method and, as the name suggests, it generally involves removing the moisture content of the food (Cahill *et al*., 2008).

However, the higher level production of powdered infant food and dairy powders may create safety and economic risks to the dairy sector, specifically when controlling microbial loads in these products.

Infant formula can be contaminated with pathogens if not properly prepared, could exposed a child to potentially harmful bacteria such as *Enterobacter sakazakii* (*Cronobacter* specie). Caregivers need to make sure that powdered infant food is prepared properly to reduce the risks of food borne illness (Fang *et al.*, 2012).
The first drying techniques involved using the sun and dry air to desiccate foods. For modern manufacturers, in addition to extending the life of the product as it is thought that organisms may not be able to survive in such a low moisture contents (Ratti *et al.*, 2001). Drying has many additional benefits, such as reducing the overall weight and space of the product.

Endospores produced by bacteria are able to survive high heat treatment. These spores can germinate in the manufactured products when conditions become favourable or they can dominate the process equipment and become a significant source of steady contamination. Sporulation of large numbers of vegetative cells in the gastrointestinal tract after consumption of dairy powder can result in the production of an enterotoxin, and the symptoms such as diarrhoea, abdominal cramps, and sometimes nausea may start 8 to 24 hours after eating the food (Cahill *et*

al., 2008).

Identification and enumeration of all spore-formers present in powdered infant food allows identification of potential harmful species whether from a hygiene, quality or pathogenic perspective. This information would allow manufacturers implement more comprehensive and/or directed preventive measures, resulting in continued economic and safety confidence in the sector (Pennacchia *et al*., 2014). Understanding the composition of total spore-formers within a product contributes to a clearer understanding of the source of potential quality or safety issues should they arise and allows faster implementation of control measures (Burgess *et al.,* 2010; Pennacchia *et al*., 2014).

Bacteria found to contaminate powdered infant formula include: *Enterobacter sakazakii, Salmonella spp, Pantoea allglomeraris, Escherichia vulneris, Hafnia alvei, Klebsiella spp, Citrobacter, Enterobacter cloacae, Bacillus cereus, Clostridium spp, Staphylococcus aureus* and *Listeria monocytogenes* These bacteria can be introduced during the manufacturing process and may cause severe invasive infections which can be fatal or cause long life disability (WHO, 2004).

Despite the large number of bacterial species that have been found in powdered infant food, the infection of infants via such contamination has only been convincingly shown for *Enterobacter sakazakii* and *Salmonella enteric*. Health authorities require that infant formula be tested for the presence of *Salmonella* species, yet outbreaks of *Salmonellosis* in infants have been caused by contaminated milks (Jourdan *et al.,* 2008).

Infant formula provides an excellent medium for bacterial proliferation and when stored inappropriately, multiplication of bacteria may result in an infectious dose being provided to the infant and inappropriate storage appears to be common practice (Agostoni *et al.,* 2004). Therefore, this study was aimed to detect and enumerate Gram positive pathogens in infant's powdered food and to carry out antibiotics susceptibility testing on the bacterial isolates obtained from samples.

2.0. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Ikot Ekpene Local Government Area, Akwa Ibom State in the Southern part of Nigeria. The city has an area of about 166km^2, with GPS coordinates of Latitude: 5.18^o N, and Longitude: 7.71 E.

2.2 Source of Sample Collection

A total of Ten (10) samples of powdered infant foods were obtained randomly from different Pharmaceutical stores, supermarkets/ retail outlets in Ikot Ekpene. Sterile techniques were applied and samples were transported immediately to Microbiology Laboratory Akwa Ibom State Polytechnic for microbiological analysis.

2.3 Preparation of samples

All samples were serially diluted; 9 ml of distilled water were added to 1g of each sample and thoroughly mixed and was homogenized by shaking for 1 minute. Tenfold serial dilution was carried out in all the samples. After dilution 0.1 ml of the appropriate dilutions were used to inoculate Nutrient Agar, Plate count Agar, Blood agar, Baid parker agar, Tryptone Sulphite Agar. The media were prepared according to the manufacturer's instruction. Pour plate method was employed and the culture plates for isolation were incubated at 37 ^{0}C for 24 hours for enumeration of colonies.

2.4 Viable Count Determination

The colonies that formed were counted and the CFU/ml was calculated using the formula:

CFU/ml = Number of Colonies x Dilution
Amount Plated

2.5 Aerobic Plate Count of the Powdered Products

Samples (0.1 ml) of the reconstituted powdered food was spread onto PCA plates and incubated at 37 ^{0}C for 48 h. The number of colonies was counted and expressed in CFU /ml .

2.6 Characterization and Identification of Bacteria Isolates

Bacterial isolates were characterized and identified based on their morphological and cultural characteristics. Confirmatory and identification was based on biochemical test carried as described by (Cheesbrough, 2006). The following biochemical test were carried out such as gram's staining, spore test, catalase, coagulase, oxidase, indole, citrate test, urease test and sugar fermentation test (glucose, galactose, sucrose and maltose).

2.7 Antibiotics Sensitivity Test

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo. Sensitivity test was carried out on each of the isolated bacteria according to the method of national committee for clinical Laboratory standard (NCCLS, 2003) and manual of antimicrobial susceptibility testing guideline (Cheesbrough, 2006). The sterile nutrient agar was aseptically poured into the sterile petri dishes and was allowed to solidify. After that, the plates were inoculated with the pure culture of bacterial isolates. With the help of a sterilized forceps, a commercial sensitivity disc was aseptically transferred to the plates and was labelled appropriately, all the plates were incubated at 37^{0}C for 24 hours. After 24 hours of incubation, the zones of inhibition were examined. Results were interpreted according to NCCLS, 2003 and Cheesbrough, 2006.

3.0. RESULTS

The Results of Detection and Enumeration of Gram Positive pathogens in Powdered Infant foods in Ikot Ekpene Metropolis were as follows:

3.1 Total Bacterial count obtained from the sample

Table 4.1: shows the total bacteria count obtained from ten (10) samples of powdered infant foods sold within Ikot Ekpene metropolis. The bacterial count ranged from 0.3 x 10^3 CFU/ml to 3.5 x 10^3 CFU/ml.

3.2 Bacterial isolates Obtained from samples

Table 4.2: Shows the bacterial isolates obtained from the samples. Some species of bacteria isolated, identified and characterized were: *Clostridium* spp, *Listeria monocytogenes, Lactobacillus* spp, *Enterococcus* spp, *Bacillus* spp, *and Staphylococcus* spp.

3.3 Percentage frequency of occurrence of bacterial isolate

Fig. 1. Shows the percentage frequency of occurrence of bacterial isolates. *Clostridium* spp had the highest percentage frequency of (20 %), followed by *Listeria monocytogenes*, *Lactobacillus* spp, *and Staphylococcus* spp which had (16 %) each, *Bacillus* spp had 12%, and the least percentage of occurrence being recoded in *Enterococcus* spp with 8 %.

3.4 Antimicrobial of Susceptibility Pattern

Table 4.4 shows the antibiotic susceptibility profile of bacterial isolates. The bacterial isolates were tested for their susceptibility to a range of antibiotics using Disc diffusion method. From the results, *Bacillus* spp was sensitive to Ciprofloxacin, Erythromycin, Gentamycin, Ampiclox, Amoxil, and Chloramphenicol but was resistant Rifampicin, Streptomycin and Norfloxacin. *Enterococcus* spp was sensitive to Ciprofloxacin, Erythromycin, Levofloxacin, Ampiclox, Streptomycin and Norfloxacin but showed resistance to Rifampicin, Amoxil and Chloramphenicol. *Listeria monocytogenes* was sensitive to Ciprofloxacin, Erythromycin, Levofloxacin, Ampiclox, Amoxil, and Streptomycin and was resistant to Gentamycin, Rifampicin, Norfloxacin and Chloramphenicol. *Staphylococcus* spp was sensitive to Ciproflaxacin., Erythromycin, Levofloaxin, Ampiclox and was resistant to Rifampicin, Amoxil, Streptomycin, Norfloxacin and Chloramphenicol. *Clostridium* spp was sensitive to Erythromycin, Gentamycin, Ampiclox, Rifampicin and Streptomycin but resistant to Ciprofloxacin, Levofloxacin, Amoxil, Norfloxacoin and Chloramphenicol. *Lactobacillus* spp was sensitive to Erythromycin, Ampiclox, Rifampicin, Amoxil and Chloramphenicol.

Table 4.1: The Total Bacterial Count of the samples

Samples	Total bacterial Count (CFU/)ml.
1	**2.5 x 10^3**
2	**2.8 x 10^3**
3.	**3.5 x 10^3**
4.	**0.4 x 10^3**
5.	**0.3 x 10^3**

6.	**1.7×10^3**
7.	**1.5×10^3**
8.	**1.1×10^3**
9.	**0.6×10^3**
10.	**1.8×10^3**

Table 4.2. Biochemical characteristics of Bacterial isolates

S/N	Biochemical Tests	Test Organisms					
		***Bacillus* spp**	***Stapylococcus aereus***	***Clostridium spp***	***Enterococcus* spp**	***Listeria Monocytogenes***	***Lactobacillus spp***
1	Gram Reaction	+	+	+	+	+	+
2	Citrate	–	+	–	–	–	–
3	MR	–	+	–	–	+	–
4	VP	–	+	–	+	+	–
5	Urease	–	+	–	–	–	
6	Indole	+	–	–		–	–
7	Spore	–	–	+	–	–	–

Key:
+ = positive
- = Negative

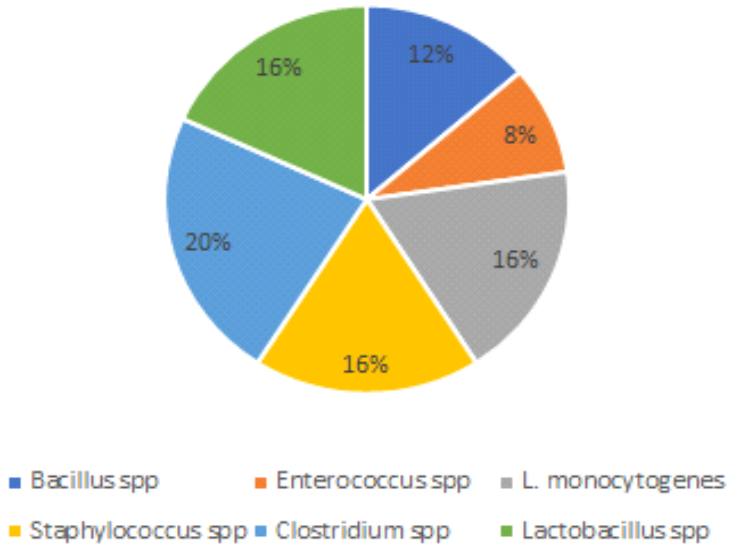

Fig. 1. Percentage frequency of bacterial isolates.

Table 4.4: Antibiotic Susceptibility profile of Bacterial Isolates (mm)

Isolates	CPX	E	LEV	CN	APX	RD	AM	S	NB	CH
Bacillus spp	S	S	R	S	S	R	S	R	R	S
Enterococcus spp	S	S	S	R	S	R	R	S	S	R
L. moncytogenes	S	S	S	R	S	R	S	S	R	R
Staphylococcus spp	S	R	S	R	S	R	R	R	R	R
Clostridium spp	R	S	R	S	S	S	R	S	R	R
Lactobacillus spp	R	S	R	R	S	S	S	R	R	S

Key:

Sensitive = 18mm, Resistant = 13-17mm, intermediate = 13mm

CPX = Ciproflaxacin,
NB = Norfloxacin,
CN = Gentamycin
AM = Amoxil,
S = Streptomycin,
RD = Rifampicin,
E = Erythromycin,
APX = Ampiclox,

CH = Chloramphenicol,
LEV = Levofloxacin

4.0. Discussion of Results

The detection and enumeration of Gram positive bacteria in powdered infant foods was carried out using Standard analytical procedures. Ten(10) samples were analysed and the following results were obtained. Results obtained indicated that samples were contaminated with pathogenic bacteria. The bacterial count obtained ranged between 0.3 x 10^3 CFU/ml to 3.5 x 10^3 CFU/ ml. Some pathogenic bacteria were isolated from the samples to include, *Lactobacillus* spp, *Bacillus* spp, *Staphylococcus* spp, *Enterococcus* spp, *Listeria monocytogenes* and *Clostridium* spp. These finding was in agreement with the work of Lesley *et al.,* (2016), who identified *Bacillus* spp in powdered milk. *Staphylococcus* spp, *Listeria monocytogenes, Clostridium* spp, and *Lactobacillus* spp have been reported to be among bacteria found to contaminate powdered infant food. The percentage frequency of occurrence of the bacterial isolates were also recorded with *Clostridium* spp having the highest percentage of occurrence of 20 %, followed by *Listeria monocytogenes* , *Lactobacillus* spp and *Staphylococcus* spp with 16 % each, *Bacillus* spp had 12 %, while *Enterococcus* spp had the lowest percentage of occurrence of 8 %. This indicates that, most of the samples were contaminated with bacteria from various sources. Contamination of powdered infant food may be as a result of poor hygiene during preparation, inadequate cleaning and poor storage of powdered food (Marino *et al*., 2007; Basher *et al*., 2006). The presence of these bacterial species encountered during the course of this work may have been as a result of poor environmental condition to include soil, dust, water and processing equipment used.

Enterococcus spp was present in this work; its presence may have resulted because of the contamination of the processing equipment's from faecal source which was in line with the work carried out by (Bredbenner *et al.,* 2003).

The presence of *Listeria monocytogenes* in the sample may probably be as a result of insufficient pasteurization of the milk sample and processing equipment's (Christianson *et al.,* 1999). The high rate of pathogens in commercially imported food samples is to be noted with concern from public health point of view, since most sample of Listeriosis and other diarrhoreal disease are attributable to consumption of contaminated foods.

Staphylococcus spp was present in the sample, its enterotoxins are considered as one of the most prevalent cause of gastroenteritis, diarrhhoea and vomiting which appears as symptoms after ingestion of food contaminated with *Staphylococcus* spp.(Pal, 2001). *Staphylococcus* spp may have occurred during food processing, storage and improper handling of food equipment's (Fein *et al*., 1999).

Clostridium spp is a Gram positive, spore forming anaerobic organism which can be found in soil and in the intestinal tracts of vertebrates (Pal, 2015). Their spores have been found to contaminate powdered food because they are resistant to cooking temperatures, the vegetative cells in the gastrointestinal tract may results in the production of enterotoxins and causes symptoms such as nausea and abdominal cramps if the food is eaten.

Lactobacillus spp was one of the isolates available in the sample which could have been

due to poor hygienic practice during processing and has been the source of disease outbreak (FAO, (2011).

The presence of *Bacillus* spp, *Micrococcus* spp, *Listeria monocytogenes* , *Clostridium* spp, *Listeria monocytogenes, Staphylococcus* spp and *Lactobacillus* spp have been reported to be among the major causes of food poisoning (Chidi, 2007).

Food poisoning cases associated with milk brand product have been reported as low as 45% of enterotoxogenic *B. cereus* has been isolated from milk and milk product (Sadek *et al.*, 2006). Occurrence of *Bacillus* spp, *Listeria monocytogenes*, *Staphylococcus* spp, *Enterococcus* spp and *Lactobacillus* spp in milk products are especially important concern in the infant milk industry.

Infants are more susceptible to food borne infection due to under-developed immune system and absence of competing microorganisms in gut micro flora (Lesley *et al.*, 2016). Moreover, infant milk powder often contain raw ingredient from various sources that are rich in nutrients, when reconstituted and left at ambient temperature for a long period, the milk products will become a suitable medium for proliferation and enterotoxin production of *Bacillus* (Tunio *et al.*, 2013), or when left at low temperature for long period, the milk product will become a suitable medium for *Listeria monocytogenes*. Hence, frequent exposure of infants and toddlers to these milk products increase the risk of contracting foodborne illness (Lesley *et al.*, 2016).

Antibiotic susceptibility profile of bacteria isolated showed that 85% of the bacterial isolate were sensitive to the commonly used antibiotic that was used for this research.

5.0. Conclusion

Based on the results obtained in this research, it is important to note that pathogenic bacteria have been a major public health concern in the food industries because they are the source of disease transmission. Sources of disease transmission of bacteria may be through unclean equipment's used during processing and packaging process. Powdered infant foods are generally considered safe for human consumption but both physical and chemical factors may reduce the shelf life of these products. Hence Microbiological monitoring of powdered infant food is highly recommended to avoid the spread of food borne disease.

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