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BACTERIOLOGICAL SAFETY OF SUYA, A READY-TO-EAT BEEF PRODUCT, AND ITS ASSOCIATION WITH ANTIBIOTIC-RESISTANT PATHOGENS IN NIGERIA

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ABSTRACT

The rapid development antibiotic-resistant food pathogens pose a heightened threat to public health. This study investigated the antibiotic resistance pattern of bacteria associated with suya meat, a ready-to-eat beef product, in Nigeria. Three hundred suya meat samples were cultured and pure isolates identified by API 20E and API 20NE. The resistance profile of isolates was determined using disc diffusion methods. Data were analysed by one-way analysis of variance and students' T-tests. The mean total plate counts (TPCs) of samples ranged from 1.0 x 10^5 to 3.7 x 10^5 CFU/g. There were no significant differences among the TPCs from zones A, B, C and D (P> 0.05). A total of 1014 isolates were obtained with *Pseudomonas aeruginosa* (13.51%) having the highest percentage occurrence and *Salmonella enteric* Typhimurium (1.48%), the lowest. A 92.90% portion of the isolates showed sensitivity to imipenem while 86.69% exhibited resistance to teicoplanin. This study revealed that the microbial quality of the ready-to-eat suya was at a borderline with reference to the microbiological guidelines for ready-to-eat animal food product. The study also revealed the presence of antibiotic-resistant bacteria in the ready-to-eat beef product which indicates a risk in food safety and a threat to public health. These findings will aid in the selection process of the right antibiotics in the treatment of food-borne infections while establishing the need for improvement on the microbial quality of the food product.

1.Introduction

Suya is a ready-to-eat spicy, barbecued, smoked or roasted meat. Its origin can be traced to the Hausa people of northern Nigeria, Sub-Saharan Africa, where their main occupation is rearing of cattle and growing of cash crops (Orogu and Oshilim, 2017). Thus, it is an important preoccupation and a major source of livelihood for the people. This generated the production of different types of beef products such as kundi, kilishi, balangu and suya, which are very popular protein-rich foods (Olayinka and Sani, 2014). However, suya is the most popular, as it is consumed in other parts of the country (El-Hassan *et al*., 2018). In the recent days, suya vendors are found in almost every nook and cranny of towns and cities with their grill stands, and are being patronized from midday to late at night. It has gradually made its way into elite circles where it has become a delicacy served at parties and other social events.

Spices such as ginger, salt, peanut cake, and other seasonings, are usually used to marinate the thinly spliced meat, and then barbecued (Egbebi and Seidu, 2011). Dried pepper mixed with spices, and sliced onions could also be added when served this delicacy.

The suya marinade is composed of complex mixture of additives and spices but there is no standard recipe for its production. The composition and types of ingredients vary from individual to individual, and according to regional preferences (Nwakanma *et al*., 2015; Amadi *et al*., 2016). However, the idea of requesting for only suya meat without the addition of the spices is becoming popular in the country. The reasons for this vary among individuals. Some simply prefer the taste of suya meat to having the spices sprinkled on it.

Barber *et al*. (2018) reported that there was an increased occurrence of disease outbreak caused by pathogenic and spoilage microorganisms in foods. The importance of foodborne diseases as a public health problem is often overlooked because their true incidence is difficult to evaluate and the severity of their health and economic impact is often not fully understood (Hassan *et al.,* 2014). Bacteria are considered the most common cause of foodborne illness representing two-thirds of foodborne disease outbreaks and wide variety of microbes with much common and less specific clinical symptoms (Bello *et al*., 2019).

The rapid development of multidrug resistance in microorganisms has become an increasingly emerging problem with serious consequences on public health (WHO, 2014). The resistance of bacteria to commonly prescribed antimicrobial agents are associated with increasing treatment failures, and which could be explained by the high frequency with which antimicrobials are used empirically to treat diseases (Ikechukwu *et al*., 2019). This implies that as resistant pathogens develop and spread, the effectiveness of the antibiotics diminishes. The aim of this study, therefore, was to investigate the antibiotic resistance profiles of bacteria of clinical importance associated with ready-to-eat suya in Nigeria.

2.Materials and methods

2.1.Study Area

Ogun State is a state in southwestern Nigeria. The estimated population is 5,217,716 according to the National Population Commission (NPC) and the National Bereau of Statistics (NBS) in 2013. The four geopolitical zones in Ogun State which include Yewa, Egba, Remo and Ijebu zones were the areas in which samplings were done.

2.3.Processing of suya meat

Sixty sticks of suya were personally prepared under aseptic conditions in accordance with standard procedures to serve as the control using beef. Putting all necessary precautions into consideration, meat samples were sliced into thin sheets and were inserted onto the suya sticks. Each stick of meat was pressed on the already prepared ingredient spread on a flat tray in order that the ingredient is evenly soaked into it. Groundnut oil was then sprinkled on the well-labeled sticks of meat before roasting was carried out.

2.2.Collection of suya meat samples

Two hundred and forty suya meat samples were purchased from different areas in the four geopolitical zones (Yewa, Egba, Remo and Ijebu zones) of Ogun State. Sixty suya samples from each of the four zones (60 x 4 = 240) were purchased from six different spots from ten different areas. The samples were collected and kept in refrigerator at 4 ^{o}C overnight to prevent contamination (as samples were purchased at night) and then transported to the laboratory for microbial analyses. Laboratory-based suya was prepared as a control each time suya meat samples were analysed in the laboratory, which summed up to 60 times. Invariably, a total of 300 suya samples were investigated in this study (Apata *et al*., 2013). For the purpose of this study, Yewa, Egba, Remo and Ijebu zones were labeled zones A, B, C and D, respectively while the samples prepared in the laboratory served as control.

2.4.Roasting of suya meat

Labeled stick meats were arranged round a glowing smokeless fire made from charcoal. The sticked meats were allowed to stay on the fire for 20 min with the distance of 22-23 cm from the centre of fire and intermittent turning

of the product. Groundnut oil was intermittently sprinkled while the meat was being roasted.

2.5.Cultivation of bacteria from suya meat

The microbiological analysis of suya meat was carried out as described by American Public Health Association (APHA, 1992) and Association of Official Analytical Chemist (AOAC, 2000). Ten grams each of suya samples were blended using a disinfected blender (model 242 NAKAI, JAPAN) with 90 ml of 0.1% (W/V) peptone water for 60 s and serial dilutions made in 0.1% peptone water (W/V) to obtain up to 10^{-5} dilution factor while 0.1 ml portion of each of the diluted samples was taken and dispensed in sterile Petri dishes containing appropriate agar medium using the spread plate method. Plate count agar (PCA) (Difco, USA) was employed for the determination of total bacterial count; violet red bile agar (VRBA) (Difco, USA) for total coliform.

Specific coliform organisms were differentiated by IMViC tests. Eosin methylene blue (EMB) agar (Oxoid, England) was used for the cultivation of *Escherichia coli* and *Enterobacter aerogenes*. For the isolation and enumeration of *Salmonella*, pre-enrichment was carried out using lactose broth incubated at 37 ^{o}C for 24 hrs. Ten (10) ml of pre-enriched medium was, under aseptic condition, pipetted and tranferred into 100 ml sterile Tetrathionate broth (Hi-media laboratories, India) incubated at 37 ^{o}C for 24 hrs. The resultant broth was streaked onto three *Salmonella* differential media which were brilliant green phenol lactose agar (Difco, USA), Bismuth sulphate agar (Difco, USA) and deoxycholate citrate agar (Oxoid, England).

For the identification of *Staphylococcus aureus* and micrococci, the sample was inoculated onto nutrient broth at 37 °C for 24 hours after which it was plated onto mannitol salt agar. Colony forming units were counted and were expressed in log10 cfu/g of samples. The microbiological guidelines for ready-to-eat food products are given in Table 1.

Table 1. Microbiological Guidelines for Ready-to-eat Food Products

Grades	TVC (total viable count)/g at 30°C	Description
I	$< 10^5$	Satisfactory
II	10^5- $< 10^7$	Borderline
III	$\geq 10^7$	Unsatisfactory

Center for Food Safety (2014).

2.6.Identification of bacterial isolates

Pure isolates on agar plates were characterized by initial morphological examination of the distinct colonies. The biochemical tests included catalase test, coagulase test, citrate utilization test, oxidase test, triple sugar iron agar, urease test, sugar fermentation test, methyl red test and indole production test (Cowan and Steel, 1985). *Salmonella* serotyping was by slide agglutination (Kauffmann-White-Le Minor scheme) based on the agglutination of bacteria with specific sera to identify variants of the somatic (O) and flagellar (H) antigens (Grimont and Weill, 2007; Guibourdenche *et al*., 2010). Specific identification of the other isolates was performed using the API 20E and API 20NE for confirmation of members of Enterobacteriaceae and non-Enterobacteriaceae, respectively.

2.7.Determination of antibiotic sensitivity of bacterial isolates

The disc diffusion assay was employed to investigate the sensitivity of isolates to the antibiotics (Clinical and Laboratory Standards Institute, CLSI, 2016). The standardized inocula of the test organisms were emulsified on the surface of Mueller Hinton Agar (Oxoid, England) using sterile cotton swab (220210 BD

SWUBE, India), and the plates was dried at room temperature for 5 min, and then incubated at 30 ^{o}C for 48 hours (Center for Food Safety, 2014). A total of 18 antibiotics representing 12 antibiotic classes were investigated against isolates from the suya samples.

The antibiotics investigated were Tetracycline (0.002–32 mg/L), Doxycycline (0.002–32 mg/L), Minocycline (0.002–32 mg/L), Erythromycin (0.016–256 mg/L), Colistin (0.064–1024 mg/L), Chloramphenicol (0.016–256mg/L), Trimethoprim/ Sulfamethoxazole (0.002–32 mg/L), Gentamicin (0.016–256 mg/L), Rifampicin (0.002–32 mg/L), Nalidixic acid (0.016–256 mg/L), Ciprofloxacin (0.002–32 mg/L), Penicillin G (0.002–32 mg/L), Ampicillin (0.016–256 mg/L), Imipenem (0.002–32 mg/L), Cefalotin (0.016–256 mg/L), Ceftriaxone (0.016–256 mg/L), Teicoplanin (0.016–256 mg/L) and Vancomycin (0.016–256 mg/L). A phase-contrast microscope with objective E.10 0.25 160/- (Nikon, France) was used (100× magnification) to read the limit of growth inhibition.

2.8.Data Analysis

Data were collated and statistically analysed using MedCalc statistical software, version 17.2. Simple means, percentages and frequencies from different locations were computed and compared using One-way Analysis of Variance (ANOVA) and independent T-test. Data were presented as mean ± standard error (SE) of triplicate data. The significance was determined at 95% level of confidence ($P \leq 0.05$).

3.Results and Discussions

3.1.Mean total bacterial counts from suya samples

Each value ($_{1} - _{10}$) in each zone represents mean of data obtained from six different suya spots in same area. The mean total plate count (TPC) from zones A, B, C and D ranged from 1.4 x 10^5 to 3.5 x 10^5 CFU/g, 1.1 x 10^5 to 3.5 x 10^5 CFU/g, 1.0 x 10^5 to 3.1 x 10^5 CFU/g and 1.0 x 10^5 to 3.7 x 10^5, respectively. The total Enterobacteriaceae counts (TECs) ranged from 1.0 x 10^3 to 2.3 x 10^3 CFU/g, 1.5 x 10^3 to 2.1 x 10^3 CFU/g, 1.1 x 10^3 to 2.5 x 10^3 CFU/g and 1.0 x 10^3 to 2.5 x 10^3 CFU/g as obtained in zones A, B, C and D, respectively. Total *Staphylococcus* count (TSCs) of suya samples ranged from 1.1 x 10^2 to 3.1 x 10^2 CFU/g, 1.1 x 10^2 to 3.0 x 10^2 CFU/g, 1.0 x 10^2 to 3.1 x 10^2 CFU/g and 1.0 x 10^2 to 2.2 x 10^2 CFU/g as encountered in zones A, B, C and D, respectively. There were no significant differences among the bacterial counts from zones A, B, C and D ($P > 0.05$) but values showed statistical differences from the control ($P < 0.05$) (Table 2).

Table 2. Mean total bacterial counts from suya samples in Nigeria

Zone	Area	Total plate count (TPC) (CFU/g)	Total Enterobacteria count (TEC) (CFU/g)	Total *Staphylococcus* count (TSC) (CFU/g)
A	A_1	1.9 x 10^5	2.3 x 10^3	1.2 x 10^2
	A_2	3.5 x 10^5	1.0 x 10^3	1.7 x 10^2
	A_3	2.3 x 10^5	2.2 x 10^3	2.1 x 10^2
	A_4	2.5 x 10^5	2.1 x 10^3	1.8 x 10^2
	A_5	2.1 x 10^5	1.8 x 10^3	3.1 x 10^2
	A_6	1.7 x 10^5	1.6 x 10^3	2.2 x 10^2
	A_7	1.4 x 10^5	1.8 x 10^3	2.1 x 10^2
	A_8	1.8 x 10^5	2.3 x 10^3	1.4 x 10^2
	A_9	2.6 x 10^5	2.1 x 10^3	2.3 x 10^2
	A_{10}	2.0 x 10^5	1.4 x 10^3	1.1 x 10^2
	Mean of means	2.18 x 10^{5a}	1.86 x 10^{3b}	1.90 x 10^{2c}

B	B_1	2.7×10^5	1.7×10^3	1.5×10^2
	B_2	1.3×10^5	1.5×10^3	3.0×10^2
	B_3	1.1×10^5	2.0×10^3	2.2×10^2
	B_4	1.9×10^5	1.8×10^3	1.5×10^2
	B_5	2.4×10^5	1.7×10^3	1.1×10^2
	B_6	2.9×10^5	1.5×10^3	1.3×10^2
	B_7	2.1×10^5	2.0×10^3	1.2×10^2
	B_8	3.5×10^5	2.1×10^3	2.1×10^2
	B_9	2.3×10^5	1.9×10^3	1.3×10^2
	B_{10}	2.2×10^5	1.5×10^3	1.2×10^2
	Mean of means	2.24×10^{5a}	1.77×10^{3b}	1.64×10^{2c}
C	C_1	3.1×10^5	1.7×10^3	1.2×10^2
	C_2	2.1×10^5	1.2×10^3	1.8×10^2
	C_3	1.2×10^5	2.3×10^3	1.6×10^2
	C_4	1.6×10^5	1.6×10^3	2.1×10^2
	C_5	1.0×10^5	2.1×10^3	2.2×10^2
	C_6	1.3×10^5	2.5×10^3	1.7×10^2
	C_7	1.7×10^5	1.3×10^3	2.1×10^2
	C_8	2.5×10^5	1.1×10^3	1.6×10^2
	C_9	2.7×10^5	1.8×10^3	1.1×10^2
	C_{10}	1.4×10^5	1.6×10^3	1.0×10^2
	Mean of means	1.72×10^{5a}	1.72×10^{3a}	1.64×10^{2b}
D	D_1	2.5×10^5	1.4×10^3	2.1×10^2
	D_2	2.3×10^5	1.9×10^3	1.9×10^2
	D_3	1.9×10^5	1.5×10^3	2.0×10^2
	D_4	1.0×10^5	2.5×10^3	1.3×10^2
	D_5	3.7×10^5	1.0×10^3	2.1×10^2
	D_6	1.2×10^5	1.6×10^3	1.4×10^2
	D_7	2.0×10^5	1.1×10^3	1.3×10^2
	D_8	1.6×10^5	1.6×10^3	2.2×10^2
	D_9	1.5×10^5	1.6×10^3	1.8×10^2
	D_{10}	1.1×10^5	1.2×10^3	1.1×10^2
	Mean of means	1.88×10^{5a}	1.54×10^{3b}	1.72×10^{2c}
Control	Cont.1	1.1×10^4	0	1.1 x 10
	Cont.2	1.5×10^4	1.0×10^2	1.0 x 10
	Cont.3	1.2×10^4	0	2.0 x 10
	Cont.4	1.6×10^4	1.1×10^2	1.3 x 10
	Cont.5	1.0×10^4	0	1.2 x 10
	Cont.6	1.3×10^4	0	1.7 x 10
	Cont.7	1.4×10^4	1.0×10^2	2.1 x 10
	Cont.8	1.2×10^4	0	1.0 x 10
	Cont.9	1.3×10^4	0	1.1 x 10
	Cont.10	1.4×10^4	1.6×10^2	1.5 x 10
	Mean	1.30×10^{4b}	0.47×10^{2c}	1.40×10^a

Each value ($_{1 - 10}$) in each zone represents mean of data obtained from six different suya spots in the area. Means of means with same superscript along same column showed no statistical difference.

Table 3. Morphological and biochemical characteristics of bacteria isolated from suya samples in Nigeria

Gram Reaction	Cellular morphology	Catalase	Oxidase	Coagulase	Indole	Motility	Methyl-Red	VogesProskauer	Urease activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Invositol	Raffinose	Frauction	No of Isolates	Isolates per zone	Most Probable Identity
-ve	R	-	+	-	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	105	A=25,B=25,C=22,D=22,CT=11	*E. coli*
-ve	R	+	-	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	55	A=11,B=11,C=13,D=13,CT=7	*E. aerogenes*
-ve	R	+	+	-	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	85	A=19,B=20,C=18,D=17,CT=11	*S. rubidaea*
-ve	R	+	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	137	A=30,B=28,C=32,D=32,CT=15	*P. aeruginosa*
+ve	C	+	-	+	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	114	A=24,B=25,C=24,D=25,CT=16	*S. aureus*
+ve	R	+	+	-	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	54	A=12,B=11,C=11,D=12,CT=8	*C. freundii*
+ve	C	+	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	ND	ND	ND	+	125	A=20,B=25,C=30,D=31,CT=19	*S. epidermidis*
+ve	R	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	107	A=24,B=24,C=25,D=22,CT=12	*B. cereus*
+ve	C	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	ND	-	85	A=20,B=18,C=18,D=18,CT=11	*M. luteus*
+ve	R	+	+	-	-	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	-	+	26	A=7,B=6,C=5,D=5,CT=3	*B. subtilis*
+ve	R	-	-	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	27	A=8,B=17,C=0,D=2,CT=0	*C. butyricum*
-ve	R	+	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	47	A=11,B=7,C=12,D=13,CT=4	*K. pneumoniae*
-ve	R	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	32	A=6,B=6,C=7,D=7,CT=6	*K. planticola*
-ve	R	+		-	-	+	-	+	-	+	+	-	-	-	+	+	-	-	+	+	-	-	-	-	-	+	15	A=4,B=3,C=3,D=5,CT=0	*S. enterica*Typhimurium

Keys: R = Rods; + = Positive reaction; - = Negative reaction; ND = Not determined; A = Zone A; B = Zone B; C =Zone C; D = Zone D and CT =Control.

Table 4. Percentage antibiotic susceptibility of bacterial isolates from suya meat samples in Nigeria

Antibiotic		*E. coli*	*Enterobacter aerogenes*	*Serratiarubidae a*	*P. aeruginosa*	*C. freundii*	*S. aureus*	*S. epidermidis*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Clostridium butyricum*	*K. pneumoniae*	*K. planticola*	*Salmonella* Typhimurium
	Status	n=105	n=55	n=85	n=137	n=54	n=114	n=125	n=107	n=26	n=85	n=27	n=47	n=32	n=15
Tetracycline	S	105 (100%)	55 (100%)	80 (94.1%)	5 (3.7%)	54 (100%)	80 (70.2%)	125 (100%)	76 (71%)	26 (100%)	85 (100%)	20 (74.1%)	29 (61.7%)	32 (100%)	15 (100%)
	I	0	0	5 (5.9%)	0	0	4 (3.5%)	0	0	0	0	0	4 (8.5%)	0	0
	R	0	0	0	132 (96.4%)	0	30 (26.3%)	0	31 (29%)	0	0	7 (25.9%)	14 (51.9%)	0	0
Doxycycline	S	105 (100%)	55 (100%)	85 (100%)	38 (27.7%)	54 (100%)	99 (86.8%)	125 (100%)	107 (100%)	26 (100%)	79 (92.9%)	27 (100%)	47 (100%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	0	0	0	0	6 (7.1%)	0	0	0	0
	R	0	0	0	99 (72.3%)	0	15 (13.1%)	0	0	0	0	0	0	0	0
Minocycline	S	105 (100%)	55 (100%)	64 (75.3%)	57 (41.6%)	54 (100%)	103 (90.4%)	125 (100%)	107 (100%)	26 (100%)	85 (100%)	27 (100%)	47 (100%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	21 (24.7%)	82 (59.9%)	0	11 (9.7%)	0	0	0	0	0	0	0	0
Erythromycin	S	77 (73.3%)	34 (61.8%)	25 (29.4%)	0	29 (53.7%)	114 (100%)	107 (85.6%)	38 (35.5%)	11 (42.3%)	71 (83.5%)	20 (74.1%)	15 (31.9%)	12 (37.5%)	4 (26.7%)
	I	5 (1.0%)	0	0	10 (7.3%)	0	0	0	0	0	0	0	6 (12.8%)	0	2 (13.3%)
	R	23 (21.9%)	21 (38.2%)	20 (23.5%)	127 (92.7%)	26 (48.2%)	0	18 (14.4%)	69 (64.5%)	15 (57.7%)	14 (16.5%)	7 (25.9%)	26 (55.3%)	20 (62.5%)	9 (60%)
Keys: S = Suseptible; I = Intermediately Susceptible and R = Resistance															

Antibiotic		*E. coli*	*Enterobacter aerogenes*	*Serratiarubidaea*	*P. aeruginosa*	*C. freundii*	*S. aureus*	*S. epidermidis*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Clostridium butyricum*	*K. pneumoniae*	*K. planticola*	*Salmonella* Typhimurium
	Status	n=105	n=55	n=85	n=137	n=54	n=114	n=125	n=107	n=26	n=85	n=27	n=47	n=32	n=15
Colistin	S	105 (100%)	55 (100%)	85 (100%)	137 (100%)	54 (100%)	11 (9.7%)	13 (10.4%)	0	0	14 (16.5%)	0	47 (100%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	14 (12.3%)	0	11 (10.3%)	0	0	0	0	0	0
	R	0	0	0	0	0	89 (78.1%)	112 (89.6%)	96 (89.7%)	26 (100%)	71 (83.5%)	27 (100%)	0	0	0
Chloramphenicol	S	105 (100%)	49 (89.1%)	85 (100%)	38 (27.7%)	54 (100%)	107 (93.9%)	91 (72.8%)	84 (78.5%)	12 (24.3%)	79 (92.9%)	16 (59.3%)	47 (100%)	32 (100%)	11 (73.3%)
	I	0	6 (10.9%)	0	19 (13.9%)	0	0	0	0	0	0	5 (18.5%)	0	0	0
	R	0	0	0	80 (58.4%)	0	7 (6.1%)	34 (27.2%)	23 (21.5%)	14 (13.1%)	6 (7.1%)	6 (22.2%)	0	0	4 (26.7%)
Trimethoprim/ sulfamethoxazole	S	105 (100%)	55 (100%)	85 (100%)	58 (42.3%)	54 (100%)	109 (95.6%)	125 (100%)	107 (100%)	26 (100%)	78 (91.8%)	9 (33.3%)	47 (100%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	5 (4.4%)	0	0	0	0	4 (14.8%)	0	0	0
	R	0	0	0	79 (57.7%)	0	0	0	0	0	7 (8.2%)	14 (51.9%)	0	0	0
Gentamicin	S	87 (82.9%)	55 (100%)	85 (100%)	73 (53.3%)	54 (100%)	80 (70.2%)	106 (84.8%)	81 (75.7%)	18 (69.2%)	85 (100%)	10 (3.7%)	39 (83%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	9 (7.9%)	7 (5.6%)	0	0	0	5 (18.5%)	0	0	0
	R	18 (17.1%)	0	0	64 (46.7%)	0	25 (21.9%)	12 (9.6%)	26 (24.3%)	18 (69.2%)	0	12 (44.4%)	8 (17%)	0	0
Keys: S = Suseptible; I = Intermediately Susceptible and R = Resistance															

Antibiotic		*E. coli*	*Enterobacter aerogenes*	*Serratiarubidaea*	*P. aeruginosa*	*C. freundii*	*S. aureus*	*S. epidermidis*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Clostridium butyricum*	*K. pneumoniae*	*K. planticola*	*Salmonella* Typhimurium
	Status	n=105	n=55	n=85	n=137	n=54	n=114	n=125	n=107	n=26	n=85	n=27	n=47	n=32	n=15
Rifampicin	S	0	0	29 (34.1%)	0	29 (53.7%)	114 (100%)	125 (100%)	39 (36.5%)	9 (34.6%)	85 (100%)	0	0	0	0
	I	0	0	9 (10.6%)	0	5 (9.3%)	0	0	14 (13.1%)	0	0	0	0	0	0
	R	105 (100%)	55 (100%)	47 (55.3%)	137 (100%)	20 (37%)	0	0	54 (50.5%)	17 (64.5%)	0	27 (100%)	47 (100%)	32 (100%)	15 (100%)
Nalidixic acid	S	76 (72.4%)	48 (87.3%)	64 (75.3%)	0	31 (57.4%)	0	0	0	0	0	27 (100%)	36 (76.6%)	32 (100%)	12 (80%)
	I	8 (7.6%)	0	0	5 (3.7%)	0	0	0	8 (7.48%)	0	0	0	0	0	0
	R	21 (20%)	7 (12.7%)	21 (24.7%)	132 (96.4%)	23 (42.6%)	114 (100%)	125 (100%)	99 (92.5%)	26 (100%)	85 (100%)	0	11 (23.4%)	0	3 (20%)
Ciprofloxacin	S	83 (79.1%)	55 (100%)	85 (100%)	68 (49.6%)	54 (100%)	99 (86.8%)	106 (84.8%)	27 (25.2%)	20 (76.9%)	31 (36.5%)	10	41 (87.2%)	32 (100%)	15 (100%)
	I	0	0	0	0	0	4 (3.5%)	7 (5.6%)	0	0	0	5 (18.5%)	0	0	0
	R	22 (21%)	0	0	69 (50.4%)	0	11 (9.7%)	12 (9.6%)	80 (74.8%)	6 (23.1%)	54 (63.5%)	12	6 (12.8%)	0	0
Penicillin G	S	0	0	34 (40%)	0	37 (68.5%)	92 (80.7%)	102 (81.6%)	77 (72%)	22 (84.6%)	85 (100%)	27 (100%)	0	0	0
	I	0	0	0	0	2 (3.7%)	0	0	0	0	0	0	0	0	0
	R	105 (100%)	55 (100%)	51 (60%)	137 (100%)	15 (27.8%)	22 (19.3%)	23 (18.4%)	30 (28.7%)	4 (15.4%)	0	0	47 (100%)	32 (100%)	15 (100%)

Keys: S = Suseptible; I = Intermediately Susceptible and R = Resistance

Antibiotic	Status	*E. coli*	*Enterobacter aerogenes*	*Serratiarubidaea*	*P. aeruginosa*	*C. freundii*	*S. aureus*	*S. epidermidis*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Clostridium butyricum*	*K. pneumoniae*	*K. planticola*	*Salmonella* Typhimurium
		n=105	n=55	n=85	n=137	n=54	n=114	n=125	n=107	n=26	n=85	n=27	n=47	n=32	n=15
Ampicillin	S	71 (67.7%)	48 (87.3%)	64 (75.3%)	58 (42.3%)	50 (92.6%)	80 (70.2%)	97 (77.6%)	54 (50.5%)	19 (73.1%)	62 (72.9%)	9 (33.3%)	26 (55.3%)	23 (71.9%)	10 (66.7%)
	I	0	0	0	7 (5.1%)	0	4 (3.5%)	8 (6.4%)	8 (7.5%)	0	7 (8.2%)	0	0	0	0
	R	34 (32.4%)	7 (12.7%)	21 (24.7%)	72 (52.6%)	4 (7.4%)	30 (26.3%)	20 (16%)	45 (42.1%)	7 (26.9%)	16 (18.8%)	18 (66.7%)	21 (44.7%)	9 (28.1)	5 (33.3%)
Imipenem	S	101 (100%)	55 (100%)	85 (100%)	137 (100%)	54 (100%)	100 (87.7%)	125 (100%)	89 (83.2%)	26 (100%)	68 (80%)	27 (100%)	32 (68.1%)	32 (100%)	11 (73.3%)
	I	0	0	0	0	0	10 (8.8%)	0	6 (5.6%)	0	0	0	0	0	0
	R	0	0	0	0	0	4 (3.5%)	0	12 (11.2%)	0	17 (20%)	0	15 (31.9%)	0	4 (26.7%)
Cefalotin	S	23 (21.9%)	0	58 (68.2%)	39 (28.5%)	25 (46.3%)	95 (83.3%)	91 (72.8%)	66 (61.7%)	13 (50%)	68 (80%)	17 (63%)	14 (51.9%)	23 (71.9%)	9 (60%)
	I	0	9 (16.4%)	0	9 (6.6%)	6 (11.1%)	9 (7.9%)	10 (8%)	0	4 (15.4%)	0	0	5 (10.6%)	0	0
	R	82 (78.1%)	46 (83.6%)	27 (31.8%)	89 (65%)	23 (42.6%)	10 (18.5%)	24 (19.2%)	41 (38.3%)	9 (34.6%)	17 (20%)	10 (37%)	28 (59.6%)	9 (28.1)	6 (40)
Ceftriaxone	S	67 (63.8%)	10 (18.2%)	79 (93%)	0	54 (100%)	85 (74.5%)	107 (85.6%)	34 (31.8%)	19 (73.1%)	62 (72.9%)	9 (33.3%)	41 (87.2%)	32 (100%)	15 (100%)
	I	11 (10.5%)	0	6 (7.1%)	0	0	9 (7.9%)	0	0	0	7 (8.2%)	0	0	0	0
	R	27 (25.7%)	45 (81.8%)	0	137 (100%)	0	20 (17.5%)	18 (14.4%)	73 (68.2%)	7 (26.9%)	16 (18.8%)	18 (66.7%)	6 (12.8%)	0	0

Keys: S = Suseptible; I = Intermediately Susceptible and R = Resistance

Antibiotic	Status	*E. coli*	*Enterobacter aerogenes*	*Serratiarubi daea*	*P. aeruginosa*	*C. freundii*	*S. aureus*	*S. epidermidis*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Clostridium butyricum*	*K. pneumoniae*	*K. planticola*	*Salmonella* Typhimurium
		n=105	n=55	n=85	n=137	n=54	n=114	n=125	n=107	n=26	n=85	n=27	n=47	n=32	n=15
Teicoplanin	S	0	0	0	0	0	30 (26.3%)	31 (24.8%)	0	0	46 (54.1%)	19 (70.4%)	0	0	0
	I	0	0	0	0	0	9 (7.9%)	0	0	0	0	0	0	0	0
	R	105 (100%)	55 (100%)	85 (100%)	137 (100%)	54 (100%)	75 (65.8%)	94 (75.2%)	107 (100%)	26 (100%)	39 (45.9%)	8 (29.6%)	47 (100%)	32 (100%)	15 (100%)
Vancomycin	S	0	0	0	0	0	49 (43%)	42 (33.6%)	0	0	46 (54.1%)	19 (70.4%)	0	0	0
	I	0	0	0	0	6 (11.1%)	9 (7.9%)	5 (4%)	0	0	0	0	5 (10.6%)	0	0
	R	105 (100%)	55 (100%)	85 (100%)	137 (100%)	48 (88.9%)	56 (49.1%)	78 (62.4%)	107 (100%)	26 (100%)	39 (45.9%)	8 (29.6%)	42 (83.4%)	32 (100%)	15 (100%)

Keys: S = Suseptible; I = Intermediately Susceptible and R = Resistance

Higher incidence of microbial contaminants in suya had been previously reported in other places (Bakobie *et al*., 2017; Ribah and Manga, 2018; Ikechukwu *et al*., 2019). Amadi *et al*. (2016) also reported the occurrence of APC and TCC values of 1.39 x 10^5 cfu/g and 6.2x10^4 cfu/g in roasted suya meat samples. Poor water and personal hygiene qualities, traditional processing techniques and exposure of suya in unhealthy environment could be attributed to this phenomenon. Similar findings on microbial biodiversity in suya had been earlier reported (Hassan *et al*., 2014; Orogu and Oshilim, 2017; Riba and Manga, 2018) and these underscore the level of contamination of the ready-to-eat food product.

3.2.Identification of bacterial isolates

One thousand and fourteen (1014) bacterial isolates were obtained. Two hundred and twenty-one (221), 226, 220 and 224 isolates were encountered from zones A, B, C and D, respectively while 123 isolates were obtained from control samples. The isolates were characterized as *E. coli*, *Enterobacter aerogenes*, *Serratia rubidaea*, *P. aeruginosa*, *C. freundii*, *S. aureus*, *S. epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Clostridium butyricum*, *K. pneumoniae*, *K. planticola* and *Salmonella enterica* Typhimurium (Table 3).

The presence of *Salmonella enterica* Typhimurium in suya is of tremendous public health concern as this puts the presumably large number of consumers at risk of gastroenteritis. *Salmonella*'s ability to grow in food is largely dependent on storage temperature. It was recently reported by dos Santos *et al.* (2019) that *Salmonella enterica Typhimurium* is a leading cause of food poisoning cases in several countries. It is a non-specifically categorized as a zoonotic bacterium associated with animals and humans, but some strains could be invasive because of the ability to cross the intestinal wall and reach the systemic circulation (Almeida *et al*., 2017). The bacterium's pathogenicity ability could be attributed to its virulence factors.

C. perfringens are found in dust, soils, vegetation among other environmental media. Its presence could be attributed to growth parameters like favourable temperature. Equipment and food handlers have also been associated with contamination of food with various types of etiologic agents. *Staphylococcus* spp are abundant in the nose and throat as well as the skin of humans. This study agrees with the report of Uzeh *et al.* (2006) who isolated *Pseudomonas* sp., *Bacillus cereus* and *Staphylococcus aureus* from tsire-suya, a Nigerian meat product. This was also buttressed by the findings of Manyi *et al.* (2014) who reported *Streptococcus* sp., *Escherichia coli*, *Bacillus* sp., *Staphylococcus aureus*, *Klebsiella* sp. and *Pseudomonas* sp. in suya samples. The existence of these organisms in the suya could be attributable to the filthy environment, poor personal hygiene of the processors and retailers, the use of contaminated utensils during processing, use of contaminated materials for packaging, activities of flies as well as the addition of spices and seasonings during processing.

3.3.Percentage frequency of bacterial isolates

Data showed that percentage contamination of the suya samples from zones A, B, C and D were 21.80%, 22.29%, 21.70% and 22.09%, respectively while the control was 12.13%. There were no statistical differences among the level of bacterial contamination from zones A, B, C and D ($P > 0.05$). The data from these zones, however, showed significant differences from the control ($P < 0.05$) (Figure 1). The most occurred bacterium from the suya samples in zone A was *P. aeruginosa* with percentage occurrence of 13.58% while the lowest was *Salmonella enteric* Typhimurium with 1.81% (Figure 2). In all, the highest occurred bacterial species was *P. aeruginosa* (137; 13.51%) while the lowest was *Salmonella enteric* Typhimurium (15; 1.48%) (Figure 3).

The results of this study differ from a study by Onuorah *et al*. (2015) who reported *Escherichia coli* (34.3%) as the most frequent

while *Streptococcus pyogenes* (8.6%) had the lowest. *P. aeruginosa* is widely spread in nature especially in the soil, water, on plants and can easily contaminate food products. This finding agrees with the study of Egbebi and Muhammad (2016) who reported *P. aeruginosa* as the most predominant organism in their study. Higher percentage of organisms had earlier been reported (Kigigha *et al*., 2017; Orogu and Oshilim, 2017). There may be a possible outbreak of food poisoning and/or food-borne infections due to the consumption of contaminated suya meat, if appropriate quality control measures are not put in place. This may lead to serious economic and public health problems.

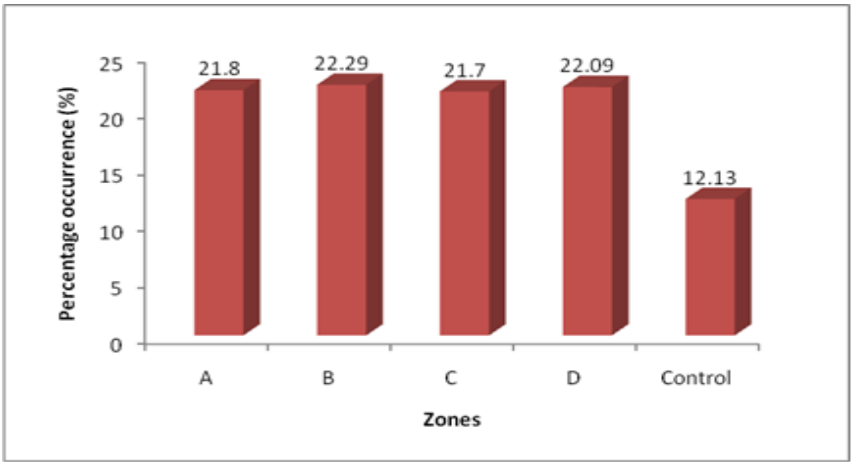

Figure 1. Percentage occurrence of bacteria in suya from different zones of Ogun State, Nigeria. Data were statistically analysed at 95% level of confidence (P<0.05) using ANOVA and paired wise sampling t-test.

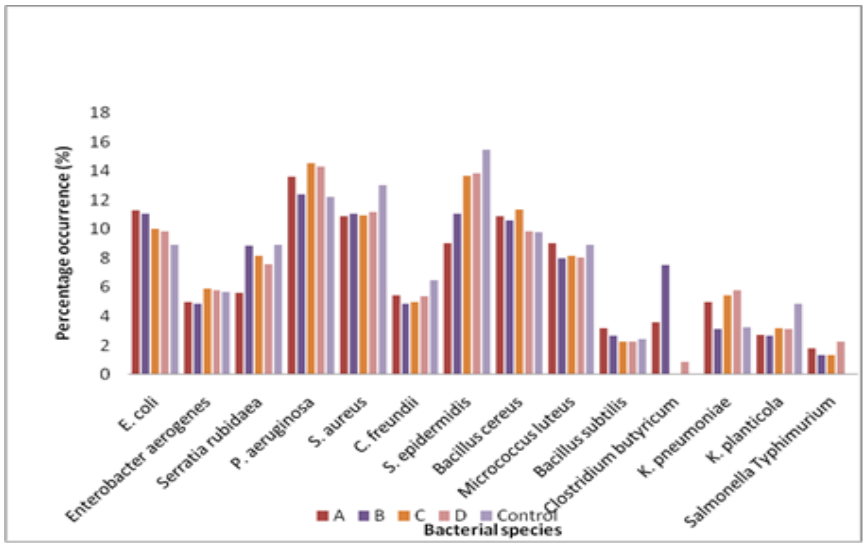

Figure 2. Percentage occurrence of individual bacterial species in suya from different geopolitical zones in Nigeria.

3.4.The antibiotic susceptibility patterns of bacterial isolates

For the purpose of simplification, a standardized, threshold-based assessment scheme has been introduced in which the degree of the effectiveness of the antibiotics investigated in this study is characterized as "susceptible (S)," "intermediate (I)," or "resistant (R)," based on their inbibition zones. The isolates showed varying degrees of

sensitivity to the antibiotics and are classified based on their zones of inhibitions (Table 4). Varying percentages including 11.20%, 15.09%, 9.86%, 18.05%, 21.10%, 34.81%, 30.57%, 36.19%, 41.50%, 36.70%, 43.49%, 52.86%, 54.83%, 68.64%, 82.15% and 86.69% of the isolated strains exhibited resistance to doxycycline, chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin, tetracycline, ciprofloxacin, ampicillin, cefriaxone, colistin, erythromycin, cefalotin, penicillin G, rifampicin, nalidixic acid, vancomycin and teicoplanin, respectively (Figure 4).

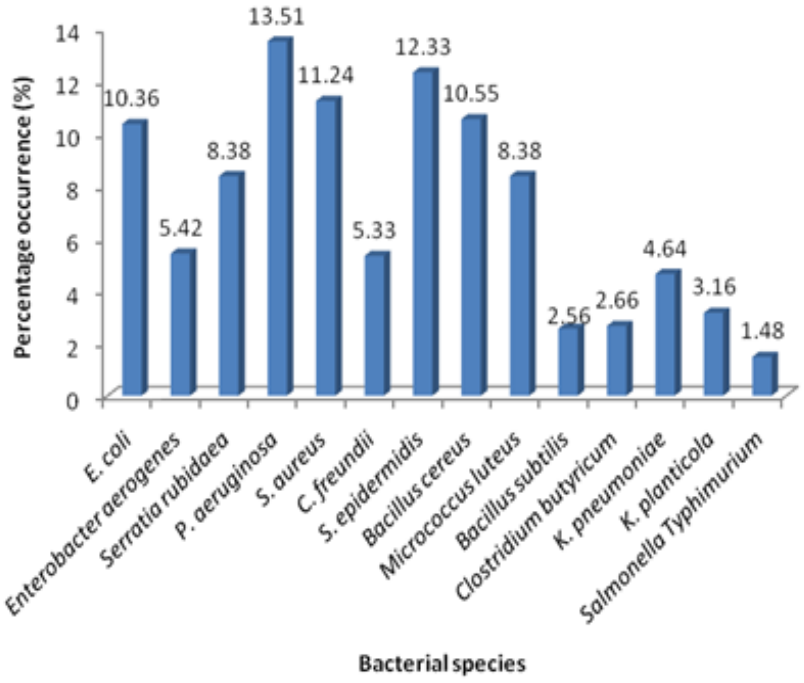

Figure 3. Cumulative percentage frequency of bacterial species from suya in Nigeria

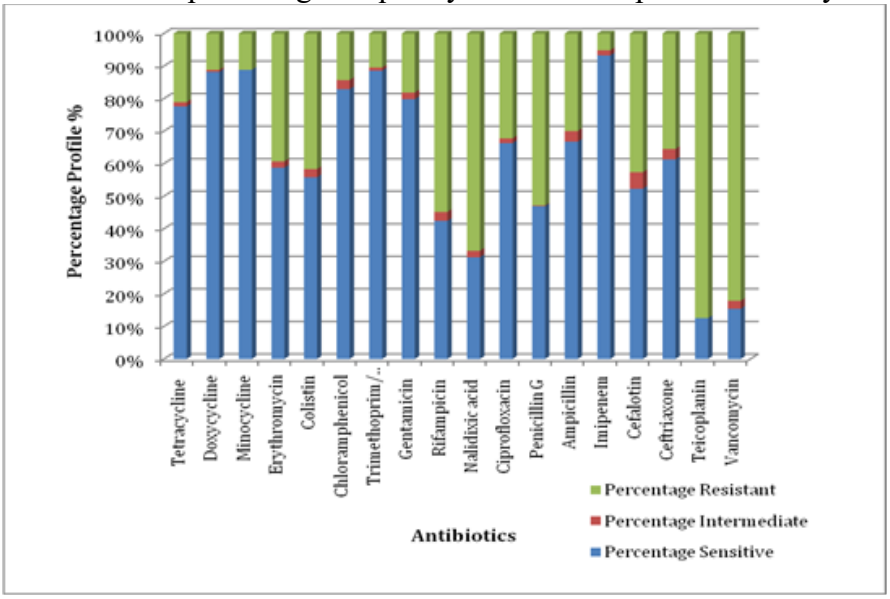

Figure 4. Cumulative percentage susceptibility and resistance profile of bacterial isolates from suya meat samples in Nigeria.

The findings on the antibiotic resistance of bacteria in this study deviated from the result of Barber *et al*. (2018) who reported that all *E. coli* was resistant to chloramphenicol and streptomycin. Nutanbala *et al*. (2011) reported the sensitivity of *E. coli* to ciproflaxain which is in line with the finding of this study. Ciprofloxacin belong to the fluoroquinolone class of antibiotics and has been known to have excellent activities against Gram-negative and Gram-positive bacteria such as *E. coli* and *S. aureus*, respectively (Cohen *et al*., 2017). The report of Sani *et al*. (2012) also buttressed the sensitivity of *S. aureus* to the fluoroquinolones. However, nalidixic acid exerted poor antimicrobial effects on the isolates as 68.64% of the bacterial isolates exhibited resistance to it in this study. The mechanism of action of the fluoroquinolones is the inhibition of bacterial DNA gyrase responsible for DNA replication and transportation (Moore, 2015). Ampicillin also inhibited the growth of 62.53% of the bacterial isolates in this study.

Minocycline, doxycycline and tetracycline exerted antimicrobial potency against 89%, 88.20% and 77.60% of the bacterial isolates. These antibiotics belong to the class tetracylines which inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. Their high potency against bacterial isolates could be attributed to the fact that they are broad-spectrum agents. However, Mhondoro *et al*. (2019) reported high percentages of resistance to this class of antibiotics in their study. The penicillin-based antibiotics, such as the imipenem, act by binding to and inactivating penicillin-binding proteins (PBPs) located on the inner membrane of a bacterial cell wall. The strength and rigidity of the bacterial cell wall are affected by the inactivation of PBPs which interferes with the cross-linkage of peptidoglycan chains. This brings about the interruption of synthesis of bacterial cell wall which weakens the bacterial cell wall and results to cell lysis (Niwa *et al*., 2016).

Cefalotin and Ceftriaxone belong to the first and third generations of cephalosporins, respectively, and they possess the same mechanism of action like the penicillin-based antibiotics. The peptidoglycan layer of bacterial cell walls is disrupted by these antibiotics through competitive inhibition on penicillin-binding proteins (Moore, 2015). More than half (62.53% and 53.35%) of the isolates were sensitive to cefalotin and ceftriaxone, respectively. This is in line with the findings of Sani *et al*. (2012) and Page (2012) who also reported the sensitivity of similar bacterial isolates to the cephalosporins.

Trimethoprim/sulfamethoxazole and colistin inhibited the growth of 83.33% and 55.60% of the bacterial isolates, respectively. Cefalotin and ceftriaxone inhibit cell wall synthesis through the inhibition of β-lactamase (Bello *et al*., 2019). Erythromycin exerted antimicrobial potency against 54.90% of the bacterial isolates in this study which was also buttressed by the report of Hardman *et al*. (2017) where over half of the organisms isolated were sensitive to same class of antibiotics. Erythromycin is a macrolide-based antibiotic which reversibly binds to the 50s ribosomal subunit to inhibit synthesis of protein (Moore, 2015).

Gentamicin belongs to the aminoglycoside class of antibiotics. The high potency exerted by the gentamicin against bacterial isolates could be associated with the mechanism of action of this class of antibiotics which enables it to bind irreversibly to the 16S rRNA subunit of the 30S ribosome, resulting to inhibition of bacterial protein synthesis. This finding is supported by the reports of Barber *et al*. (2018), Mhondoro *et al*. (2019) and Breijyeh *et al*. (2020). A high percentage of the bacterial isolates (87.50%) were sensitive to chloramphenicol. Chloramphenicol belongs to the phenicol class whose mode of action is to interfere with bacterial protein synthesis.

The production of chloramphenicol acetyltransferase (CAT) is responsible for the resistance of bacteria to chloramphenicol while some resistance occur as a result of inability of certain bacteria to reach their target sites.

Only 15.39% and 12.43% of the bacteria isolated in this study were sensitive to vancomycin and teicoplanin, respectively. This is attributed to the fact that vancomycin and teicoplanin are narrow spectrum and exert very weak action against many Gram-negative bacteria. Vancomycin and teicoplanin belong to the glycopeptides and their modes of action are same as the β-lactam antibiotics. However, glycopepetides differ from β-lactams in that they interact with different molecular targets as they bind to acyl-D-alanyl-D-alanine in peptidoglycan and, hence, inhibit the function of glycosyltranfereases in susceptible bacteria. The hydrophilic antibiotics like β-lactams pass through porins, and glycopeptides cannot cross the outer membrane due to their structures that hinder it from using any of these passages (Breijyeh *et al*., 2020).

4.Conclusions

The microbial loads encountered in suya meat from this study were at the borderline based on the microbiological guidelines for ready-to-eat food products. The study revealed the presence and distribution of multidrug-resistant food pathogens in the food product which is of tremendous public health concern.

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