Prevalence of multidrug-resistant extended spectrum beta-lactamase-producing Salmonella strains in commercial raw chicken meat

G. I. Ogu1*, J. A. Odoh1, J. C. Okolo2, J. C. Igborgbor3 and F. I. Akinnibosun4

1Department of Microbiology, Federal University Lokoja, Lokoja, Kogi State, Nigeria
2Department of Environmental Biotechnology and Bio-conservation, National Biotechnology Development Agency, Abuja, Nigeria
3Department of Biology, University of Delta, Abgor, Delta State, Nigeria
4Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria

Abstract

The incidence of extended spectrum beta-lactamase (ESBL)-producing pathogens is worrisome because it confers multiple drug resistance (MDR). Considering their serious clinical significance, the study investigated the prevalence of MDR-ESBL-producing Salmonella strains isolated from raw chicken meat in Southern Nigeria. A total of 240 raw chicken meat were sampled and the recovered Salmonella strains were characterized for MDR and ESBL-genes using Kirby Bauer disc diffusion and molecular techniques. Of the 52 confirmed Salmonella enterica serotypes, 67.31% (35/52) were Salmonella enterica subspp. enterica serovar Typhimurium, 32.68% (17/52) were Salmonella enterica subspp. enterica serovar Enteritidis, 78.85% (41/52) were ESBL-producer and 88.45% (46/52) multidrug resistant. Ampicillin (96.15%) and gentamycin (40.39%) were the most and least antibiotics. The most prevalent MDR-ESBL-genes were bla CTX-M (92.68%), followed by bla SHV genes (68.29%) and bla TEM (31.71%). This study showed that Salmonella serotypes with high ESBL-genes and MDR were prevalent in raw chicken meat vended in southern Nigerian markets.

Keywords: ESBL-producing Salmonella; Multidrug-resistance; Chicken meat; Ampicillin

Introduction

World Health Organization (WHO) and the Food Agriculture Organization (FAO) have separately declared Salmonella as the most prevalent and important zoonosis since 1950 (Antunes et al., 2016; Mouttou, et al., 2017). This has culminated to its inclusion of the bacterium in the terrestrial animal health code of WHO (Mouttou, et al., 2017). Coburn et al. (2007) stated that In humans, typhoid disease is known to manifest one to two weeks following bacterial inoculation with generalized fever and malaise, abdominal pain with or without other symptoms which include: headache, myalgias, nausea, anorexia and constipation. Majovicz et al. (2010) estimated that annually about 94 million non-typhoid Salmonella gastroenteritis cases culminating in 155,000 deaths occurred. The authors also reported that the majority of the disease burden was in the South-East Asian and the Western Pacific Regions respectively. Most human salmonellosis cases are foodborne, but each year, infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoos, farm environments or other public, professional or private settings. Majovicz et al. (2010) observed that approximately 80.3 million of 93.8 million human Salmonella-linked gastroenteritis cases—have been diagnosed yearly worldwide; are foodborne, thus representing about 86% of human salmonellosis cases. Mouttou et al. (2017) estimated that approximately 55% of human Salmonella incidences were of foodborne origin, 14% were travel-related, 13% are transmitted through environmental sources,

*Corresponding author e-mail: gideonigou@gmail.com
9% occurred due to direct human-to-human transmission and 9% were inked to direct animal contact. A majority of Salmonella outbreaks in the last ten years have been linked to poultry reservoir (Dao et al., 2016; Lamas et al., 2018). Meremo et al. (2012) observed that although infections triggered by Salmonella spp. are self-limiting in healthy individuals, some become serious infections in immune compromised individuals such as HIV/AIDS victims, elders, pregnant women and children where care must be under taken. However, treatment with antibiotics is vital for the proper management of severe or invasive human salmonellosis (Yhiler and Bassey, 2015).

Antibiotics have been utilized in both humans and animal feeds for treatment, disease prevention and control, and growth promotion (Dao et al., 2016). The occurrence of resistance in Salmonella isolates to routinely utilized antimicrobials is on the rise both in the veterinary and public health area due to their extensive usage both in humans and in veterinary medicine (Ata et al., 2015; Noenchat, 2018). Molla et al. (2003) and Van Duijkeren et al. (2003) observed that several Salmonella serotypes could acquire resistance to antibiotics in food animals before transmission to humans through the food chain. The authors also reported that the increased resistance rates and growing up of multidrug resistant strains (MDR) has made the treatment of Salmonella infections more difficult. Multidrug resistance and reduced susceptibility of non-typhoidal Salmonella (NTS) to ciprofloxacin have been reported from humans and chicken products in some African countries including Nigeria (Gordon et al., 2008). Eng et al. (2015) and Douglas et al. (2015) described several risk factors associated with the transmission of MDR Salmonella from poultry to humans include; the consumption of food or water contaminated with Salmonella spp. or the consumption of salmonellosis infected poultry products.

Fig. 1. Map of Nigeria showing the study area (Ogu and Akinibosun, 2019)
Recent reports on the isolation of Salmonella serotypes with large extended spectrum beta-lactamases (ESBLs) plasmid genes from processed meat and poultry (Ata et al., 2015; Ziecha et al., 2016; Parvinet et al., 2020) is of great public health importance because of the possible transmission of their plasmid-encoded resistant genes to humans (Evers et al., 2017). The presence of extended spectrum beta-lactamases (ESBLs) genes confer on them multi-drug resistances abilities up to the third and fourth generations of cephalosporins, monobactams and carbapenems (Bradford, 2001). ESBLs are reported to be associated with high multidrug resistance and consequently higher mortality, morbidity and cost of chemotherapy (Bradford, 2001; Leverstein-van Hall et al., 2011; Seiffert et al., 2013). In Nigeria, there is still scanty data on the characterized levels of multidrug resistant and ESBL-producing Salmonella strainsin chicken meat. Hence, this study investigated the extended-spectrum beta-lactamase-producing Salmonella in raw chicken meat vended in within southern Nigerian markets.

Materials and methods

Study area

The study areas included four major open markets in South-Southern Nigeria (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, 5.1478°E) 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.

Collection of sample

The sampling regime was carried out between October 2017 and September 2020. The samples were collected using the simple random sampling methods from open markets in Delta, Edo, Ondo and Ekiti State, southern Nigeria. The portions of the chilled raw chicken meat used for bacteriological analysis included both the skin and its muscle tissues. Two hundred and forty chicken (240) meat samples were collected from all the markets that were sampled. The samples were placed in sterile stomacher bags and sealed appropriately. All the samples were conveyed to the laboratory after collection in black polyethylene bags placed within ice packs (WHO, 2010).

Isolation of Salmonella

Isolation of Salmonella spp. in the raw chicken meat was performed by the meat rinse centrifugation-plating technique as previously described (Line et al., 2001; Rodrigo et al., 2006; Cox et al., 2014). Twenty-five gram portions of each chicken meat sample were cut into small pieces with sterile forceps/scissors and placed in a sterile bag containing 150 ml sterile 0.1% buffered peptone water (Becton and Dickinson, USA). The chicken meat was massaged and rotated in the sterile bag for at least 2 minutes to rinse the meat into the peptone water. Twenty-five milliliter (25 ml) of the rinsate was collected in a sterile bottle and centrifuged at 4470 g for 20 minutes, followed by the removal of 1 ml sediment that was used to make serial dilutions up to 10⁶. Ten micro liters of each of the dilutions was spread plated on to sterile duplicate Petri dishes containing Xylose Lysine Deoxycholate (XLD) agar supplemented with novobiocin (15 mg/l). The inoculated plates were then incubated at 37°C for 48 hours. After incubation, colonies were sub-cultured and characterized

Phenotypic characterization of Salmonella isolates

The phenotypic techniques employed for the genus-level identification of presumptive Salmonella colonies were performed by standard methods (Krieg and Holt, 1984). Phenotypic tests performed in distinct presumptive Salmonella colonies that were picked from the Petri plates 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.

Serological characterization of Salmonella isolates

Confirmed Salmonella isolates that were identified by the phenotypic tests and 16SrRNA gene sequence analyses were used for the serological examination. The antigenic formula of a pure Salmonella culture was identified by a slide agglutination test that was performed by separately mixing one drop of the different Salmonella O and H antisera with a saline emulsion of the pure culture on a slide for 1 minute followed by observing for agglutination using indirect lighting over a dark background. The antigenic formula derived upon completion of the agglutination tests was used to identify the Salmonella serotype by referring to a Kauffmann-White reference scheme (Kauffmann, 1974).

Molecular characterization of Salmonella isolates

Species-level identification employed a technique which involved partial 16SrRNA gene analysis that was performed by polymerase chain reaction (PCR) and sequencing methods (Lane, 1991). Ultrapure DNA templates were extracted from the identified Salmonella isolates using the Zymo-Spin
column as prescribed by the manufacturer (Zymo Research Corporation, Irvine, CA, USA). Universal 16S rRNA bacterial primers (27F 5’AGAGTTTGATCCTGGCTCAG; 1492R 5’GGTACCTTGTAGCTACGCTT3; 1466 base pair) (Lane, 1991) often employed for bacterial taxonomy was used to determine the presence of 16S rRNA gene in the salmonella isolates and Salmonella entericasubsp. entericaserovar Typhimurium ATCC 14028 used as a positive control strain for the PCR. The DNA sequencing of PCR products was performed by the dideoxy chain termination method (Sanger et al., 1977). The PCR products were cleaned up with ExoSAP-IT (ThermoFisher Scientific, Waltham, MA) and subjected to cycle sequencing with the Big Dye Terminator version 3.1 (Applied Biosystems) using standard cycling conditions followed by quality checking and proofreading with Sequencer 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 performed with BLASTN 2.8.0+ program (National Center for Biotechnology Information [NCBI]) to confirm the species of the Salmonella isolates.

**Antibiotics susceptibility test**

Each of the Salmonella isolates used for phenotypic tests and 16S rRNA gene analysis was tested for multidrug resistance with the Kirby Bauer disc diffusion test as prescribed by the Clinical and Laboratory Standards Institute (CLSI, 2014). Inhibitory zone diameter around each of the Salmonella colonies was 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 reference strains to detect any potential errors in the disc diffusion susceptibility test. Ampicillin (10 μg), Amoxicillin/Clavulanic acid (20 μg), Amikacin (30 μg), Ceftazidime (30 μg), Cefotaxime (30 μg), Ceftiraxone (30 μg), Streptomycin (10 μg), Tobramycin (20 μg), Gentamycin (10 μg), Nalidixic acid (30 μg), Ofloxacine (5 μg), Ciprofloxacine (5 μg), Sulfamethoxazole/trimethoprim (25 μg), Tetracycline (30 μg) and Chloramphenicol (30 μg) were the antibiotic discs (Abtek Biologicals Ltd., UK) that were tested.

**Estimation of multiple antibiotic resistance indices**

The multiple antibiotic resistance indices (MAR) of the Salmonella isolates were determined according to the method prescribed by Krumperman (1983). Applying Equation 1, a calculated MAR value of greater than 0.2 indicated a high-risk source of acquiring multidrug-resistant Salmonella from these samples.

\[
\text{MAR} = \frac{\sum (AR)}{A \times B}
\]

MAR is the mean multiple antibiotic resistance indices. AR is the antibiotic resistance scores of each Salmonella isolate (AR 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. B is the total count of Salmonella isolates examined.

**Phenotypic characterization ESBL**

The Phenotypic characterization of Extended-Spectrum Beta-lactamas (ESBL)-producing Salmonella isolates was determined by double disc synergy test (DDST) and the combination disc test as previously described (CLSI, 2014). Salmonella entericasubsp. entericaserovar Typhimurium ATCC 14028 was used as a positive control strain for the ESBL production test.

**Molecular Characterization of the ESBL genes**

Characterization of the Salmonella isolates exhibiting ESBL phenotypes were further analyzed to detect their ESBL gene variants by PCR and DNA sequencing of the ESBL-encoding genes (blaSHV, blaTEM, and blaCTX-M). The primers employed for ESBL confirmation are shown in (Table I). The PCRs were performed in a MyCycler PCR system (Bio-Rad, Hercules, CA). The PCR assay was carried out in a 0.2 ml thin wall tube. Each tube consisted of a 25 μl mixture containing 1.5 mM MgCl₂, 0.2 μM of each primer, 200 μM of each of the deoxynucleoside triphosphates (dNTPs), 1.5 U of Taq polymerase (Cinna Gen, Tehran, Iran) and 2.0 μl of DNA template. The PCR cycling condition for blaCTX-M and blaSHV was maintained as follows: initial denaturation at 94°C for 7 minutes; 30 cycles of amplification with denaturation at 94°C for 30 seconds; annealing at 57°C for 30 seconds; extension at 72°C for 30 seconds and a final extension at 72°C for 5 minutes. For the blaTEM gene, the PCR cycling condition was the same as those for blaCTX-M and blaSHV except that the annealing temperature for blaTEM gene was maintained at 53°C.

The PCR products were subsequently run on a 2% agarose gel and sequencing performed as previously described. Comparison of the experimentally derived nucleotide sequences...
Table I. Primers used for detection and sequencing of target genes

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>27F</td>
<td>AGAGTTTGATCMTGTTGCTCAG</td>
<td>1466</td>
<td>Lane (1991)</td>
</tr>
<tr>
<td></td>
<td>1492R</td>
<td>GTTACCTTGTACGACTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaSHV</td>
<td>blaSHV-F</td>
<td>ATGCCGTATATATCCTG</td>
<td>774</td>
<td>Schmitt et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>blaSHV-R</td>
<td>TGCTTTGTCGCCAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaTEM</td>
<td>blaTEM-F</td>
<td>ATAAAATCTGGAAGACGAAA</td>
<td>1080</td>
<td>Weill et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>blaTEM-R</td>
<td>GACAGTTCACTGAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaCTX-M</td>
<td>blaCTX-M-F</td>
<td>CCCATGTTTAAAAAAACACTGC</td>
<td>950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaCTX-M-R</td>
<td>CAGCCGTTTGGCCGTCTAAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bp: base pair

(query sequences) against the reference sequence database (non-redundant protein sequences) was performed with BLASTX 2.8.0+ program (NCBI) to identify the specific class A extended-spectrum beta-lactamases expressed by the ESBL genes in the multidrug-resistant Salmonella isolates. All non-redundant GenBank CDS translations + PDB + SwissProt + PIR + PRF excluding environmental samples from WGS projects were searched for protein sequences that were homologous to the translated nucleotide query sequences of each of the multidrug-resistant Salmonella isolates.

Data analysis

Descriptive statistics of Salmonella counts and prevalence datasets was performed with NCSS version 12 data analysis software. Also performed with NCSS ver. 12 data analysis was the Shapiro–Wilk normality test and Fisher (F) one-way ANOVA test for normally distributed datasets. The test of hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

Results and discussion

A total of 240 raw chicken meat samples were investigated for the presence of Salmonella species. Of the 229 presumptive Salmonella isolates characterized, 52 isolates were confirmed as Salmonella species. The phenotypic characteristics of the isolates are shown in Table II. These were in agreement with the reported characteristics expected of the Genus Salmonella (Cheesbrough, 2000). Salmonella enterica has been reported as bacterium that constantly prevalent in raw chicken meat and retailing materials/environments (Ogu and Akinnibosun, 2019) and thus our finding is in line with the findings of previous workers who also detected S. enterica in the commercial raw chicken meat samples that they investigated (Akbar and Kumar 2013; Pedro et al., 2016; Ugwu et al., 2019). Moreover, our finding is in discordance with the findings of Cretu et al. (2009) reported that Salmonella was absent in poultry products collected from Sweden. The reason for the variation was attributed majorly to the stringent compliance of chicken meat processors and poultry breeders to the governmental statutory programs established by the Swedish government.

Table II. Phenotypic characterization of Salmonella isolates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grams reaction</td>
<td>(-) short rods</td>
</tr>
<tr>
<td>Motility</td>
<td>(+)</td>
</tr>
<tr>
<td>Catalase</td>
<td>(+)</td>
</tr>
<tr>
<td>Urease</td>
<td>(-)</td>
</tr>
<tr>
<td>Coagulase</td>
<td>(-)</td>
</tr>
<tr>
<td>Citrate</td>
<td>(-)</td>
</tr>
<tr>
<td>Indole</td>
<td>(-)</td>
</tr>
<tr>
<td>Triple sugar iron</td>
<td>Alkali ne slant with</td>
</tr>
<tr>
<td></td>
<td>acid but and gas</td>
</tr>
<tr>
<td>H₂S production</td>
<td>(+)</td>
</tr>
</tbody>
</table>
Table III shows the antigenic formula of the serological test carried out on the confirm isolates. It was observed that all the confirmed *Salmonella* isolates investigated belonged to two serotypes, namely, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Salmonella enterica* subsp. *enterica* serovar Enteritidis. *Salmonella enterica* subsp. *enterica* serovar Typhimurium occurred more frequently (35/52; 67.31%) in relation to *Salmonella enterica* subsp. *enterica* serovar Enteritidis (17/52; 32.69%). The results of serological assay from this study significantly agreed with the work of Abdel-Aziz (2016) who identified *S. Typhimurium, S. Enteritidis* and *S. Kentucky* in the chicken meat samples that were examined. *S. enterica* subsp. *enterica* serovar Typhimurium and *S. enterica* subsp. *enterica* serovar Enteritidis have been variously asserted to be the most frequently isolated serovars that cause foodborne outbreaks in the world (Herikstad *et al.*, 2002; Ibrahim *et al.*, 2014). GenBank accession numbers for representative *Salmonella* serotypes isolated from the raw chicken meat samples were *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain OGUAKINNIBOSUN 234 (MW426267), *Salmonella enterica* subsp. *enterica* serovar Enteritidis strain OGUAKINNIBOSUN 235 (MW426268), *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 236 (MW426269), *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 237 (MW633955), *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 238 (MW639905), *Salmonella enterica* subsp. *enterica* serovar Enteritidis strain OGUAKINNIBOSUN 239 (MW641980).

**Table III. Serological characterization of Salmonella isolates**

<table>
<thead>
<tr>
<th>Antigenic formula</th>
<th>Isolate (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4,[5],12:i:1,2</td>
<td><em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Typhimurium (n=35)</td>
</tr>
<tr>
<td>1,9,[f],g,m,[p]:[1,7]</td>
<td><em>Salmonella enterica</em> subsp. <em>enterica</em> serovar Enteritidis (n=17)</td>
</tr>
</tbody>
</table>

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

S1: (Salmonella enterica subsp. entericaserovar Typhimurium strain OGUAKINNIBOSUN 237 16S rRNA gene); S2: (Salmonella enterica subsp. entericaserovar Typhimurium strain rRNA gene); S4: (Salmonella enterica subsp. entericaserovar Typhimurium 16S rRNA gene); S5: (Salmonella enteri OGUAKINNIBOSUN 238 16S rRNA gene); S3: (Salmonella enterica subsp. entericaserovar Enteritidis strain OGUAKINNIBOSUN 239 16S rRNA gene); S6: (Salmonella enterica subsp. entericaserovar Typhimurium 16S rRNA gene); PC: Positive control (Salmonella enterica subsp. entericaserovar Typhimurium ATCC 14028 16S rRNA gene); NC: Negative control (Sterile water); bp: Base pair; L: Molecular ladder (100 base ladder).
Fig. 3. Phylogenetic tree of the Isolates in relation with other bacteria

(*) is used to indicate some novel Salmonella enterica strains isolated from the raw chicken meat samples examined in this study. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50% are displayed on branches

Table IV. Antibiotic resistance pattern of the *Salmonella* isolates

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

AMC: Amoxycillin/Clavulanic acid; AMP: Ampicillin; AK: Amikacin; CTX: Cefotaxime; CAZ: Cefazidine; 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. Zone diameter interpretive standards stipulated by the Clinical and Laboratory Standards Institute were used to determine the susceptibility or resistance of the selected antibiotics to the *Salmonella* species isolated from the raw chicken meat samples.
The molecular characterization of the isolates using the 16S rRNA gene PCR sequencing test showed that *S. enterica* were the main species present in the chicken meat samples as shown in Fig. 2 and 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, (1991).

The antibiotic resistance profile of *Salmonella* isolates obtained from the chicken meat samples is presented in Table IV. Of the 52 *Salmonella* isolates tested, 46 *Salmonella* isolates were found to be multidrug-resistant. Multidrug-resistant *Salmonella* isolates were most prevalent in raw chicken meat samples vended in Edo State and least prevalent in Ekiti State. Overall, the *Salmonella* isolates were most resistant to ampicillin (96.15%) but were more sensitive to gentamicin (40.39%). Amongst the *Salmonella* isolates obtained from the chicken meat samples collected from the different sampling locations, MAR ranged from 0.69 to 0.87. Overall, MAR was estimated at 0.83. These MAR values in the raw chicken meat samples collected from all the sampling locations exceeded the recommended limit of 0.2, thus, indicating that raw chicken meat from South Southern Nigeria were a source of multidrug-resistant *Salmonella* with a probable significant health risk. Antunes *et al.* (2016), Ugwu *et al.* (2019) and 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. The huge data on the association of multidrug-resistant *Salmonella* with chicken meat is extremely worrying due to the probable resistance of *Salmonella* to an array of antibiotics that are clinically relevant (Antunes *et al.*, 2016).

The prevalence of presumptive *Salmonella*, multidrug-resistant *Salmonella* and multidrug-resistant ESBL-producing *Salmonella* present in the raw chicken meat samples are presented in Table V. Overall, the prevalence of presumptive *Salmonella*, multidrug-resistant *Salmonella* and multidrug-resistant ESBL-producing *Salmonella* were respectively estimated at 22.71%, 20.09% and 17.90%. The datasets of counts of multidrug-resistant *Salmonella* and multidrug-resistant ESBL-producing *Salmonella* were also found to be normally distributed (p = 0.61 and 0.54 respectively). Based on the results of the normality test, parametric Fisher one-way analysis of variance (ANOVA) tests within each of the datasets indicated no significant difference (p = 0.72, 0.55 and 0.52 for presumptive *Salmonella*, multidrug-resistant *Salmonella* and multidrug-resistant ESBL-producing *Salmonella* respectively). The prevalence reported in this study were lower than those reported by Zeich *et al.* (2016) and Parvin *et al.* (2020) who worked conveyor belts of broiler cutting rooms in Brazilian broiler processing plants and with frozen chicken meat in Bangladesh, respectively. Similarly, higher prevalence rate of multiple drug resistant and ESBL producing *Salmonella* from chicken at retail markets in Guangdong, China (Zhang *et al.*, 2018). The difference could be attributed to the differences in distribution of the serotypes in the chicken samples. The ANOVA test between the *Salmonella* datasets also indicated no significant difference (p = 0.54). Overall, the prevalence of chilled raw chicken meat contaminated with *Salmonella* was estimated at 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 plant, as well as the meat retailing environment are the probable sources of contamination of chicken meat sold in the open markets (Maharjan *et al.*, 2019). Improper slaughtering and manual evisceration process of the raw chicken meat intestinal contents could also be an important source of contamination of the meat with *Salmonella* species.

**Table V. Prevalence of Salmonella (S), multidrug-resistant (MRS) and multidrug-resistant ESBL-producing Salmonella (MREPS) present in the raw chicken meat**

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Total samples</th>
<th>F/X</th>
<th>P (%)</th>
<th>F/X</th>
<th>P (%)</th>
<th>F/X</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta State</td>
<td>60</td>
<td>14/64</td>
<td>21.88</td>
<td>12/64</td>
<td>18.75</td>
<td>10/64</td>
<td>15.63</td>
</tr>
<tr>
<td>Ondo State</td>
<td>60</td>
<td>9/41</td>
<td>21.95</td>
<td>8/41</td>
<td>19.51</td>
<td>7/41</td>
<td>17.07</td>
</tr>
<tr>
<td>Edo State</td>
<td>60</td>
<td>23/91</td>
<td>25.28</td>
<td>21/91</td>
<td>23.08</td>
<td>20/91</td>
<td>21.98</td>
</tr>
<tr>
<td>Ekiti State</td>
<td>60</td>
<td>6/33</td>
<td>18.18</td>
<td>5/33</td>
<td>15.15</td>
<td>4/33</td>
<td>12.12</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
<td>52/229</td>
<td>22.71</td>
<td>46/229</td>
<td>20.09</td>
<td>41/229</td>
<td>17.90</td>
</tr>
</tbody>
</table>
The double disc diffusion synergy and combination tests confirmed 41 isolates as multidrug-resistant ESBL-producing Salmonella species out of the 46 multidrug-resistant Salmonella isolates tested. The sequence analysis of the PCR products (Fig. 4, 5, and 6) with BLASTX software revealed the presence of blaTEM, blaSHV and blaCTX-M genes in the Salmonella isolates examined. The blaCTX-M genes were found to be the most prevalent genes since they occurred in 92.68% of the multidrug-resistant Salmonella isolates examined, while blaTEM were the least prevalent beta-lactamase genes (31.71%) amongst the multidrug-resistant Salmonella isolates examined. The blaSHV genes occurred in 68.29% of the multidrug-resistant Salmonella isolates examined. Fifty percent of the multidrug-resistant Salmonella isolates co-carried the blaCTX-M and blaSHV genes, while 34.15% of the isolates co-carried the blaCTX-M and blaTEM genes. Friese et al. (2013), Huijbers et al. (2014), Valentin et al. (2014), Abdel-Aziz et al. (2016), Qiao et al. (2017) and Saliu et al. (2017) have reported the presence of ESBL in chicken meat. Friese et al. (2013) documented that ESBL-producing bacteria relatively occurred in more in poultry meat than other types of meat. As was also reported in this study, Huijbers et al. (2014) and Valentin et al. (2014) indicated that CTX-M-1 appeared to be the most prevalent ESBL in poultry meat. They also reported the presence SHV and TEM in poultry meat. However, an important controversy is whether poultry only serves as a reservoir of ESBL-producing bacteria or is also connected with human infections (Saliu et al., 2017). Gen Bank accession numbers for representative ESBL genes obtained from multidrug-resistant ESBL-producing Salmonella serotypes isolated from the chicken meat samples were S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 237 beta-lactamase CTX-M-1 gene (MW662674), S. entericasubsp. entericaserovar Typhimurium strain OGUKINNIBOSUN 236 beta-lactamase CTX-M-61 gene (MW662668), S. enterica subsp. entericaserovar Enteritidis strain OGUKINNIBOSUN 235 beta-lactamase CTX-M-1 gene (MW662673), S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 234 beta-lactamase CTX-M-1 gene (MW662672), S. enterica subsp. enterica serovar Enteritidis strain OGUKINNIBOSUN 239 beta-lactamase CTX-M-1 gene (MW662676), S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 238 beta-lactamase CTX-M-1 gene (MW662675), S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 237 beta-lactamase SHV-11 gene (MW662671), S. enterica subsp. enterica serovar Enteritidis strain OGUKINNIBOSUN 235 beta-lactamase SHV-11 gene (MW662670), S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 234 beta-lactamase SHV-11 gene (MW662669), S. enterica subsp. enterica serovar Typhimurium strain OGUKINNIBOSUN 236 beta-lactamase TEM gene (MW678648) and S. enterica subsp. enterica serovar Enteritidis strain OGUKINNIBOSUN 239 beta-lactamase TEM gene (MW678649).

Fig. 4. PCR showing beta-lactamase CTX-M gene amplification in some of the Salmonella isolates obtained from commercial raw chicken meat

[A1: (Salmonella enterica subsp. entericaserovar Typhimurium strain OGUKINNIBOSUN 237 beta-lactamase CTX-M-1 gene); A2: (Salmonella entericasubsp. entericaserovarTyphimuriumstrain OGUKINNIBOSUN 236 beta-lactamase CTX-M-61 gene); A3: (Salmonella entericasubsp. entericaserovarEnteritidisstrain OGUKINNIBOSUN 235 beta-lactamase CTX-M-1 gene); A4: (Salmonella entericasubsp. entericaserovarTyphimurium strain OGUKINNIBOSUN 234 beta-lactamase CTX-M-1 gene); A5: (Salmonella entericasubsp. entericaserovarEnteritidis strain OGUKINNIBOSUN 239 beta-lactamase CTX-M-1 gene); A6: (Salmonella entericasubsp. entericaserovarTyphimurium strain OGUKINNIBOSUN 238 beta-lactamase CTX-M-1 gene); PC: Positive control (Salmonella entericasubsp. entericaserovar Virchow strain 75-22438-1 beta-lactamase CTX-M gene); bp: Base pair; L: Molecular ladder (100 base ladder).
prevalent ESBL in poultry meat. They also reported the.

(2014) indicated that CTX-M-1 appeared to be the most

isolates examined, while

occurred in 92.68% of the multidrug-resistant

were found to be the most prevalent genes since they

bla

Salmonella

ing

CTX-M

isolates examined. Fifty percent of the multidrug-resistant

et al

bla

bla

et

Enteritidis strain OGUAKINNIBOSUN 239 DNA template); A6: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 238 DNA template); PC: Positive control (Salmonella enterica subsp. enterica serovar Keurmassar

strain DAK-2 beta-lactamase SHV gene); bp: Base pair; L: Molecular ladder (100 base ladder);]

Fig. 5. PCR showing beta-lactamase SHV gene amplification in some of the Salmonella isolates obtained from commercial raw chicken meat

[A1: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 237 beta-lactamase SHV-11 gene); A2: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 236 DNA template); A3: (Salmonella enterica subsp. enterica serovar Enteritidis strain OGUAKINNIBOSUN 235 beta-lactamase SHV-11 gene); A4: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 234 beta-lactamase SHV-11 gene); A5: (Salmonella enterica subsp. enterica serovar Enteritidis strain OGUAKINNIBOSUN 239 DNA template); A6: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 238 DNA template); PC: Positive control (Salmonella enterica subsp. enterica serovar Keurmassar

strain DAK-2 beta-lactamase SHV gene); bp: Base pair; L: Molecular ladder (100 base ladder);]

Fig 6: PCR showing beta-lactamase TEM gene amplification in some of the Salmonella isolates obtained from commercial raw chicken meat

[A1. (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 237 DNA template); A2: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 236 beta-lactamase TEM gene); A3: (Salmonella enterica subsp. enterica serovar Enteritidis strain OGUAKINNIBOSUN 235 DNA template); A4: (Salmonella enterica subsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 234 DNA template); A5: (Salmonella enterica subsp. entericas serovar Enteritidis strain OGUAKINNIBOSUN 239 beta-lactamase TEM gene); A6: (Salmonella entericusubsp. enterica serovar Typhimurium strain OGUAKINNIBOSUN 238 DNA template); PC: Positive control (Salmonella enterica subsp. enterica serovar Typhimurium ATCC 13311

beta-lactamase TEM gene); bp: Base pair; L: Molecular ladder (100 base ladder).]
Conclusion

This study has shown multidrug resistant and EBSL-producing *Salmonella* serotypes are prevalent in raw chicken meat retailed within southern Nigerian open markets. The widespread prevalence of MDR and ESBL-producing serotypes could be associated with the extensive use of antibiotics during chicken rearing/production among other unhygienic retailing practices. It is of public health importance because consumers are exposed to the risk of infection by MDR-ESBL-producing *Salmonella* strains from the contaminated chicken meat. Hence, this further highlights the need for rational use of antibiotics in livestock/poultry farming, proper meat handling/cooking practices, and enforcement of standard food safety by governmental regulatory agencies so as to stem or prevent the risk of MDR-ESBL-bacterial mediated foodborne diseases.

References


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Friese A, Schulz J, Laube H, Von Salviati C Hartung J and Roesler U (2013), Faecal occurrence and emissions of livestock-associated methicillin-resistant Staphylococcus aureus (laMRSA) and ESBL/AmpC-producing E. coli from animal farms in Germany, Berliner und Munchener Tierarztliche Wochenschrift 126: 175-180.


reservoir of ESBL-producing bacteria or is also connected reported in this study, Huijbers et al.


