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Multidrug-resistance and biofilm formation profiles of *Salmonella* spp. isolated from raw chicken meat

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Abstract

Available data showed that the recovery of multidrug-resistant and biofilm-producing *Salmonella* spp. from chicken meat is still scanty in Nigeria. Consequently, this study aimed to characterize the probable multidrug-resistant and biofilm producing *Salmonella* spp. prevalent in chicken meat vended in southern Nigerian markets. About 240 randomly sampled chilled raw chicken meats were collected from open markets in Delta, Edo, Ekiti and Ondo States, and then were analysed for detecting the presence of *Salmonella* spp.; using rinse centrifugation-plating technique, serological examination and 16S rRNA gene sequencing. The confirmed *Salmonella* isolates were tested for multidrug-resistance and biofilm formation using Kirby Bauer disc diffusion test and tissue culture plates, respectively. Out of 229 presumptively examined *Salmonella* isolates, 52 isolates were confirmed as *Salmonella* spp., while 46 isolates were recorded as multidrug-resistant. The main serotypes recovered were; *S. enterica* subsp. *enterica* serovar *Typhimurium* (35/52; 67.31%), and *S. enterica* subsp. *enterica* serovar *Enteritidis* (17/52; 32.69%). Biofilm characterization of the recovered *Salmonella* isolates were; strong (OD > 0.240), 6 (11.5 %); moderate (0.120-0.240), 13 (25.0 %); weak (OD < 0.120), 19 (36.5 %), and non-biofilm producers (OD < 0.120), 14 (26.9 %). This study showed that multidrug-resistant and biofilm-producing *Salmonella* spp. were prevalent in raw chicken meat; vended within southern Nigerian open markets. Thus, there is an urgent need for relevant regulatory agencies to enforce consumer's safety.

Keywords: Biofilm, Multidrug-resistant *Salmonella*, Chicken meat, Tissue culture plates

1. Introduction

Globally, chicken meat is recognized as a good source of animal protein, with low levels of cholesterol, and thus widely preferred over other types of meat (Ogu *et al*., 2017a; 2017b). Contamination of chicken meat occurs during slaughtering; feather plucking, evisceration,

washing, use of unhygienic processing and storage equipment (Ogu *et al*., 2017b). Consumption of such poorly processed and undercooked meat could lead to food-borne illnesses (Antunes *et al*., 2016; Ogu and Akkinibosun, 2019). Several studies conducted by Eng *et al*., (2015); Antunes *et al*., (2016); Mouttotou, *et al*., (2017) have described chicken products as principal reservoirs of *Salmonella* spp., which are principle foodborne pathogens.

Salmonella spp. contain more than 2500 known serotypes; with *S. enterica* serovars *Enteritidis* and *Typhimurium* types (the non-typhoidal *Salmonella* group) being the most frequently implicated serovars in salmonellosis outbreaks (Eng *et al*., 2015; Heredia and García, 2018). Gastroenteritis is the most clinical feature of Salmonellosis, which is usually self-limiting; but might be invasive and severe, particularly in children, elderly and immune-compromised patients (WHO. 2014; Eng *et al*., 2015). Treatment with antibiotics is vital for management of severe or invasive human Salmonellosis. Recently, effective treatments of salmonellal infections have been negatively affected by the rising cases of multidrug resistant strains (Okeke *et al*., 2007; Browne, *et al*., 2018; Xu *et al*., 2020). The occurrence of resistance in *Salmonella* isolates is detrimental to both the veterinary and public health areas, due to the extensive usage of antimicrobials (Eng *et al*., 2015).

In addition to the worldwide concern of antimicrobial resistance, biofilm formation is a rising challenge. According to several studies of Flemming *et al*., (2016); Ziech *et al*., (2016); Farahani, *et al*., (2018); Abebe, (2020), biofilm is a matrix of extra-cellular biomolecules, which bind communities of microbes together on the surfaces of living and inanimate substrates, in order to survive unfavorable, hostile or stressed conditions, caused by the use of antibiotics, sanitizers and\or detergents. Clinically, biofilm formation is important because they confer the pathogens extra-tolerance and resistance to antibiotics and host immune system, thereby impeding their ease of eradication, as reported recently by Li and Lee, (2017); Oxaran *et al*., (2018); Tasneem *et al*., (2018); Sharma *et al*., (2019). Biofilms formation has been also reported in poultry farms and meat processing environments (Schonewille *et al*., 2012; Wang *et al*., 2013). Currently, different serotypes of *S. enterica* recovered from clinical and poultry samples have been identified to produce biofilms (Ćwiek *et al*., 2020).

In spite of the relatively high cost of the local chicken meat and the ban on importation of frozen chicken into Nigeria, the majority of Nigerian citizens still patronize chicken meat sold in open markets without recourse to their wholeness and\or processing conditions (Ogu *et al*., 2017b). This issue is of a public health relevance considering the possibility of exposure to the resistant strains of Salmonella, as well as biofilm producers.

Therefore, the objectives of this study were to assess the antibiotic resistance profiles and biofilm production potentials of several *Salmonella* spp.; isolated from raw chicken meat vended within southern Nigerian open markets.

2. Material and methods

2.1. Study area

The studied areas included four major open markets in Southern Nigeria, as presented in Fig. (1), namely; Oja-Oba and Effurun main markets situated in Delta State (5.7040°N, 5.9339°E) and Ondo State (6.9149°N, 5.1478oE); respectively, as well as Oja-Oba and Oba markets situated in Ekiti State (7.7190°N, 5.3110°E) and Edo State (6.6342°N, 5.9304°E), respectively.

2.2. Collection of samples

The sampling regime was carried out between October, 2017 and September, 2020. A total of 240 chicken meat samples were collected from open markets located in Delta, Edo, Ondo and Ekiti State, southern Nigeria, using simple random sampling methods. Portions of the sampled chilled raw chicken

meat used for bacteriological analysis included both the skin and its muscle tissues. The samples were placed in sterile stomacher bags and sealed appropriately. All samples were conveyed to the Microbiological laboratory after collection in black polyethylene bags placed within ice packs (WHO. 2010).

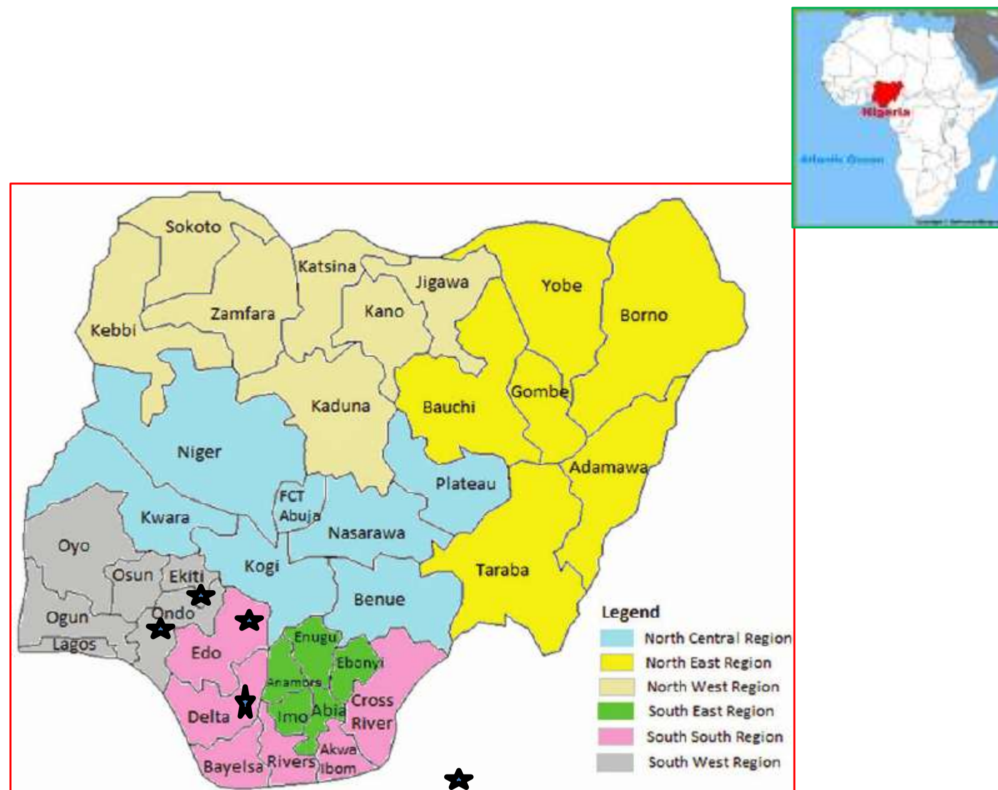

Fig. 1: Geographical map of Nigeria showing the currently studied areas (Ogu and Akinibosun, 2019)

2.3. Isolation and phenotypic characterization of *Salmonella* spp.

Isolation and enumeration of *Salmonella* spp. from the raw chicken meat were carried out using the meat rinse centrifugation-plating technique, as previously described by Line *et al*., (2001); Rodrigo *et al*., (2006); Cox *et al*., (2014). About 25 portion of each chicken meat sample was cut into small pieces using sterile forceps/scissors, and then placed in a sterile glass container containing 150 ml of sterile 0.1 % buffered peptone water (Becton and Dickinson, USA). The chicken meat was massaged and rotated individually in the sterile container for at least 2 min., to rinse the meat into the peptone water. After that, 25 ml of the rinse was collected in a sterile flask and then centrifuged at 4470 g for 20 min., followed by the removal of 1 ml of the sediment that was used to make serial dilutions up to 10-6. An aliquot of 10 µl of each dilution was spread on the surface of Xylose Lysine Deoxycholate (XLD) agar plates supplemented with Novobiocin (15 mg/ l), using a sterile glass spreader. The inoculated plates were then incubated at 37°C for

48 h. After incubation, colonies on the Petri plates were counted. The colony counts were used to deduce the presumptive Salmonella counts (PSC); expressed as presumptive *Salmonella* colony-forming units per ml (cfu/ ml) of the rinse.

The phenotypic techniques employed for the Genus-level identification of the presumptive Salmonella colonies were performed using the standard methods, according to Krieg and Holt, (1984). Phenotypic assays carried out on the distinct presumptive *Salmonella* colonies included; Gram staining, Triple sugar iron utilization, Citrate utilization, Urea utilization, Indole production, Methyl red test, Voges Proskauer test, Oxidase test, Motility test, Coagulase test, Catalase test and Haemolysis test (Krieg and Holt, 1984; Cheesbrough, 2000).

2.4. Serological characterization

Confirmed *Salmonella* isolates that were identified by the phenotypic tests were subjected to serological examination. The antigenic formula of a pure *Salmonella* culture was identified using a slide agglutination test, as described previously by Cheesbrough, (2000). One drop of the different *Salmonella* O and H antisera (Oxoid, UK) was mixed individually with a saline emulsion of the tested pure *Salmonella* culture on a glass slide for 1 min., followed by observation of agglutination formation under indirect lighting over a dark background. The patterns of agglutination reactions were used to identify *Salmonella* serotype, by referring to the Kauffmann-White reference scheme (Kauffmann, 1974; Guibourdenche *et al*., 2010).

2.5. Molecular characterization of *Salmonella* isolates

Species-level identification of the Salmonella isolates was carried out using 16S rRNA gene analysis; followed by polymerase chain reaction (PCR), according to Lane, (1999). Ultrapure DNA templates were extracted from the tested *Salmonella* isolates using the Zymo-Spin column, as prescribed by the manufacturer (Zymo Research Corporation, Irvine, CA, USA). Universal 16S rRNA bacterial primers mainly; 27F 5'AGAGTTTGATCCTGGCTCAG';1492R 5'GGTTACCTTGTTACGACTT3'; 1466 base pair (Lane, 1999), often employed for bacterial taxonomy were used to determine the presence of 16S rRNA gene in the *Salmonella* isolates. *S. enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028 was used as a positive control strain for the PCR. DNA sequencing of the PCR products was performed using the Dideoxy chain termination method, in reference to Sanger *et al*., (1977). The PCR products were cleaned up with ExoSAP-IT (ThermoFisher Scientific, Waltham, MA), and then subjected to cycle sequencing with the Big Dye Terminator version 3.1 (Applied Biosystems) using the standard cycling conditions, followed by quality checking and proofreading with Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, MI). Comparison of the experimentally derived nucleotide sequences (query sequences) against the reference sequence database (rRNA_typestrains/prokaryotic_16S_ribosomal_RNA) was carried out with BLASTN 2.8.0+ program (National Centre for Biotechnology Information [NCBI]), to confirm the species level of the *Salmonella* isolates.

2.6. Antibiotic susceptibility testing

Each of the *Salmonella* isolates used for phenotypic tests and 16SrRNA gene analysis was tested for multidrug resistance using the Kirby Bauer disc diffusion assay, as prescribed by the Clinical and Laboratory Standards Institute (CLSI. 2014). Inhibition zone diameters around each of the Salmonella colonies were interpreted as; sensitive, intermediate or resistant based on zone diameter interpretive standards stipulated by the Clinical and Laboratory Standards Institute. *Staphylococcus aureus* ATCC 25923 was used as a reference strain to detect any potential errors in results of the disc diffusion susceptibility assay. Ampicillin (10 µg), Amoxycillin/Clavulanic acid (20 µg), Amikacin (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg),

Ceftriaxone (30 µg), Streptomycin (10 µg), Tobramycin (20 µg), Gentamycin (10 µg), Nalidixic acid (30 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), Sulfamethoxazole/trimethoprim (25 µg), Tetracycline (30 µg) and Chloramphenicol (30 µg), represent the tested antibiotic discs (Abtek Biologicals Ltd., UK).

2.7. Estimation of the multiple antibiotic resistance indices (MAR)

The multiple antibiotic resistance indices (MAR) of the *Salmonella* isolates were determined according to the method prescribed by Krumperman, (1983). Using the following equation, obtaining a MAR value greater than 0.2 indicated a high-risk source of acquiring multidrug-resistant Salmonella from the tested samples

$$MAR = \frac{\Sigma(AR)}{A \times B}$$

Where; MAR is the mean multiple antibiotic resistance indices, while AR is the antibiotic resistance scores of each Salmonella isolate, which is defined as the sum of antibiotic classes to which a particular Salmonella isolate exhibited resistance. A is the total number of antibiotic classes tested, and B is the total count of Salmonella isolates examined.

2.8. Detection of biofilm production

In vitro characterization of biofilm production by the *Salmonella* isolates was carried out using the Tissue culture plates (TCP) methods, described recently by Beshiru *et al*., (2018); Orjih *et al*., (2021) with slight modification. The *Salmonella* isolates were cultured individually overnight in Trypton soy broth (Merck, Darmstadt, Germany) at 37°C before adjusting the cell suspension to cell density similar to 0.5 McFarland standards. An aliquot of 200 µl of each standardized isolate cell's was dispensed into each well of 96 well tissue culture plate. Sterile broth medium was used as negative control, while *S. enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028; included in separate well, served as a positive control strain. The tissue culture plate was incubated aerobically at 37 °C for 24 h. Thereafter, the wells were carefully tapped to remove the contents before washing thrice with 250 µl of sterile phosphate buffer saline (pH 7.2), to completely release the free-hanging bacterial cells. Sodium acetate (2 %) was used to fix the biofilms produced by the adherent cells before applying 0.2 % crystal violet stain. Excess stain was carefully rinsed off using deionized water, and then the dye incorporated by the adherent cells was solubilized by adding 200 µl of 33 % glacial acetic acid (Merck, Darmstadt, Germany) (Orjih *et al*., 2021). A Microplate reader (Molecular Devices San Jose, CA) set at wave length 570 nm was employed to read the optical density (OD) of each well. This was carried out in triplicates. Absorbance was determined by subtracting the OD_{570} of the control from that of the test assays OD_{570}, with a mean value determined for each isolate. Results of biofilm formation obtained were rated as OD_{570} values; (< 0.120) as non-biofilm producers, (0.120-0.240) as moderate biofilm producers, and (> 0.240) as strong biofilm producers (Orjih *et al*., 2021).

2.9. Statistical analysis

Descriptive statistics of Salmonella counts and prevalence datasets were performed using NCSS version 12 data analysis software. Biofilm characterization was assayed using descriptive statistics and expressed as OD_{570} values. Moreover, NCSS ver. 12 data analysis through the Shapiro–Wilk normality test and Fisher (F) one-way ANOVA test were used for normally distributed datasets. The test of hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

3. Results and Discussion

3.1. Isolation and prevalence of *Salmonella* spp.

The prevalence and counts of presumptive *Salmonella* spp. recovered from the raw chicken meat samples are presented in Table (1). Overall, the prevalence of presumptive *Salmonella* is 22.71 %, while the mean counts are estimated as 6.19 ± 6.42 $\log_{10}$. Shapiro-Wilk test showed that the datasets of counts of presumptive *Salmonella* are normally

distributed (p= 0.79; α= 0.05). Based on results of the normality test, the parametric Fisher one-way analysis of variance (ANOVA) tests within each of the datasets indicated no significant difference (p= 0.72) for presumptive *Salmonella*. The ANOVA test between the *Salmonella* datasets also indicated no significant difference (p = 0.54). The counts reported in this study are higher than those reported by previous work of Briehta-Harhay *et al.* (2008), who worked with chicken meat collected from the United States. However, studies conducted by Vaidya *et al*., (2005); Lindblad *et al*., (2006); Maharjan *et al*., (2019) did not detect *Salmonella* spp. in the chicken meat samples that were examined. Overall, the prevalence of chilled raw chicken meat contaminated with *Salmonella* spp. is estimated as 0.17 (40/240). This value of prevalence exceeded the limits (≤ 0.1) set by the Meat Industry Guide, United Kingdom (MIG. 2017). Improper handling by workers and poor hygienic conditions of meat processing plants, in addition to the meat retailing environment are the probable sources of contamination of chicken meat sold in the open markets (Ogu and Akinnibosun, 2019; Maharjan *et al*., 2019). Moreover, improper slaughtering and manual evisceration process of the raw chicken meat intestinal contents are considered as important sources of contamination of the chicken meat samples with *Salmonella* spp.

Table 1: Prevalence and counts of *Salmonella* spp. recovered from the raw chicken meat

		Presumptive *Salmonella* spp.			
				Counts (PSC)	
Sampling locations	**N**	**Prevalence**		**Mean ± SD (log_{10} cfu/ml)**	**95%CI (log_{10} cfu/ml)**
		F/X	**P (%)**		
Delta State	60	14/64	21.88	5.79 ± 5.22	4.47 - 7.11
Ondo State	60	9/64	21.95	4.48 ± 4.61	3.31 - 5.65
Edo State	60	23/91	25.28	6.74 ± 6.78	5.03 - 8.46
Ekiti State	60	6/33	18.18	4.74 ± 4.15	3.69 - 5.79
Total	240	52/229	22.71	6.19 ± 6.42	3.38 - 7.00

Where; N: Counts of the raw chicken meat samples examined; F: Counts of *Salmonella* isolates that were identified as *Salmonella* spp.; X: Total presumptive *Salmonella* isolates; P: Percentage prevalence of *Salmonella* spp. in the raw chicken meat; PSC: Presumptive *Salmonella* counts; CI = Confidence Interval; SD: Standard deviation. The counts are presented as mean ± standard deviation.

3.2. Phenotypic and molecular identification of the *Salmonella* isolates

Results of phenotypic identification of all the recovered *Salmonella* isolates showed that they were; Gram-negative, motile, short rods, catalase (+), urease (-), coagulase (-), citrate (-), indole (-), red slope/yellow butt reaction in triple sugar iron (alkaline slope/acid butt), and produced enough H_2S. These characteristics agreed with the expected standard results of Genus Salmonella (Cheesbrough, 2000).

The 16S rRNA gene sequencing analysis showed that *S. enterica* is the main species detected currently in the chicken meat samples, as indicated in Fig. (2, 3). PCR amplifications yielded products of 1466 bp for the selected isolates. This is the anticipated base pair (bp) size of the samples recorded positive for

Salmonella, according to the Genus-specific PCR reaction applied in this study, in reference to Lane, (1999). Out of the 229 presumptive *Salmonella* isolates examined, 52 isolates are confirmed as *Salmonella* spp. Similarly, previous studies conducted by Akbar and Kumar, (2013); Pedro *et al*., (2016); Ugwu *et al*., (2019) also detected *S. enterica* in the raw chicken meat samples that they examined. However, Cretu *et al*., (2009) reported that *Salmonella* was absent in poultry products collected from Sweden. This was largely attributed to the strict compliance of chicken meat processors and poultry breeders to the regulatory programs set by the Swedish government.

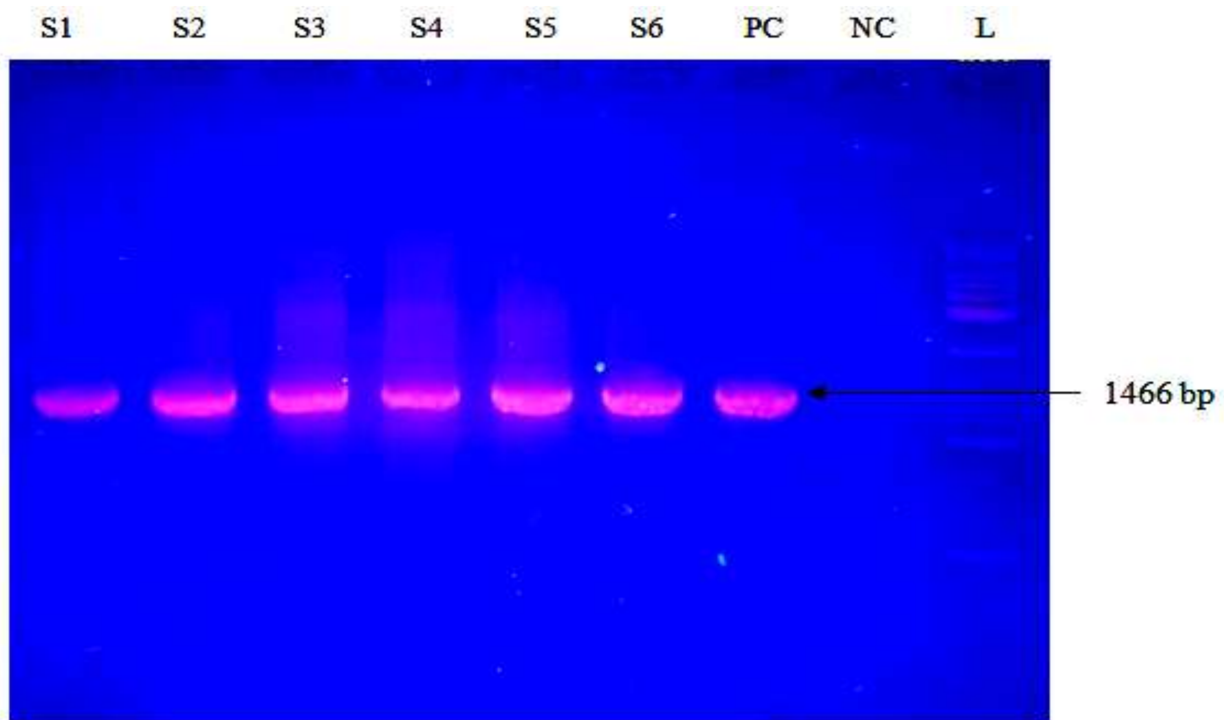

Fig. 2: PCR showing 16S rRNA gene amplification in some of the bacterial isolates obtained from commercial raw chicken meat

Where; S1: (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain OGUAKINNIBOSUN 237 16SrRNA gene); S2: (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain OGUAKINNIBOSUN 238 16SrRNA gene); S3: (*Salmonella enterica* subsp. *enterica* serovar *Enteritidis* strain OGUAKINNIBOSUN 239 16S rRNA gene); S4: (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* 16S rRNA gene); S5: (*Salmonella enterica* subsp. *enterica* serovar *Enteritidis* 16S rRNA gene); S6: (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* 16S rRNA gene); PC: Positive control (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028 16S rRNA gene); NC: Negative control (Sterile water); bp: Base pair; L: Molecular ladder (100 base ladder).

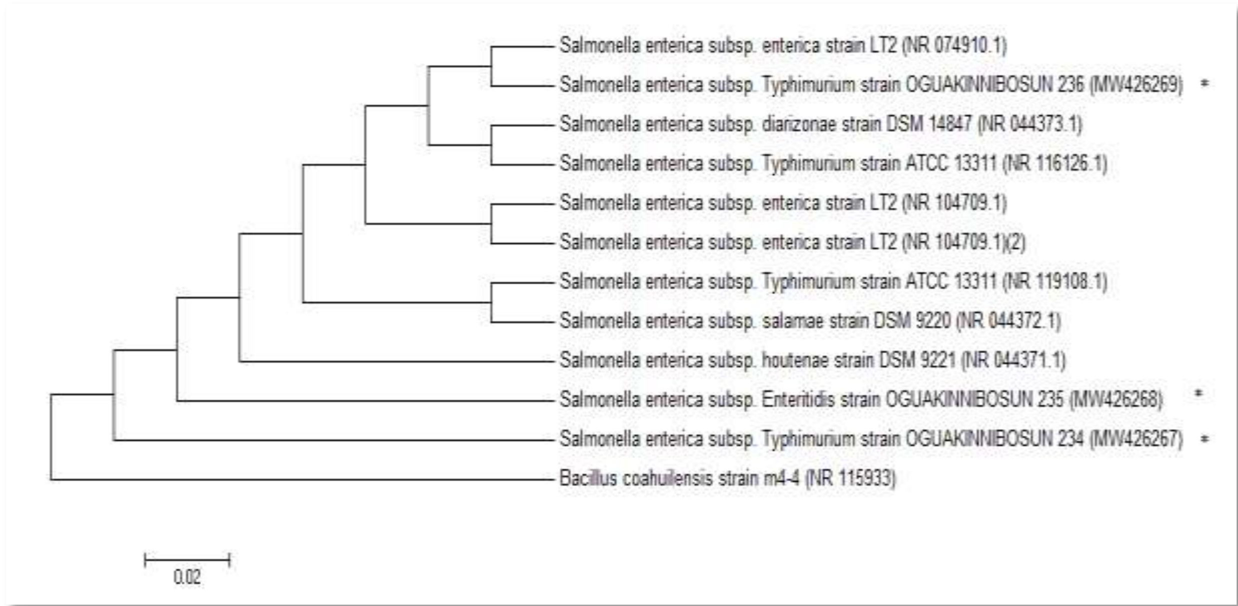

Fig. 3: Phylogenetic tree constructed using the neighbour-joining method

Where; (*) indicates certain novel *S. enterica* strains isolated from the raw chicken meat samples examined in the current study. The GenBank accession numbers of all the recovered strains that are indicated in parenthesis are used to implement the phylogenetic tree

3.4. Antibiotic susceptibility of the *Salmonella* spp.

The antibiotic resistance patterns of the *Salmonella* spp. recovered from the chicken meat samples are presented in Table (2). Of the 52 *Salmonella* isolates tested, 46 *Salmonella* isolates are recorded to be multidrug-resistant. The multidrug-resistant *Salmonella* isolates are most prevalent in raw chicken meat samples vended in Edo State, and are least prevalent in Ekiti State. Overall, according to the recorded diameters of inhibition zones, the *Salmonella* isolates are most resistant to Ampicillin (96.15%), but are more sensitive to Gentamycin (40.39%). Amongst the tested *Salmonella* isolates, the MAR ranged from 0.69 to 0.87. Generally, MAR is estimated at 0.83. These MAR values detected in the raw chicken meat samples exceeded the recommended limit of 0.2. This indicates that raw chicken meat samples collected from Southern Nigeria are potential sources of multidrug-resistant *Salmonella* spp., with a probable significant health risk. Similarly, Antunes *et al*., (2016), Ugwu *et al*., (2019); Parvin *et al*., (2020) have also detected multidrug-resistant *Salmonella* in chicken meat samples. Thus, poultry products are currently identified as a public health concern. In accordance, previous study of Antunes *et al*., (2016) reported that the association of multidrug-resistant *Salmonella* with chicken meat is extremely disturbing, due to the probable resistance of *Salmonella* spp. to multiple arrays of antibiotics that are clinically relevant.

3.5. Biofilm formation

Biofilm characterization of the 52 strains revealed that 73.1 % of the isolates produced biofilm detected using the tissue culture plate techniques. The production rate is classified: as strong biofilm producers (OD > 0.240), 6 (11.5 %); moderate (0.120-0.240), 13 (25.0 %), weak and non-biofilm producers (OD < 0.120), 19 (36.5 %) and 14 (26.9 %); respectively, as demonstrated in Table (3). Similarly, previous studies showed varying rates of biofilm productions; 98.3 % (Oliveira *et al*., 2014), 14.8 % (Laviniki *et al*., 2015), 100 % (Ziech *et al*., 2016) and 72.7 % (Sereno *et al*., 2017), were reported from poultry products, pig feed mills and processing materials in Brazil, respectively. Moreover, Portuguese *S. enterica* serovars yielded 100 % biofilms formation (Seixas *et al*., 2014). The

differences in biofilm production by *Salmonella* species was recently attributed to a number of factors, mainly; species diversity, differences in incubation temperature, growth medium and dynamism of the environment (Bashiru *et al*., 2018). Findings from this study supported a previous report that biofilm production in poultry products is on the rise and this represent a threat to the public health (Burmølle *et al*., 2010; Tasneem *et al*., 2018), due to the clinical implications of the biofilm-mediated antimicrobial resistance.

Table 2: Antibiotic resistance patterns of the *Salmonella* isolates

	Percentage of antibiotic resistance (%)				
Antibiotic	**Delta (n=14)**	**Ondo (n=9)**	**Edo (n=23)**	**Ekiti (n=6)**	**Total (n+52)**
AMC (20 µg)	64.29	77.78	82.61	50.00	73.08
AMP (10 µg)	100.00	100.00	95.65	83.33	96.15
AK (30 µg)	50.00	77.78	73.91	0.00	59.62
CTX (30 µg)	50.00	77.78	52.17	0.00	50.00
CAZ (30 µg)	50.00	77.78	82.61	50.00	69.23
CRO (30 µg)	50.00	77.78	65.22	0.00	55.77
CN (10 µg)	50.00	77.78	30.44	0.00	40.39
TOB (20 µg)	71.43	77.78	82.61	50.00	75.00
STR (10 µg)	100.00	100.00	100.00	83.33	98.08
CIP (5 µg)	50.00	77.78	56.52	0.00	51.92
NAL (30 µg)	64.29	77.78	82.61	50.00	73.08
OFX (5 µg)	85.71	88.89	91.30	83.33	88.46
SXT (25 µg)	57.14	77.78	82.61	50.00	71.15
TET (30 µg)	85.71	88.89	91.30	83.33	88.46
CAM (30 µg)	57.14	77.78	82.61	50.00	71.15
MR	12	8	21	5	46
∑AR	75	55	144	29	303
A	7	7	7	7	7
MAR	0.77	0.87	0.89	0.69	0.83

Where; AMC: Amoxycillin/Clavulanic acid; AMP: Ampicillin; AK: Amikacin; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; CN: Gentamycin; TOB: Tobramycin; STR: Streptomycin; CIP: Ciprofloxacin; NAL: Nalidixic acid; OFX: Ofloxacin; SXT: Sulfamethoxazole/Trimethoprim; TET: Tetracycline; CAM: Chloramphenicol; MR: Counts of multidrug-resistant *Salmonella*; AR: Antibiotic resistance scores; A: Counts of antibiotic classes; MAR: Mean multiple antibiotic resistance indices. Currently, the inhibition zone diameters interpretive standards stipulated by the Clinical and Laboratory Standards Institute were used to determine the susceptibility and\or resistance of the tested *Salmonella* spp. to the different antibiotics.

Table 3: Characterization of biofilm formation by *Salmonella* spp.

	Biofilm formation capacity [n (%)]*				
Parameter	**Delta (n=14)**	**Ondo (n=9)**	**Edo (n=23)**	**Ekiti (n=6)**	**Total (n=52)**
Strong	2 (33.3)[b]	0 (0.0)[c]	4 (66.7)[a]	0 (0.0)[c]	6 (11.5)
Moderate	3 (23.1)[b]	2 (15.4)[c]	6 (46.2)[a]	2 (15.4)[c]	13 (25.0)
Weak	3 (15.8)[c]	4 (21.1)[b]	9 (47.4)[a]	3 (15.8)[c]	19 (36.6)
Non-producer	6 (42.9)[a]	3 (21.4)[c]	4 (28.6)[b]	1 (7.1)[d]	14 (26.9)

Where; *OD_{570} values < 0.120: non-biofilm producers; OD_{570} values between 0.120-0.240: moderate biofilm producers, and OD_{570} values > 0.240: strong biofilm producers. *Values with different letters across the same rows are not significantly different ($p< 0.05$).

Conclusion

This study revealed that multidrug-resistant *Salmonella* spp. were prevalent in raw chicken meat vended within southern Nigerian open markets. Extensive exploitation of antibiotics in poultry farms could probably be the key factor for this. Moreover, this study characterized remarkably high percentages of biofilm production by the multidrug-resistant-*Salmonella* strains, thus posing a serious public health concern of the probable transmission of multidrug-resistant biofilm-producing *Salmonella* strains to the consumers of chicken meat. Accordingly, there is an urgent need for vigorous enlightenment campaigns and sanctions against antibiotics abuse in the poultry sector, coupled with appropriate monitoring and implementations of requisite food safety policy.

Conflict of interest

The authors declare no conflict of interests.

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Ethical approval

None applicable, as birds used in this study were non-living, thus ethical approval was not necessary.

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