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ENUMERATION OF BACTERIAL LOAD AND ISOLATION OF COLIFORM BACTERIA IN CANNED FOODS SOLD WITHIN ZARIA METROPOLIS OF KADUNA STATE, NIGERIA

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ABSTRACT
Canned foods have potentials for contamination with microbes. To determine the bacteria load of canned foods sold within Zaria metropolis, 24 samples of eight brands of canned foods were pour-plated on nutrient agar. Salmonella isolation was by enrichment on tetrathionate broth followed by Salmonella –Shigella agar plating and biochemical characterization of isolates. Twenty (83.3%) of the cans were positive for bacterial growth. Baked beans had the highest bacterial load of 1.9 x 10^5 CFU/g while sweet corn had the least (1.7 x 10^3 CFU/g). No Salmonella (0%) was isolated but there were three coliform bacteria as follows: Enterobacter spp (10; 41.7%), Citrobacter spp (2; 8.3%) and Shigella spp (1; 4.2%). In addition, Pseudomonas spp was isolated (3; 12.5%). There was statistical significant difference between bacterial growth and pH of the can's content (χ^2 =7.2, df= 2. P= 0.027). The study has shown that the canned foods examined had low bacterial load < 10^6 but the isolation of other pathogenic organisms is of public health importance.

Key words: Canned foods, bacterial load, Zaria

INTRODUCTION
Foods can be preserved by canning, which help retain the original taste and flavour of the food and hence this method is commonly used for food preservation especially for commercial purposes (Desrosier, 2004; Ogbulie *et al*., 2014). Foodborne pathogens encompass a wide spectrum of microorganism that contaminates food and water at different points during their preparation (Hanson *et al*., 2012). The incidence of spoilage in canned foods is low, but when it occurs it must be investigated properly. Spoilage is usually caused by growth of microorganisms following leakage or under processing. Some microorganisms that grow in canned foods, however, do not produce gas and therefore cause no abnormal appearance of the can; nevertheless, they cause spoilage of the product.

Canned foods or shelf stable canned foods are packed in hermetically sealed containers and are commercially sterile (ICMSF, 1986). Sealed and sterilized canned vegetables remain microbiologically stable for years at ambient temperature as the heat process inactivates mesophilic microorganisms. Spoilage in low-acid (pH N4.5) canned vegetables occurs mainly at high incubation temperatures (N40 °C) and is caused by the survival and further multiplication of thermophilic spore-forming bacteria (Durand *et al*., 2015). Canned foods are sterilized before being placed on the grocery shelf but if the sterilization has been unsuccessful, contamination or food spoilage may occur (Desrosier, 2004).

Bacteria attach to available surfaces in industrial environments, and can develop into extensive biofilm, which is a potential source of contamination of foods that may lead to spoilage or transmission of foodborne pathogens (Gunduz and Tuncel, 2006). Investigated microflora forming biofilms include *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Campylobacter* spp., *Escherichia coli* and *Listeria* spp. These bacteria are of special significance in ready-to-eat and minimally-processed food products, where microbiological control is not conducted in the terminal processing step (Herald & Zottola, 1988;

Cabanes *et al*., 2002; Gunduz & Tuncel, 2006; Kim *et al*., 2006).

Canned foods have been reported to be contaminated mainly by spore forming bacteria of the genera Bacillus, *Clostridium and Desulfotomaculum (*ICMSF, 1986). Food poisoning by *C. perfringens* has been associated most often with meat and gravies, however, *C. perfringens* spores are also found in milk and cheese which could grow to cause food poisoning (Donnelly and Busta, 1981; Granun, 1990). *Bacillus cereus* and *B. licheniformis* contaminate milk causing broken cream and soft coagulum with blown cans (Oomus *et al*, 2007; Arun, 2008). This study was therefore aimed at determining the bacterial quality of selected canned foods sold within Zaria, Kaduna State, to know if they are safe for consumption.

MATERIALS AND METHODS

Study Area

The study was carried out in Samaru area of Zaria metropolis. Zaria is an ancient and major city in Kaduna State, Northern Nigeria, and renowned as center of learning. Zaria is located between the Latitude 11°-04°N and 7°-42°E Longitude. It has a total area of about 300km^2 and a population 408,198 (Mortimore, 1970; Agbogu *et al*., 2006)

Samples Collection

A total of 24 samples of eight brands of canned foods comprising of canned chicken luncheon, sweat corn, liquid milk, red kidney beans, giant peas, tomatoes, baked beans and sardine were bought from selected shops within Samaru market. All selected cans were checked to ensure they were within the expiry date. Labeling information of each can was recorded and this included the NAFDAC (National Agency for Food and Drug Administration and control) number, manufacture and expiry dates, batch number, manufacturer's address, preservatives and ingredients. Cans were examined for evidence of bloating, leakage and physical damage. The samples were then taken to the bacteria zoonosis laboratory of Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for microbiological analysis.

Microbiological analysis

The laboratory procedures for the determination of the contamination of canned foods are as follows:

Total Aerobic Plate Count

Prior to analysis, the surface of each container was cleaned with 70% ethanol and tincture of iodine. Containers were opened aseptically near the flame of the Bunsen burner to avoid contamination. The pH of the samples was taken using pH meter (Jenway 3505, England). For each sample, 10g was weighed aseptically into stomacher bag containing 90ml of Peptone Water (PW) and mixed for one to two minutes. Thereafter, the PW primary mixtures (10^{-1}) were incubated at 37^{0}C for 24 hours. Enumeration of total viable bacteria was carried out using the total plate count (TPC) technique. A tenfold serial dilution (10^{-2}) was prepared by transferring 0.1ml of primary dilution into test tubes containing 9.9mls physiological saline. For the determination of TPC, 0.1ml of 10^{-2} dilution of the homogenate was inoculated into nutrient agar plate and spread using sterile glass spreaders which was then incubated at 37^{0}C for 24 hours. The number of colonies in the dilution was multiplied by the dilution factor to obtain the TPC. The TPC was expressed as Colony Forming Unit per gram (CFU/g).

Detection of *Salmonella*

For *Salmonella* detection, 1ml of the homogenate as previously described under total aerobic plate count was pre enriched with 9mls of tetrathionate broth and incubated at 37°C for 24 hours. A loopful of the tetrathionate broth culture was streaked on *Salmonella –Shigella* agar (SSA) and also incubated at 37°C for 24 hours. Suspected *Salmonella* colonies appeared colourless and these were subjected to biochemical characterization using triple sugar iron, indole, urease, methyl red, citrate, Voges proskeur, SIM motility, oxidase. Lactose, rhannose, maltose, sucrose and mannitol were used for the sugar fermentation tests carried out according to manufacturers' instructions. Gram staining was carried out with Gram negative

organisms appearing pinkish while Gram positive ones appeared purple or blue.

Data analysis

Statistical Package for Social Science Version 16 was used for data analysis. Chi square and Fishers exact tests were used to check for association between microbial load of the canned foods and factors such as use of preservatives, expiry date and pH. P≤ 0.05 was considered significant at 95% confidence onterval.

RESULTS

Of the 24 samples of canned foods analysed, 20 (83.3%) were positive for microbes. A 100% positivity was recorded each for the baked beans, liquid milk, giant peas, tomatoes, chicken luncheon and sardine while only 1 (33.3%) out of the 3 cans each of kidney beans and sweet corn was positive for growth. Baked beans had the highest contamination of 1.9 x 10^5 CFU/g of the sample while the least was sweet corn (1.7 x 10^3 CFU/g). Kidney beans and liquid milk had microbial loads of 9.3 x 10^4 and 8.3 x 10^3 CFU/g respectively (Table 1).

The biochemical characterization of the isolates that were suspected to be *Salmonella* is shown in Table 2. A total of 16 (66.7%) out of the 24 isolates that were non lactose fermenters upon characterization gave three positive coliform bacteria as follows:: *Enterobacter* spp (10; 41.7%), *Citrobacter* spp (2; 8.3%) and *Shigella* spp (1;4.2%) and also 3 (12.5%) isolates of *Pseudomonas* spp. *Pseudomonas* spp was found in kidney beans and baked beans, *Citrobacter* spp. in tomato paste and sweet corn, *Shigella* spp. in giant peas while *Enterobacter* spp, was more widely distributed as it was found in sweet corn, giant peas, baked beans, tomato paste chicken luncheon, sardine and liquid milk.

The physical assessment of the labeling of the canned foods showed no external damages or leakages and all had NAFDAC number, manufacture and expiry dates, batch number, manufacturer's address and ingredients. Most of the canned foods had salt listed as part of the ingredient which also served as natural preservative, only chicken luncheon had Sodium Nitrate as a chemical preservative (Table 3).

All the three chicken luncheon cans that had chemical preservatives were positive (100%) for microbial growth while 17 (81%) out of the 21 cans without chemical preservatives had microbial growth. There was no statistical significance difference between microbial growth in the content of the cans and use of chemical preservatives (Fishers exact P= 1.000) (Table 4).

Table 5 shows the effect of pH on microbial contamination of the cans. The highest contamination was in the acidic cans 9 (100%) followed by alkaline 10 (83.3%) while the least was in the neutral cans (1; 33.3%). There was statistical significance between microbial growth in the content of the cans and pH of the contents (χ^2 =7.2, df= 2. P= 0.027).

The result from this study also showed that the longer the expiry date, the least the microbial growth as cans within 2018 expiry date were least contaminated (66.7%), followed by the 2016 group (77.8%) while the highest (100% each) was in the 2015 and 2017 groups. There was no statistical significant difference between microbial growth in the content of the cans and expiry date of the cans (χ^2 =3.2, df= 3. P= 1.725) (Table 6).

Table 1: Mean pH and total plate counts of canned foods sold within Zaria metropolis, Kaduna State, Nigeria.

S/No	Type of Canned food	Number examined	Number positive (%)	Mean CFU Per g	Mean pH
1	Kidney beans	3	1(33.33)	9.3 x 10^4	7.00
2	Sweat corn	3	1(33.33)	1.7 x 10^3	7.40
3	Giant peas	3	3(100)	4.3 x 10^3	6.50
4	Baked beans	3	3(100)	1.9 x 10^5	6.80
5	Tomatoes	3	3(100)	1.5 x 10^4	5.00
6	Chicken luncheon	3	3(100)	8.8 x 10^4	7.20
7	Sardine	3	3(100)	1.1 x 10^4	7.70
8	Liquid milk	3	3(100)	8.3 x 10^3	7.30
	Total	24	20(83.3)		

CFU/g= Colony forming unit per gram
pH= Potential of hydrogen

Table 2: Isolation of coliform bacteria and *Pseudomonas* spp. from canned foods sold within Zaria metropolis, Kaduna State, Nigeria (N=24).

Isolates	No positive	Prevalence (%)
Enterobacter	10	41.7
Citrobacter	2	8.3
Shigella	1	4.2
Pseudomonas	3	12.5
Total	16	66.7%

Table 3: Physical assessment of the labeling compliance of canned foods sold within Zaria metropolis, Kaduna State, Nigeria.

Canned food	Canned labels				
	NAFDAC Number	Production Date	Expiry date	Batch Number	Preservatives Used
Kidney beans					
1	-	+	+	+	+
2	-	+	+	+	+
3	-	+	+	+	+
Sweat corn					
1	-	+	+	+	-
2	-	+	+	+	-
3	-	+	+	+	-
Giant peas					
1	+	-	+	+	+
2	+	-	+	+	+
3	+	-	+	+	+
Baked beans					
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
Tomato					
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
Meat					
1	+	+	+	_	+
2	+	+	+	_	+
3	+	+	+	_	+
Sardine					
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
Milk					
1	+	+	+	+	-
2	+	+	+	+	-
3	+	+	+	+	-

Key: Present (+), Absent (-)

Table 4: Association between chemical preservatives and microbial contamination of canned foods sold within Zaria metropolis, Kaduna State, Nigeria.

Chemical preservatives	Number examined	Number positive (%)	Fishers exact test
Yes	3	3 (100)	P = 1.000
No	21	17 (81.0)	
Total	24	20 (83.3)	

Table 5: Association between pH and microbial contamination of canned foods sold within Zaria metropolis, Kaduna State, Nigeria.

pH	Number examined	Number of positive (%)	Test statistics
Acidic	9	9 (100)	Chi square (χ^2) =7.2
Neutral	3	1 (33.3)	Df= 2
Alkaline	12	10 (83.3)	P= 0.027
Total	24	20 (83.3)	

Table 6: Association between expiry date and microbial contamination of canned foods sold within Zaria metropolis, Kaduna State, Nigeria.

Expiry date	Number examined	Number positive (%)	Test statistics
2015	1	1 (100)	Chi square (χ^2) =3.2
2016	9	7 (77.8)	Df= 3
2017	8	8 (100)	P= 1.725
2018	6	4 (66.7)	
Total	24	20 (83.3)	

DISCUSSION

The canned foods examined had low microbial loads (< 10^6) which was within acceptable microbiological limit (ICMSF, 1986). The combined effects of high temperature treatment, pH, preservatives and anaerobic condition of canning could have been responsible for the low microbial loads (Oranusi *et al*., 2012). Baked beans had the highest counts probably because of its high moisture content and condensed nature which may have been a good medium for the growth of microorganisms. It may also be a reflection of the quality of the raw materials, under processing, pre-process contamination and to the level of stringency in their production (Oranusi *et al*., 2012). The absence of swollen and leaky cans may have also contributed to the low microbial load as all the cans were in perfect shape.

Though *Salmonella* was not detected in this study, but the isolation of *Pseudomonas* species, *Enterobacter* species, *Citrobacter* species and *Shigella* species are still of public health significance. Most of the isolates are of human flora and are known to be opportunistic pathogens. They are facultative and hardy organisms, thus their survival in canned foods could be explained as previous studies (Saadia and Hassanein, 2010; Durand *et al*. 2015) on isolation of pathogenic bacteria in food samples also indicated that some gram negative and gram positive bacteria were isolated as discovered in this investigation. The result in this study is similar to the findings made by Kay *et al.* (1994) who found some pathogenic bacteria found in fast foods and traditional fast foods. Most investigators indicated that bacteria, fungi and yeasts may exert their pathogenic action either through infection of body, or as a source of toxic substances as has been demonstrated in contaminated foods (Saadia and Hassanein, 2010). Food poisoning and other diseases of man are those associated with contamination of food products by infectious organisms (Frazier and Westhoff, 1994).

The higher contamination in the cans that had contents which were highly acidic is not farfetched from the truth as there is a pH optimum for each microorganism at which growth is maximal. Moving away from the optimal pH in either direction slows microbial growth. Shifts in pH of a food with time may reflect microbial activity, and foods that are poorly buffered (i.e., do not resist changes in pH), such as vegetables, may shift pH values considerably. A food may start with a pH which precludes bacterial growth, but as a result of the metabolism of other microbes (yeasts or molds),

pH shifts may occur and permit bacterial growth (www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm071351.htm) (Accessed 8th May, 2016 at 3.00pm). Small amount of acetic acid can cause bacteria to become stressed, making them to react by producing more toxin. However, if a large amount of acetic acid is added, as was done in the past, the acidity is greatly increased and the bacteria do not survive () (Acessed 8th May, 2016 at 3.00pm)

The higher contamination in the cans that had preservatives may mean that not enough concentrations were used for the prevention of Microbial growth. In order for an antimicrobial preservative to work, it must be used at the right concentration. Ideally, it will disrupt microbial growth while at the same time preserving most of the nutritional value of the food. Thus the amount and type of preservative used will determine how much microbial control of the preservative in use (Dalton, 2002).

It is better to consume canned foods within expiry date as shown in this study or better still if the expiry date is further away. Some products loose freshness and taste if closer to expiry date and may even deteriorate faster as compared to those further away from expiry date.

CONCLUSION

This study has shown that the canned foods sampled, though with minimal microbial load within acceptable limit, depending on an individual's varying tolerance ability, may still pose a health problem especially those related to gastrointestinal symptoms when the contents are consumed. The isolation of other bacterial organisms such as *Enterobacter*, *Citrobacter*, *Shigella* and *Pseudomonas* is of public health significance. Hence, consumers of canned foods should ensure that the can's contents are heated to reduce chances of food borne illness.

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