# Prevalence, characterization and antibiotic resistance of Shiga toxigenic *Escherichia coli* serogroups isolated from fresh beef and locally processed ready-to-eat meat products in Lagos, Nigeria

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## Abstract

Fresh beef and meat products have been implicated in outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) worldwide. This study investigated the prevalence of *E. coli* O157: H7 and non-O157 STEC serogroups in fresh beef in the open market and street vended meat products ($n$ = 180) in Lagos metropolis, Nigeria. A combination of culture media and immunomagnetic separation followed by typing for associated virulence factors and serotypes was performed. Antimicrobial susceptibility testing was performed on the isolated STEC serotypes using the disk diffusion method. A total of 72 STEC serogroup isolates were detected from 61 out of 180 samples. The O157 STEC serotypes were detected in fresh beef, *suya* , minced meat and *tsire* with prevalence of 20.8% while non-O157 STEC serogroups were detected in all the samples. Molecular typing revealed 25% ($n$ = 18) of the STEC serogroups showed presence of all the s *tx1*, *stx2*, *eaeA*, *fliCH7* and *rfbE* O157 virulence factors while 54.2% ($n$ = 39) possessed a combination of two virulence genes. Multidrug resistance was discovered in 23.6% ($n$ = 17) of the total STEC serogroups. Locally processed ready-to-eat meat products in Lagos metropolis, Nigeria harbour potentially pathogenic multi-drug resistant STEC serogroups that can constitute public health hazard.

## Highlights

- Prevalence of STEC in locally processed ready-to-eat meat products in Lagos metropolis, Nigeria was established.
- STEC O157 serotypes were found in 20.8% of the meat products while nonO157 STEC serogroups were isolated in all the samples.
- *stx* 1, *stx* 2, *eaeA*, *fliCH7* and *rfbE* O157 were found in 25% of the STEC serogroups while 54.2% had at least two virulence genes.
- Multidrug resistance was discovered in 23.6% of the total STEC serogroups

## 1 Introduction

Shiga toxin producing *Escherichia coli* (STEC), often referred to as enterohaemorrhagic *E. coli* (EHEC) is a group of pathogenic *E. coli* strains producing one or more Shiga toxins (stx) (Monaghan et al., 2011). Pathogenicity in STEC is linked to several virulence factors which allow the organism to attach and colonize the bowel, invade tissues, and produce toxins that contribute to disease symptoms and progression (Grant et al., 2011). The ability of STEC to cause serious disease in humans depends on their ability to produce stx, attaching and effacing (A/E) and plasmid mediated factors (Farrokh et al., 2013; Grant et al., 2011). The diversity of the serotypes, the infective dose, ability to survive in food matrices and in the host's gut influences the pathogenicity of STEC (Grant et al., 2011; Mellies et al., 2007).

Nigeria ranks first in the health burden of zoonotic diseases in Africa (Grace et al., 2012). The major pathogenic microorganisms frequently associated with foods of animal origin is the Enterohemorrhagic *E. coli* O157: H7; however, implication of non-O157 STEC serotypes in foodborne infections has been on the increase worldwide (Bettelheim and Goldwater, 2014; Beyi et al., 2017; Gould et al., 2013; Wang et al., 2013). More than 380 non-O157 STEC serotypes have been associated with human diseases (Rahal et al., 2015), while over 100 serotypes have been associated with foodborne outbreaks and sporadic cases of gastrointestinal diseases and haemolytic uremic syndrome (HUS) (Smith et al., 2014). Therefore, non-O157 STEC serotypes pose great risk to public health as *E. coli* O157: H7 (Mathusa et al., 2010), but their infections have been under-recognized and under-reported in Africa (Lupindu, 2018). Information about human infections with *E. coli* O157 and non-O157 STEC serogroups is limited in the Nigeria.

The microbiological safety of meat and meat products is an important public health concern worldwide. Meat products have been implicated in foodborne disease outbreaks caused by toxigenic E. coli all over the world (Robertson et al., 2016). Suya (spiced grilled beef), *kilishi* (biltong-like beef), roasted beef and tsire (spiced smoked beef) are widely consumed ready-to-eat (RTE) meat products in Nigeria. These indigenous RTE meat products are mostly processed under poor sanitary conditions by the informal sector of the economy with little or no food safety regulations in place (Obadina et al., 2014). It has been shown that only 2% of meat samples in Nigeria complied with acceptable meat standards (Okike et al., 2011). In Nigeria, for instance fresh beef and meat products are mostly display in the open for sale without any control and at ambient temperature in the open markets. Sellers of such meat and meat products usually do not exhaust their product sales in a day. The unsold meat products are not properly kept due to poor storage facilities and are usually kept at ambient temperature till the second day for sale, thereby increasing the risk of microbial contaminations and proliferations (Okike et al., 2011). Likewise, the unregulated use of antibiotics for livestock farming, unhygienic practices that predominates animal slaughtering and processing of meat carcass in Nigeria; potentially enhances cross contamination of meat products with pathogenic multi-drug resistant (MDR) STEC (Adenipekun et al., 2015; Ojo et al., 2010).

Studies have highlighted the occurrence of *E. coli* O157:H7 in meat products in Nigeria (Olatoye, 2010; Ojo et al., 2010); however, there is little or no emphasis on the occurrence of non-O157 STEC serogroups; which has been known to account for a substantial portion of all STEC infections worldwide (Smith et al., 2014). Antimicrobial resistance in STEC serogroups is a matter of increasing concern and there is a paucity of data on the prevalence of multi-drug resistant STEC from foods in developing countries such as Nigeria (Ojo et al.,

2010). Therefore, this study determined the prevalence, molecular characteristics and antimicrobial resistance of *E. coli* O157:H7 and non-O157 STEC serotypes in fresh beef and commonly consumed locally processed meat products in the open market in Lagos, Nigeria.

## 2 Materials and methods

### *2.1 Study area and sample collection*

A total of 180 meat samples (30 samples each) of fresh beef, minced meat, *suya* , *kilishi* , roasted beef and *tsire* were purchased from various open markets covering selected vital areas within Lagos State, Nigeria (6.498 º N 3.38285°E) using simple random sampling technique. The locations of sampled fresh beef and meat products are indicated on the map of Lagos State ( Fig. 1 ). The areas covered are noted for high population density with middle-low-income earners and the sampling was carried out for a period of 12 weeks (June – August 2019). Samples were purchased from vendors at open market stalls and were placed in sterile polyvinyl bags, then kept on ice packs in cooler boxes during transportation to the laboratory. Cross contamination of samples was avoided during sample collection, transportation and processing. The microbiological examination of the samples was carried out within 24 h of collection.

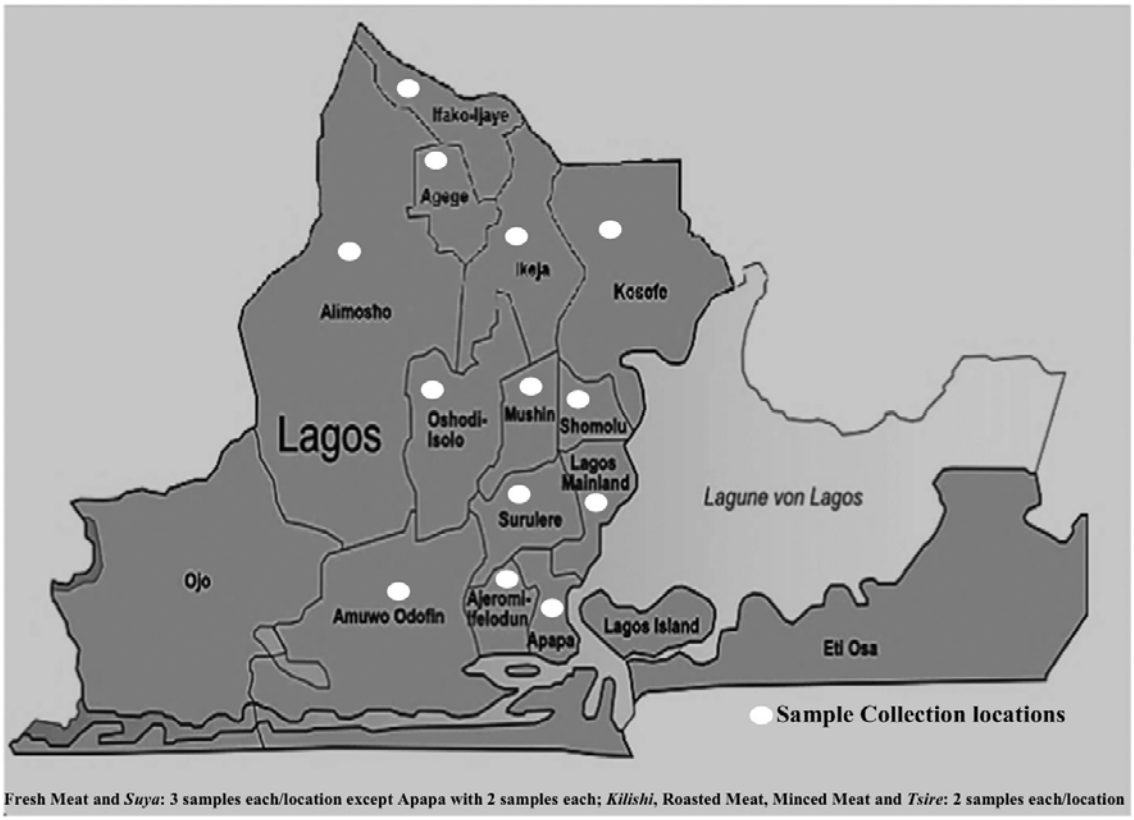


**Fig. 1.** Map of Lagos State showing sampling locations (LGA) of fresh beef and meat products within Lagos Metropolis ($n$ = 180). Three (3) samples per location for each of fresh meat and *suya* except from Apapa with 2 samples each. Two (2) samples per location for each of *kilishi* , roasted meat, minced meat and *tsire* .

### *2.2 Isolation of STEC serogroups from fresh beef and meat products*

An enrichment and recovery of STEC was performed as previously described (Cagney et al., 2004). Briefly, ten grammes (10 g) of each meat sample was thoroughly homogenised with 90 ml of sterile modified tryptic soy broth (mTSB) (Oxoid, Basingstoke, UK) supplemented with vancomycin (final concentration of 40 μg/ml); the homogenate was then incubated overnight at 37 °C. After overnight incubation at 37 °C, the pre-enrichment broth was divided into two portions, a part was plated onto Sorbitol MacConkey (SMAC) agar supplemented with cefixime (0.5 mg/l) and potassium tellurite (2.5 mg/l) (CT-SMAC) and incubated at 37 °C for 24 h for isolation and presumptive identification of O157 and non-O157 STEC. After incubation, all suspected *E. coli* O157 colonies (colourless non-sorbitol fermenting) and pink colonies of presumptive non-O157 STEC (sorbitol fermenting) were selected and further purified by streaking onto Tryptone Soy Agar (TSA) (Merck, Darmstadt, Germany). The pure cultures of the suspected STEC isolates were then preserved in sterile brain heart infusion broth (CM0225, Oxoid, Hampshire, UK) containing 20% glycerol (Sigma-Aldrich Chemie GmbH) at −80 °C for use in subsequent analyses. The other portion of the pre-enriched homogenate was used for the automated immunomagnetic separation (IMS) assay as described by Njage et al. (2012); using a Bead-Retriever instrument (Thermo Fisher Scientific, Vantaa, Finland). Briefly, one disposable sample tube strip was placed into a BeadRetriever rack for each sample to be processed. The dynabeads anti *E. coli* O157 (Invitrogen AG, Basel, Switzerland) were re-suspended by vortexing until the pellet in the bottom disappeared. Suspended dynabeads were mixed with the sample and wash buffer composed of 0.15 M NaCl and 0.01 M Sodium Phosphate buffer, pH 7.4, with 0.05% Tween 20. The filled tubes were inserted into the sample racks and then sterile protective tip combs were inserted into the instrument. The IMS was then performed by running the EPEC/VTEC program.

### *2.3 Identification of presumptive STEC serogroups*

All presumptive STEC isolates were biochemically confirmed for conventional identification of *E. coli* using the API 20E system (BioMerieux, Marcyl-l' Etoile, France). After biochemical identification, the O157 isolates were serologically screened for the presence of the serogroup O157:H7 antigen using *E. coli* O157 Latex Test Kit (DR0620, Oxoid) according to the manufacturer's instructions. The non-O157 STEC isolates were also serotyped as previously described by Kok et al. (1996) using rapid diagnostic *E. coli* antisera kits (Difco Laboratories, Detroit Michigan, USA). *E. coli* polyvalent antisera 2, 3 and 4 were used together with O monovalent antisera for mostly available O (O1 – O185) antisera.

### *2.4 Molecular characterization of STEC serotypes*

#### *2.4.1 Bacterial DNA extraction and amplification*

The DNA of all the presumptive STEC isolates and the positive control strain (*E. coli* O157:H7 – ATCC 43895) was extracted as previously described by Choo et al. (2007). Five millilitre (5 ml) STEC culture in nutrient broth (Merck) was incubated overnight at 37 °C and 1 ml from each cultured isolate was then taken and centrifuged at 17000 × *g* for 2 min using a MiniSpin micro centrifuge (Merck). The pellet was then washed with 1 ml of sterile distilled water. The bacterial pellet was resuspended in 200 ml of sterile distilled water and heated at 95 °C for 10 min in a dry-heating block, then centrifuged at 17000 × *g* for 10 min; the

**Table 1.** Oligonucleotide primers used for amplification of the various targeted genes in isolated Shiga-toxin producing *E. coli* (STEC).

| Target gene | Primer pair | Oligonucleotide primer sequences (5′–3′) | Size of the amplified DNA (bp) | References |
|---|---|---|---|---|
| *stx1* | *Stx*1F | ATAAATCGCCATTCGTTGACTAC | 180 | Paton and Paton, 1998 |
| | *Stx*1R | AGAACGCCCACTGAGATCATC | | |
| *stx2* | *Stx*2F | GGCACTGTCTGAAACTGCTCC | 255 | Paton and Paton, 1998 |
| | *Stx*2R | CGCCAGTTATCTGACATTCTG | | |
| *eaeA* | *eaeA*F | GACCCGGCACAAGCATAAGC | 384 | Paton and Paton, 1998 |
| | *eaeA*R | CCACCTGCAGCAACAAGAGG | | |
| *rfb*O157:H7 | O157F | CGGACATCCATGTGATATGG | 259 | Paton and Paton, 1998 |
| | O157R | TTGCCTATGTACAGCTAATCC | | |
| $fliC_{H7}$ | $fliC_{H7}$-a | TACCATCGCAAAAGCAACTCC | 247 | Wang et al., 2002 |
| | $fliC_{H7}$-b | GTCGGCAACGTTAGTGATACC | | |

supernatant was transferred to sterile tube and used as template DNA for PCR detection of the target virulence genes.

*2.4.2 Detection of virulence factors*

Presence of shiga-toxin genes as virulence markers were detected using a multiplex PCR reaction targeting the *stx* 1, s *tx* 2, *eaeA, fli* CH7 and *rfbE* O157 genes in all the presumptive STEC isolates. The detection of the virulence factors was performed as previously described by China et al. (1996). The primers for the detection of *stx* 1, *stx* 2 and *eaeA* were used for the PCR assays as previously described by Paton and Paton (1998). The oligonucleotide primers sequences for this three virulence factors and PCR product sizes are shown in Table 1 . The condition for amplification was an initial denaturation at 94 °C for 3 min followed by 34 cycles of denaturation at 94 °C for 1 min, 1 min of annealing at between 53 °C and 59 °C, depending on the primer, 1 min of elongation at 72 °C and then final elongation at 72 °C for 3 min using a thermo-cycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). The amplified DNA products were visualised on 2% agarose gel electrophoresis stained with ethidium bromide. Strains of *E. coli* O157:H7 ATCC 43895 and *E. coli* O157:H7 ATCC 43888 were used as positive and negative controls, respectively ( Fig. 3 ).

***2.5 Antibiotic susceptibility test***

The STEC isolates were tested for susceptibility to Ampicillin (AMP), Chloramphenicol (CHL), Ciprofloxacin (CIP), Enrofloxacin (ENF), Nalidixic Acid (NA), Norfloxacin (NOR), Streptomycin (STR) and Tetracycline (TET). The susceptibility test was performed using Kirby-Bauer disk diffusion technique (Bauer et al., 1966) on Mueller Hinton agar (Oxoid, UK) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015). The standard procedure was adhered and the zones of inhibition were measured based on the breakpoints of the zone diameters for respective antibiotic agents. The antibiotic susceptibility results were interpreted in accordance with the interpretive criteria provided by CLSI (2015). *Escherichia coli* (ATCC 25922) and *E. coli* (ATCC 43895) were included as quality control organisms in antimicrobial susceptibility determination.

**3 Results and discussion**

***3.1 Prevalence of STEC O157 and non-O157 serogroups in fresh beef and meat products***

Highest microbial counts (5.6 log $_{10}$ cfu/g) of presumptive STEC were observed in fresh beef followed by *Suya* and *Tsire* while the lowest counts were recorded in *kilishi* ( Fig. 2 ). The prevalence of STEC serogroups is based on the number of fresh beef and meat products that were positive for presumptive STEC. Fresh beef and *suya* had the highest STEC prevalence of 76.7% and 56.7% respectively, while roasted beef and minced meat had the lowest occurrence ( Table 2 ). The high prevalence could be attributed to the unhygienic practises which is the norm in the meat processing sector in Nigeria. Reports have indicated that slaughtering of animals, evisceration and splitting of carcass is usually carried out on abattoir floor; poor quality water used for washing carcasses ((Bello et al., 2015, Enabuele and Uraih, 2009; Fasanmi et al., 2018; Ojo et al., 2010). These aforementioned reasons in addition to high ambient temperature, humidity and poor handling practices dispose raw meat to deterioration and contamination during transportation, processing and sale. In the addition, the unhygienic condition of the tables on which the meats were displayed for sale in the open markets could also contribute to the contamination of the fresh beef. These hygiene breaches

encourage cross-contamination of animal carcass with intestinal contents harbouring STEC (Abong'o and Momba, 2009; Adenipekun et al., 2015).

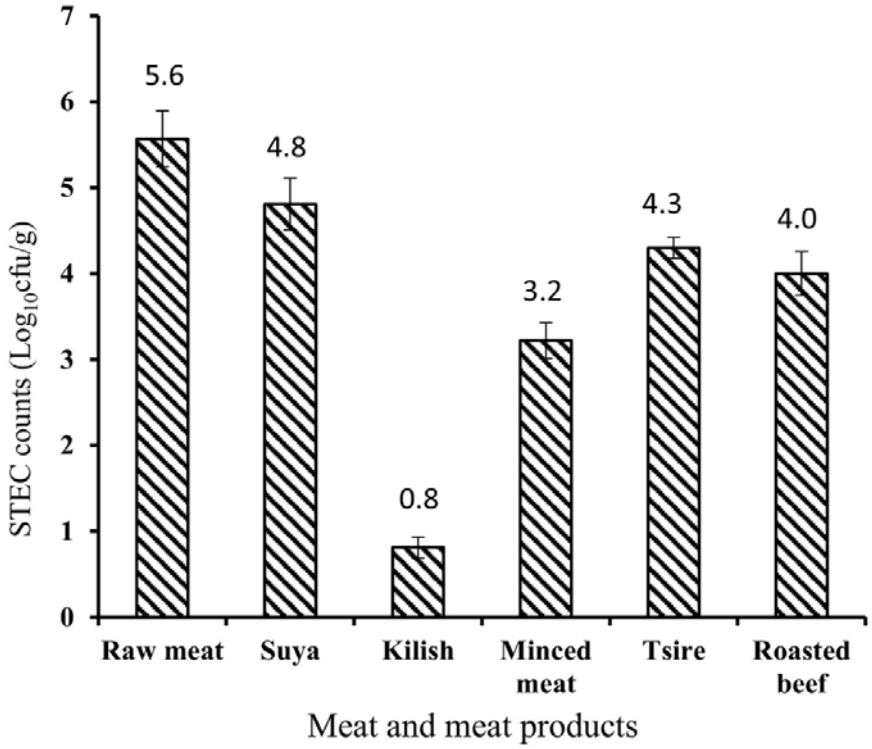


**Fig. 2**. Microbial counts of Shiga toxin-producing *Escherichia coli* (STEC) serogroups isolated from fresh meat and meat products in Lagos metropolis ($n$ = 180).

**Table 2**. Prevalence of O157 and non-O157 Shiga toxin-producing *E. coli* serogroups in the meat and meat products sold in Lagos Metropolis ($n$ = 180).

| Meat and meat products investigated | Number of samples | Number of samples positive for STEC | Prevalence (%) |
|---|---|---|---|
| Fresh beef | 30 | 23 | 76.7 |
| *Suya* | 30 | 17 | 56.7 |
| *Kilishi* | 30 | 5 | 16.7 |
| Minced meat | 30 | 4 | 13.3 |
| Roasted beef | 30 | 3 | 10.0 |
| *Tsire* | 30 | 9 | 30.0 |
| Total | 180 | 61 | 33.7 |

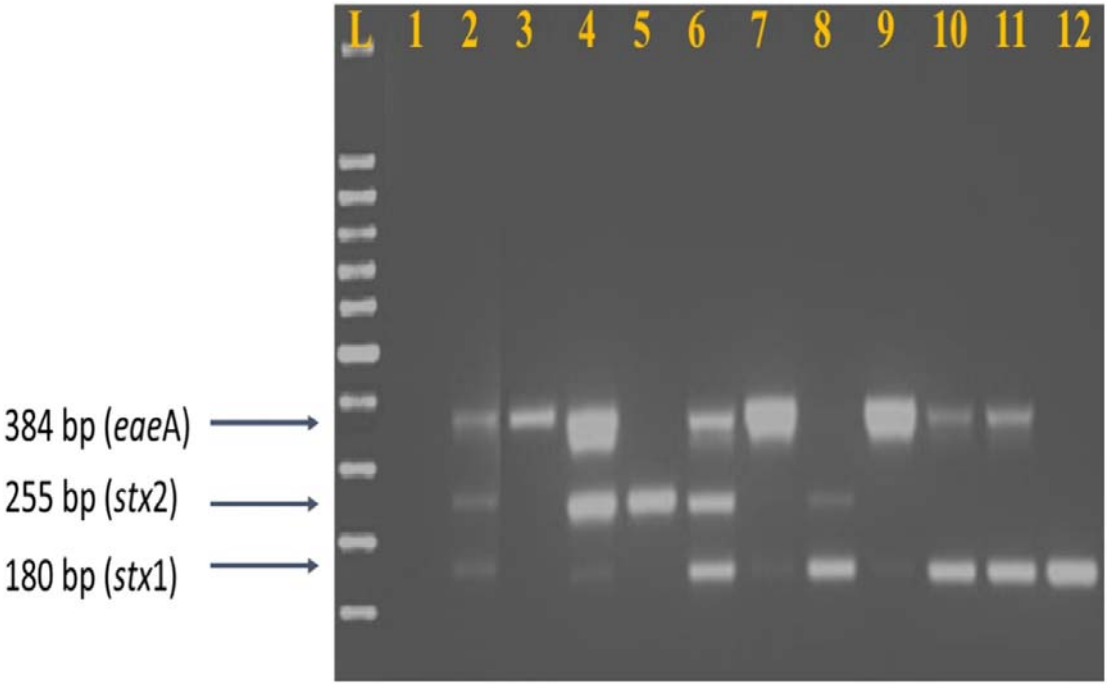


**Fig. 3**. Illustrative agarose gel electrophoresis image of multiplex-PCR products ( *stx* 1, stx2, *eae* A). Lane L: marker (100-bp ladder), lane 1: negative control, lane 2: positive control (180, 285, and 384 bp) and STEC isolates lanes: 3 ( *eae* A), 4 ( *eae* A, *stx* 2, *stx* 1), 5 ( *stx* 2), 6 ( *eae* A, *stx* 1, *stx* 2), 7 ( *eae* A, *stx* 1), 8 ( *stx* 2, *stx* 1), 9 ( *eae* A), 10 ( *eae* A, *stx* 1), 11 ( *eae* A, *stx* 1) and 12 ( *stx* 1).

Most significant and of a great concern in this study is the prevalence of STEC in the ready-to-eat meat products such as *suya, kilishi* and *Tsire* which are consumed directly without further processing. Consumption of such contaminated meat products can result in public health hazard as infections caused by STEC have been linked mostly to the consumption of beef and related meat products (EFSA, 2013; Robertson et al., 2016; Toro et al., 2018). In the overall, 61 out of 180 (33.7%) fresh beef and meat products tested were positive for STEC. Although it is difficult to compare the prevalence of STEC obtained in this study with the previously reported data due to the different hygienic levels and variations in the isolation procedures, nevertheless, the prevalence reported in this study is higher than values reported for *E. coli* O157:H7 in similar studies conducted in Nigeria (Olatoye, 2010; Ojo et al., 2010). However, these authors did not test for the presence of non-O157 STEC serotypes as those studies were only focused on *E. coli* O157:H7. In comparison with prevalence of STEC in meat carcasses and meat products from other parts of the world; this study recorded higher prevalence of STEC than reported in Argentina (32%), United Kingdom (27%), USA (16.2%), France (11%), Italy (8.4%), Canada (5.4%), Poland (3%) and the European Union (1.3%) (Brusa et al., 2020; EFSA, 2014; Monaghan et al., 2012). Thus, corroborating the high prevalence is linked to lack of hygiene standards and poor quality of wash in most abattoirs in Nigeria.

### *3.2 Serological identification and distribution of STEC O157 and non-O157 in fresh beef and meat products*

Out of the 72 STEC isolates that were recovered in the fresh beef and meat products, 15 (20.8%) were found to be positive for the three target genes ( *fli* CH7, *rfb* EO157 and *eae* A)

**Table 3.** Distribution of isolated Shiga toxin-producing *Escherichia coli* serotypes among the meat and meat products investigated.

| Serogroups | Prevalence of O157 and non-O157 STEC serogroups in the meat and meat products ($n = 72$) | | | | | | % occurrence of STEC |
|---|---|---|---|---|---|---|---|
| | Fresh meat | *Suya* | *Kilishi* | Minced meat | Roasted meat | *Tsire* | |
| O157 ($n = 15$) | 7 (46.7[a]) | 4 (26.7) | 0 (0.0) | 1 (6.7) | 0 (0.0) | 3 (20.0) | 15 (20.8) |
| O26 ($n = 12$) | 6 (50.0) | 2 (16.7) | 2 (16.7) | 0 (0.0) | 1 (8.3) | 1 (8.3) | 12 (16.7) |
| O83 ($n = 8$) | 1 (12.5) | 0 (0.0) | 1 (12.5) | 2 (25.0) | 2 (25.0) | 2 (25.0) | 8 (11.1) |
| O91 ($n = 3$) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (33.3) | 2 (66.6) | 3 (4.2) |
| O103 ($n = 6$) | 5 (83.3) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6 (8.3) |
| O111 ($n = 12$) | 5 (41.6) | 3 (25.0) | 2 (16.7) | 1 (8.3) | 0 (0.0) | 1 (8.3) | 12 (16.6) |
| O128 ($n = 8$) | 3 (37.5) | 4 (50.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (12.5) | 8 (11.1) |
| O138 ($n = 3$) | 0 (0.0) | 2 (66.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (33.3) | 3 (4.2) |
| O145 ($n = 3$) | 0 (0.0) | 3 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (4.2) |
| O147 ($n = 2$) | 2 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (2.8) |
| Total isolates | 29 (40.3) | 19 (26.4) | 5 (6.9) | 4 (5.6) | 4 (5.6) | 11 (15.3) | 72 |

[a] Represents the prevalence of isolated STEC serotype in the meat and meat products.

that are characteristics of *E. coli* O157:H7 while the remaining 57 (79.2%) isolates tested positive for the non-O157:H7 STEC serotypes ( Table 3 ). The occurrence of STEC O157:H7 was highest (46.7%) in the fresh beef examined while among the meat products tested, its prevalence was highest in suya (26.7%) and was not detected in kilish and roasted meat. In addition, the prevalence rate of STEC O157:H7 in the minced meat was relatively low compared to other meat products examined. However, this level of prevalence still poses a great threat to the consumers due to the low infectious dose of this pathogen (<100 cells) (Doyle et al., 2001; Strachan et al., 2005; Tuttle et al., 1999). Although *E. coli* O157:H7 was not isolated in the roasted meat and *kilish* , the prevalence of non-O157 STEC serotypes in these meat products poses just as great risk to the consumers as *E. coli* O157:H7 (Eblen, 2007; Cooley et al., 2013).

Among the 9 non-O157 STEC serotypes identified, O111 and O26 were the most prevalence which accounted for 16.6% and 16.7%, respectively ( Table 3 ). These two serotypes have been identified as the most common non-O157 STEC associated with foodborne illness cases worldwide (Kappeli et al., 2011). Shiga toxin producing *E. coli* O103 serotype was detected in fresh beef and *suya* while O145 and O147 serotypes were isolated only from *suya* and fresh beef, respectively. STEC serogroups O26, O111, O103 and O145 detected in this study are considered to be highly pathogenic to humans and are frequently implicated in food poisoning outbreaks worldwide with public health implications (Garvey et al., 2009; Hale et al., 2012; Smith et al., 2014). It is important to note that with the low level of sanitary practices observed in the abattoirs and among the meat processors coupled with inadequate data on infections outbreak in Nigeria, the non-O157 STEC identified in this study could spread easily without early detection. It is therefore, imperative for the Government and food safety stakeholders in the country to take seriously the prevalence of these STEC serotypes in *ready-to-eat* meat products in order to avoid public health hazard and possible outbreak of STEC.

In the overall, 79.2% of the STEC isolated were non-O157 STEC serotypes while 20.8% were *E. coli* O157:H7 ( Table 3 ). This finding is a confirmation that consumers are more exposed to non-O157 STEC from food sources than *E. coli* O157:H7 STEC in Nigeria, as it is the trend in other part of the world (Grant et al., 2011; Johnson et al., 2006); yet the reports on the incidence of non-O157 STEC infection in Nigeria is far lower than that of *E. coli* O157:H7 (Olowe et al., 2014). The high prevalence of non-O157 STEC in the current study supported previous reports on high level of these pathogens in the animals from which the meat and meat products are sourced in the southwestern Nigeria (Adenipekun et al., 2015; Ajayi et al., 2011; Nsofor et al., 2013; Ojo et al., 2010).

### *3.3 Virulence genes distribution among STEC O157 and non-O157 serogroups from fresh beef and meat products*

The presence of three virulence genes (s *tx* 1, s *tx* 2 and *eaeA* ) were detected in 25% of the total isolates while 66.7% of *E. coli* O157:H7 isolates harboured the three virulence genes ( Table 4 ). Among the isolated STEC serogroups, 8.3% and 12.5% tested positive for either s *tx* 1 or *stx* 2 only, respectively, while 23.6% of the isolates carried a combination of *stx1* and *stx* 2. None of the isolates harboured *eaeA* only, rather 2.8% and 27.8% of the isolates carried *eaeA* in association with *stx* 1 or *stx* 2, respectively. Detection of the three virulence genes were observed in non-O157 STEC serotypes O26, O111, O103 and O147.

**Table 4.** Distribution of virulence genes (*Stx1*, *Stx2*, and *eaeA*) of STEC serotypes isolated from the meat and meat products sold in Lagos metropolis, Nigeria.

| Virulence genes | | | % distribution of virulence genes in the STEC isolate | | | | | | | | | | Total isolates ($n = 72$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *stx1* | *stx2* | *eaeA* | O157 ($n = 15$) | O26 ($n = 12$) | O83 ($n = 8$) | O91 ($n = 3$) | O103 ($n = 6$) | O111 ($n = 12$) | O128 ($n = 8$) | O138 ($n = 3$) | O145 ($n = 3$) | O147 ($n = 2$) | |
| + | − | − | 0 (0.0) | 1 (8.3) | 0 (0.0) | 1 (33.3) | 0 (0.0) | 2 (16.7) | 0 (0.0) | 1 (33.3) | 1 (33.3) | 0 (0.0) | 6 (8.3[a]) |
| − | + | − | 1 (6.7) | 2 (16.7) | 1 (12.5) | 0 (0.0) | 1 (16.7) | 2 (16.7) | 0 (0.0) | 0 (0.0) | 2 (66.7) | 0 (0.0) | 9 (12.5) |
| + | + | − | 2 (13.3) | 1 (8.3) | 2 (25.0) | 2 (66.7) | 3 (50.0) | 4 (33.3) | 0 (0.0) | 2 (66.7) | 0 (0.0) | 1 (50.0) | 17 (23.6) |
| − | − | + | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| + | − | + | 0 (0.0) | 1 (8.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (8.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (2.8) |
| − | + | + | 2 (13.3) | 4 (33.3) | 5 (62.5) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 8 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 20 (27.8) |
| + | + | + | 10 (66.7) | 3 (25.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 3 (25.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (50.0) | 18 (25.0) |

[a] Represents the percentage distribution of the virulence genes.

The main virulence factors that contribute to pathogenicity in STEC is the production of shiga-toxin by one of *stx* 1, *stx* 2 or both, each including several variants of toxin produced (Lorenz et al., 2013). These virulence factors enable the organism to attach and colonize the bowel, invade tissues, and produce toxins that contribute to disease symptoms and progression (Grant et al., 2011). Shiga toxins are also capable of binding to the cellular receptors and inhibiting the protein synthesis in several organs such as kidney, brain and liver thereby causing severe diseases (Smith et al., 2014). Therefore, the presence of non-O157 STEC that harboured virulence genes in the fresh beef and meat products sold in Lagos Metropolis should be of great concern to the health authorities as consumption of such products may constitute a serious public health risk to the consumers. Non-O157 STEC O26, O103, and O111 serotypes isolated in the examined meat products are capable of inducing HUS (Bielaszewska et al., 2013; Soborg et al., 2013) and can also cause severe foodborne illness to the consumers as thermal treatment of the meat products may not inactivate the toxins (Rasooly and Do, 2010). In addition, 44.4% of the STEC serotypes did not possess *eaeA* gene, albeit, production of intimin is not required for STEC to be considered pathogenic as some reported severe cases of HUS have been caused by *eae* -negative STEC (Barlow et al., 2006). The combination of *eaeA* with *stx1* or *stx 2* genes by some of the non-O157 STEC serotypes isolated in this study is of serious public health concern because among various virulence factors involved in non-O157 STEC pathogenicity, the combined effect of *eaeA* with *stx1* and *stx 2* genes has been associated with enhanced virulence (Mathusa et al., 2010).

**Table 5.** Antimicrobial susceptibility of Shiga toxin-producing *Escherichia coli* (STEC) isolated in the fresh beef and meat products sold in Lagos metropolis, Nigeria.

| Antibiotics | Disc code | Antibiotic disc content (µg/disc) | Number of STEC isolates ($n$ = 72) | | |
|---|---|---|---|---|---|
| | | | R | I | S |
| Ampicillin | AMP | 10 | 58 (80.6[a]) | 6 (8.3) | 8 (11.1) |
| Chloramphenicol | CHL | 30 | 40 (55.6) | 5 (6.9) | 27 (37.5) |
| Ciprofloxacin | CIP | 5 | 20 (27.8) | 9 (12.5) | 43 (59.7) |
| Enrofloxacin | ENF | 5 | 18 (25.0) | 6 (8.3) | 48 (66.7) |
| Nalidixic Acid | NA | 30 | 22 (30.6) | 7 (9.7) | 43 (59.7) |
| Norfloxacin | NOR | 10 | 24 (33.3) | 4 (5.6) | 44 (61.1) |
| Streptomycin | STR | 10 | 52 (72.2) | 4 (5.6) | 16 (22.2) |
| Tetracycline | TET | 30 | 60 (83.3) | 3 (4.2) | 9 (12.5) |
| Total (%) | | | 51.04 | 7.6 | 41.3 |

R – Resistant, I – Intermediate, S – Sensitive.
[a] Percentage of the isolates tested.

### *3.4 Antimicrobial susceptibility of STEC serogroups from fresh beef and meat products in Lagos Metropolis*

The antimicrobial resistance of the STEC isolated in fresh beef and meat products is presented in Table 5 . The STEC isolates were highly resistant to 3 (Ampicillin, Streptomycin and Tetracycline) out of the 8 antimicrobial agents tested. Some isolates were either

**Table 6.** Antimicrobial resistant of O157 and non-O157 Shiga toxin-producing *Escherichia coli* serogroups isolated from meat and meat products sold in Lagos Metropolis, Nigeria.

| Serogroup | Number and % of STEC serogroups resistant to antimicrobial agents ($n = 72$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AMP | CHL | CIP | ENF | NA | NOR | STR | TET |
| O157 ($n = 15$) | 13 (86.7[a]) | 10 (66.7) | 6 (40.0) | 4 (26.7) | 6 (40.0) | 5 (33.3) | 11 (73.3) | 12 (80.0) |
| O26 ($n = 12$) | 10 (83.3) | 8 (66.7) | 2 (16.7) | 3 (25.0) | 2 (16.7) | 3 (25.0) | 8 (66.7) | 11 (91.6) |
| O83 ($n = 8$) | 6 (75.0) | 4 (50.0) | 4 (50.0) | 1 (12.5) | 1 (12.5) | 1 (12.5) | 6 (75.0) | 7 (87.5) |
| O91 ($n = 3$) | 2 (66.7) | 1 (33.3) | 0 (0.0) | 1 (33.3) | 2 (66.7) | 2 (66.7) | 1 (33.3) | 2 (66.7) |
| O103 ($n = 6$) | 5 (83.3) | 3 (50.0) | 2 (33.3) | 3 (50.0) | 4 (66.7) | 6 (100.0) | 4 (66.7) | 5 (83.3) |
| O111 ($n = 12$) | 11 (91.7) | 7 (58.3) | 3 (25.0) | 3 (25.0) | 6 (50.0) | 1 (8.3) | 7 (58.3) | 10 (83.3) |
| O128 ($n = 8$) | 6 (75.0) | 4 (50.0) | 2 (25.0) | 2 (25.0) | 1 (12.5) | 3 (37.5) | 8 (100.0) | 6 (75.0) |
| O138 ($n = 3$) | 2 (66.7) | 1 (33.3) | 2 (66.7) | 0 (0.0) | 0 (0.0) | 1 (33.3) | 2 (66.7) | 3 (100.0) |
| O145 ($n = 3$) | 2 (66.7) | 2 (66.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (33.3) | 2 (66.7) | 2 (66.7) |
| O147 ($n = 2$) | 1 (50.0) | 0 (0.0) | 0 (0.0) | 1 (50.0) | 0 (0.0) | 1 (33.3) | 1 (33.3) | 2 (100.0) |
| Total Resistance | 58 (80.6) | 40 (55.6) | 21 (29.2) | 18 (25.0) | 22 (30.6) | 24 (33.3) | 50 (69.4) | 60 (83.3) |

Key abbreviations of the antimicrobials are presented in Table 5.

[a] Percentage of the total isolates tested.

**Table 7.** Multidrug resistance (MDR) profile of O157 and non-O157 Shiga toxin-producing *Escherichia coli* serogroups isolated from meat and meat products sold in Lagos metropolis, Nigeria.

| Antimicrobial resistance patterns | Number of STEC serotypes isolates showing multidrug resistance (MDR) ($n = 72$) | | | | | | | | | | Total isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | O157 | O26 | O83 | O91 | O103 | O111 | O128 | O138 | O145 | O147 | |
| AMP, CHL, CIP, ENF, NA, NOR, STR, TET | 2 | 1 | 2 | 1 | 1 | 2 | 1 | – | – | – | 10 (13.9[a]) |
| AMP,CIP, ENF, NA, NOR, STR, TET | 1 | 2 | 1 | – | – | 1 | – | – | – | – | 5 (6.9) |
| AMP, CIP, STR, CHL, ENF, NA, TET | – | – | – | – | – | – | – | 1 | – | – | 1 (1.4) |
| AMP, CIP, STR, CHL, NA, NOR | 1 | – | – | – | – | – | – | | – | – | 1 (1.4) |
| AMP, STR, CHL, NA, TET | – | 1 | – | – | – | – | – | 1 | – | – | 2 (2.8) |
| AMP, STR, CHL, TET | 1 | – | 1 | – | 1 | 2 | 1 | – | – | 1 | 7 (9.7) |
| AMP, CIP, STR, CHL | – | – | – | – | | 1 | – | – | – | – | 1 (1.4) |
| AMP, ENF, NOR, TET | – | 1 | – | – | 1 | – | – | – | – | – | 2 (2.8) |
| AMP, CIP, NA, TET | – | – | 1 | – | – | – | – | – | – | – | 1 (1.4) |
| CIP, ENF, NA, NOR | – | 1 | – | – | – | – | – | 1 | – | – | 2 (2.8) |
| CHL, STR, TET | 1 | 1 | – | – | – | 1 | 1 | – | – | – | 4 (5.6) |
| AMP, STR,TET | 3 | 2 | – | 1 | – | 2 | 2 | – | 3 | 1 | 14 (19.4) |
| AMP, CHL,TET | – | 1 | | 1 | – | – | – | – | – | – | 2 (2.8) |
| AMP, CHL,STR | 1 | – | 1 | – | 1 | – | 1 | – | – | – | 4 (5.6) |
| AMP, TET | 2 | 1 | – | – | – | – | – | – | – | – | 3(4.2[a]) |
| CHL, NA | – | – | – | – | – | 1 | – | – | – | – | 1 (1.4) |
| CIP, TET | – | – | – | – | 2 | – | – | – | – | – | 2 (2.8) |
| AMP, STR | 2 | – | 2 | – | – | 2 | 2 | – | – | – | 8(11.1) |
| CHL, TET | 1 | 1 | – | – | – | – | – | – | – | – | 2 (2.8) |
| Total isolates | 15 | 12 | 8 | 3 | 6 | 12 | 8 | 3 | 3 | 2 | 72 |

[a] Represents the percentage of the MDR of the total isolate tested, Key abbreviations of the antimicrobials are presented in Table 5.

intermediately susceptible (I) and/or fully susceptible (S) while others are resistant to some antimicrobial agents. In the overall, 51.04% of the isolates exhibited resistance to at least one of the antimicrobial agents while 41.3% were susceptible to one or more antimicrobial agents.

All the non-O157 STEC serogroups had at least one isolate that is resistant to Ampicillin, Norfloxacin, Tetracycline and Streptomycin (Table 6). Highest resistance was to tetracycline (83.3%) while only 25% of the isolates were resistant to enrofloxacin. There were also double and multiple resistances among the tested isolates. A total of 72 isolates were analysed for antimicrobial resistance, 56 (77.8%) of the STEC can be categorised as multi-drug resistant (MDR) strains being resistant to 3 or more unrelated antimicrobial agents and 19 antimicrobial resistance phenotypes were recognized (Table 7). All the enrofloxacin, nalidixic acid and norfloxacin resistant isolates were multi-drug resistant. However, the most predominant resistance phenotype ($n$ = 14) was AMP-STR-TET.

This study has established that antimicrobial-resistant STEC is prevalent in the fresh beef and meat products sold in Lagos Metropolis. Studies have also confirmed the incidence and characterization of antimicrobial resistant *E. coli* O157 and non-O157 STEC in meat producing animals in Nigeria (Adenipekun et al., 2015; Ojo et al., 2010; Olatoye, 2010). It has been shown that application of antimicrobial agents in animal husbandry which are often at a sub-therapeutic level is the major potential driving force for antimicrobial resistance in foodborne pathogens that are associated with meat and meat products (Ojo et al., 2010). In addition, the high rates to antibiotics resistance in bacteria especially those associated with meat and meat products have been attributed to inappropriate or uncontrolled use of antibiotics in farming practices such as in animal feeds (Van et al., 2007). Though antibiotics are not generally recommended in the treatment of STEC infections in humans due to the concern that they will induce *stx* production and worsening the symptoms, their use is still highly significant in the management of life-threatening foodborne infections like diarrhoea (Mohsin et al., 2015; Rund et al., 2013). Resistance to these groups of first-line antimicrobial agents will reduce the effectiveness of the antimicrobials at early empirical treatment, as well as restriction of treatment choice, even after confirmation of microbiological diagnosis (Rasheed et al., 2014). Therefore, the high level of resistance of the non-O157 STEC serotypes isolated in this study to Tetracycline, Amplicilin and Streptomycin is an indication of a widespread resistance of these pathogens to most commonly used antimicrobial agents in both human and animal health practice in Nigeria (Adenipekun et al., 2015; Ajayi et al., 2011; Nsofor et al., 2013; Ojo et al., 2010). The public health significance of these findings is that these MDR isolates may pose a threat to the life of the consumers of these meat products by limiting therapeutic options. The ability of these resistant STEC strains to transfer the antibiotic resistance genes to other enteric bacteria within the gastrointestinal tract could enhance antibiotic resistance within the population in Nigeria thereby complicating therapy (Rasheed et al., 2014). High prevalence of antibiotics resistance bacteria in Nigeria has been attributed to free and unregulated access of non-professionals to different classes of antimicrobial coupled with indiscriminate use of antibiotics (Olatoye, 2010).

## 4 Conclusions

This study established high prevalence rate of non-O157 STEC serotypes in the fresh beef sold at open markets and street vended meat products in Lagos, Nigeria. The presence of these pathogens indicates that meat hygiene is being compromised in the city, a situation that

calls for public health concern. Implementation of effective food safety management system that will ensure drastic reduction in contamination of meat and meat products with foodborne pathogens is required. Compliance to Good Manufacturing practice (GMP) at every stage of the meat supply and processing chain, from the abattoir, to the retailer, and especially at the small scale or local meat processors should be enforced. It is therefore imperative for the health authorities in the state to embark on regular training and health education of meat retailers, butchers and local meat processors. Conditions of sanitation and hygiene in processing and sales of fresh beef and meat products to the public should also be closely monitored most importantly in the open market in order to reduce or minimize prevalence of foodborne pathogens. In addition, the findings of this study provide fundamental information that would aid in the surveillance of antimicrobial resistance of STEC in the Nigeria.

**Declaration of competing interest**

The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

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