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ISOLATION OF SHIGA TOXIN O157:H7 *ESCHERICHIA COLI*, ANTIBIOGRAM, AND PCR DETECTION OF *PHOA* GENE AMONG *ESCHERICHIA COLI* STRAINS ISOLATED FROM RAW BEEF AND ASSOCIATED CONTACT SURFACES IN OTA, OGUN STATE

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ABSTRACT
Several studies have linked *Escherichia coli* to the onset of diseases in both healthy and immunosuppressed individuals. This study examines the isolation of *Escherichia coli* strain O157:H7, antibiotic resistance pattern, and the detection of *phoA* gene in *E. coli* isolated from raw beef and its associated contact surfaces. A total of thirty (30) samples were collected from raw beef vendors and were cultured on nutrient agar, MacConkey agar, eosin methylene blue, and sorbitol for isolation of *Escherichia coli.* The isolated *E. coli* strains were tested against eight antibiotics. Furthermore, the isolates were identified using the *phoA* gene that codes for alkaline phosphatase. The results revealed *E. coli* in ten (33.3%) samples tested, while seven (70%) of the ten *E. coli* isolated harboured the *phoA* gene. This study showed high resistance (100%) of *E. coli* to meropenem, gentamicin, ceftriaxone, aztreonam, and cefepime. Followed by nitrofurantoin (85.7%), amikacin (42.9%), and ciprofloxacin (14.3%). Also, this study revealed multidrug-resistant *E. coli* contamination of raw meat and associated contact surfaces, which implies a breakdown in hygiene among meat vendors. Therefore, meat vendors should be monitored and educated on proper hygiene measures in order to control the spread of pathogenic and resistant strains of *Escherichia coli*.

Keywords: Cattle, Alkaline phosphatase, *Escherichia coli*, Meat, Sorbitol.

INTRODUCTION

A key component of global public health is food safety, but foodborne infections are becoming a major public health threat (Fayemi et al, 2026). Globally, an estimated 600 million people are affected as a result of food-borne illnesses, and 420,000 deaths due to food poisoning are reported every year (Havelaar, 2015). One of the most crucial microbes for food and water safety monitoring is *E. coli*, and Shiga toxin-producing *E. coli* (STEC) O157:H7 is often linked to outbreaks of food poisoning (Manning *et al*, 2008). Many of the virulence factors that *E. coli* possesses are either localised in pathogenicity islands or encoded on mobile genetic elements and plasmids. These virulence factors manifest as adhesion, invasion, endotoxins, exotoxins, or iron acquisition factors (Sora *et al*, 2021).

Often, Shiga toxin-producing *Escherichia coli* (STEC) causes various foodborne disease outbreaks, which often lead to morbidity and mortality. STEC infections are typically acquired by consuming contaminated food or water (Gyles, 2007). A significant percentage of STEC infections have been linked to eating contaminated, undercooked food, typically dairy and meat (Hussein, 2007; Ferens and Hovde, 2011). Cattle are recognized as the principal reservoir of *Escherichia coli* that produces Shiga toxin (STEC), and humans usually contract the infection by eating contaminated food, especially raw or undercooked meat. Symptoms most commonly associated with this serotype include abdominal cramps, bloody diarrhea, thrombotic thrombocytopenic purpura, hemorrhagic colitis, and hemolytic uremic syndrome in both outbreaks and sporadic cases (Karmali *et al*, 2010).

Across Africa, several studies have confirmed the emergence of antimicrobial resistance in STEC O157:H7 isolates from various sources. In Egypt, multi-drug resistant strains have been recovered from humans, animals, and the environment (Selim *et al*, 2013). South Africa has also reported similar findings from cattle (Iweriobor *et al*, 2015). In Nigeria, Chigor *et al* (2010) found comparable resistance patterns, further underscoring the widespread nature of this threat.

Indiscriminate use of antibiotics in animals has led to the emergence of multidrug-resistant *E. coli* strains, thereby endangering public health. Thus, it is necessary to monitor *E. coli* resistance patterns as well as the accurate detection of *E. coli* using molecular methods. *Escherichia coli* can be differentiated from other closely related Enterobacteriaceae using the genetic marker *phoA*, an alkaline phosphatase gene, which offers a more accurate identification in polymerase chain reaction than conventional biochemical tests (Luo *et al*, 2023).

Food safety is considered one of the most pressing issues in the global food sector. Since Shiga toxin-producing *E. coli* (STEC) infection is associated with eating beef, it is crucial to control and avoid STEC contamination of raw beef. Therefore, it is essential to accurately identify and determine the resistance pattern of *E. coli* from raw beef. This study will raise awareness of the necessity of stringent measures in meat handling and the need to limit the extensive usage of antibiotics in animals. This can significantly reduce the spread of multidrug-resistant *E. coli*.

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MATERIALS AND METHODS

Samples collection

Thirty (30) samples of raw beef and associated contact surfaces were aseptically collected from local meat vendors in Ota, Ogun State. A total of five (5) samples each from hand swabs, knife swabs, table swabs, and 15 from raw beef swab samples were

obtained between the periods of February and March 2025 in Ota, Ogun State, Nigeria. Sample codes with the sample sites are included in Table 1. The samples were collected in sterile zip-lock bags and were quickly taken to the laboratory for further analysis. The samples were collected aseptically and stored in the refrigerator.

Table 1: Sample codes for the collection of raw beef and associated contact surfaces

NUMBER	TYPE	CODE
5	Cow beef	1Ba
5	Fillet mignon	1Bb
5	Cow hump	1Bc
5	Knife swab	1Bd
5	Hand swab	1Be
5	Table swab	1Bf

Microbiological analysis of raw beef samples
Samples were cut and processed using sterilized scalpels. The samples were cut into slices using a sterile knife and weighed at 25.0 grams each, and placed in a McCartney bottle with 225ml of buffered peptone water. Subsequently, the mixtures were homogenized for 1 minute. The homogenized samples were incubated at 37 °C for 18 hours. Then 1 mL of the pre-enrichment culture was transferred to 9 mL of modified tryptone (Oxoid, UK) soy broth. It was incubated at 37 °C for 24 hours.

Isolation and identification of *Escherichia coli*
After processing the collected samples, each was then streaked onto MacConkey agar (Huankai Ltd., Guangzhou, China) and Eosin Methylene Blue agar (EMB), where it was incubated for 24 hours at 37°C. Growth was observed on MacConkey and EMB agar; *E. coli* appeared as pink colonies (lactose fermenters) on MacConkey, and *E. coli* produced colonies with a metallic sheen on EMB agar.

After the isolation of *Escherichia coli*, the isolates were then streaked onto nutrient agar, and it was incubated for 24 hours at 37°C. After the period of incubation, the isolates were inoculated onto Sorbitol MacConkey agar, where they were incubated for 24 hours at 37°C. Growth was observed on Sorbitol MacConkey Agar. Non-sorbitol-fermenting *Escherichia coli* appeared as a colourless /pale colony.

Biochemical identification of strains of *Escherichia coli*
After the isolation of *Escherichia coli*, the isolates were then streaked onto nutrient agar and incubated for 24 hours at 37°C. After the period of incubation, different biochemical tests were carried out for preliminary identification of *Escherichia coli* isolates. The biochemical tests carried out on the samples include; gram staining, Sulfide Indole Motility (SIM) test, citrate, urease, and Voges-Proskauer tests.

Genomic DNA extraction by the boiling method
All isolates were subjected to molecular confirmation via PCR. Genomic DNA was extracted from the isolates using the boiling technique (Ribeiro *et al*, 2016). The isolates' overnight cultures were harvested and washed with Phosphate Buffer Saline (PBS) in 1 ml. The cells were washed, 200 µl of sterile distilled water was added, they were vortexed to homogenize, and then heated for 10 minutes at 100°C. The suspension was immediately put into ice after boiling, vortexed one more time, and centrifuged at 10,000 rpm for 10 minutes. 2 µl of the supernatant was used for PCR after being transferred to another tube.

Determination of DNA quantity and quality
Following the DNA extraction process, the quantity and quality of the DNA extract were precisely measured. This was done by randomly choosing some samples and using an ND1000 Nanodrop spectrophotometer to assess their concentration and purity. The range of purity was between 1.65 and 2.0, while the average concentration was between 100 ng and 200 ng.

Polymerase chain reaction (PCR) using phoA primers
The alkaline phosphatase gene of the *Escherichia coli* was amplified by polymerase chain reaction using the primer pairs *phoA*-F (5'-CGTGATCAGCGGTGACTATGAC-3') and *phoA*-R (5'-CGATTCTGGAAATGGCAAAAG -3') (Hu *et al*, 2011). The PCR reaction was performed in 20 µl of a reaction mixture, and the reaction concentration included 1X Blend Master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 M of each deoxynucleoside triphosphate (dNTP) (Solis Biodyne), 25 pMol of each primer (BIOMERS, Germany), and 2 units of FIREPol DNA polymerase (Solis Biodyne). Thermal cycling was carried out in an Eppendorf Vapo Protect thermal cycler (Nexus Series) for 35 amplification cycles lasting 30 seconds at 94°C, 1 minute at 55°C, and 1 minute and 30 seconds at 72°C. The initial denaturation was carried out at 95°C for 15 minutes, and a final extension phase for 10 minutes at 72°C. On a 1.5% agarose gel, the amplification product was separated, and electrophoresis was then carried out at 80V for one hour and 30 minutes. DNA bands were visible after electrophoresis with ethidium bromide staining. A 100 bp DNA ladder was used.

Antibiotic susceptibility test
Only seven (7) isolates with expected (720 bp) amplicon were classified as *E. coli* and progressed to Antibiotic Susceptibility Testing (AST). Nitrofurantoin (300 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Aztreonam (30 µg), and Meropenem (10 µg) by HiMedia Laboratories, India, were used for antibiotic susceptibility using the Kirby-Bauer disk diffusion method. To compare the turbidity of the test and control inocula, a standard McFarland's solution (0.5) was prepared.
Each antimicrobial disc was positioned on Mueller-Hinton Agar (MHA) plates containing the streaked *E. coli*. The plates were then incubated at 37°C for 20 hours. The diameters of the zone of inhibition were measured and reported in accordance with EUCAST recommendations (EUCAST, 2025).

RESULTS

Isolation of *Escherichia coli*
Out of thirty (30) samples cultured, overall 10 (33.3%) *Escherichia coli* strains were observed (Figure 1 and Table 2). Table swabs had the highest isolation rate (60%) of *E. coli*, followed by cow-beef (40%), knife swabs (40%), hand swabs (40%), and cow hump (20%). However, there was no growth of *E. coli* observed on agar plates from tenderloin/fillet mignon samples (Table 2).

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Isolation of Shiga toxin (O157:H7) *Escherichia coli* in raw beef and associated contact surfaces.

Using Sorbitol MacConkey agar (SMAC), all the *Escherichia coli* (100 %) isolated from raw beef and associated contact surfaces were observed to be Shiga toxin O157:H7. They all formed colourless/pale colonies on SMAC.

Molecular detection of *Escherichia coli*

Out of the 10 presumptive *E. coli* isolated, 7 (70%) were confirmed positive by polymerase chain reaction using the *phoA* primers. The electrophoresis gel for the PCR amplification product of the *phoA* gene (720 bp) is shown in Figure 2.

Antibiotic Susceptibility Testing

Seven confirmed *Escherichia coli* isolates tested against eight antibiotics from raw beef and other contact surfaces revealed the highest resistance (100%) of *E. coli* to 5 different antibiotics, namely, meropenem (100%), gentamicin (100%), ceftriaxone (100%), aztreonam (100%), and cefepime (100%), followed by nitrofurantoin (85.7%), amikacin (42.9%), and lastly ciprofloxacin (14.3%) (Tables 3 and 4).

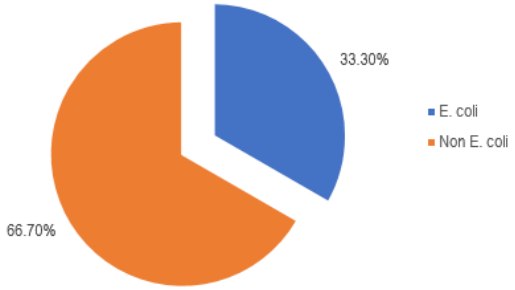

Figure 1: Percentage isolation of *Escherichia coli* from raw beef and the associated contact surfaces among Meat sellers in Ota, Ogun-State.

Table 2: Isolation rate of Presumptive *Escherichia coli* from raw beef and associated contact surfaces

Sample	No of different samples collected	Number of isolates	Isolation rate of *E. coli*
1.Cow-beef	5	2	40%
2.Tenderloin/fillet mignon	5	0	0%
3.Cow hump/hump steak	5	1	20%
4. Knife swab	5	2	40%
5.Hand swab	5	2	40%
6.Table swab	5	3	60%
Total	**30**	**10**	**33.30%**

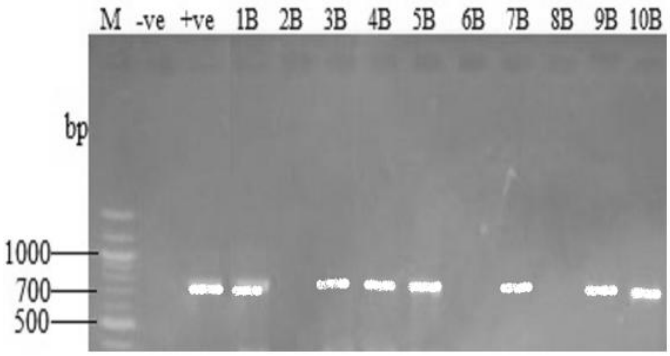

Figure 2: Electrophoresis gel for the PCR amplification product of the *phoA* gene (720 bp). Lane 1, 100 bp DNA ladder; lane 2, negative control without template DNA; lane 3, *E. coli* reference strain K12 Mg1655; lanes 4-13, samples

Table 3: Zones of Inhibition in millimeters (mm) of Antibiotic Susceptibility test of *Escherichia coli* from raw beef and associated contact surfaces

No.	PCR code	Isolate code	CIP (5 µg)	AMK (30 µg)	MEM (10 µg)	NIT (300 µg)	GEN (10 µg)	CTR (25 µg)	AT (30 µg)	CPM (30 µg)
1	1B	1Ba1	30 mm (S)	17 mm (R)	0 mm (R)	0 mm (R)	8 mm (R)	11 mm (R	8 mm (R)	9 mm (R)
2	3B	1Bd1	24 mm (I)	20 mm (S)	0 mm (R)	0 mm (R)	0 mm (R)	16 mm (R)	10 mm (R)	0 mm (R)
3	4B	1Be1	20 mm (R)	20 mm (S)	6 mm (R)	10 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	6 mm (R)
4	5B	1Be2	28 mm (S)	22 mm (S)	9 mm (R)	18 mm (S)	12 mm (R)	12 mm (R	0 mm (R)	0 mm (R)
5	7B	1Bf1	26 mm (S)	15 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)
6	9B	1Bf2	28 mm (S)	20 mm (S)	0 mm (R)	0 mm (R)	0 mm (R)	10 mm (R	9 mm (R)	14 mm (R)
7	10B	1Bf3	30 mm (S)	15 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)	0 mm (R)

CIP-Ciprofloxacin, AMK- Amikacin, MEM- Meropenem, NIT- Nitrofurantoin, GEN- Gentamicin, CTR- Cotrimoxazole, AT- Aztreonam, and CPM- Cefepime, S – Susceptibility, I- Intermediate, R- Resistance

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Table 4: Antibiotic resistance pattern of *Escherichia coli* isolated from different raw beef samples and associated contact surfaces

Antibiotics	No of *E. coli* Resistance (%)	No of *E. coli* Intermediate (%)	No of *E. coli* Susceptible (%)
1.Ciprofloxacin	1 (14.3)	1 (14.3)	5 (71.4)
2.Amikacin	3 (42.9)	0 (0)	4 (57.1)
3.Meropenem	7 (100)	0 (0)	0 (0)
4.Nitrofurantoin	6 (85.7)	0 (0)	1(14.3)
5.Gentamicin	7 (100)	0 (0)	0 (0)
6.Ceftriaxone	7 (100)	0 (0)	0 (0)
7.Aztreonam	7 (100)	0 (0)	0 (0)
8.Cefepime	7 (100)	0 (0)	0 (0)

DISCUSSION

In recent years, the frequency of documented outbreaks of human diseases connected to the consumption of contaminated beef has increased. In this study, out of all the samples tested, 33.3% were positive for *E. coli*. This detection rate aligns with previous reports from similar studies in Africa and other developing regions. For instance, Shah *et al* (2024) found *E. coli* in 34% of goat meat samples collected in Pakistan, while Taye *et al* (2013) reported a comparable *E. coli* contamination rate (30.97%) in Ethiopia. The relatively high presence of *E. coli* can often be attributed to poor hygiene practices, such as the use of contaminated water, unclean tools, or improper handling during slaughtering, processing, and selling (Obiukwu *et al*, 2015). Furthermore, there have been reports of pathogenic *E. coli* O157:H7 strains recovered from clinical samples (humans), food, animals, and the environment in different countries. (Muller *et al.*, 2001; Chahed *et al.*, 2006).

More notably, all the *E. coli* isolates obtained in this study were identified as shiga toxin-producing *E. coli* (STEC) O157:H7 based on selective and differential media. STEC is known as one of the most dangerous strains to cause foodborne illness. STEC O157:H7 has been implicated in serious outbreaks worldwide and is known for causing symptoms such as bloody diarrhea, abdominal cramps, and, in severe cases, hemolytic uremic syndrome (HUS), which can lead to kidney failure (Karmali, 2004). The fact that this strain was found on both the meat and associated contact surfaces strongly suggests cross-contamination and insufficient cleaning practices (Gutema *et al*, 2021). This reinforces the idea that interventions must go beyond just the meat itself and focus on the whole processing environment.

From these findings, out of the culture-positive isolates tested for the *phoA* gene, 70% were PCR-confirmed to be *E. coli*. This suggests that *phoA* may not be present in some strains due to sequence polymorphism (Jackson *et al*, 2007), highlighting the drawbacks of identifying *E. coli* using a single marker. Although the *phoA* gene is known to be highly specific for *E. coli* identification. This gene was also used for molecular identification of *E. coli* in a study in Egypt (Saeed *et al*, 2022).

In this study, the results of the antibiotic susceptibility testing were alarming. All isolates (100%) showed resistance to five major antibiotics: meropenem, gentamicin, ceftriaxone, aztreonam, and cefepime. These are antibiotics typically reserved for serious bacterial infections in both humans and animals. Resistance to meropenem in particular is concerning, as it belongs to the carbapenem class, often considered the last line of defense against multidrug-resistant gram-negative infections (Nordmann *et al*, 2012). The fact that *E. coli* isolates from raw meat are resistant to this drug points to a disturbing trend that mirrors what has been reported globally regarding the rise of carbapenem-resistant Enterobacteriaceae (CRE) (Logan and Weinstein, 2017). Beyond that, we also observed high resistance to nitrofurantoin (85.7%), moderate resistance to amikacin (42.9%), and comparatively lower resistance to ciprofloxacin (14.3%). Although ciprofloxacin is still relatively effective in this context, the growing resistance to multiple antibiotics across various drug classes suggests a high rate of multi-drug resistant (MDR) *E. coli* strains. According to the classification by Magiorakos *et al* (2012), MDR bacteria are resistant to at least one agent in three or more antimicrobial categories.

These findings raise concerns about the limited options for treating infections that might arise from these strains. The high rate of resistance could be due to the misuse of antibiotics in both animal husbandry and human medicine. In many developing countries, antibiotics are still freely available without prescription, and they are often added to animal feed as growth promoters or used proactively without proper veterinary guidance (Boeckel *et al*, 2015). Such practices create the perfect environment for resistant bacteria to thrive and multiply.

In the agricultural sector, the improper administration of antibiotics for treating animal or plant diseases and for enhancing food-animal growth is believed to contribute to the continuing increase of antibiotic-resistant strains (Magiorakos *et al*, 2012; Boeckel *et al*, 2015). The emergence of novel antimicrobial resistance in zoonotic *E. coli* isolated from both domestic animal hosts and animal-derived products has been highlighted in research articles over the past years.

The findings from this research highlight serious risks to public health and food safety, particularly in settings where meat is processed and sold under informal or poorly regulated conditions. Collectively, these findings paint a worrying picture. The simultaneous presence of strains of virulent *E. coli* O157:H7 and resistance to multiple critical antibiotics in raw meat and contact surfaces shows how easily foodborne pathogens can become public health threats, especially in environments with poor hygiene, limited surveillance, and law enforcement of food safety standards. Consumers who handle or eat undercooked or contaminated meat are at significant risk, particularly those with compromised immune systems, young children, and the elderly.

Conclusion

These findings provide clear evidence of *E. coli*, particularly strains of O157:H7 contamination, and high levels of antibiotic resistance among isolates from raw beef and contact surfaces in the meat-selling environment, which is an indication of poor hygiene while handling beef and insanitary practices in slaughterhouses. The identification of multidrug-resistant isolates highlights the urgent need for coordinated efforts to improve hygiene practices, regulate antibiotic use in agriculture, and strengthen food safety policies in local markets.

These results highlight the need for interventions that help meat handlers better understand meat safety and hygiene. Regular food safety training can help improve hygienic practices in meat handling, and improved abattoir infrastructure can help promote safer meat handling and lower the risk of contamination.

Competing Interest

The authors have declared that there are no conflicting interests.

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