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Antibiotics susceptibility pattern and occurrence of bacteria in ready-to-eat roasted spiced chicken sold along road sides in Makurdi town

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Abstract

Antibiotics susceptibility pattern and the bacterial load in ready to eat roasted spiced chicken sold along road sides in Makurdi town were determined, and also, the associated bacteria were identified and isolated. Samples (10) of roasted spiced chicken, were collected each from Wurukum, Wadata, Old GRA, Highlevel and Northbank areas, which were evaluated for their bacterial load and antibiotic susceptibility using pour plate and the disc diffusion method. Bacteria isolated were tested against antibiotic to determine their sensitivity. Results showed mean total viable bacterial counts of $3.57x10^3 \pm 2.78\times10^3$cfu/g, $4.71 \times 10^3 \pm 3.81 \times 10^3$cfu/g, $2.90 \times 10^3 \pm 4.43\times10^3$cfu/g,$1.82x10^3 \pm 1.61 \times 10^3$ cfu/g and $3.49x10^3 \pm 2.97\times10^3$cfu/g for Wurukum, Wadata, Old GRA, Highlevel and Northbank respectively. All the counts were within the tolerable limit of $<10^6$. There was a significant difference ($P < 0.05$) in the bacterial count across the locations. On the percentage prevalence of isolates across samples from the various location, Wurukum had the highest prevalence of 22.36%, while High level had the least with 16.15%. *Staphylococcus* species*, Proteus, Salmonella, Shigella, Escherichia coli, Klebisella* and *Bacillus* species were isolated and identified as contaminating bacteria in this study. The bacterial isolates showed highest percentages of susceptibility for Ciprofloxacin, with over 90% susceptibility for most of the bacterial genera except for *Shigella* and *Klebsiella* species, which serve as a wake-up call to pharmaceutical industries to develop higher susceptibility antibacterial agent against those bacteria that shown higher resistance. Ampiclox and amoxicilin were the least active drugs. Therefore, the results of this study suggests serious public health concern because*, Escherichia coli,* which is an indicator of fecal contamination, its presence in these food samples is a pointer of serious contamination of the analyzed roasted chicken, making it a possible route of food-borne disease outbreak

Keywords: Susceptibility; Antibiotic; Bacteria; Contamination; Prevalence; Chicken

1. Introduction

Chicken meat is mostly preferred by consumers around the world because of its desirable nutritional qualities, such as low fat content and relatively high concentration of polyunsaturated fatty acid (Patsias et al., 2008). Ready to eat roasted spiced meat are traditional barbecue, smoked or roasted commonly obtained from beef or chicken and are marinated with various spices such as clove, ginger, pepper, salt, peanut, vegetable, oil, as well as foods additives and flavouring (Akpamu, 2011; Egbebi and Seidu, 2011). Roasted spiced chicken is used a lot when preparing Nigerian recipes, especially the rice recipes and stew recipes and it can also be eaten as snack and also washed down with chilled drinks (Madubike, 2010). Roasted spiced chicken is also a street vended food which provides a source of inexpensive,

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convenient and often nutritious menu for cities, urban and rural areas, a major source of income for vast number of persons and creates opportunity for self-employment, more also, street vending of food is a common characteristic of countries with high unemployment, low salaries and poor social security program (Bryan et al., 1988).

However, most of the people who patronize and consumed roasted chicken meat sold along road sides are ignorant of high health risk associated with consuming this product, this is because such meat and other meat products are exposed to various degrees of contamination at every stage of handling and preparation (Ologhobo et al., 2010). Reports by some researchers have shown that food poisoning bacteria such as *Campylobacter jejuni*, *Salmonella* sp, *Clostridium perfringens* and *Staphylococcus aureus*, live in the gut and skin of the birds, this can get on to raw chicken during slaughter and processing due to inadequate cooking or re-contamination. Cross contamination of cooking utensils, or packaging materials, through these routes and as such, pathogens of this source can infect the consumers (Scott, 2010). Though *Salmonella* sp and *Campylobacter* sp accounted for most of the cases of food poisoning associated with chicken (Scott, 2010). *Escherichia coli*, *Salmonella* sp, *Staphylococcus aureus* and *Shigella* sp were reported to be isolated from ready-to-eat roasted chicken (Ologhobo et al., 2010).

The sources of these contaminations have been linked to poor hygienic conditions of handlers, environment, raw meats, spices and packaging material, as well, as cross-processing contamination (Food and Agricultural Organization, 1999; Odey et al., 2013; Hassan et al., 2014). One of the food safety measures was subjected to the disease causing organism isolated from food to treatment with appropriate antibiotics (Amadi et al., 2016). Although irrational use of antibiotics has led to antibiotics resistant bacteria, this has resulted to a serious health problem worldwide, which is now trying to be solved by many approaches (Pavyde et al., 2015).

1.1. Statement of the Problem

Street-vended meat and meat products are exposed to various forms of contaminants such as *Staphylococcus aureus*, *Shigella* sp, *Campylobacter* sp, *Escherichia coli*, *Salmonella* sp and *Klebsiella* sp at every stage of handling, which include cross-contamination, insufficient roasting, uneven temperature distribution and exposure to unhygienic environment (Etok, 1998; Ologhobo et al., 2010).

Gastroenteritis and other food-borne infections such as Salmonellosis, Shigellosis and Staphylococcus food poisoning are as a result of contaminants found in street-vended meat.

1.2. Justification for the Study

This study shall necessitate the impacts of microbiologist in reduction of morbid conditions that can lead to death and economic loss among the general populace due to food-borne diseases associated with consumption of contaminated ready to eat roasted spiced chicken sold along road sides in Makurdi, Benue state, North-central Nigeria. The exposure of roasted spiced chicken to various levels of contamination, unhygienic method of preparation and packaging materials, and also, high increase in acquisition of antibiotic resistance endemic strains of bacteria in roasted spiced chicken have all justified the need for this study.

1.3. Aim of the Study

The aim of this study was to determine antibiotics susceptibility pattern, the bacterial load and identification of bacteria associated with ready-to-eat roasted spiced chicken sold along road side in Makurdi town, Benue state, North-central Nigeria.

Objectives of the Study

- To enumerate bacterial counts of ready-to-eat roasted spiced chicken from different locations in Makurdi town.
- To isolate and identify bacteria associated with roasted spiced chickens sold along road sides in Makurdi town.
- To determine antibiotics susceptibility profiles of bacteria isolated from ready-to-eat roasted spiced chicken in Makurdi town.

2. Materials and methods

2.1. Study Area

Makurdi, the capital city of Benue State, has grown into a town since its inception in the early 1930s and is experiencing rapid urbanization like many urban centers in the Nigeria. Makurdi has an estimated population of 500, 797 (National

Population Commission 2009). The town is located in the central region of Nigeria between latitudes 7037' and 7047' north of the equator and longitude 8027' and 8040'. Makurdi town is a major link between the north and south of Nigeria. The River Benue, which is the second largest River in Nigeria bisects the town into two parts, that is, north and south banks. The climate of Makurdi is hot and humid according to Koppens classification. High temperatures are experienced especially in the month of March and April, and it ranges between 35°C and 21°C in the summer, 37°C and 16°C in the winter. Rainfall occurs between the months of May and October with average rainfall amount ranging between 750mm to 2000mm spread over 5-6 months (Iorliam, 2008).

The vegetation is mainly of savannah, mixed with grasses and trees that are generally of average height. Most of the indigenes are farmers and the inhabitants of the river areas engage in fishing as their primary or important secondary occupation while others are civil servants, teachers, businessmen and a few are craft men (Msoo and Goodness, 2014; Terwase and Terese, 2013).

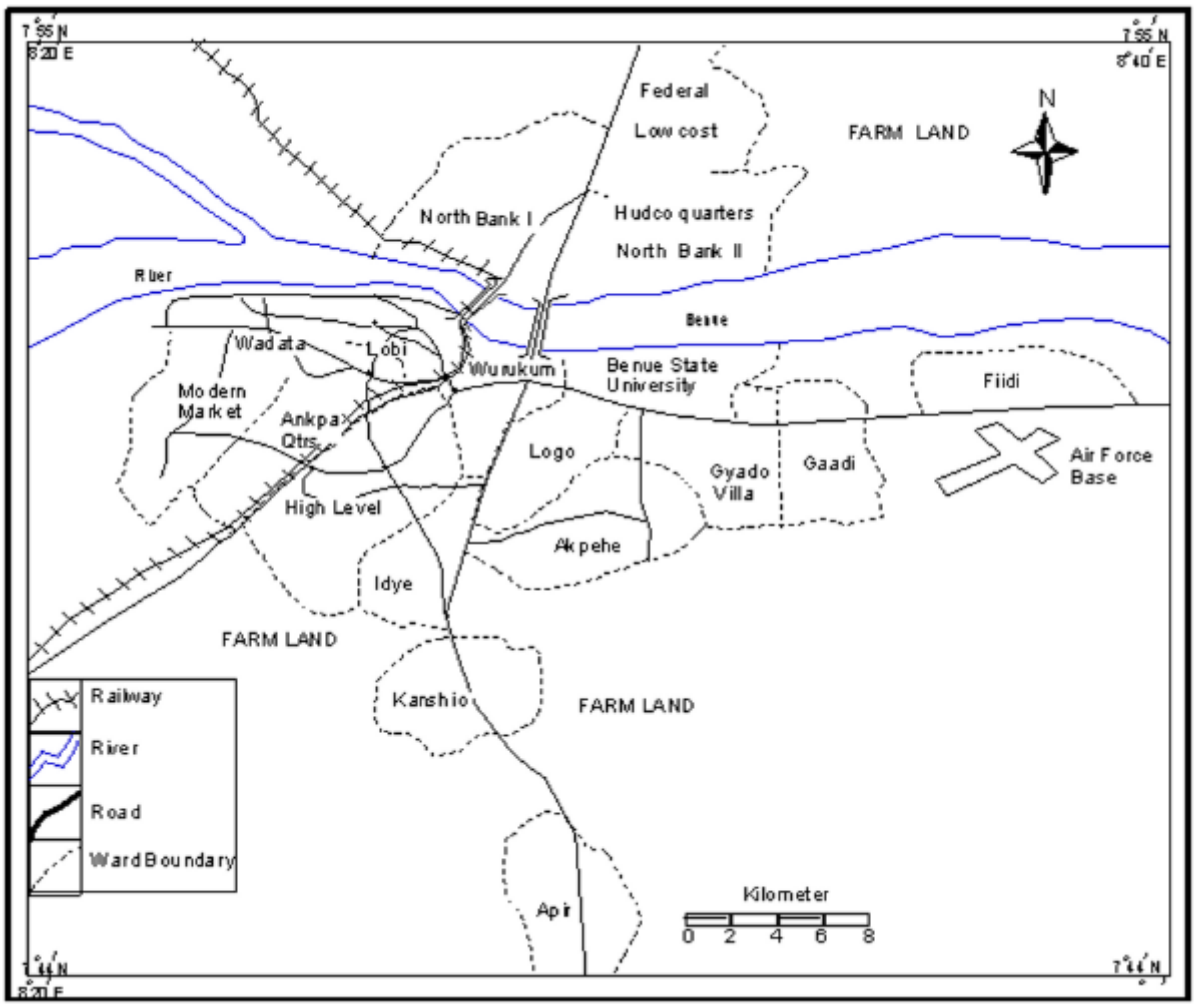

Figure 1 Map of Makurdi Showing Samples Collection sites in Shades (Benue-State Ministry of Land and Survey, 2011)

2.2. Sample Collection

Roasted spiced chicken samples were obtained from five different locations in Makurdi town, Benue-State: Wurukum (A), Wadata (B), Northbank(C), Old GRA (D), and High Level (E). All the samples were collected from each location, between 7pm-8pm whereby, roasted chicken samples were on top one feet above flames coming out of charcoal burner prepared by the vendor. Different parts of the roasted spiced chicken were taken into the plastic container (one sample part per one plastic container) that has already be cleaned with ethyl alcohol in the presence of open flames to ensure sterility of the surrounding air.

2.3. Sample identification

Each sample per location were identified using biometric method, where alphabets and numbers were used to identified and differentiated between samples from different locations, and also, within locations such as A_1-A_{10} which indicate samples within location A (Wurunkum) accordingly while B_1 (first sample taken from Wadata), this is done in repeated order till the last location and sample E_{10} (High level).

2.4. Sample Preparation

The roasted spiced chicken (RSC) sample was cut into smaller pieces with the aid of knife clean with ethyl alcohol and passed over flames to maintain sterility, which enabled the bones to be completely removed. It was thoroughly blended using sterile blender. Exactly 10 grams from the blended sample was placed in 90ml of distilled water and mixed thoroughly to give a good homogenate, which was used as a stock. Several dilutions were carried out up to ten folds (10^{-10}) for each sample that was prepared using 1ml from stock homogenate. It was transferred into 9ml of sterile distilled water in a test-tube for the serial dilution experiment. This was carried out in order to obtained discrete colony for enumeration (Ologhobo et al., 2010).

2.5. Experimental Design

Ten treatments in a completely randomized design of roasted spiced chicken that were collected in each location (A, B, C, D, E) and packaged in each sterile plastic container per sample, which were labeled accordingly (A_1,A_2, A_3, A_4, A_5,A_6, A_7, A_8, A_9, A_{10}). Same procedure was repeated for sites B, C, D and E. Samples were taken immediately to the Department of Microbiology Laboratory, University of Agriculture, Makurdi for analysis.

2.6. Data collection

Colony enumeration and the data obtained were carried out using the method described by Collins et al., (1989), for estimating bacteria count was used to enumerate the viable count of the colonies and expressed in colony forming units per ml (cfu/ml10^{-1}).

2.7. Data Analysis

Data recorded from the results were subjected to analysis of variance (ANOVA, one way method) and to Duncan's Multiple Range Test (DMRT) to determine the significant differences among treatments.

3. Results

The mean bacterial counts of samples from the five spots (A_1-A_5) at Wurukum, Makurdi in (Table 1). The bacterial counts ranged from 2.83 ± 2.16 x10^3cfu/g in spot A_3 to 4.79 ± 3.68 x 10^3cfu/g in spot A_2. The results showed significant difference between spots A_2 and A_3 ($P < 0.05$) while there was no significant difference between A_1 and A_4, A_3 and A_5. The results indicated no significant difference between pairs of mean between spots A_1, A_2, A_3, A_4 and A_5.

Table 2 shows the results of bacterial counts from Wadata samples (B_1-B_5). The overall bacterial mean counts from the five spots was 4.71 x 10^3cfu/g. There was significant difference between B_1, B_2 and B_3, B_4 ($P< 0.05$) while there is no significant difference between B_1 and B_4 and B_3 and B_5. Sample from B_4 had the highest bacterial count of 6. 55 x 10^3± 4. 85 x10^3cfu/g while B_3 had lowest bacterial mean count of 3. 17 x 10^3cfu/g.

Table 1 Bacterial Counts of Roasted Spiced Chicken Collected at Wurukum (A) Location

SPOT	BACTERIA (cfu/g)
A_1	$3.70 \pm 3.69 \times 10^{3ab}$
A_2	$4.79 \pm 3.68 \times 10^{3a}$
A_3	$2.83 \pm 2.16 \times 10^{3b}$
A_4	$3.36 \pm 2.03 \times 10^{3ab}$
A_5	$3.15 \pm 2.54 \times 10^{3b}$
Mean	$\mathbf{3.57 \pm 2.78 \times 10^3}$

A_1- A_5 represent the first to the fifth samples at Wurukum.

Table 2 Bacterial Counts of Roasted Spiced Chicken Collected at Wadata (B) Location

SPOT	BACTERIA (cfu/g)
B_1	$5.54 \pm 4.74 \times 10^{3a}$
B_2	$4.85 \pm 3.08 \times 10^{3ab}$
B_3	$3.17 \pm 2.48 \times 10^{3b}$
B_4	$6.55 \pm 4.85 \times 10^{3a}$
B_5	$3.43 \pm 2.8 \times 10^{3b}$
Mean	$\mathbf{4.71 \pm 3.81 \times 10^{3}}$

B1- B5 represent the first to the fifth samples at Wadata

Table 3 presents bacterial counts from old G.R.A, which is location C. The bacterial counts ranged from 1.41 x 10^3 cfu/g in spot C_3 to 6.38 x10^3cfu/g in spot C_1. There was no significant difference between C_1 and C_4, C_2 and C_3, while C_1 and C_5 differed significantly ($P<0.05$). Table 4 presents results of bacterial count for location D. The overall mean bacterial counts for samples from North Bank (D_1-D_2) was 1.82 ± 1.61 x 10^3cfu/g of the roasted spiced chicken. The highest bacterial count was in sample D_4 with 2.90 x 10^3 ± 2.43 x10^3 cfu/g while spot D_3 had the lowest count (1.01 x 10^3 ± 0.73 x 10^3 cfu/g). There was no significant difference ($p<0.05$) between four (4) spots, out of the five (5) spots in this location, which were D_1, D_2, D_3 and D_5, however D_4 differed significantly.

Table 3 Bacterial Counts of Roasted Spiced Chicken Collected at Old GRA (C) Location

SPOT	BACTERIA (cfu/g)
C_1	$6.38 \pm 7.46 \times 10^{3a}$
C_2	$4.53 \pm 4.50 \times 10^{3ab}$
C_3	$1.41 \pm 1.16 \times 10^{3bc}$
C_4	$2.18 \pm 2.35 \times 10^{3bc}$
C_5	0.00 ± 0.00^{c}
Mean	$\mathbf{2.90 \pm 4.43 \times 10^{3}}$

C_1- C_5 represent the first to the fifth samples at Old GRA

Table 4 Bacterial Counts of Roasted Spiced Chicken Collected at NorthBank (D) Location

SPOT	BACTERIA (cfu/g)
D_1	$1.33 \pm 0.94 \times 10^{3b}$
D_2	$1.55 \pm 1.22 \times 10^{3b}$
D_3	$1.01 \pm 0.73 \times 10^{3b}$
D_4	$2.90 \pm 2.43 \times 10^{3a}$
D_5	$1.82 \pm 1.61 \times 10^{3b}$
Mean	$\mathbf{1.82 \pm 1.61 \times 10^{3}}$

D_1- D_5 represent the first to the fifth samples at North bank

Whereas, Table 5 presents the bacterial counts from High Level (E_1-E_5) with an overall mean bacterial count of 3.49 x 10^3cfu/g. There was no significant difference across the locations. Though no significant difference between E_1, E_2 and E_4. Also, there is no significant difference between E_3 and E_5.

Table 6 presents the mean bacterial counts across samples locations. Wadata (B) had the highest mean bacterial count of $4.71\times10^3\pm3.81\times10^3$cfu/g while Northbank (D) had the least mean bacterial count of $1.82\times10^3\pm1.61\times10^3$cfu/g. There was a significant difference ($P < 0.05$) in the bacterial count across the locations.

Table 5 Bacterial Counts of Roasted Spiced Chicken Collected at High Level (E) Location

SPOT	BACTERIA (cfu/g)
E_1	$4.13 \pm 3.42 \times 10^{3a}$
E_2	$4.54 \pm 3.58 \times 10^{3a}$
E_3	$3.11 \pm 2.55 \times 10^{3ab}$
E_4	$2.65 \pm 2.92 \times 10^{3a}$
E_5	$3.03 \pm 2.97 \times 10^{3ab}$
Mean	$\mathbf{3.49 \pm 2.97 \times 10^3}$

E_1- E_5 represent the first to the fifth samples at High level.

Table 6 Bacterial Counts (cfu/g) Across Samples Locations

SPOT	WURUKUM	WADATA	OLD GRA	NORTH BANK	HIGHLEVEL
A_1-E_1	$3.70 \pm 3.69 \times 10^{3b}$	$5.54 \pm 4.74 \times 10^{3a}$	$6.38 \pm 7.46 \times 10^{3a}$	$1.33 \pm 0.94 \times 10^{3b}$	$4.13 \pm 3.42 \times 10^{3a}$
A_2-E_2	$4.79 \pm 3.68 \times 10^{3a}$	$4.85 \pm 3.08 \times 10^{3ab}$	$4.53 \pm 4.50 \times 10^{3ab}$	$1.55 \pm 1.22 \times 10^{3b}$	$4.54 \pm 3.58 \times 10^{3a}$
A_3-E_3	$2.83 \pm 2.16 \times 10^{3b}$	$3.17 \pm 2.48 \times 10^{3b}$	$1.41 \pm 1.16 \times 10^{3ab}$	$1.01 \pm 0.73 \times 10^{3b}$	$3.11 \pm 2.55 \times 10^{3ab}$
A_4-E_4	$3.36 \pm 2.03 \times 10^{3ab}$	$6.55 \pm 4.85 \times 10^{3a}$	$2.18 \pm 2.35 \times 10^{3a}$	$2.90 \pm 2.43 \times 10^{3b}$	$2.65 \pm 2.92 \times 10^{3a}$
A_5-E_5	$3.15 \pm 2.54 \times 10^{3b}$	$3.43 \pm 2.8 \times 10^{3b}$	$0.00 \pm 7.46 \times 10^{3b}$	$1.82 \pm 1.61 \times 10^{3b}$	$3.03 \pm 2.97 \times 10^{3ab}$
Mean	$\mathbf{3.57 \pm 2.78 \times 10^3}$	$\mathbf{4.71 \pm 3.81 \times 10^3}$	$\mathbf{2.90 \pm 4.43 \times 10^3}$	$\mathbf{1.82 \pm 1.61 \times 10^3}$	$\mathbf{3.49 \pm 2.97 \times 10^3}$

Mean on the same row with different superscript, differ significantly ($P<0.05$)

Table 7 present the antibiotic susceptibility pattern of the isolated bacteria to some common antibiotics. The results showed that Ciprofloxacin was the most active among the antibiotics tested with 100% activity against most of the bacterial isolates except *Shigella* species, which had 55.56% susceptibility to the antibacterial agents. Amoxycillin and ampiclox were least in terms of activity against the bacterial isolates.

Table 7 Antibiotic Susceptibility Pattern of Bacteria Isolated from Samples Across Locations. (Figure in Parenthesis are Percentages)

	Staphyloccus sp	*Baccillus sp*	*Escherichia coli*	*Salmonella sp*	*Proteus sp*	*Klebsiella sp*	*Shigella sp*
	N=25	N=20	N=17	N=16	N=32	N=33	N=18
PEF	12 (48.001)	16(80)	3(17.65)	2(12.50)	21(65.63)	26(78.79)	11(61.11)
CPX	24(96.00)	20(100)	17(100)	16(100)	31(96.88)	29(87.88)	10(55.56)
OFX	20(80.00)	13(65.00)	16(94.12)	14(87.50)	28(87.50)	23(69.70)	14(77.78)
SXT	10(40.00)	12(60.08)	0(0.00)	3(18.75)	12(37.50)	26(78.79)	12(66.67)
CN	18(72.00)	18(90.00)	12(70.59)	6(37.50)	28(87.50)	31(93.93)	12(66.67)
AU	21(84.00)	19(95.08)	11(64.71)	15(93.75)	30(93.75)	33(100)	15(83.33)
AM	0(0.00)	2(10.00)	1(5.88)	4(25.00)	10(31.25)	11(33.33)	3(16.67)
CHL	16(64)	12(60.00)	9(52.94)	14(87.50)	32(100)	31(93.93)	16(88.89)
APX	0(0.00)	0.(0.00)	1(5.88)	0(0.00)	3(9.38)	12(36.36)	0(0.00)

Key: PEF = Pefloxacin; CPX = Ciprofloxacin; OFX = Ofloxacin; SXT = Septrin; CN = Gentamycin; AU = Augmentine; AM = Amoxicillin; CHL = Chlorophenicol; APX = Ampiclox

4. Discussion

Bacteria contamination of street vended ready-to-eat meat, such as roasted chicken, is of serious public health concern in Makurdi metropolis due to increase in the consumption of the product in recent years (Bryan et al, 1988). The results of the bacterial analysis in this study showed that, the bacterial count of samples ranged from 1.01×10^3 cfu/g (Sample D_3 in Northbank) to 6.55×10^3 cfu|g for sample B_4 of Wadata. These values fall within tolerable limits (less than 10^6cfu|g) set out by Centre for Food Safety (CFS, 2014 and NAFDAC reported by Oyebode 2005). The mean viable and total coliform counts were from 3.07×10^3cfu/g(Northbank) to 7.97×10^3cfu/g(Wadata), 2.08×103cfu/g (Old GRA) to 5.78×103cfu/g (Wadata) and 2.00×10^2(Highlevel) to 4.42×10^2cfu/g(Wurukum) respectively. These values disagree with that of Bryan et al., 1997 who reported as 10^7 in Zambia.

The cultural, morphological and biochemical characteristics of the bacteria isolated and identified gave seven genera of bacteria, which included *Proteus*, *Staphylococcus*, *Salmonella*, *Shigella*, *Escherichia coli*, *Klebsiella* and *Bacillus*. These findings agreed with Djoulde et al., (2015) in Cameroon and little et al., (2002) who, in their separate studies, implicated those genera of bacteria. Though, the bacteria counts of these samples fall within tolerable limits (CFS, 2014 and NAFDAC reported by Oyebode, 2005). However, the presence of coliforms like *Salmonella*, *Shigella*, *Proteus*, *Klebsiella* and *Escherichia coli* are of serious public health concern. *Escherichia coli,* which is an indicator of fecal contamination, its presence in this food samples suggested serious contamination of the analyzed roasted chicken, making it a possible route of food-borne disease outbreak. In addition, the presence of several coliforms suggest poor hygienic practiced by vendors or handlers of these products. *Salmonella* and *Shigella* species are causative agents of food poisoning, hence, their presence is a threat to food safety. Meat, being a good source of protein is also a rich medium for bacteria proliferation, including harmful microorganisms. It has been reported that Street-vended foods have been implicated in the outbreaks of food-borne illnesses (Bryan et al, 1988). The presence of specific bacteria such as *Escherichia coli, Staphylococcus aureus* and *Salmonella* in foods served by the street vendors is indicative of a degree of ignorance on the part of the handlers towards proper hygienic practices. *Staphylococcus* species are common environmental bacteria and could thus have been introduced into the food after cooking through cross contamination, for instance, from utensils used by the vendors (Mosupy and Holy, 1999). Their presence in food indicates poor personal hygiene and poor processing by the vendors. The presence of *Staphylococcus aureus* in heat treated food is a pointer to largely poor personal hygiene, improper storage facilities and unhygienic environment.

On the percentage prevalence of bacteria in the samples analyzed, *Klebsiella* species was the most prevalent of the bacterial isolates, accounting for 20.50% of the total bacterial isolates obtained. This was followed closely by *Proteus* species (19.88%). Conversely, *Salmonella* specie was least with 9.98%. This is in line with Djoulde, (2015).

The antibiotic susceptibility of the bacteria isolates to antibacterial agents shows that most of the bacteria isolates showed high level of resistance against common antibiotics like amoxicillin, ampiclox, septrin and streptomycin. On the other hand, gentamycin, chloramphenicol, ofloxacin, pefloxacine and ciprofloxacin showed moderate activity against the tested bacterial isolates.

5. Conclusion

From the results of this work, it can be concluded that *Staphylococcus*, S*almonella*, *Proteus, Escherichia coli*, *Klebsiella* and *Shigella* species were the bacterial genera associated with contamination of ready-to-eat roasted spiced chicken sold along road sides in Makurdi town. The bacterial counts ranged from $1.01\times10^3\pm0.73\times10^3$cfu/g to $6.55\times10^3\pm4.85\times10^3$cfu/g indicating high bacteria contamination of the samples analyzed. The antibiotic susceptibility pattern showed that most of the bacterial isolated were highly susceptible to ciprofloxacin, augmentin, ofloxacin, chloramphenicol and gentamicin while they showed high level of resistance to ampiclox, amoxicillin, pefloxacin and septrin.

More importantly, stringent measures should be recommended to prevent bacteria contamination of ready-to-eat roasted spiced chicken sold along road sides, such as, enteric pathogens, so as to curb possible outbreak of food-borne diseases.

There is need for better packaging of ready-to-eat roasted spiced chicken (using aluminium foil for packaging rather than newspaper that had passed through different stages of contamination) to reduce their level of contamination, and also, there is need for public health officer to carry out point of sales exercise to educate the chicken vendors to stop or avoid using same kitchen utensils such as knife, used in cutting the raw chicken during preparation to chop the ready to serve chicken for customers, as this will prevent cross contamination . However, where packaging is not possible, they should be kept in safer places like show glasses in airtight container to prevent contamination from air, roadway dust and automotive pollutant.

The use of wrong dosage of antibiotics (drug abuse) should be discouraged to prevent antibiotics resistance bacteria.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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