BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **56(4)**, 271-284, 2021

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

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

G. I. Ogu[1]*, J. A. Odoh[1], J. C. Okolo[2], J. C. Igborgbor[3] and F. I. Akinnibosun[4]

[1]*Department of Microbiology, Federal University Lokoja, Lokoja, Kogi State, Nigeria*
[2]*Department of Environmental Biotechnology and Bio-conservation, National Biotechnology Development Agency, Abuja, Nigeria*
[3]*Department of Biology, University of Delta, Abgor, Delta State, Nigeria*
[4]*Department 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 *Salmonellaenterica* serotypes, 67.31% (35/52) were *Salmonella enterica*subsp. *enterica*serovar Typhimurium, 32.68% (17/52) were *Salmonella enterica*subsp. *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.

Received: 21 June 2021
Revised: 31 October 2021
Accepted: 04 November 2021

DOI: https://doi.org/10.3329/bjsir.v56i4.57200

Keywords: ESBL-producing *Salmoenlla*; 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; Mouttotou, *et al*., 2017). This has culminated to its inclusion of the bacterium in the terrestrial animal health code of WHO (Mouttotou, *et al*., 2017). Coburn *et al.* (2007) stated that In humans, typhoid disease is known to manifest one to twoweeks following bacterial inoculation with generalized fever and malaise, abdominal pain with or without other symptoms which include; headache, myalgias, nausea, anorexia and constipation. Majowicz *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. Majowicz *et al.* (2010) observed that approximately 80.3 million of 93.8 million human *Salmonella*-linked gastroenteritis cases-that have been diagnosed yearly worldwide; are foodborne, thus representing about 86% of human salmonellosis cases. Mouttotou *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: gideoniogu@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.

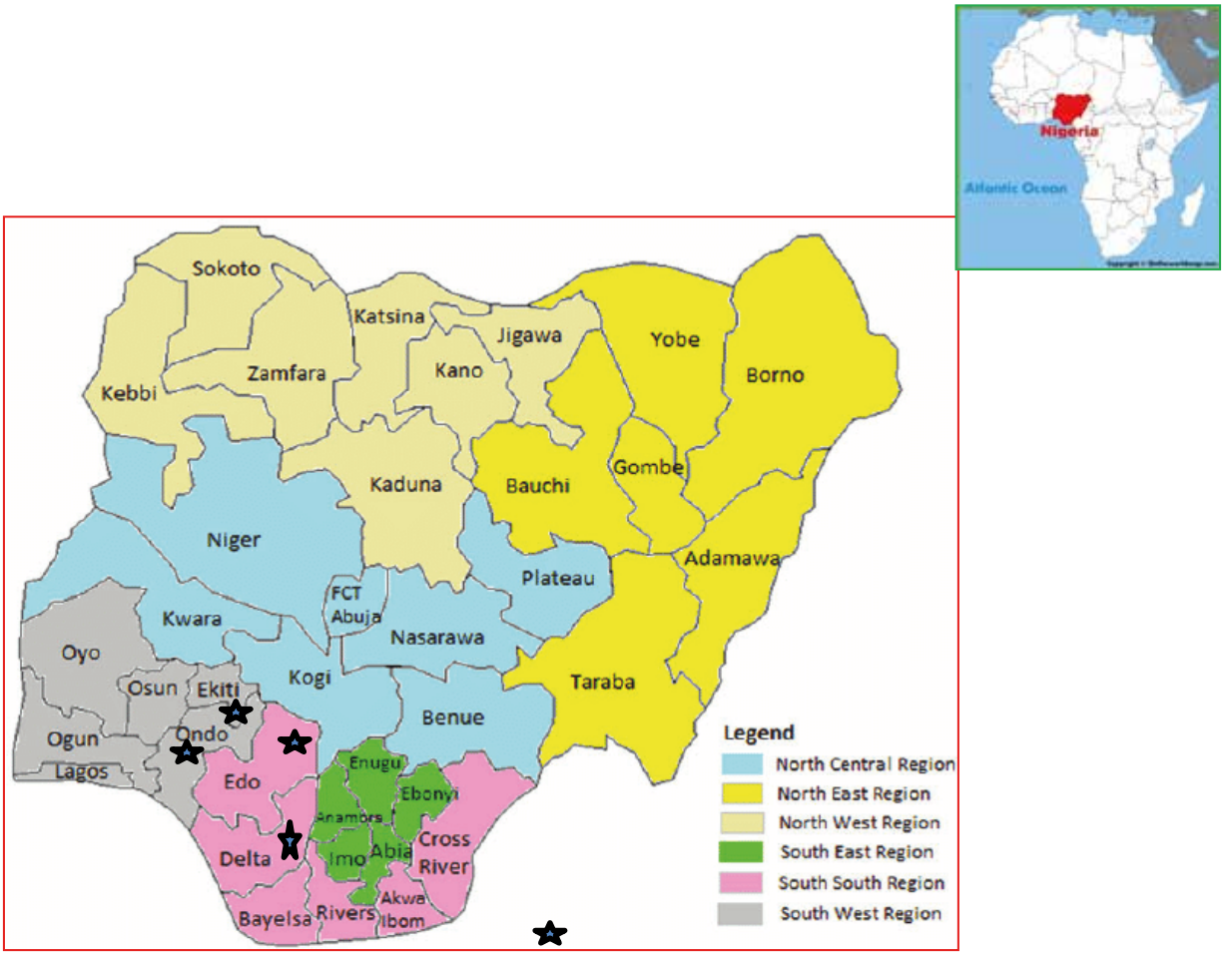


Fig. 1. Map of Nigeria showing the study area (Ogu and Akinibosun, 2019)

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; Parvin*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^{o}N, 5.9339^{o}E) and Ondo State (6.9149^{o}N, 5.1478^{o}E) respectively, as well as Oja-Oba and Oba markets situated in Ekiti State (7.7190^{o}N, 5.3110^{o}E) and Edo State (6.6342^{o}N, 5.9304^{o}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 10^{-6}. 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^{o}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 analysiswere 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 16SrRNA bacterial primers (27F 5'AGAGTTTGATCCTGGCTCAG'; 1492R 5'GGTTACCTTGTTACGACTT3'; 1466 base pair) (Lane, 1991) often employed for bacterial taxonomy was used to determine the presence of 16SrRNA gene in the *salmonella* isolates and *Salmonella enterica*subsp. *enterica*serovar 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 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 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), 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) 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.

$$A \times R \quad \frac{\sum(AR)}{A \times B} \qquad (1)$$

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-lactamases (ESBL)-producing *Salmonella* isolates was determined by double disc synergy test (DDST) and the combination disc test as previously described (CLSI, 2014). *Salmonella enterica*subsp. *enterica*serovar 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 (bla_{SHV}, bla_{TEM}, and bla_{CTX-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 $MgCl_2$, 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 *bla*CTX-M and *bla*SHV 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 *bla*TEM gene, the PCR cycling condition was the same as those for *bla*CTX-M and *bla*SHV except that the annealing temperature for *bla*TEM 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

Target	Primer	Sequence (5'–3')	Size (bp)	References
16SrRNA	27F 1492R	AGAGTTTGATCMTGGCTCAG GGTTACCTTGTTACGACTT	1466	Lane (1991)
bla$_{SHV}$	*bla*$_{SHV}$-F *bla*$_{SHV}$-R	ATGCGTTATATTCHCCTGTG TGCTTTGTTCCGGGCCAAAC	774	Schmitt *et al*. (2007)
bla$_{TEM}$	*bla*$_{TEM}$-F *bla*$_{TEM}$-R	ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATC	1080	Weill *et al*. (2004)
bla$_{CTX-M}$	*bla*$_{CTX-M}$-F *bla*$_{CTX-M}$-R	CCCATGGTTAAAAAACACTGC CAGCGCTTTTGCCGTCTAAG	950	

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). *Salmonellaenterica* 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

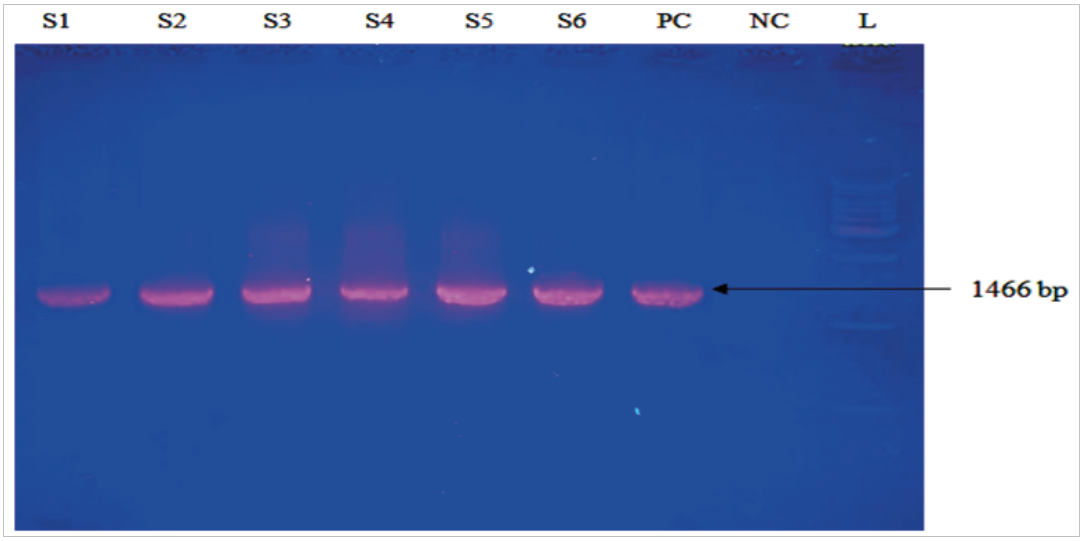
Parameter	Test Results
Grams reaction	(-) short rods
Motility	(+)
Catalase	(+)
Urease	(-)
Coagulase	(-)
Citrate	(-)
Indole	(-)
Triple sugar iron	Alkali ne slant with acid butt and gas
H_2S production	(+)

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*serovarTyphimurium strain OGUAKINNIBOSUN 234 (MW426267), *Salmonella enterica*s ubsp. *enterica*serovar Enteritidisstrain OGUAKINNIBOSUN 235 (MW426268), *Salmonella enterica*subsp. *enterica*serovar Typhimuriumstrain 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

Antigenic formula	Isolate (n=52)
1,4,[5],12:i:1,2	*Salmonella enterica* subsp. enterica serovar Typhimurium (n=35)
1,9,12:[f],g,m,[p]:[1,7]	Salmonella enterica subsp. enterica serovar Enteritidis (n=17)

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 16SrRNA gene); S2: (*Salmonella enterica* subsp. *entericaserovar Typhimurium* strain rRNA gene); S4: (*Salmonella enterica* subsp. *entericaserovar Typhimurium* 16S rRNA gene); S5: (*Salmonella enteri* OGUAKINNIBOSUN 238 16SrRNA gene); S3: (*Salmonella enterica* subsp. *entericaserovar Enteritidis* strain OGUAKINNIBOSUN 239 16S ca subsp. *entericaserovar Enteritidis* 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).

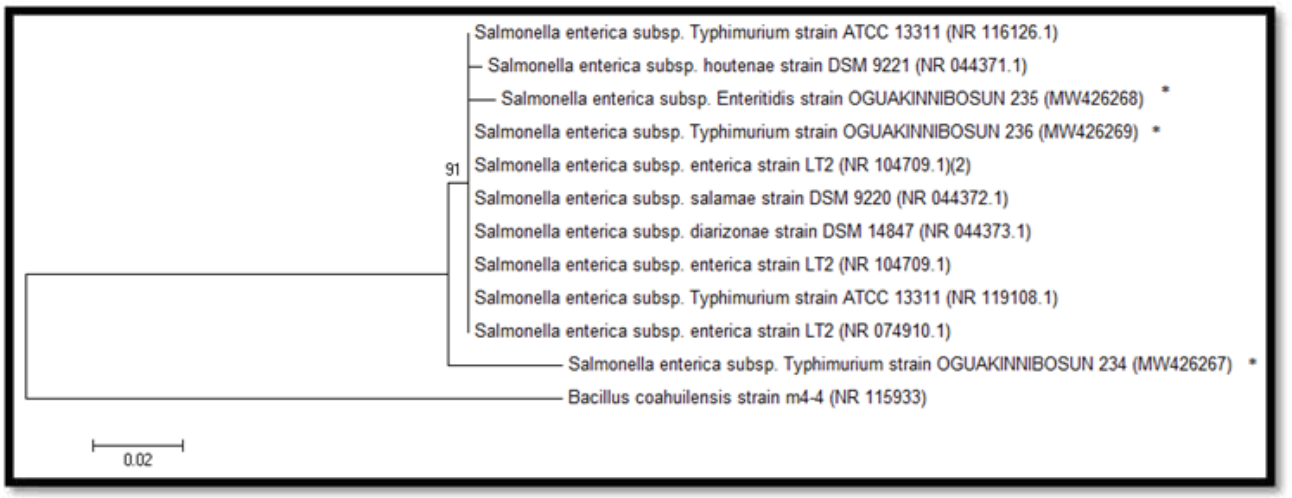


Fig. 3. Phylogenetic tree of the Isolates in relation with other bacteria

(*) 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

		Prevalence 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
$\sum AR$	75	55	144	29	303
A	7	7	7	7	7
MAR	0.77	0.87	0.89	0.69	0.83

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. 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 16SrRNA 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 gentamycin (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 potential 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 ofthe 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

Sampling Location	Total samples	Prevalence					
		S		MRS		MREPS	
		F/X	P (%)	F/X	P (%)	F/X	P (%)
Delta State	60	14/64	21.88	12/64	18.75	10/64	15.63
Ondo State	60	9/41	21.95	8/41	19.51	7/41	17.07
Edo State	60	23/91	25.28	21/91	23.08	20/91	21.98
Ekiti State	60	6/33	18.18	5/33	15.15	4/33	12.12
Total	240	52/229	22.71	46/229	20.09	41/229	17.90

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 *bla*TEM, *bla*SHV and *bla*CTX-M genes in the *Salmonella* isolates examined. The *bla*CTX-M genes were found to be the most prevalent genes since they occurred in 92.68% of the multidrug-resistant *Salmonella* isolates examined, while *bla*TEMwere the least prevalent beta-lactamase genes (31.71%) amongst the multidrug-resistant *Salmonella* isolates examined. The *bla*SHV genes occurred in 68.29% of the multidrug-resistant *Salmonella* isolates examined. Fifty percent of the multidrug-resistant *Salmonella* isolates co-carried the *bla*CTX-M and *bla*SHV genes, while 34.15% of the isolates co-carried the *bla*CTX-M and *bla*TEM 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 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 OGUAKINNIBOSUN 237 beta-lactamase CTX-M-1 gene (MW662674), *S. enterica*subsp. *enterica*serovar *Typhimurium* strain OGUAKINNIBOSUN 236 beta-lactamase CTX-M-61 gene (MW662668), *S. enterica* subsp. *enterica*serovar Enteritidis strain OGUAKINNIBOSUN 235 beta-lactamase CTX-M-1 gene (MW662673), *S. enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 234 beta-lactamase CTX-M-1 gene (MW662672), *S. enterica* subsp. *enterica* serovar Enteritidis strain OGUAKINNIBOSUN 239 beta-lactamase CTX-M-1 gene (MW662676), *S. enterica*-subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 238 beta-lactamase CTX-M-1 gene (MW662675), *S.enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 237 beta-lactamase SHV-11 gene (MW662671), *S. enterica* subsp. *enterica* serovar Enteritidis-strain OGUAKINNIBOSUN 235 beta-lactamase SHV-11 gene (MW662670), *S. enterica* subsp. *enterica* serovar Typhimurium strain OGUAKINNIBOSUN 234 beta-lactamase SHV-11 gene (MW662669), *S. enterica* subsp. *enterica* serovarT yphimuriumstrain OGUAKINNIBOSUN 236 beta-lactamase TEM gene (MW678648) and *S. enterica*-subsp. *enterica* serovar Enteritidis strain OGUAKINNIBOSUN 239 beta-lactamase TEM gene (MW678649).

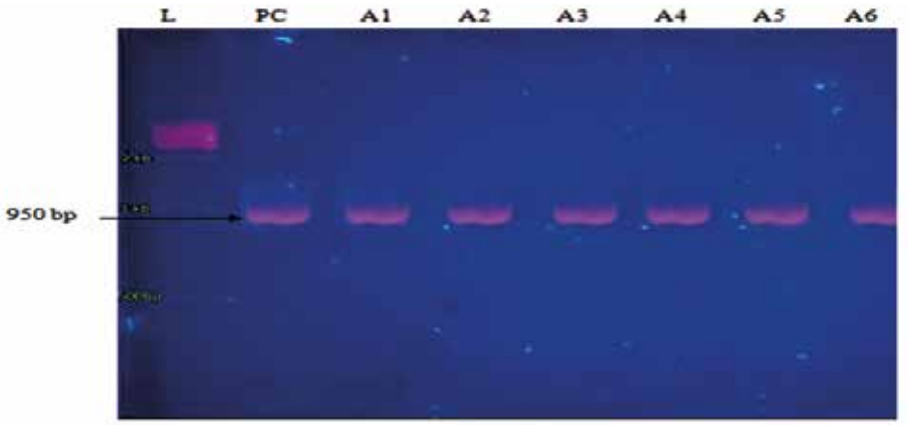


Fig. 4. PCR showing beta-lactamase CTX-M gene amplification in some of the *Salmonella* isolates obtained from commercial raw chicken meat

[A1: (Salmonella enterica subsp. entericaserovar Typhimurium strain OGUAKINNIBOSUN 237 beta-lactamase CTX-M-1 gene); A2: (Salmonella entericasubsp. entericaserovarTyphimuriumstrain OGUAKINNIBOSUN 236 beta-lactamase CTX-M-61 gene); A3: (Salmonella entericasubsp. entericaserovarEnteritidisstrain OGUAKINNIBOSUN 235 beta-lactamase CTX-M-1 gene); A4: (Salmonella entericasubsp. entericaserovarTyphimurium strain OGUAKINNIBOSUN 234 beta-lactamase CTX-M-1 gene); A5: (Salmonella entericasubsp. entericaserovarEnteritidis strain OGUAKINNIBOSUN 239 beta-lactamase CTX-M-1 gene); A6: (Salmonella entericasubsp. entericaserovarTyphimurium strain OGUAKINNIBOSUN 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).

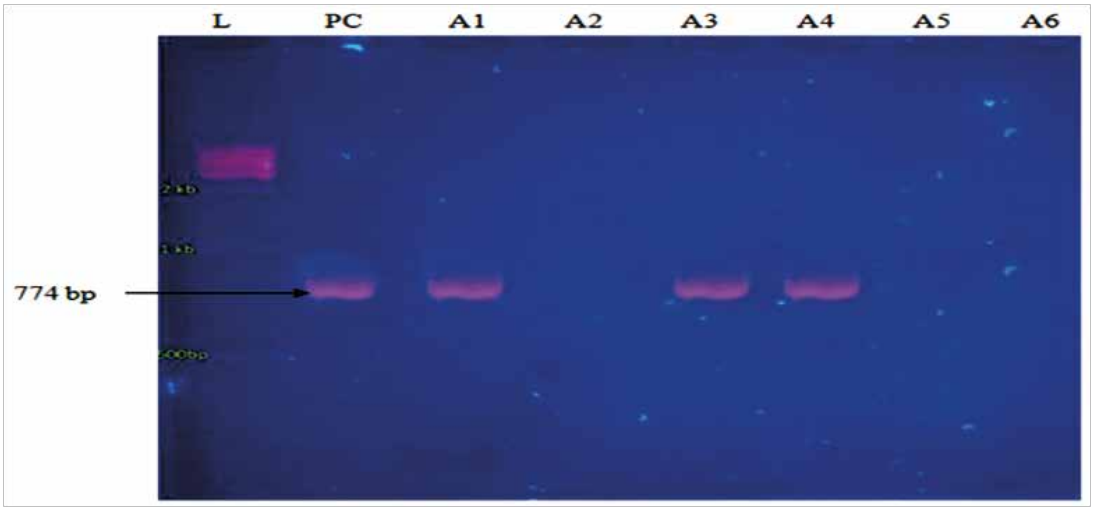


Fig. 5. PCR showing beta-lactamase SHV gene amplification in some of the *Salmonella* isolates obtained from commercial raw chicken meat

[A1: (*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* strain OGUAKINNIBOSUN 237 beta-lactamase SHV-11 gene); A2: *(Salmonella enterica* subsp. *enterica* serovar *Typhimuriumstrain* OGUAKINNIBOSUN 236 DNA template); A3: (*Salmonella enterica* subsp. *entericaserovar Enteritidisstrain* 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-lactamse SHV gene); bp: Base pair; L: Molecular ladder (100 base ladder);]

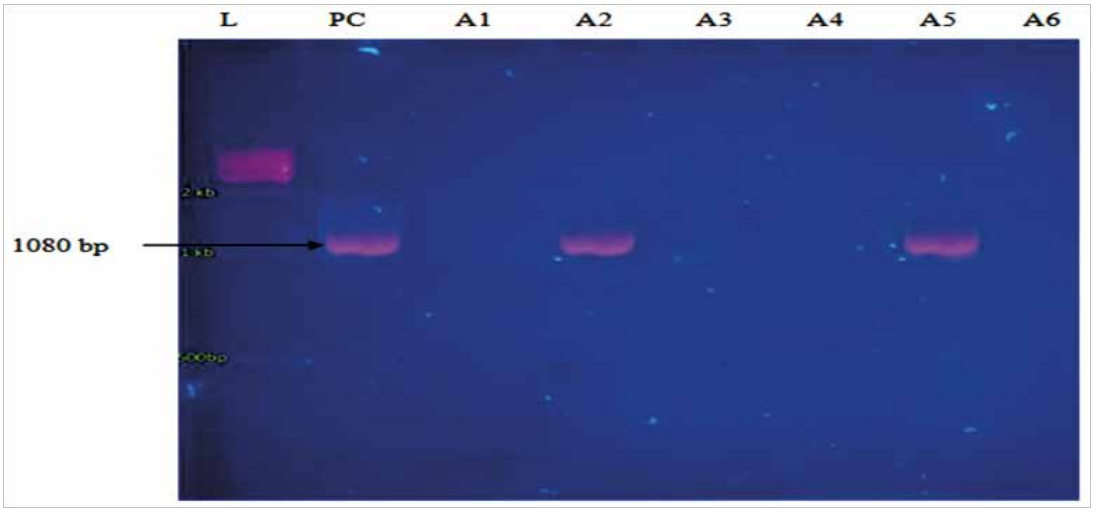


Fig 6: PCR showing beta-lactamase TEM gene amplification in some of the Salmonella isolates obtained from commercial raw chicken meat

[A1. (*Salmonella enterica* subsp. *entericaserovar 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* erovar *Enteritidis* strain OGUAKINNIBOSUN 239 beta-lactamase TEM gene); A6: (*Salmonella enterica*subsp. *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, propermeat 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

Abdel-Aziz NM (2016), Detection of *Salmonella* species in chicken carcasses using genus specific primer belong to invA gene in Sohag city, Egypt, *Veterinary World.* **9:** 1125-1128. DOI: 10.14202/ vetworld. 2016. 1125-1128

Akbar A and Kumar A (2013), Prevalence and antibiogram study of *Salmonella and Staphylococcus aureus* in poultry meat, *Asia Pacific Journal of Tropical Biomedicine* **3:** 163 – 168. DOI: org/10.1016/S2221-1691(13)60043-X

Antunes P, Mourão J, Campos J and Peixe L (2016), Salmonellosis: the role of poultry meat, *Clinical Microbiology and Infection* **22:** 110–121.

Ata Z, Dinç G, Yibar A, Müştak HK and Şahan O (2015), Extended spectrum beta-lactamase activity and multidrug resistance of *Salmonella* serovars isolated from chicken carcasses from different regions of Turkey, *Ankara Üniversitersi Veteriner Fakultesi Dergisi* **62:** 119-123.

Bradford PA (2001), Extended-spectrum b-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat, *Clinical Microbiology Reviews* **14:** 933-951. DOI: org/10. 1128/CMR.14.4.933-951.2001

Cheesbrough M (2000), District laboratory practice in tropical countries, E.C.B.S. Cambridge University Press 2nd Ed., pp 256-267.

Clinical and Laboratory Standards Institute (CLSI) (2014), Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Fourth Informational Supplement, CLSI document M100-S24. Wayne, PA: CLSI.

Coburn B, Grass GA and Finlay BB (2007), Salmonella, the host and disease: a brief review, *Immunology and Cell Biology* **85:** 112–118.

Cox NA, Buhr RJ, Smith DP,Cason JA, Rigsby LL and Bourassa DV (2014), Sampling naturally contaminated broiler carcasses for *Salmonella* by three different methods, *Journal of Food Protection* **77:** 493-495. DOI: org/10.4315/0362-028X. JFP-13-320

Cretu C, Floristean V, Carp-Carare M, Bradatan G. and Isan E (2009), The Influence of pH and temperature on Salmonella spp. from fresh, chilled and frozen poultry carcasses. *Cercetări Agronomiceîn Moldova* XLII(2): 79-84.

Dao T, Masashi K, Phuc DN, Hien TL, Phong TN, Doan NM, Ninh HL, Chinh VD, Takao K, Ryuji K, Shinya Y, Yuji H, Michio J and Shinji Y (2016), Prevalence, antibiotic resistance, and extended-spectrum and AmpCβ–Lactamase productivity of *Salomonella* isolates from raw meat and seafood samples in Ho Chi Minh City, Vietnam, *International Journal of Food Microbiology* **236:** 115-122.

Douglas EC, Nelson AC, Mark AH, Jeanna LW, Buhr RJ and Fedorka-Cray PJ (2015), *Salmonella* and antimicrobial resistance in broilers: A review, *The Journal of Applied Poultry Research.* 00: 1-19. DOI: org/10.3382/japr/pfv038

Eng S-K, Pusparajah P, Mutalib N-S, Ser H-L., Chan, K-G and Lee L-H (2015), *Salmonella:* A review on pathogenesis, epidemiology antibiotic resistance, *Frontiers in Life Science* **8**(3): 284-293.

Evers EG, Pielaat A, Smid JH, Van Duijkeren E, Vennemann FBC, Wijnands LM and Chardon JE

(2017). Comparative Exposure Assessment of ESBL-Producing Escherichia coli through Meat Consumption, *PLoS ONE*. **12:** e0169589.

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.

Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS, Hart CA and Molyneux ME (2008), Epidemics of invasive Salmonella entericaserovar Enteritidis and S. entericaserovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi, *Clinical Infectious Diseases* **46:** 963-969. DOI: org/10.1086/529146

Herikstad H,Motarjemi Y and Tauxe RV (2002), Salmonella surveillance: A global survey of public health serotyping, *Epidemiology and Infection* **129:** 1-8.

Huijbers PM, Graat EA, Haenen AP, Van Santen MG, Van Essen-Zandbergen A, Mevius DJ, Van Duijkeren E and Van Hoek AH (2014), Extended-spectrum and Amp Cbetalactamase-producing *Escherichia* coli in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics, *Journal of Antimicrobial Chemotherapy* **69:** 2669-2675.

Ibrahim W, Abd El-Ghany W, Nasef S and Hatem M (2014), A comparative study on the use of real time polymerase chain reaction (RT-PCR) and standard isolation techniques for the detection of *Salmonella* in broiler chicks, *International Journal of Veterinary Sciences and Medicine* **2:** 67-71.

Kauffmann G (1974), Kauffmann white scheme, *Journal of Acta Pathologica Microbiologica.* **61:** 385.

Krieg NR and Holt JC (1984), Bergey's Manual of Systematic Bacteriology, 1st Ed., Vol. 1, Williams and Wilkins, *Baltimore.* United States, pp 150-180

Krumperman PI (1983), Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of faecal contamination of foods, *Applied and Environmental Microbiology* **46:** 165-170.

Lamas A, Miranda JM, Regal P, Vázquez B, Franco CM and Cepeda A (2018), A comprehensive review of non-enterica subspecies of Salmonella enterica, *Microbiological Research* **206:** 60-73.

Lane DJ (1991), 16S/23S rRNA sequencing. ***In:*** nucleic acid techniques in bacterial systematic, Eds. Stackebrandt E. and Goodfellow M, John Wiley and Sons. New York.

Leverstein-Van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, Van Den Munckhof M. P, Van Essen-Zandbergen A, Platteel T, Fluit AC, Van De Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ. and National ESG (2011), Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains, *Clinical Microbiology and Infection* **17:** 873–880. DOI: org/10.1086/529146

Line JE, Stern NJ, Lattuada CP and Benson ST (2001), Comparison of methods for recovery and enumeration of Campylobacter from freshly processed broilers, *Journal of Food Protection* **64:** 982-986. DOI: org/10.4315/0362-028X-64.7.982

Maharjan S, Chhetri VS, Sherchan SP, Panta OP and KarkiTB (2019), Microbial quality of poultry meat in an ISO 22000:2005 certified poultry processing plant of Kathmandu valley, *International Journal of Food Contamination* **6**(1): 1-9. DOI: org/10. 1186/ s40550-019-0078-5

Majowicz S, Musto J, Scallan E, Angulo F, Kirk M, O'Brien S, Jones T, Fazil A and Hoekstra R (2010), The global burden of non-typhoidal *Salmonella* gastroenteritis, *Clinical Infectious Diseases* **50:** 882-889.

Meremo M, Mshana SE, Kidenya BR, Kabangile R, Peck R and Kataraihya JB (2012), High prevalence of non-typhoid Salmonella bacteramia among febrile HIV adult patients admitted at tertiary Hospital, North-Western Tanzania, *International Archieve of Medicine* **17**(5): 28.

MIG (Meat Industry Guide) (2017), Microbiological criteria, United Kingdom, P 32.

Molla B, Mesfin A and Alemayehu A (2003), Multiple antimicrobial resistant *Salmonella* serotypes isolated from chicken carcasses and giblets in DebreZeit and Addis Ababa, Ethiopia, *Ethiopian Journal of Health Development* **17:** 131-139.

Mouttotou N, Ahmad S, Kamran Z and Koutoulis KC (2017), Prevalence, risks and antibiotic resistance of *Salmonella* in poultry production chain ***In:*** Current Topics in *Salmonella* and Salmonellosis, Intech Europe, pp 216-234.

Noenchat P (2018), Characterization of Drug Resistance in *Salmonella* spp. Isolated from Broiler Chicken Production, *MSc.* Thesis, Veterinary Public Health, Graduate School, Khon Kaen University, p 72.

Ogu GI and Akinnibosun FI (2019), Occurrence of Salmonella in Raw Chicken Meat from Retail Equipment and Environments in Southern Nigeria Open Markets, *Notulae Scientia Biologica* **11**(2): 175-182. DOI: org/10.15835/nsb11210469

Parvin S, Hasan M, Ali Y and Haque E (2020), Prevalence and multidrug-resistance pattern of *Salmonella* carrying extended-spectrum β-lactamase in frozen chicken meat in Bangladesh, *Journal of Food Protection* **83:** 2107-2121. DOI: org/10.4315/JFP-20-172

Pedro H, Waldemir SA, Beatriz DF, Virginia LA, Dayse LA, Dalia DP, Elmiro RD and Maria HC (2016), Prevalence and fluoroquinolones resistance of *Campylobacter* and *Salmonella* isolates from poultry carcasses in Rio de Janeiro, Brazil. *Food Control* **61:** 243-247.

Qiao J, Zhang, Q, Alali WQ, Wang J, MengL and Xiao Y (2017), Characterization of extended-spectrum beta-lactamases (ESBLs)-producing Salmonella in retail raw chicken carcasses, *International Journal of Food Microbiology* **248:** 72-81. DOI: org/10.1016/j.ijfoodmicro.2017.02.016

Rodrigo S, Adesiyun A, Asgarali Z and Swanston W (2006), Occurrence of selected foodborne pathogens on poultry and poultry giblets from small retail processing operations in Trinidad, *Journal of Food Protection* **69:** 1096-1105. DOI: org/10.4315/0362-028X-69.5.1096

Saliu E,Vahjen W and Zentek J (2017), Types and prevalence of extended–spectrum beta–lactamase producing Enterobacteriaceae in poultry, *Animal Health Research Reviews* **18:** 46-57. DOI: org/10.1017/S1466252317000020

Sanger F, Nicklen S and Coulson AR (1977) DNA sequencing with chain-terminating inhibitors, Proceedings of the National Academy of Science USA. **74:** 5463-5467.

Seiffert SN, Hilty M, Perreten V and Endimiani A (2013), Extended-spectrum cephalosporin- resistant gram-negative organisms in livestock: an emerging problem for human health?, *Drug Resistance Update* **16:** 22-45.

Schmitt J, Jacobs E and Schmidt H (2007), Molecular characterization of extended- spectrum beta-lactamases in Enterobacteriaceae from patients of two hospitals in Saxony, Germany, *Journal of Medical Microbiology* **56:** 241-249. DOI: org/10.1099/jmm.0.46670-0

Ugwu MC, Omanukwue C, Chimezie C, Okezie U, Ejikeugwu CP, Nnnabuife-Iloh E, and Esimone CO (2019), Poultry farm and poultry products as sources of multiple antimicrobial-resistant *Salmonella* and *S. aureus, Journal of Tropical Diseases* **7:** 100-308.

Van Duijkeren E, Wannet WJ, Houwers DJ, and Pelt W (2003), Antimicrobial susceptibilities of Salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001, *Journal of Clinical Microbiology* **41:** 3574-3578.

Valentin L, Sharp H, Hille K, Seibt U, Fischer J, Pfeifer Y, Michael GB, Nickel S, Schmiedel J, Falgenhauer L and Friese A (2014), Subgrouping of ESBL-producing Escherichia coli from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs, *International Journal of Food Microbiology* **304:** 805-816. DOI: org/10.1016/j.ijmm.2014.07.015

Weill FX, Demartin M, Fabre L and Grimont PA (2004), Extended-spectrum-beta-lactamase (TEM-52)-producing strains of *Salmonella enterica* of various serotypes isolated in France, *Journal of Clinical Microbiology* **42:** 3359-3362.

WHO (World Health Organization) (2010), Laboratory protocol ***In:*** Isolation of Salmonella spp. From food and animal faeces, 5th Ed. pp 4-8, 13. Accessed on 27 April, 2017. http://www.antimicrobialresistance.dk/data/images/protocols/isolation_of_salm_22060.pdf.

Yhiler NY and Bassey BE (2015), Antimicrobial Susceptibility Patterns of *Salmonella* species in Poultry Production Setting in Calabar, Cross River State, Nigeria, *American Journal of Health Research* **3**(2): 76-81.

Zhang L, Fu Y, Xiong Z, Ma Y, Wei Y, Qu X, Zhang H, Zhang J and Liao M (2018), Highly Prevalent Multidrug-Resistant *Salmonella* From Chicken and Pork Meat at Retail Markets in Guangdong, China, *Frontiers in Microbiology* **9:** 2104. DOI: 10.3389/fmicb.2018.02104

Ziech RE, Lampugnani C, Perin AP, Sereno MJ, Sfaciotte RAP, Viana C, Soares VM, Nogueira Pinto JPN and Bersot LS (2016), Multidrug resistance and ESBL-producing Salmonella spp. isolated from broiler processing plants, *Food Microbiology* **47**(1): 191-195. DOI: org/10.1016/j.bjm.2015.11.021