

Antimicrobial Sensitivity, Shiga toxin-producing *Escherichia coli* (STEC) and virulence genes of isolates in some selected sites of Port Harcourt, Nigeria

ABSTRACT

Aims: To assess the antimicrobial sensitivity, Shiga toxin-producing *Escherichia coli* (STEC) and virulence genes of isolates in some selected sites of Port Harcourt, Nigeria.

Study design: Case-controlled study.

Place and Duration of Study: Selected places in Port Harcourt, Rivers State, Nigeria, between November, 2020 to November, 2021.

Methodology: Three hundred and fifty-two (352) samples were analyzed, 80 meat and 63 waste waters from the abattoir, 46 meat sample from roadside butchers, 109 clinical stool samples, 30 stool samples from food sellers, 20 stool samples from healthy subjects and 4 commercial bottled waters which served as control samples. A combination of methods was employed: conventional culture using Tryptone soya broth as an enrichment media, chromagar^{STEC} and serology with O157 latex reagents. Multiplex polymerase chain reaction analysis was used to screen for the presence of specific virulence genes in representative isolates and 16SrRNA sequence data was used to confirm the identity of isolates. GraphPad Prism 2.01 was used to perform the statistical analysis and p values < 0.05 were considered statistically significant.

Results: The results showed that 100% of the selected isolates profiled for virulence gene harbored a combination of stx1 + stx2, while 45% carried the eaeA gene. The culture and molecular methods used in STEC detection showed a good level of agreement comparatively. The result of isolates sequenced for identification by targeting the 16S gene and compared with publicly available sequences on NCBI for phylogenetic analysis showed 100% identities to the ribosomal RNA gene of seven uncultured *Escherichia coli* strains with Gene Accession numbers (CP049290, MN208208, KY780343, CP058233 MK641327, CP082357, CP082129, MH346270). Isolates were resistant to Ceftazidime 98.9%, Cefuroxime 100%, Cefixime 100%, Augmentin 100% and sensitive to the quinolones and nitrofurantoin. The virulence gene combination pattern, the antibiogram pattern and the phylogenetic analysis result indicated genetic relatedness of isolates.

Conclusion: Multi-drug resistance among STEC isolates was wide spread. All the isolates were resistant to the cephalosporins used and Augmentin. The representative isolates profiled for virulence gene, all harbored stx₁ and stx₂ combination, only 45% harbored stx₁, stx₂ and eaeA gene combination. Phylogenetic analysis of the representative isolates confirmed the genetic similarity of isolates as it showed 100% relatedness coverage.

4. CONCLUSION

Multi-drug resistance among STEC isolates was wide spread. All the isolates were resistant to the cephalosporins used and Augmentin. The representative isolates profiled for virulence gene, all harbored stx₁ and stx₂ combination, only 45% harbored stx₁, stx₂ and eaeA gene combination. Phylogenetic analysis of the representative isolates confirmed the genetic

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Keywords: Antimicrobial Sensitivity, Shiga toxin-producing *Escherichia coli* (STEC), virulence genes, Port Harcourt, Nigeria

1. INTRODUCTION

Shiga toxin-producing *Escherichia coli* (*E. coli*), STEC is one of the most important food-borne pathogens in the world; the bacterium can cause large outbreaks and comprises a large group of organisms capable of causing severe gastrointestinal disease in humans. Infections may in most severe cases progress to life-threatening complications such as; hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS) [1,2]

Cattles are the principal reservoir of STEC and are resistant to infection by the pathogen [3]. However, humans are highly susceptible to infection following direct contact with contaminated animal faeces or consumption of contaminated animal products such as beef, milk, vegetable or water [4].

Within the STEC family, certain strains appear to be more predominant and of greater virulence for humans. For instance, those belonging to serogroup O157:H7 and those with particular combinations of other putative virulence traits generally referred to as non-O157 STEC. Illnesses caused by non-O157:H7 rivaling that of O157 STEC in certain geographic regions, over 400 STEC serotypes have been associated with human disease in the world [5]. Options to test for both O157:H7 and non-O157: H7 STEC infection, are necessary for early recognition and appropriate treatment of these infections. Regrettably, there is no definitive biochemical characteristic which distinguishes STEC strains belonging to serogroups other than O157 from other fecal *E. coli* strains, a fact which significantly complicates the isolation of such organisms [2].

Studies reveal an increasing antibiotic resistance of STEC organisms in animals and meat [6]. The presence of antibiotic resistant strain in meat may represent a threat to human health because such strains can be transmitted to humans through the consumption of contaminated meat. Therefore, surveillance of antimicrobial resistance in STEC is very important for preventing the spread of antimicrobial resistance in organisms and future disease management [6].

E. coli and related bacteria possess the ability to transfer DNA via bacterial conjugation or transduction, which allows genetic material to spread horizontally through an existing population. The process of transduction, which uses the bacterial virus called a bacteriophage, [7] is where the spread of the gene encoding for the Shiga toxin from the *Shigella* bacteria to *E. coli* helped produce *E. coli* O157:H7, the Shiga toxin-producing strain of *E. coli*. [7].

The pathogenicity of *E. coli* O157:H7 and other non-O157 STEC is associated with several virulence factors. Shiga toxins 1 and 2 (encoded by *stx*₁ and *stx*₂ genes) are the most important virulence factors, and play a major role in the pathogenesis of HC and HUS [8]. Besides Shiga toxins, intimin (encoded by *eaeA* gene) and enterohaemolysin (encoded by *E-hlyA* genes) are also two important virulence factors. Intimin was found to be responsible for attachment of the bacteria to the intestinal epithelial cells, causing attaching and effacing (A/E) lesions in the intestinal mucosa [8]. Enterohaemolysin has been demonstrated to cause enterocyte and leukocyte lysis in humans, promoting iron acquisition for bacterial nutrition [9].

Molecular typing is a useful tool for determining the genetic characteristics of food-borne bacteria and identifying probable sources of infections. Currently, there are no public health surveillance data on the occurrence of STEC in the study area. The laboratory diagnosis of STEC infections is not routinely carried out in the clinical laboratories. Under such a scenario, it is difficult to establish events and direction of transmission, as well as to quantify the risk of pathogen transmission between humans, livestock and the environment [10]. The aim of this study therefore was to assess the antimicrobial sensitivity, Shiga toxin-producing *Escherichia coli* (STEC) and virulence genes of isolates from samples in some selected sites of Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Port Harcourt which is the capital of Rivers State, is the oil hub of Nigeria. It is highly congested due to industrialization. Port Harcourt lies along the Bonny River, 66 kilometers upstream from the Gulf of Guinea and is located in the Niger Delta area. Geographically it lies on the coordinates, latitude 4.75°N and longitude 7°E [11]. Its population was estimated at 2million, making it one of the largest metropolitan areas in Nigeria [11]. Port Harcourt features a tropical wet climate with lengthy and heavy rainy season and very short dry season. Its topography ranges from flat plains with a network of rivers to tributaries [11]. The people of the state depend on different sources of water supply for their drinking water and domestic water needs.

2.2 Sampling Locations

Samples were collected from Rumuokoro, Egbelu, Igwuruta, Mile 3 and Mbuogba Abattoirs; some roadside butchers; some roadside casual restaurants (Buka)/street stalls that sell food prepared in advance; and some hospital, clinics and laboratories all in Port Harcourt. Majority of the stool samples were collected at Obio Cottage Hospital in Port Harcourt. Stool samples were also collected from Ebony Clinic, one of the most popular and well attended clinic located at Rumuokwuta, Port Harcourt. Few additional stool samples were collected from two private laboratories, Antel Medical Laboratory and Healthwise Medical Laboratory located at Rumuigbo and Rumuokwuta in Port Harcourt, which conducted medical test for private patients.

2.3 Study Population

The study was conducted between November 2020 and November 2021. A total of three hundred and fifty-two (352) samples were collected. The clinical stool samples from patients with gastrointestinal complaints and food sellers stool samples were collected from both sexes comprising different age groups between the ages of 20 and above 40 years. It is a mixed socio-economic, population including the poor and rich, those living in well-organised settlements and those living in areas with poor sanitary conditions. Also, individuals without gastrointestinal complaints, who do not eat outside their homes and are not regularly in contact with raw beef were included as control population. Again, samples were also collected from cow meat due to high rate of beef consumption by residence in Port Harcourt.

2.4 Sample Collection

2.4.1 Meat and waste abattoir water Samples

A total of sixty-seven (67) water samples were collected, 63 waste water were sampled from five different abattoir sites as follows: Rumuokoro 20, Egbelu 5, Igwuruta 19, Mgbuoba 10 and Mile III 10. Four (4) water samples from Eva commercial bottled water representing control sample were included. One hundred and twenty- nine (129) meat samples were

purchased from roadside butchers and abattoirs. Forty-six (46) meat samples were purchased from roadside butchers at different locations as follows: Mgbuoba 10, Rumuola 9, Mile 3 9, Mile 4 9. Also, sixteen (16) meat samples each was purchased from five (5) abattoirs sites namely: Egbelu, Igwuruta, Mgbuoba, Mile 3 and Rumuokoro, giving a total of 80 abattoir meats samples. Different parts of the beef were sampled. Microbiological analyses were conducted within 1-2 hours of sample collection. Samples were collected aseptically with sterile universal bottles (Smart diagnostics 2019, China).

2.4.2 Stool Sample

A total of one hundred and fifty-nine stool samples (159) were collected. Eighty-nine (89) were obtained from Obio-cottage hospital, and twenty (20) samples from private laboratories in the city. Another thirty (30) stool samples were collected from food vendors in the city. Samples were collected from both sexes of all age groups. Twenty (20) samples from healthy subjects representing control population were also included. The samples were collected using sterile stool bottles (Smart diagnostic, China). The stool macroscopy was observed and noted immediately after collection.

2.5 Sample Analysis

2.5.1 Media Preparation

All the agar-based media and broth used in this study were reconstituted and sterilized according to the manufacture's instruction. The molten agar was allowed to cool to about 45°C, supplemented (where applicable) and poured into sterile plastic petri dishes (about 20ml per Petri dish). They were allowed to solidify, packed and stored in the refrigerator for subsequent uses.

2.5.2 Enrichment and Recovery

2.5.2.1 Meat Sample

Each of the meat sample was macerated on the butchers table and 2.5g of the macerated meat sample was suspended in 22.5ml of tryptone soya broth (TSB) (Oxoid CM0129) using sterile universal bottle mixed by shaking and incubated at 37°C for 18-24hours.

2.5.2.2 Waste Abattoir Water Sample

1millilitre of waste abattoir water sample was added to 9.0ml of tryptone soya broth (TSB) (Oxoid,) contained in Bijou bottles, mixed by shaking and incubated at 37°C for 18-24hours.

2.5.2.3 Stool Sample

Pea size stool or 1ml stool sample was inoculated into sterilized Bijou bottle containing 10ml of TSB mixed or emulsified by shaking, incubated at 37°C for 18-24 hours.

2.5.3 Microbiological Analysis

A loopful of each sample from the enrichment broth was plated on Eosin methylene blue agar (TM, India) and incubated at 37°C for 24hours. A colony was picked from colonies exhibiting characteristic deep red *E. coli* colonies with metallic greenish sheen appearance and sub cultured into nutrient agar plates, for biochemical test. Thereafter, the *E. coli* isolates were sub cultured into supplemented Chromagar STEC (France). About two Mauve colonies typical of Shiga toxin producing *E. coli* were picked and again sub cultured into sorbitol MacConkey agar (SMAC) (Bio mark, India) supplemented with 2.5mg¹⁻¹ of potassium tellurite (Bio mark, India). All colourless colonies from SMAC were further tested using *E. coli* O157 latex agglutination (Oxoid DR0620) test for O157.H7 identification. In addition, antibiogram (Kirby- Bauer method) of the STEC strains was performed and noted

2.5.3 Identification of isolates

The identification of isolated bacteria was done by examining the cultural morphology and colour of the bacterial colonies, cultured on indicator agar plates, serology testing, and conventional biochemical tests such as citrate utilization, indole reactions, methyl red tests.

2.5.4 Antimicrobial Susceptibility Testing

The antibiotic sensitivity of *E. coli* O157:H7 and non- O157 STEC isolates were determined using the Kirby- Bauer disk diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI). MacFarland 0.5 standard was used to adjust the turbidity of the bacterial suspension. Muller- Hinton agar and 8 antimicrobial agents were used for the assay. The 8 antibiotics tested were as follows: Ciprofloxacin (CPR) 5ug, Ofloxacin (OFL) 5ug, Nitrofurantoin (NIT) 300ug, Gentamycin (GEN) 10ug, Ceftazidime (CAZ) 30ug, Cefuroxime (CRX) 30ug, Cefixime (CXM) 5ug, Augmentin (Aug) 30ug. The isolates were classified as sensitive, intermediate, and resistant.

2.5.5 Molecular Identification of Isolates

2.5.5.1 DNA Extraction (Boiling Method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) were spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

2.5.5.2 DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

2.5.5.3 Detection of Shiga-Toxin Producing Gene

Twenty (20) representative positive STEC isolates from culture were characterized for the presence of virulence genes *eae*, *stx*₁, and *stx*₂ by multiplex PCR. DNA was extracted for each individual isolate as described above.

The multiplex PCR was carried out by combining three specific pairs of primer *Stx*₁F – ATAAATCGCCATTCGTTGACTAC/*Stx*₁R-AGAACGCCCACTGAGATCATC, *Stx*₂F- GGCACGTCTGAACTGCTCC/*Stx*₂R-TCGCCAGTTATCTGACATTCTG and EAEAF- GACCCGGCACAAGCATAAGC / EAEAR- CCACCTGCAGCCAAGAGG. The multiplex reaction was performed in a 50 µl final volume containing 1 µl of the template DNA, 0.2 mM DNTPs, 10 mM Tris-HCl (pH8.8), 1.5 mM MgCl₂, 50 mM KCl, 2 U Taq DNA polymerase (Biolabs), and 5µml of each primer (Biosynthesis). DNA templates from the *E. coli* isolates were submitted to multiplex PCR. The thermo cycling conditions which were as follows: an initial denaturation step of 94°C for 5 min, 94°C for 45 min, 50°C for 1 min, and 2 min and 72°C for 35 cycles, with a final elongation step of 9 min extension at 72°C. Amplified samples were evaluated by 1% agarose gel electrophoresis in Tris-borate- EDTA buffer (Pronadisa, Madrid, Spain) and stained with 2 µg of ethidium bromide (Sigma-Aldrich, Madrid, Spain) per ml and photographed under UV light.

2.5.5.4 16S rRNA Amplification

The twenty representative isolates were identified as *E. coli* using the 16SrRNA PCR sequencing described briefly thus; The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

2.5.5.5 Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

2.6 Statistical Analysis

GraphPad Prism 2.01 was used to perform the statistical analysis. Prevalent rates were presented in percentages. Pearson Chi-Square was used for the analysis of categorical data and p-values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Antimicrobial Susceptibility Pattern of *E.coli* O157(n=29) and non-O157 (n=69) Isolates.

Antimicrobial Agent (ug)	<i>E.coli</i> O157:H7		Non <i>E.coli</i> O157		All STEC	
	S(%)	R(%)	S(%)	R(%)	S (%)	R (%)
Ciprofloxacin (5)	27(93)	0(0)	56(81)	5(7.0)	83(85)	7(7)
Ofloxacin (5)	23(79.0)	0(0)	55(80)	5(7.0)	78(80)	7(7)
Nitrofurantoin (30)	23(79.0)	2(7)	62(90)	2(7.)	85(88)	14(14.2)
Gentamycin (10)	18(62)	1(3.0)	20(29)	8(12)	38(39)	9(9.1)
Ceftazidime(30)	0	28(97)	0(0)	69(100)	0(0)	97(98.9)
Cefuroxime (30)	0	29(100)	0(0)	69(100)	0(0)	98(100)
Cefixime (5)	0	29(100)	0(0)	69(100)	0(0)	98(100)
Augmentin (30)	0	29(100)	0(0)	69(100)	0(0)	98(100)

Table 2: Frequency of detection of Virulence genes in 20 Representative STEC Isolates

Premise/sample types (n)	Virulence genes		
	Stx ₁	Stx ₂	eaeA
Food sellers Stool 6	6	6	2
Abattoir Meat 3	3	3	0
Clinical Stool 7	7	7	4
Waste Water 2	2	2	1

Roadside Meat 2	2	2	2
Total 20	20 (100%)	20 (100%)	9 (45%)

Table 3: Genetic Relatedness of the Representative STEC Isolates

R.S.I	Virulence Gene			Antibiotic Profile				Sample ID	16SrRNA PCR result
	Stx ₁	Stx ₂	eae	CAZ	CRX	CXM	AUG		
Non O157 STEC	+	+	-	R	R	R	R	FS 16	<i>E.coli</i>
Non O157 STEC	+	+	+	R	R	R	R	FS 17	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	FS 19	<i>E.coli</i>
<i>E.coli</i> O157	+	+	+	R	R	R	R	FS 20	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	WW 21	<i>E.coli</i>
Non O157 STEC	+	+	+	R	R	R	R	WW 22	<i>E.coli</i>
<i>E.coli</i> O157	+	+	+	R	R	R	R	RS/M 25	<i>E.coli</i>
Non O157 STEC	+	+	+	R	R	R	R	RS/M 26	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	FS 26	<i>E. coli</i>
Non O157 STEC	+	+	-	R	R	R	R	A/M 32	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	A/M 33	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	A/M 39	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	C/S 35	<i>E.coli</i>
Non O157 STEC	+	+	+	R	R	R	R	C/S 37	<i>E.coli</i>
<i>E.coli</i> O157	+	+	+	R	R	R	R	C/S 65	<i>E.coli</i>
<i>E.coli</i> O157	+	+	+	R	R	R	R	C/S 77	<i>E.coli</i>
Non O157 STEC	+	+	+	R	R	R	R	C/S 79	<i>E.coli</i>
Non O157 STEC	+	+	-	R	R	R	R	C/S 85	<i>E.coli</i>
<i>E.coli</i> O157	+	+	-	R	R	R	R	C/S 103	<i>E. coli</i>
<i>E.coli</i> O157	+	+	-	R	R	R	R	FS 11	<i>E.coli</i>

Key: CAZ = Ceftazidine
CRX = Cefuroxime
CXM = Cefixime
AUG = Augmentin

FS = Food Sellers Stool
A/M = Abattoir Meat
RS/M = Roadside Butchers Meat
WW = Waste Water
C/S = Clinical Stool

STEC: Shiga Toxin Producing *E. coli*, R.S.I: Representative STEC Isolates.

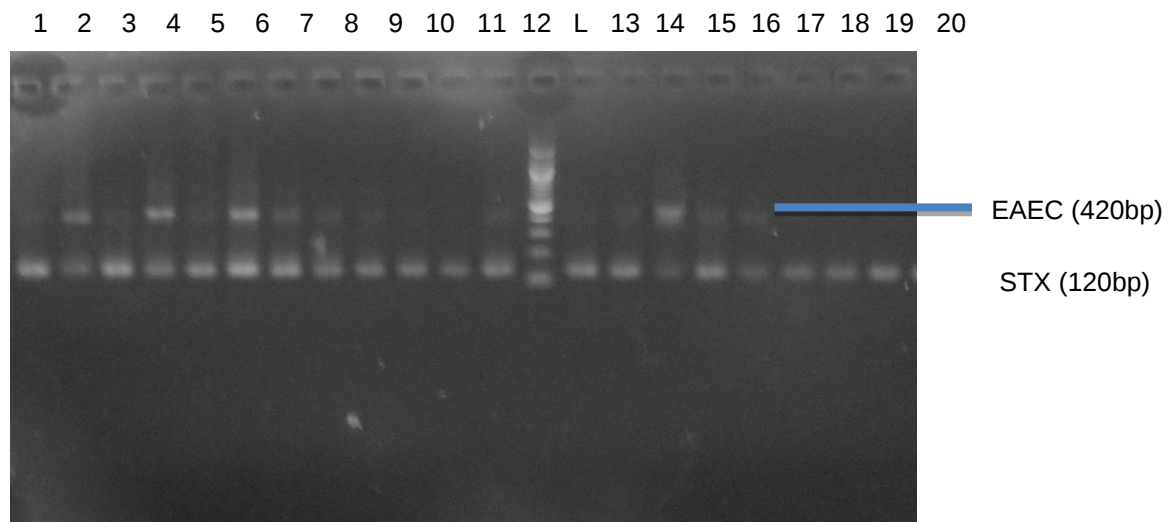


Plate 1: Multiplex agarose gel showing the eaeA and stx genes from the *E. coli* isolates. Lanes 2, 4, 6, 7, 8 14, 15, 16 and 17 represent the eaeA genes. Lanes 1-20 shows stx 1&2 genes on the same reading frame.

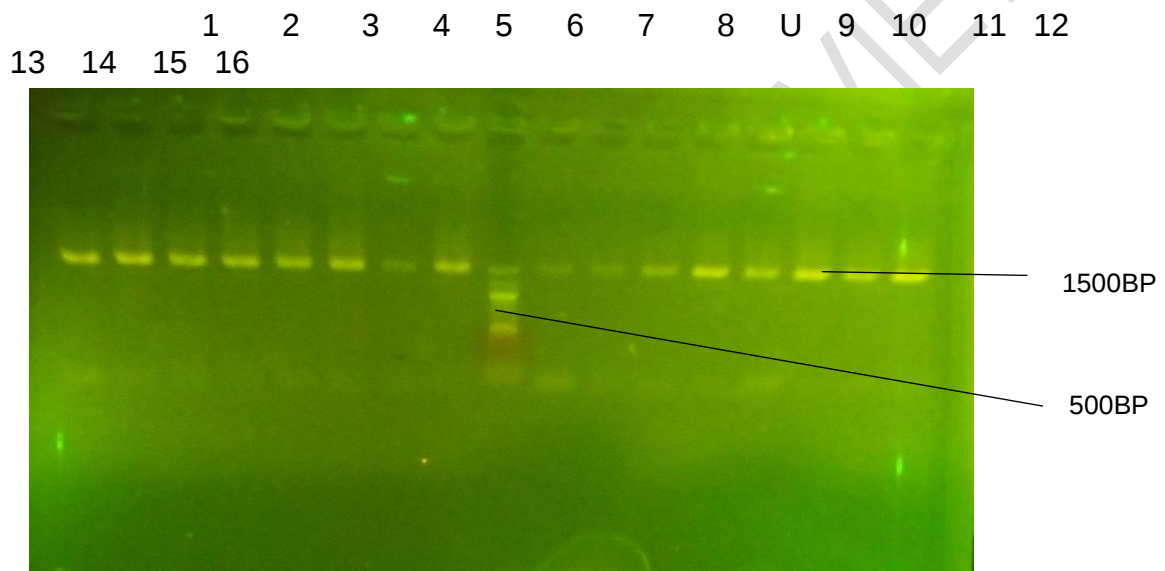


Plate 2 shows Agarose gel electrophoresis of some selected *Escherichia coli* isolates. Lane 1 – 16 represents 16SrRNA gene bands (1500bp). Lane U represents the 500bp Molecular ladder.

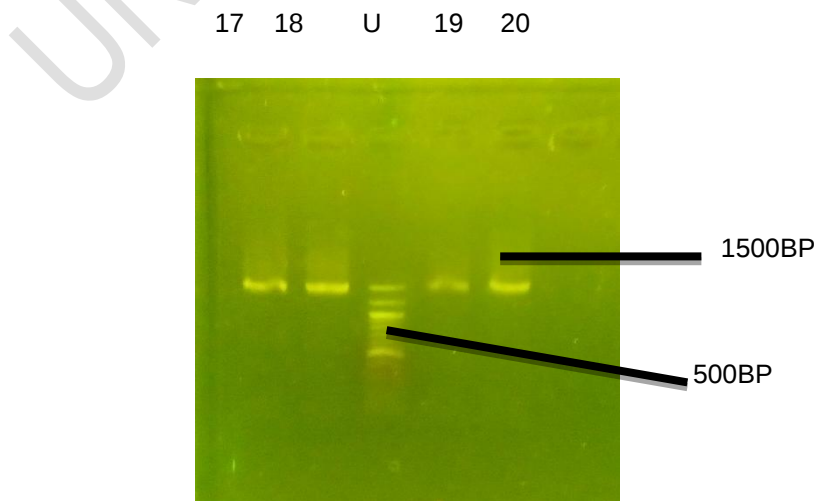


Plate 3: Agarose gel electrophoresis of some selected *Escherichia coli* isolates. Lane 17–20 represents 16SrRNA gene bands (1500bp). Lane U represents the 500bp Molecular ladder.

In this study, the antimicrobial susceptibility of *E. coli* O157 and non-O157 STEC serogroup isolated from Abattoir fresh meat, waste water, clinical stool, food sellers' stool and Roadside butchers' meat samples (Table 1) showed that of the eight antimicrobials commonly used in the treatment of human infections used to determine the antibiotic susceptibility of *E. coli* O157 and non-O157 STEC, all the 98 (100%) isolates were resistant to ceftazidime 97(98.9%), cefuroxime 98(100%), cefixime 98(100%) and Augmentin 98(100%). Susceptibility to antimicrobials gave: gentamycin 38(39%), Nitrofuratoin 85(88%), Ofloxacin 78(80%) and Cipicfloxacin 83(85%). The isolates displayed a wide spread multi-drug resistance of 60% (Table 1).

Antimicrobial agents primarily play a vital role in the lives of both humans and animals worldwide [12]. Antimicrobials are used in food animals to prevent and treat diseases and to promote the growth of food-producing animals. The increased use of antimicrobial agents in food animal production and in humans is a significant factor in the emergence of antimicrobial resistant bacteria. Antibiotic resistance may occur either spontaneously by selective pressure or over use by farmers on their beef cattle [12]. An investigation carried out by Govaris et al [6] gave evidence of antibiotic resistance among STEC organism. In this study all of the STEC serogroups were resistant to the Cephalosporins, resistance to Beta-lactam antimicrobial agents in *E. coli* are primarily mediated by Beta- lactamase, which hydrolyses the beta- lactam ring and thus inactivates the antibiotics. The 60% wide spread multi-drug resistance of the total STEC isolates means that, potential pathogenic, multi-drug resistant STEC isolates were recovered from meat, human stool and in the environment of the study area. STEC isolates from meat, wastewater and stool were shown to be genetically related and hence provide evidence on the possible transfer of the pathogens between different study materials.

The antimicrobial susceptibility pattern among STEC isolates investigated in this study reveals a genetic similarity among the isolates. The result of isolates sequenced for identification by targeting the 16SrRNA gene in phylogenetic analysis showed 100% coverage similar to the *E. coli* strains they were compared with in the publicly available sequence on NCBI. However, phylogenetic analysis using 16SrRNA does not provide a good resolution that help to determine relatedness among isolates.

Molecular diagnostic approach is one of the most specific, sensitive, yet relatively rapid diagnostic technique, though it is expensive but offers complete assessment compared to the conventional method. It is important to note that isolates belonging to the non-O157 serogroup would have not been detected if the regular O157-biased conventional method of detection only was used. The inclusion of methods targeting serogroups, and stx genes in context with culture techniques would allow clinical laboratories to detect most STEC serogroups. The centers for Disease Control and Prevention USA, released a guideline in 2009 for the detection of STEC in relation to acute community – acquired diarrhea, which include specific testing for shiga – toxin (STX) or their genetic determinants in addition to traditional culture [13]. Another observation in this study that is of importance is seen in the comparative evaluation of the culture technique and the molecular method of detection as the gold standard. Culture method compared favorably well with the molecular method in this study. It is therefore suggestive that the use of chromagar^{STEC} and the recovery media tryptone soy broth (TSB) is reliable in the detection of STEC pathogens, however, the final results can be obtained at the earliest three days after specimen collection.

Recently, it was assumed that incidence of infection and outbreaks were more common in the developed countries. Results from isolation of STEC in this study showed that this may not be the case but could be due to improved clinical awareness of STEC infections, well equipped laboratories that could test for serogroups other than O157 serogroups, a shift in diagnostic methods from only detection by culture-to-culture independent methods, with PCR and other molecular packages commonly used. They also have in place, good reporting and surveillance systems to document incidence and occurrences of STEC infections and outbreaks. This present study and others conducted by researches all over Nigeria revealed that STEC pathogens and especially non-O157 serogroup are prevalent in the country at large [14,15].

The detection of either *Stx*₁ or *Stx*₂ genes confirm the presence of STEC pathogens (Table 2, Plate 1). It has been reported that strains harboring *stx*₂ genes are more virulent than strains carrying either *stx*₁ or combinations of *Stx*₁ and *Stx*₂ (Farrokh *et al.*, 2013; Toro *et al.*, 2018). In this study, all the isolates profiled possessed a combination of *stx*₁ and *stx*₂ genotype. These findings cannot be underestimated since strains carrying *Stx*₁ only may cause diarrhea in immune compromised individuals [16].

Furthermore, the principal adherence factor in STEC is the intimin protein coded by the *eaeA* gene. Strains carrying *eaeA* genes are considered potential pathogens. STEC serogroups known to cause severe intestinal disease must intimately attach to the enterocyte of the intestinal membrane. Therefore, adherence factors are critical factors for STEC pathogenicity, 45% of the profiled isolates possessed the *eaeA* genes, implying that pathogenic STEC isolates were detected from some of the specimens. However, there are other factors that can aid attachment in the absence of the *eaeA* gene, example include *saa*, *aggR*, *aidA*, *ehaA*, and *iha* genes [17,18]. Testing for the presence of additional gene can provide clinically and epidemiologically important information about the infecting strain.

4. CONCLUSION

Multi-drug resistance among STEC isolates was wide spread. All the isolates were resistant to the cephalosporins used and Augmentin. The representative isolates profiled for virulence gene, all harbored *stx*₁ and *stx*₂ combination, only 45% harbored *stx*₁, *stx*₂ and *eaeA* gene combination. Phylogenetic analysis of the representative isolates confirmed the genetic similarity of isolates as it showed 100% relatedness coverage.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this research and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Ethical approval was obtained from Rivers State Ministry of Health, the state hospital board ethical review committee and the state ministry of environment. Informed consent was obtained from food vendors after the purpose of the study was explained to them.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND

PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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