
Occurrence and antibiotic resistance profile of extended spectrum beta lactamase producing *Escherichia coli* O157:H7 isolated from fresh and grilled beef meant for human consumption in Osun State, Nigeria

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Abstract

Zoonotic and multidrug resistant extended spectrum beta-lactamase (ESBL) *E. coli* O157:H7 found in raw and processed meat can contribute to the increased chances of infections and drug resistance in humans. The present study assessed the occurrence and antibiotic resistance profile of ESBL-*E. coli* O157:H7 isolated from fresh and grilled beef meant for human consumption in Osun State, Nigeria. A cross-sectional survey was conducted on fresh beef sampled at slaughter slabs and grilled beef sourced from three senatorial districts in Osun State, Nigeria. 120 fresh beef and 60 grilled beef samples were surveyed. Isolation and characterization of ESBL-*E. coli* O157:H7 followed standard procedures on eosin-methylene blue agar, sorbitol-MacConkey agar and MacConkey agar supplemented with 4 mg/L of cefotaxime, and was confirmed by slide agglutination test using *E. coli* O157: H7 anti-serum. Antibiotic susceptibility testing of ESBL-*E. coli* O157:H7 isolates against amoxycillin-clavulanic acid, tetracycline, gentamicin, ceftriaxone, ciprofloxacin and penicillin was performed by disc diffusion method. Results showed that the occurrence of ESBL-*E. coli* O157: H7 in the fresh and grilled beef sampled were 14% (17 out of the 120 sampled) and 5% (3 out of the 60 sampled), respectively. Significantly ($p = 0.0001$) higher occurrence of ESBL-*E. coli* was recorded in fresh beef samples obtained from government owned slaughter slabs than those obtained from private slaughter slabs. The occurrence of ESBL-*E. coli* O157: H7 in both the fresh and grilled beef sampled did not significantly ($p > 0.05$) vary among the three senatorial zones of Osun State, Nigeria. All (100%) of the ESBL-*E. coli* O157:H7 isolates were resistant to ceftriaxone, penicillin and amoxicillin-clavulanic acid. 95% of the isolates were resistant to tetracycline, while 40% and 25% of the isolates were susceptible to gentamicin and ciprofloxacin, respectively. It was concluded that multidrug-resistant ESBL-*E. coli* O157: H7 occur in fresh and grilled beef in Osun State, Nigeria. This is considered to be of public health importance. It is recommended that slaughter slabs and beef grilling points in Osun State Nigeria should cultivate necessary hygienic practices and implement hazard analysis and critical control point (HACCP) measures.

Keywords: *Escherichia coli*; ESBL-*E. coli* O157: H7; Occurrence; Fresh and grilled beef; Antibiotic resistance; Osun State, Nigeria.

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Introduction

Meat and meat products constitute important sources of protein, fat, essential amino acids (such as lysine, methionine, and tryptophan), bioavailable heme, iron, zinc and B-complex vitamins like B12 (Olayinka and Sani, 2014; Pighin *et al.*, 2016; Wyness, 2016). In Nigeria beef is consumed traditionally as either fresh/boiled beef or as processed meat preserved by salting, smoking, curing, or sun-drying, with locally named examples such as suya, kilishi, dambu nama, kundi/tinko (dried or smoked beef), ponmo or kanda (processed cow skin) (Wyness, 2016; Erhirhie *et al.*, 2020).

Muscle tissue from healthy live animals is largely sterile, but carcass surfaces become exposed to diverse microflora during slaughter, dressing, processing, and handling (Rouger *et al.*, 2017) and these microbial contaminants may represent risks to human health. Such risk includes contamination with several major foodborne zoonotic pathogens such as extended spectrum beta lactamase (ESBL) producing *Escherichia coli*, shiga toxin-producing *E. coli* (STEC), *Salmonella spp.*, *Staphylococcus aureus* and *Campylobacter spp.* (Rouger *et al.*, 2017; Ali and Alsayeqh, 2022; Iwuagwu *et al.*, 2023; Kadi *et al.*, 2026).

Extended spectrum beta lactamase (ESBL) producing bacteria are found in the gut of food animals and humans (Rawat and Nair, 2010). Food animals such as cattle, sheep and goats harboring ESBL-producing bacteria can aid in the spread of the bacteria within the community (Bortolaia *et al.*, 2010) where present, via consumption of contaminated animal products, utilization of animal wastes for agriculture and animal-human interaction. The presence of beta lactamase producing *E. coli* (BLPE) in Nigerian ruminants, particularly cattle has earlier been reported by Ejikeugwu *et al.* (2019) and Onuoha *et al.* (2023). ESBL-producing microbes belong to the family *Enterobacteriaceae* (Mahamat *et al.*, 2019), with complex epidemiology involving food-

borne pathogens like *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumoniae* (Sanjit *et al.*, 2017; Lawan *et al.*, 2024). *Enterobacteriaceae* is a large gram negative bacteria family, responsible for several types of infections including respiratory, intestinal, urinary tract, bloodstream and intra-abdominal infections (Ullah, *et al.*, 2021). *Enterobacteriaceae* responsible for producing ESBL were first reported in Germany in 1983 and since then, the frequency has been reported to be increasing rapidly worldwide (Ullah, *et al.*, 2021).

The enzyme, extended spectrum beta-lactamase (ESBL), can be found in some strains of *E. coli* and other Gram-negative bacteria (Lawan *et al.*, 2024). Beta-lactam antibiotics are bactericidal antibiotics that contain a beta-lactam ring in their chemical structure: they include penicillins, cephalosporins, monobactams, carbapenems and carbacephems. Beta-lactam antibiotics bind to and inhibit penicillin binding proteins (PBPs) (Lawan *et al.*, 2024). Beta lactamase had been shown to be sensitive to β -lactamase inhibitors such as sulbactam, clavulanic acid, and tazobactam (Mukherjee *et al.*, 2013).

Extended spectrum β -lactamases are group of β -lactamases that hydrolyze a broad range of β -lactam antibiotics, like oxyimino-cephalosporins and aztreonam, but are repressed by β -lactam inhibitory substances such as clavulanic acid, tazobactam and sulbactam. These enzymes are encoded by the genes mediated by plasmids and are common amongst the species of *Enterobacteriaceae* (Ullah, *et al.*, 2021). The extensive utilization of broad-spectrum antibiotics is the reason for the entrance of ESBL enzymes. Transferable enzymes has been shown to be resistant to different antibiotics such as penicillin, cephalosporins, and monobactams, but are unable to hydrolyze the cephamycin or carbapenem (Lawan *et al.*, 2024).

Antimicrobial resistance (AMR) is a global health problem with regards to the treatment of infectious diseases (Chukwu *et al.*, 2021). AMR is of significant public health concern with societal and economic consequences (Dhingra *et al.*, 2020). It is a rising problem for public and community health in developing countries (WHO, 2020). There are different forms of antimicrobial resistance, such as resistance to beta lactam antibiotics and extended spectrum cephalosporins (Lawan *et al.*, 2024). The ESBLs producers have been identified among members of the *Enterobacteriaceae* which may harbor several determinants of antibiotic resistance, making treatment of infections caused by these pathogens more difficult and challenging (Rottier *et al.*, 2012).

There is paucity of information in available literature on the occurrence and antibiotic resistance profile of ESBL-*E. coli* O157:H7 in beef meant for human consumption in Osun State, Nigeria despite the fact that beef is the major meat consumed in the State. This study assessed the occurrence and antibiotic resistance profile of ESBL-*E. coli* O157:H7 in fresh and grilled beef meant for human consumption in Osun State, Nigeria.

Materials and Methods

Study design and sample collection: A cross-sectional survey design and a purposive sampling method were utilized in this study. Fresh and grilled beef from government-owned and private slaughter slabs in the three senatorial zones (Osun central, Osun west and Osun east) of Osun State Nigeria were sampled between May and June, 2022. Osun State is situated in the tropical rain forest zone covering an area of approximately 14, 875sqkm and lies between latitude 7° 30' 0" N and longitude 4° 30' 0" E (Ojurongbe *et al.*, 2015) with a population census figure of 3,423,535 (NPC, 2006).

A total of 120 fresh beef (20 from government-owned slaughter slabs and 100 from private slaughter slabs; 40 from each of the three senatorial zones) and 60 grilled beef (suya) (20 from each senatorial zones) were surveyed in the study. The sample size (n) was calculated using the Thrusfield formula (1995) by considering expected prevalence of 6.94% ($p = 0.0694$) for fresh beef and 4.17% ($p = 0.0417$) for grilled beef (suya) as recorded by Enabulele and Nduka (2009), at 95% confidence interval (CI) ($Z = 1.96$) and level of significance of 5% ($d = 0.05$). Negativity or attrition was also adjusted for.

The beef samples were aseptically collected into sterile zip lock bags and stored on ice packs after which they were immediately transported to the Milk and Meat Hygiene Laboratory of Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria for analysis. Fresh beef samples were collected from slaughter slabs within 6 hours of slaughter, while grilled beef samples were collected after seasoning and packaging for the consumers.

Sample preparation: Homogenization of each fresh or grilled beef (1g) was performed in 9 ml of buffered peptone water (BPW; Himedia) for 2 minutes, and the homogenates were incubated at 37°C for 18 – 24 hours for enrichment according to Sodagari *et al.* (2015).

Bacterial growth and examination: The method described by Ullah *et al.* (2021) was used for bacterial growth and examination, with some modifications. After 18 hours incubation in peptone water, 10 µl (loop full) of each enriched homogenate was plated on eosin methylene blue (EMB) agar (Oxoid) and incubated at 37°C for 24 hours, after which growth were examined for (a typically green metallic sheen appearance). Samples with typical green metallic sheen appearance were marked as positive for *E. coli*. The positive colonies were subjected to biochemical tests

(indole, catalase and oxidase tests), and were sub-cultured on sorbitol-MacConkey (SMAC) agar (Oxoid) and incubated at 37°C for 24 hours and then observed for typical colourless colonies on SMAC characteristic of *E. coli* O157:H7. Presumptive *E. coli* O157:H7 colonies were sub-cultured onto MAC agar (Oxoid) plates containing 4 mg/L cefotaxime (CTX) and incubated at 37°C for 24 hours. Colonies with typical *E. coli* growth (pink colonies on MAC agar) containing 4 mg CTX were considered positive for ESBL production and were individually tested for agglutination using a commercial *E. coli* O157:H7 slide agglutination kit for confirmation (Pro-Lab).

Antibiotic sensitivity testing of ESBL-*E. coli* O157:H7: Antibiotic susceptibility test was performed on all the twenty isolates recovered using Kirby Bauer's disk diffusion method on Muller-Hinton agar (MHA) (Oxoid) medium as described in the Guidelines of the Clinical Laboratory Standard Institute (CLSI 2020). The antibiotics used include amoxicillin-clavulanic acid (30 µg), tetracycline (30 µg), gentamicin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg) and penicillin (10 µg) (Deben Diagnostics, UK).

A standardized suspension of each isolate equivalent to 0.5 McFarland standards was inoculated onto MHA plates and the antibiotics disc was aseptically placed on the agar plates at distance of 24 mm centre to centre and 15mm from the edge of petri dish and incubated at 37°C for 18 – 24 hours. After incubation 24 hours, the diameter of the zone of inhibition was measured and recorded according to CLSI guidelines (CLSI, 2020).

Data Analysis and Presentation: Data generated were subjected to descriptive statistics, and frequencies of occurrence were calculated and presented in percentages. Non-parametric data were analyzed using Pearson's Chi-Square.

Results

Out of the total 180 samples collected from both slaughter slabs and beef grilling points, the prevalence of non-ESBL *E. coli* isolated by using eosin methylene blue agar (EMBA) and biochemical tests were 85.8% and 95.0%, respectively. Grilled beef had a higher occurrence of non-ESBL *E. coli* (57 out of 60 = 95%) than fresh beef (103 out of 120 = 85.8%) (Table 1). Occurrence of ESBL-*E. coli* O157:H7 in samples collected from the slaughter slabs (fresh beef) and beef grilling points (grilled beef) were 14.2% (17 out of 120) and 5.0% (3 out of 60), respectively (Table 1).

There was a significantly ($p < 0.05$) higher occurrence of non-ESBL *E. coli* in fresh beef collected from privately-owned slaughter slabs (94 out of 100 = 94.0%) than the ones collected from government-owned (9 out of 20 = 45.0%) slaughter slabs (Table 2). However, the occurrence of ESBL-*E. coli* O157:H7 in the fresh beef collected from government slaughter slabs was higher (11 out of 20 = 55.0%) than its occurrence in the fresh beef collected from privately owned slaughter slabs (6 out of 100 = 6.0%) (Table 2).

Out of 120 fresh beef collected from all the three senatorial districts, Osun West had the highest occurrence for non-ESBL *E. coli* (36 out of 40 = 90.0%), while Osun East had the highest occurrence for ESBL *E. coli* (7 out of 40 = 17.5%) (Table 3). For grilled beef, out of 60 grilled beef collected from the three senatorial districts, Osun West had the highest occurrence for non-ESBL *E. coli* (20 out of 20 = 100.0%), while Osun Central had the highest occurrence for ESBL *E. coli* (2 out of 20 = 10.0%) (Table 4).

Table 1. Distribution of the occurrence of ESBL-*E.coli* O157:H7 in beef meant for human consumption in Osun State, Nigeria, based on meat type.

Sample type	Number of samples	Number of non-ESBL <i>E. coli</i> , with % in bracket	Number of ESBL <i>E. coli</i> O157:H7, with % in bracket	Chi square value with P-value in brackets
Fresh beef	120	103 (85.8%)	17 (14.2%)	3.403 (p = 0.065)
Grilled beef	60	57 (95.0%)	3 (5.0%)	
Total	180	160 88.9%)	20 (11.1%)	

Table 2. Distribution of the occurrence of ESBL-*E.coli* O157:H7 in fresh beef meant for human consumption in Osun State, Nigeria, based on sources of samples (government or privately owned slaughter slabs).

Source of meat sample	Number of samples	Number of non-ESBL <i>E. coli</i> , with % in bracket	Number of ESBL <i>E. coli</i> O157:H7, with % in bracket	Chi square value with P-value in brackets
Government owned slaughter slab	20	9 (45.5%)	11 (55.5%)	32.909 (p = 0.0001)
Privately owned slaughter slab	100	94 (94.0%)	6 (6.0%)	
Total	120	103 (85.8%)	17 (14.2%)	

Table 3. Distribution of the occurrence of ESBL-*E.coli* O157:H7 in fresh beef meant for human consumption in Osun State, Nigeria among the senatorial zones from which samples were collected.

Zone from which samples were collected	Number of samples	Number of non-ESBL <i>E. coli</i> , with % in bracket	Number of ESBL <i>E. coli</i> O157:H7, with % in bracket	Chi square value with P-value in brackets
Osun Central	40	34 (85.0%)	6 (15.0%)	2.959 (p = 0.2278)
Osun West	40	36 (90.0%)	4 (10.0%)	
Osun East	40	33 (82.5%)	7 (17.5%)	
Total	120	103 (85.8%)	17 (14.2%)	

Table 4. Distribution of the occurrence of ESBL-*E.coli* O157:H7 in grilled beef meant for human consumption in Osun State, Nigeria among the senatorial zones from which samples were collected.

Zone from which samples were collected	Number of samples	Number of non-ESBL <i>E. coli</i> , with % in bracket	Number of ESBL <i>E. coli</i> O157:H7, with % in bracket	Chi square value with P-value in brackets
Osun Central	20	18 (90.0%)	2 (10.0%)	2.105 (p = 0.349)
Osun West	20	20 (100.0%)	0 (0.0%)	
Osun East	20	19 (95.0%)	1 (5.0%)	
Total	60	57 (95.0%)	3 (5.0%)	

Table 5. Antibiotic resistance profile of ESBL-*E.coli* O157:H7 isolated from fresh and grilled beef meant for human consumption in Osun State, Nigeria (n = 20).

Antibiotic	Concentration (µg/disc)	Number susceptible, with % in bracket.	Number with intermediate susceptibility, with % in brackets.	Number resistant, with % in brackets.
Gentamicin	10	8 (40%)	10 (50%)	2 (10%)
Ciprofloxacin	5	5 (25%)	7 (35%)	8 (40%)
Tetracycline	30	0 (0%)	1 (5%)	19 (95%)
Ceftriaxone	30	0 (0%)	0 (0%)	20 (100%)
Penicillin	10	0 (0%)	0 (0%)	20 (100%)
Amoxicillin-clavulanic acid	30	0 (0%)	0 (0%)	20 (100%)

All the ESBL-*E. coli* O157:H7 isolates (100%) were resistant to ceftriaxone, penicillin and amoxicillin-clavulanic acid, while 95% were resistant to tetracycline (Table 5). However, 40% of the isolates were resistant to ciprofloxacin, while only 10% of the isolates were resistant to gentamicin (Table 5). The following percentages of the isolates exhibited intermediate susceptibility to these antibiotics: Gentamicin – 50% Ciprofloxacin – 35%, and Tetracycline – 5% (Table 5). On the other hand, 40% of the isolates were susceptible to gentamicin, and 25% were susceptible to ciprofloxacin, while none of the isolates were susceptible to tetracycline, ceftriaxone, penicillin and amoxicillin-clavulanic acid (Table 5).

Discussion

This study recorded a higher occurrence of non-ESBL *E. coli* (88.9%) than ESBL-*E. coli* O157:H7 20 (11.1%) in fresh and grilled beef in the study area, and also a higher occurrence of ESBL-*E. coli* O157:H7 in fresh beef (14.2%) than grilled beef (5.0%) in the study area. This higher occurrence of ESBL-*E. coli* O157:H7 in fresh beef than in grilled beef in the study area is thought to be because cattle among other animals harbour ESBL-*E. coli* O157:H7 in their guts and during processing as commonly

occurs in most slaughter slabs in the study area, slaughtering, evisceration, removal of rumen and intestinal contents and cutting are done on the same slabs, and this allows for cross contamination of other body parts with gut content. Further, it is believed that grilled beef had lowest occurrence because the process of direct heating and seasoning exerts reductive effects on ESBL-*E. coli* O157:H7 load of the grilled beef.

The present study found ESBL-*E. coli* O157:H7 occurrence to be significantly higher in fresh beef obtained from government-owned slaughter slabs than private slaughter slabs. The probable reason for this could be due to the fact that in most government owned slaughter slabs, there is no separation between clean operation and dirty operation areas. Thus dirty operations like slaughtering is done on the same slabs as clean operations like evisceration and cutting and this may have led to cross contamination of beef with gut contents. In contrast, in most of the private slaughter slabs surveyed, clear distinction existed between areas for dirty operations and clean operations, and this most probably minimized cross contamination.

The results of the antibiotics susceptibility testing revealed that most of the isolates were

resistant to ceftriaxone, penicillin, amoxicillin-clavulanic acid and tetracycline. The isolation of multidrug resistant ESBL-*E. coli* O157:H7 in this study is consistent with earlier reports of multiple antimicrobial resistance especially the beta-lactam group of antibiotics (Raji, 2015). These important antibiotics are the most frequently used antibiotics in human medicine in Nigeria (Akindele *et al.*, 2010). This finding suggests that animals contribute reasonably to antimicrobial resistance in the population. Amoxicillin clavulanic acid and ceftriaxone are one of the most important drugs used for the treatment of drug-resistant strains of bacteria in human medicine (Bottenfield *et al.*, 1998; Clark *et al.*, 2001). Hence, the high occurrence of resistant strains of ESBL-*E. coli* O157:H7 in this study is of both human medical and veterinary importance (Schmiedel *et al.*, 2014).

The probable reason for the high rate of multidrug resistant strains of ESBL-*E. coli* O157:H7 in this study may be because of the indiscriminate use of antimicrobials as growth promoters and for disease prevention purposes in Nigerian livestock industry (Olaitan *et al.*, 2011; Ejeh *et al.* 2017). The high rate of multidrug resistance among the ESBL-*E. coli* O157:H7 in this study to ceftriaxone is presumed to be largely due to the earlier reported extra-label use of this drug in food animals to treat infections without appropriate dosage calculation (Azabo *et al.*, 2022). This extra-label use contribute largely to abuse of this drug.

The isolation of multidrug resistant extended spectrum beta-lactamase producing ESBL-*E. coli* O157:H7 from beef meant for human consumption in Osun State Nigeria poses significant public health risks to the residents of the State. The consumption of multidrug resistant ESBL-*E. coli* O157:H7 laden beef can lead to food borne zoonotic transmission of ESBL-*E. coli* O157:H7 to humans. Occupational exposure of humans (farmers, abattoir and slaughter house workers) to such multidrug

resistant *E. coli* via direct contact with reservoir ruminants can also lead to zoonotic infections in humans, because food animals such as ruminants (cattle and sheep) can carry ESBL-*E. coli* O157:H7 in their gastrointestinal tract without exhibiting clinical signs.

Of importance also is the environmental contamination resulting from improper animal waste disposal that can pollute water and soil, leading to contaminated environments and another source of exposure for humans. The use of cattle dungs as manure by farmers for crop cultivation which is very predominant in Northern Nigeria, can also lead to transfer of multidrug resistant ESBL-*E. coli* O157:H7 and their genes to crops and vegetables and hence the transfer of multidrug resistant genes to humans (Ogunleye *et al.*, 2013; Ejeh *et al.*, 2017) via consumption and processing. This will limit the effectiveness of commonly used antibiotics in humans, limiting treatment options to potentially less effective or more expensive alternatives. This limited treatment options can lead to increased rates of complications and mortality from infections (Lawan *et al.*, 2024),

The isolation of multidrug resistant ESBL-*E. coli* O157:H7 poses a threat to the entire residents of Osun State, not just individuals with direct exposure to the cattle but everyone that uses by-products from the cattle being slaughter (Lawan *et al.*, 2024). The spread of resistance genes can complicate public health challenges and may lead to outbreaks of untreatable infections and overburdening healthcare challenges (Lawan *et al.*, 2024). Antibiotic resistance has significant economic implications, such as increased healthcare costs due to prolonged illnesses and ineffective treatments and loss of productivity due to worker absenteeism and ill health.

Negative impacts on the livestock industry due to potential restrictions on trade and antibiotic use in animal production (Lawan *et al.*, 2024) can also result.

Conclusion: The isolation of multidrug resistant extended ESBL-*E. coli* O157:H7 from beef meant for human consumption in Osun State, Nigeria poses a serious public health threat to people and the animal population. The potential zoonotic and reverse zoonotic transmission of resistant strains of ESBL-*E. coli* O157:H7 (Lawan et al., 2024) and their genes among human and animal population in Osun State is of serious public health concern. Animals had been marked as the common source of zoonotic transfer of resistant strains of *E. coli* especially in resource poor countries (Daszak et al., 2000; Cleaveland et al., 2001; Ejeh et al., 2017). The control and regulation of the use of beta-lactam antibiotics in animals in Nigeria will therefore help to ameliorate the problems of antimicrobial resistance in Nigeria. Proper attention should be given to improve on antimicrobial stewardship, especially the β -lactams and cephalosporins.

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Conflict of interest

The authors declare no conflict of interest.

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