Antimicrobial Sensitivity of Shiga toxin-producing *Escherichia coli* (STEC) and Virulence Genes of Representative Isolates in Port Harcourt, Nigeria

Ujoh, N. A. [a*], Nwokah, E. G. [a], Amala S. E. [a] and Wachukwu, C. K. [a]

[a] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors Authors WCK, NEG and ASE designed and supervised the study, author ASE wrote the protocol, author UNA wrote the first draft of the manuscript, managed the analyses and the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: To assess the antimicrobial susceptibility, Shiga toxin-producing *Escherichia coli* (STEC) and virulence genes of representative 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 forty-nine (349) samples were analyzed, 80 meat and 63 waste waters from five abattoirs cited in the city, 46 meat samples from five selected roadside butchers sites in the city, 109 patients stool samples, 30 stool samples from food sellers, 20 stool samples from healthy subjects and 1 commercial bottled water 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.

_____

Corresponding author: E-mail: ngujor69@gmail.com;

Results: The results showed that 20 (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 nitrofuratoin. The virulence gene combination pattern, the antibiogram pattern and the phylogenetic analysis result indicated genetic relatedness of isolates.

Conclusion: The representative isolates harbored stx_1 and stx_2 combination, only 45% harbored stx_1, stx_2 and eaeA genes combination. Phylogenetic analysis of the representative isolates confirmed the genetic similarity of isolates.

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].

Cattle 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 identified with few 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 virulence characteristics additional to the presence of *stx* genes of this 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, of Shiga toxin-producing *Escherichia coli* (STEC) and virulence genes of representative 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 4.75^0N and longitude 7^0E [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 forty-nine (349) 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 less than 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-four (64) 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. One (1) water sample from Eva commercial bottled water representing control sample was 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, Mile3 9, Mile4 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; and 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, 2019, China). The stool macroscopy was observed and reported 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 20 ml 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 and incubated at 37°C for 6-18hours.

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 6-18 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 $37^{\circ c}$ 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 2.5mg$^{1-1}$ of potassium tellurite (Bio mark, India). All colourless colonies from SMAC were further tested using *E. coli* O157 latex agglutination (Oxoid DR0620) test kit for O157.H7 identification. In addition, antibiogram (Kirby-Bauer method) of the STEC strains was performed and reported.

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, Nitrofuratoin (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 lunched 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 and EaeA gene

Twenty (20) representative STEC isolates from culture were characterized for the presence of virulence genes eae, stx_1, and stx_2 by multiplex PCR. DNA was extracted from each individual isolate as described above.

The multiplex PCR was carried out by combining two specific pairs of primer StxUFW-GAACGAAATAATTTATATGT/StxUREV-TTTGATTGTTACAGTCAT and EaeFW-CTGGCGAAAGACTGTATCAT/EaeREV-CAATGTATAGAAATCCGCTGTT. 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 5uml 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'-CGGTTACCTTGTTACGACTT-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, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extention, 72ºC 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 µl BigDye® terminator v1.1/v3.1, 2.25 µl of 5 x BigDye sequencing buffer, 10 µM Primer PCR primer, and 2-10ng PCR template per 100 bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5 s and 60°C for 4 min.

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

In this study, the antimicrobial susceptibility of *E. coli* O157 and non-O157 STEC serogroup isolated from Abattoir fresh meat, abattoir 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).

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

| Antimicrobial Agent (ug)	*E.coli* O157:H7		Non *E.coli* 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)
Nitrfuratoin (30)	23(79.0)	2 (7)	62 (90)	2 (7.)	85 (88)	14 (14.2)
Gentanycin (10)	18(62)	1 (3.0)	20 (29)	8 (12)	38 (39)	9(9.1)
Ceftazicdime(30)	0	28 (97)	0 (0)	69 (100)	0 (0)	97 (98.9)
Cefuromixe (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)
Augurentin (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
Foodsellers 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	*E.coli*
Non O157 STEC	+	+	+	R	R	R	R	FS 17	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	FS 19	*E.coli*
E.coli O157	+	+	+	R	R	R	R	FS 20	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	W/W 21	*E.coli*
Non O157 STEC	+	+	+	R	R	R	R	W/W 22	*E.coli*
E.coli O157	+	+	+	R	R	R	R	RS/M 25	*E.coli*
Non O157 STEC	+	+	+	R	R	R	R	RS/M 26	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	FS 26	*E. coli*
Non O157 STEC	+	+	-	R	R	R	R	A/M 32	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	A/M 33	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	A/M 39	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	C/S 35	*E.coli*
Non O157 STEC	+	+	+	R	R	R	R	C/S 37	*E.coli*
E.coli O157	+	+	+	R	R	R	R	C/S 65	*E.coli*
E.coli O157	+	+	+	R	R	R	R	C/S 77	*E.coli*
Non O157 STEC	+	+	+	R	R	R	R	C/S 79	*E.coli*
Non O157 STEC	+	+	-	R	R	R	R	C/S 85	*E.coli*
E.coli O157	+	+	-	R	R	R	R	C/S 103	*E. coli*
E.coli O157	+	+	-	R	R	R	R	FS 11	*E.coli*

Key: CAZ = Ceftazidine FS = Food Sellers Stoo CRX = Cefuroxime A/M = Abbattoir Meat CXM = Cefixime RS/M = Roadside Butchers Mea, AUG = Augmentin W/W = Waste Wate, C/S = Clinical Stool STEC: Shiga Toxin Producing E. coli, R.S.I: Representative STEC Isolates.
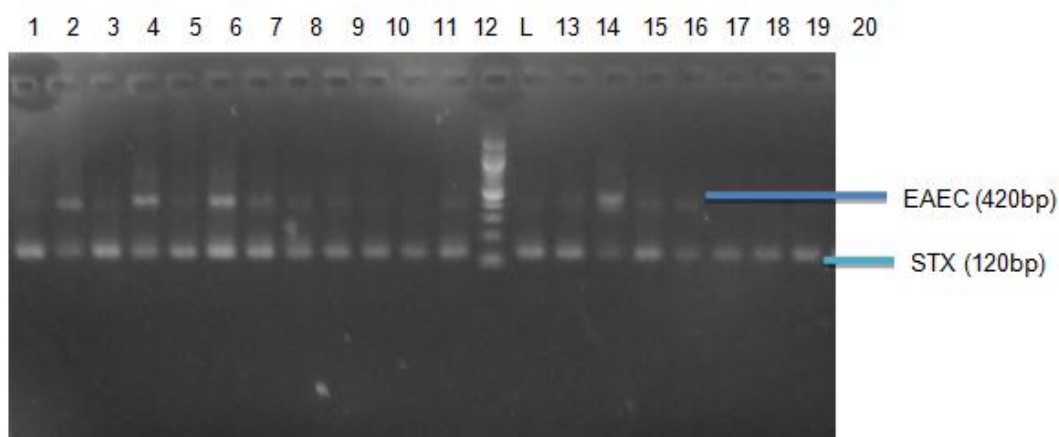
Plate 1. Multiplex agarose gel showing the eaeA and stx genes from the *E. coli* isolates. Lanes 2, 4, 6, 7, 8 14, 15, 16 and 17 represent the eaeA genes. Lanes 1-20 shows stx 1&2 genes on the same reading frame
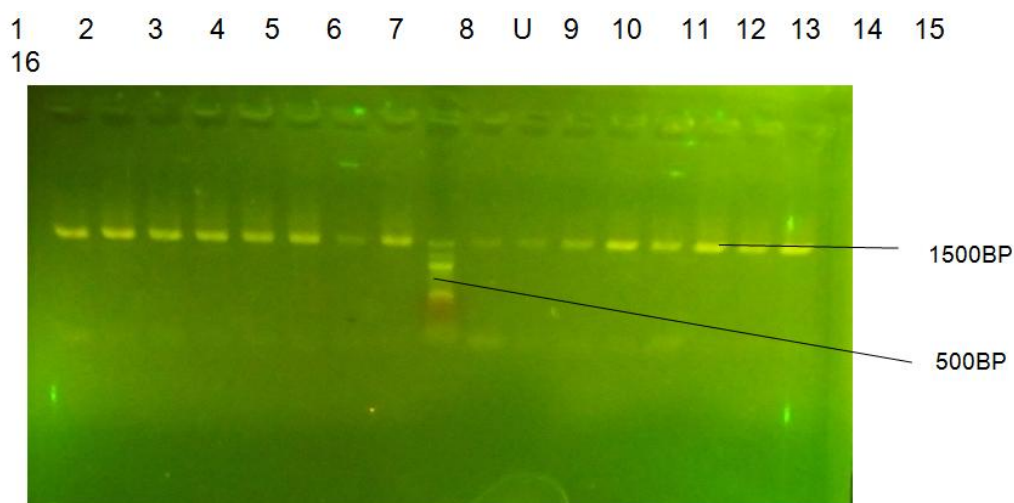
Plate 2 shows Agarose gel electrophoresis of some selected *Escherichia coli* isolates. Lane 1 – 16 represents 16SrRNA gene bands (1500bp). Lane U represents the 500bp Molecular ladder
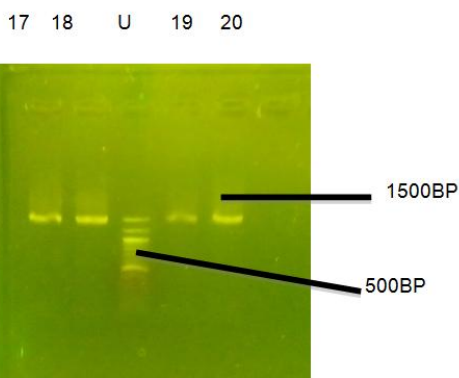
Plate 3. Agarose gel electrophoresis of some selected *Escherichia coli* isolates. Lane   17–20 represents 16SrRNA gene bands (1500bp). Lane U represents the 500bp Molecular ladder
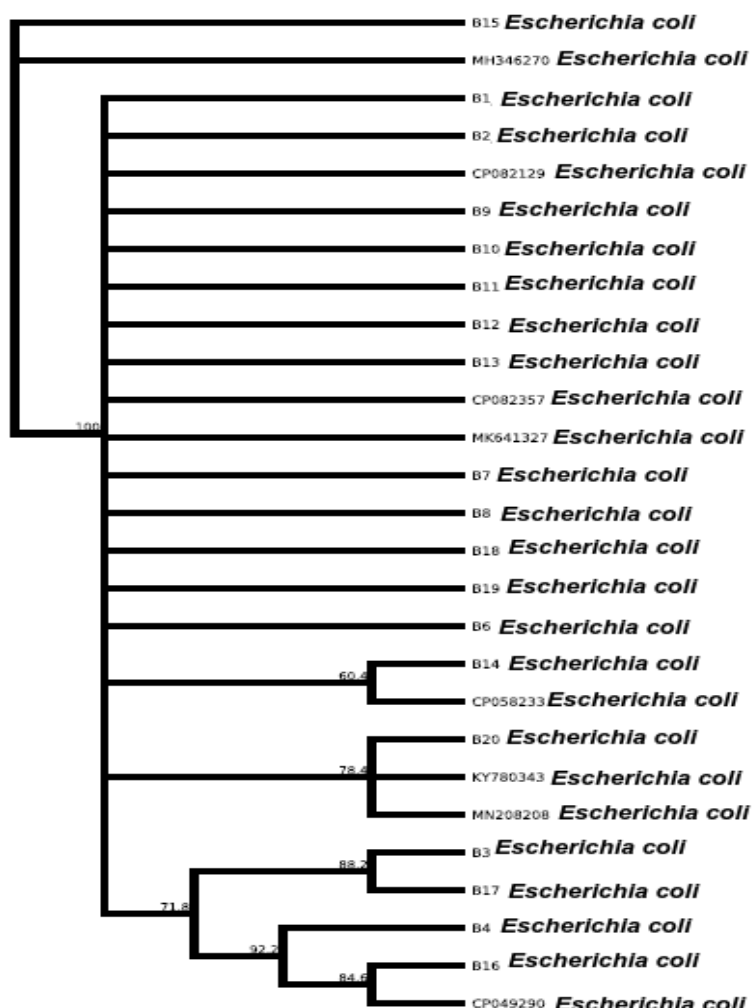
Fig. 1. Pylogenetic tree showing evolutionary distances between the bacterial

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 occurrence of multidrug- resistant bacteria with intrinsic and acquired bacterial resistant genes which may act in synergy, transfered by genetic mobility elements,paired with inappropriate use of antibiotics have simultaneously enhanced the emergence of resistance to various antibiotics [19].Antibiotic resistance may occur either spontaneously by selective pressure as consequence of 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 local transfer of the pathogens from animal reservoir or raw meat to the environment and to humans.

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 relatedness to the *E. coli* strains they were compared with in the

publicly available sequence on NCBI (Fig. 1). However, phylogenetic analysis using 16SrRNA does not provide a good resolution that help to identify isolates on the bases of their strains. 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 PCR-based method of detection.Culture method compared favorably well with the molecular method in this study. Noll et al. however highlighted the importance of utilizing both methods for accurate detection of STEC serogroups.

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 survellience 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].

Furthemore, 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.

DISCLAIMER

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.

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.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors Have Declared That They Have No Known Competing Financial Interests Or Non-Financial Interests Or Personal Relationships That Could Have Appeared To Influence The Work Reported In This Paper.

REFERENCES

1. Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, Yeung DH, Kirk MD. Global incidence of human Shiga toxin–producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne pathogens and disease. 2014 Jun 1;11(6):447-55.
2. Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clinical microbiology reviews. 1998 Jul 1;11(3):450-79.
3. Pruimboom-Brees IM, Morgan TW, Ackermann MR, Nystrom ED, Samuel JE, Cornick NA, Moon HW. Cattle lack vascular receptors for Escherichia coli O157: H7 Shiga toxins. Proceedings of the National Academy of Sciences. 2000 Sep 12;97(19):10325-9.
4. Savoye F, Feng P, Rozand C, Bouvier M, Gleizal A, Thevenot D. Comparative evaluation of a phage protein ligand assay with real-time PCR and a reference method for the detection of Escherichia coli O157: H7 in raw ground beef and trimmings. Journal of Food Protection. 2011 Jan;74(1):6-12.
5. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal. 2013 Apr;11(4):3138..
6. Govaris A, Angelidis AS, Katsoulis K, Pournaras S. Occurrence, virulence genes and antimicrobial resistance of Escherichia coli O157 in bovine, caprine, ovine and porcine carcasses in Greece. Journal of Food Safety. 2011 May;31(2):242-9.
7. Brüssow H, Canchaya C, Hardt WD. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiology and molecular biology reviews. 2004 Sep 1;68(3):560-602.
8. Abu-Ali GS, Ouellette LM, Henderson ST, Whittam TS, Manning SD. Differences in adherence and virulence gene expression between two outbreak strains of enterohaemorrhagic Escherichia coli O157: H7. Microbiology. 2010 Feb;156(Pt 2):408.
9. Lorenz SC, Son I, Maounounen-Laasri A, Lin A, Fischer M, Kase JA. Prevalence of hemolysin genes and comparison of ehxA subtype patterns in Shiga toxin-producing Escherichia coli (STEC) and non-STEC strains from clinical, food, and animal sources. Applied and environmental microbiology. 2013 Oct 15;79(20):6301-11.
10. Lupindu AM. Isolation and characterization of Escherichia coli from animals, humans, and environment. Escherichia Coli-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications. London, United Kingdom: IntechOpen Limited. 2017 Jul 12:187-206.
    Available at: http://dx.doi.org/10.5772/67390. 2017.
11. Britannica. The Editors of encyclopaedia 'Port Harcourt'. Encylopedia Britannica; 2019.
12. Hudson JA, Billington C, Cornelius AJ, Wilson T, On SL, Premaratne A, King NJ. Use of a bacteriophage to inactivate Escherichia coli O157: H7 on beef. Food microbiology. 2013 Oct 1;36(1):14-21.
13. European Centre for Disease Prevention and Control (ECDC). Surveillance of seven

priority food- and waterborne diseases in the EU/EEA – 2010−2012. Stockholm; 2012.
Available:http://ecdc.europa.eu/publications-data/surveillance-seven-priority-food-and-waterborne-diseases-eueea2010-2012. 2015.

14. Oluyege AO, Famurewa O. Shiga toxin and non-shiga toxin-producing Escherichia coli O157 from cattle, goats and chicken in Ado-Ekiti, South West, Nigeria. International Journal of Tropical Disease and Health. 2015;6(4):108-18.

15. Ayoade F, Oguzie J, Eromon P, Omotosho OE, Ogunbiyi T, Olumade T, Akano K, Folarin O, Happi C. Molecular surveillance of shiga toxigenic Escherichia coli in selected beef abattoirs in Osun State Nigeria. Scientific reports. 2021 Jul 7;11(1):1-9.

16. Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H, Raynaud S, Thevenot D, Condron R, De Reu K, Govaris A, Heggum K. Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. International journal of food microbiology. 2013 Mar 15;162(2):190-212.

17. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal. 2013 Apr;11(4):3138.

18. Toro M, Rivera D, Jiménez MF, Díaz L, Navarrete P, Reyes-Jara A. Isolation and characterization of non-O157 Shiga toxin-producing Escherichia coli (STEC) isolated from retail ground beef in Santiago, Chile. Food Microbiology. 2018;75:55-60.