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Detection of virulence genes and antibiogram of Shiga toxin-producing *Escherichia coli* O157:H7 from slaughtered cattle and abattoir effluent in Zaria, Nigeria

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Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a formidable human pathogen causing food-borne diseases worldwide. The present study investigates virulence genes and determines the antimicrobial characteristics of STEC O157:H7 isolates from faecal and effluent samples at Zaria abattoir in Zaria. PCR assays were used to detect the virulence genes of STEC O157:H7 isolates. The isolates that produced *stx* genes were subjected to antibiotic susceptibility testing using Kirby Bauer disc diffusion technique. Out of the 13 STEC O157:H7 isolates evaluated, *E. coli* 16SrRNA gene was detected in 12 isolates (92.3%) and the distribution of the virulence genes showed that of the 12 *E. coli* 16SrRNA gene positive isolates, 10 (83.3%) had *stx*$_1$ gene, 2 (16.7%) produced *stx*$_2$ gene, 2 (16.7%) harbored both *stx*$_1$ and *stx*$_2$ genes, and 2 (16.7%) lacked *stx* genes. Similarly, 8.3% had *hlyA* gene, 58.3% were positive for *fliC* gene and none produced *eaeA* gene. The ten isolates that produced *stx* genes were highly susceptible to Imipenem 100%, Ciprofloxacin 100%, and Ofloxacin 100% while strong resistance was observed against Cefepime 90.0%, Streptomycin 70.0% and Ampicillin 70.0% and Tetracycline 60.0%. Also, 80.0% of the ten *stx* genes positive isolates exhibited Multiple Antibiotic Resistance (MAR), with the Multiple Antibiotic Resistance Index (MARI) ranging from 0.083 to 0.67. 60.0% exhibited Multiple Drug Resistance (MDR) traits. These high MAR and MDR values suggest that there may be abuse of antibiotics in the study area. Thus, good hygienic practices should be adopted in abattoirs to prevent the transmission of STEC O157:H7 pathogens responsible for many foodborne diseases in humans.

Key words: Abattoir, effluent, virulence genes, antibiotic susceptibility, Shiga toxin-producing *Escherichia coli* (STEC).

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen that causes diseases by producing Shiga toxins (Pruimboom-Brees et al., 2000). These cytotoxins inhibit protein synthesis which results in necrotic or apoptotic cell death in humans and animals (Pruimboom-Brees et al., 2000; Iwu et al., 2021). Shiga

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toxin-mediated vascular damages in colon and kidney have been reported to cause bloody diarrhea, gastroenteritis, enterocolitis, cystitis, hemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS) (Wang et al., 2002; Bitrus et al., 2011; Brandelli et al., 2015). HUS is characterized by haemorrhagic anaemia and thromobocytopenia, which cause kidney infections, neurological issues and acute renal failure cases in children (Bitrus et al., 2011). Epidemiological data of human infection showed that the HUS caused by STEC is on the increase, that is, from 3 to 5% globally, with the highest mortality rate in children of about 2 years old (Loconsole et al., 2020).

Domestic and wild ruminants have been identified as reservoirs of STEC O157:H7. Cattle are important carriers of STEC O157:H7 (Nadya et al., 2016). Feces of clinically normal cattle are important source of STEC O157:H7. Cattle spread infection by shedding STEC O157:H7 pathogen in their feces. Similarly, abattoir effluent is sewage discharge flowing out of a slaughterhouse into the environment and usually consists of wastewater, blood, animal body fluids, faeces, dirt, meats, soaps and detergents to mention but a few. One of the problems with abattoir effluent is that it harbours STEC O157:H7. This slaughterhouse waste is eventually discharged into the environment and therefore poses a serious threat to air, water, soil quality, microorganisms, humans, and animals if not well-treated (Bamidele and Okoya, 2015).

STEC O157:H7 pathogen is associated with various virulence genes that promote its colonization of the host and determine the ability of the pathogen to multiply within the host cells or cause diseases. Some of these virulence genes include Shiga toxin 1 and 2 (encoded by *stx*$_1$, *stx*$_2$), mucus-activatable Shiga toxin 2d genotype (encoded by *stx*$_{2d}$), enterohemolysin (encoded by *hlyA*, also known as *ehxA*) and intimin (encoded by *eaeA*). Apart from genes coding for virulence, there are identification or specific genes of STEC O157:H7 such as *rfbE*$_{O157}$, which codes the GDP perosamine synthetase (*rfb* $_{O157}$), O-antigen specific for STEC O157:H7; flagellin (encoded by *fliC*$_{H7}$), H-antigen specific for *E. coli* O157:H7, which is responsible for producing H7 flagellum of STEC O157:H7 serotype and *uidA*, which encodes β-glucuronidase. Other genes associated with STEC O157:H7 include the genes for the cytotoxic necrotizing factors, heat-labile toxin, heat-stable toxin, enteroinvasive toxin, and the enteroaggregative protein (Wang et al., 2002; Al-Ajmi et al., 2020).

The emergence of antibiotic resistance has become a global threat, with serious adverse effect on the economy and public health. Antibiotic resistance can occur naturally, but it is usually caused by misuse of antibiotics in humans and animals. Antibiotics are not approved therapeutic drugs for treatment of human infections caused by STEC O157:H7 because of easy transmission of resistance genes horizontally to other pathogens in hosts and environment (Moses et al., 2018). Disposal of abattoir effluents serves as an important vehicle for community wide dissemination of antibiotic resistance of STEC O157:H7 strains. Person to person transmission of STEC O157:H7 is responsible for the epidemiology and maintenance of the disease in a population. Therefore, the present study was designed to determine virulence genes present in STEC O157:H7 isolates using Polymerase Chain Reaction and conduct antibiogram on STEC O157:H7 isolates using Kirby diffusion test. The study exposed the occurrence of STEC O157:H7 pathogen in slaughtered cattle and effluent at Zaria abattoir. The research findings added information to the baseline data and stimulated prompt Public Health action in preventing and controlling the spread of the pathogen.

METHODOLOGY

Collection of bacterial isolates

A total of 13 pre-identified STEC O157:H7 isolates were used in the present study. The isolates obtained from this study were sourced from reference specimens stored in the microbiology laboratory of the Faculty of Veterinary Medicine, University of Abuja. The isolates were subjected to rigorous microbiological growth protocols involving growth on broth media, selective media, differential media, preliminary biochemical tests and confirmation using Microbact™ GNB 24 E; it was identified to be STEC using serology, verotoxin assay, and molecular identification of *stx* gene.

These isolates comprised 8 and 5 isolates from slaughtered cattle faeces and abattoir effluent waste samples collected at Zaria abattoir. The isolates had been in storage for less than a month and were then transferred to Molecular Laboratory of National Veterinary Research Institute (NVRI), Vom, Nigeria for further studies.

DNA extraction

Overnight culture of bacterial cells was used for this investigation. The genomic DNA of STEC O157:H7 was extracted and purified using commercially available DNA extraction kit, the qiagen kit {QIAamp DNA Mini Kit (250), Catalogue number 51306; Qiagen} for Gram-negative organisms, according to the manufacturer's instructions, and the total genomic DNA was determined by running on 1.0% agarose gel.

Polymerase chain reaction (PCR) amplification of 16S rRNA genes

The number colonies of STEC O157:H7 isolates were determined by plating serial dilutions of cultures on agar plates. The isolates were vortexed for 30 s and then added directly to PCR for amplification of the 16S rRNA genes. DNA of STEC O157:H7 isolates were purified using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and used as a PCR template. PCR amplification of 16S rRNA genes was performed using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) containing the 27F/1492R primer set (**Figure 1**) and LongAmp™ Taq 29 Master Mix (New England Biolabs, Ipswich, MA, USA). Amplification was conducted using an Applied Biosystems Veriti™ Thermal Cycler (Thermo Fischer Scientific, Waltham, MA, USA)

Table 1. List of primers sequences, amplicon sizes and target genes.

Primer set	Primer	Sequence (5′- 3′)	Target genes	Size of amplicon (bp)	References
A	E16S-F E16S-R	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA	*16SrRNA*	401	Wang et al. (2002)
B	Stx1-F Stx1-R	GAAGAGTCCGTGGGATTACG AGCGATGCAGCTATTA	stx_1	130	Bitrus et al. (2011)
C	Stx2-F Stx2-R	TTAACCACACCCACGGCAGT GCTCTGGATGCATCTCTGGT	stx_2	346	Bitrus et al. (2011)
D	HylA-F HylA-R	AGCTGCAAGTGCGGGTCTG TACGGGTTATGCCTGCAAGTTCAC	*EHEC hylA*	569	Wang et al. (2002)
E	FliC-F FliC-R	TACCATCGCAAAAGCAACTCC GTCGGCAACGTTAGTGATACC	$fliC_{H7}$	247	Wang et al. (2002)
F	EAE-F EAE-R	GCAAATTTAGGTGCGGGTCAGCGTT GGCTCAATTTGCTGAGACCACGGTT	*eaeA*	494	Bitrus et al. (2011)

Source: Wang et al. (2002) and Bitrus et al. (2011).

with the following PCR conditions initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 20 s, 55°C for 30 s, and 65°C for 2 min, followed by a final extension at 65°C for 5 min (Kai et al., 2019). *E. coli* O157:H7 strain EDL 933 (EDL 933) was used as a positive control and the following primers were used: forward, 5'-CCCCCTGGACGAAGACTGAC-3'; and reverse, 5'-ACCGCTGGCAACAAAGGATA-3' (Bitrus et al., 2011).

PCR amplification of Shiga-toxin genes

Stx_1 and Stx_2 primers sets were used to detect Stx_1 and Stx_2 genes (Bitrus et al., 2011). The PCR reaction mixture was a total of 25 µl containing 5.0 µl DNA extract, 2.5 µl of 10 × buffer (Fermentas®), 0.5 µl of 10 mM dNTPs mix (Fermentas®), 0.5 µl of 20 µM primers (Table 1), 1.5 µl of 25 mM $MgCl_2$ and 2.5 U of Taq Polymerase (Fermentas®). PCR amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 94°C for 5 min. This was followed by 40 cycles of denaturation at 94°C for 1 min; annealing at 53°C for Stx_1 and at 55°C for Stx_2 for 1 min; and extension at 72°C for 1 min. Ten microlitres of the PCR product were electrophoresed in an agarose gel (1.5%) containing 5 µl of 10 mg/ml ethidium bromide at 80 V for 60 min. 50 bp DNA marker (Fementas) was used as molecular size marker. DNA amplifications were examined under UV transilluminator and results documented using Gel Documentation System (Synegene®) (Bitrus et al., 2011).

Multiplex PCR amplification of *fliC, hlyA* and *eaeA* genes

A set of primer mixture was used to run the Multiplex PCR (Table 1). The reagents mixture was as follows: 5 µl of genomic DNA extract was used as template with Mg^{2+} free 2.5 µl 10x PCR reaction buffer (Fermentas®); 0.5 µl of 10 mM dNTPs; 1.5 µl of 25 mM $MgCl_2$ and 2.5 units of Taq polymerase (Fementas®). The set contained 0.6 µM *hlyA*, 0.4 µM of *fliC* and 0.75 µM *eaeA* primers. PCR amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 95°C for 8 min. This was followed by 30 cycles of denaturation at 95°C for 30 s; annealing at 58°C for 30 s; and extension at 72°C for 30 s. Final extension was at 72°C for 7 min. Ten microlitres of the PCR product were electrophoresed in an agarose gel (1.5%) containing 5 µl of 10 mg/ml ethidium bromide at 80 v for 60 min. 50 bp DNA marker (Fementas®) was used as molecular size marker. DNA amplifications were examined under UV transilluminator (Sigma) and results documented using Gel Documentation System (Synegene®) (Bitrus et al., 2011).

Antibiotic susceptibility testing

Kirby Bauer disc diffusion test was performed to screen STEC O157: H7 isolates for *in vitro* antimicrobial susceptibility using the protocol in CLSI (2020). Mueller-Hinton agar was prepared according to the manufacturer's instructions. The medium was cooled to 45-50°C and poured into plates. Plates could set on a level surface to a depth of approximately 4 mm. When the agar has solidified, plates could dry before use. An 18 to 24 h old broth culture of the *E. coli* isolate was standardized by diluting to 0.5 Mcfarland's standard. A sterile swab stick was inserted into the standardized STEC O157: H7 inoculum, drained to remove excess inoculum load, and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. After this, the inoculated Mueller-Hinton agar plate could dry for a few minutes at room temperature with the lid closed. After the agar surface has dried for few minutes, antibiotic impregnated discs (Oxoid, UK) of known concentrations: Ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), cefepime (FEP, 30 µg), ofloxacin (OFX, 5 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GM, 10 µg), imipenem (IPM, 10 µg), streptomycin (S, 10 µg), and tetracycline (TE, 30 µg) were carefully applied on the inoculated

Table 2. List of antimicrobial categories, antimicrobial agents, disc contents, and zone diameters breakpoints (nearest whole millimeters).

S/N	Antimicrobial categories	Antimicrobial agents	Disc contents (µg)	Zone diameters breakpoints (mm)			
				S	SDD	I	R
1	Aminoglycosides	Gentamicin	15	≥ 15	-	13-14	≤ 12
		Streptomycin	10	≥15	-	12-14	≤ 11
2	β-lactam combination agents	Amoxicillins-Clavulanic acid	20/10	≥ 18	-	14-17	≤ 13
3	Carbapenems	Imipenem	10	≥ 23	-	20-22	≤ 19
4	Cephems	Cefepime,	30	≥ 25	19-24	-	≤ 18
		Ceftazidime	30	≥21	-	18-20	≤ 17
		Ceftriaxone	30	≥23	-	20-22	≤ 19
5	Penicillins	Ampicillin	10	≥17	-	14-16	≤ 13
6	Phenicols	Chloramphenicol	30	≥18	-	13-17	≤ 12
7	Quinolones and Fluoroquinolones	Ciprofloxacin	5	≥26	-	22-25	≤ 21
		Ofloxacin	5	≥16	-	13-15	≤12
8	Tetracyclines	Tetracycline	30	≥15	-	12-14	≤11

I=Intermediate, R=Resistance, S=Susceptibility, SDD=Susceptible-Dose-Dependent.
Source: CLSI (2020).

Mueller-Hinton agar plates using sterile forceps. The plates were then incubated at 37°C for 24 h. After incubation, the diameters of the zones of inhibition were measured with a ruler to the nearest millimeter and recorded. *E. coli* ATCC 25922 (American Type Culture Collection, Manassas, VA, USA) strains were used as controls in each assay. The results were recorded as resistant, intermediate and susceptible according to the guidelines of Clinical Laboratory Standards Institute criteria for Enterobacteriaceae (Table 2) (Moses et al., 2018; Ayandele et al., 2020; CLSI, 2020).

Determination of multiple antibiotic resistance (MAR) and multiple drug resistance (MDR)

MAR of the STEC O157:H7 isolates was calculated as antimicrobial resistance to two or more antibiotics while multiple antibiotic resistance indices (MARI) were calculated using the standard method (Moses et al., 2018; Ayandele et al., 2020). This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b). It was calculated using its formula as: MARI = a/b.

MDR of the STEC O157:H7 isolates was calculated using the standard method (Ibrahim et al., 2012). MDR was of STEC O157:H7 taken as antimicrobial resistance to three or more antimicrobial classes or categories. Antimicrobial categories are classifications of antimicrobial agents based on their mode of action and specific to target organisms (Table 2) (Ibrahim et al., 2012).

Statistical analyses

Descriptive statistics such as tables, figures, charts and frequencies were used in data analyses.

RESULTS

PCR amplification of 16S rRNA

The results of PCR amplification of 16Sr RNA genes (Table 1 and Figure 1) showed that out of thirteen STEC O157:H7 isolates investigated, 12 (92.3%) harboured 16Sr RNA gene. The analysis of the results showed that out of the twelve positive isolates, 7 (87.5%) were from faecal sources while 5 (100.0%) were from effluents samples.

PCR amplification of Shiga-toxin genes

The PCR results (Table 1 and Figures 2 and 3) indicated that STEC O157:H7 harboured the stx_1 10 (83.3%) and stx_2 2 (16.7%) genes, respectively. Of the ten isolates that possessed stx_1 gene, 6 (85.7%) were from faecal samples and 4 (80.0%) originated from effluent samples. Similarly, 1 (14.3%) of the isolates that harboured stx_2 genes was from faecal samples while the remaining 1 (20.0%) was from the effluent samples.

Multiplex PCR amplification of *fliC, hlyA* and *eaeA* genes

The PCR results (Table 1 and Figure 4) revealed that

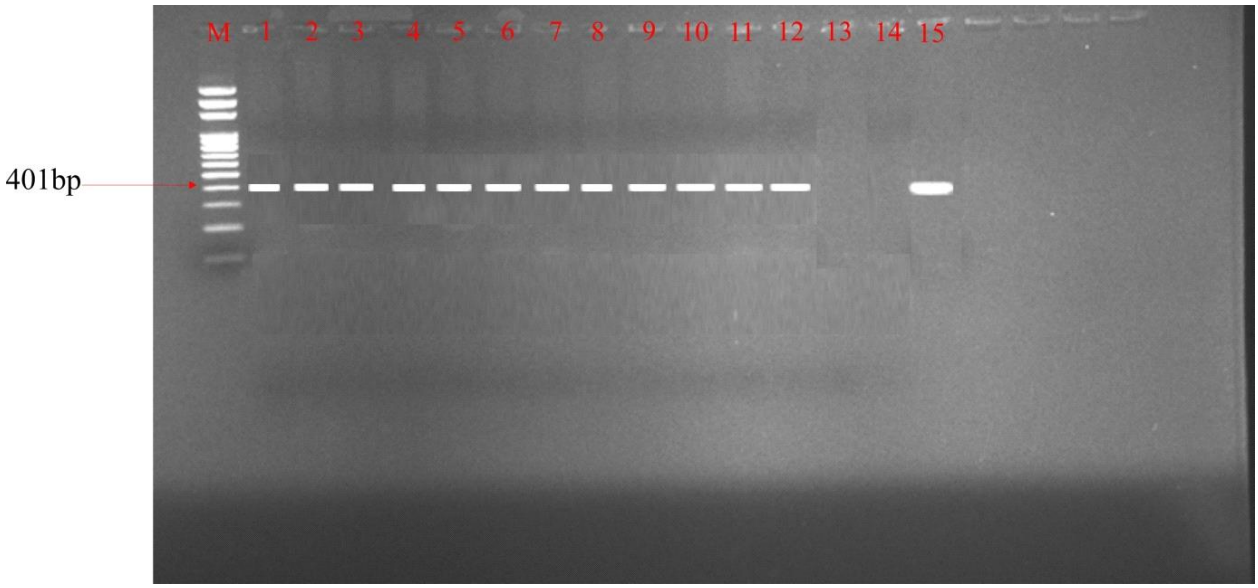

Figure 1. Electrophoretogram showing PCR detection of *16SrRNA* gene in STEC O157:H7 isolates. Lane M is 401 bp DNA marker/ladder. Lanes 1-12 show the amplified product *16SrRNA* gene in STEC O157:H7 isolates recovered in this study. Lane 13 shows negative amplification for *16SrRNA* gene. Lane 14 is negative control (nuclease free water). Lane 15 is positive control (E16S primers set that were used to amplify *E. coli* 16S rRNA gene).

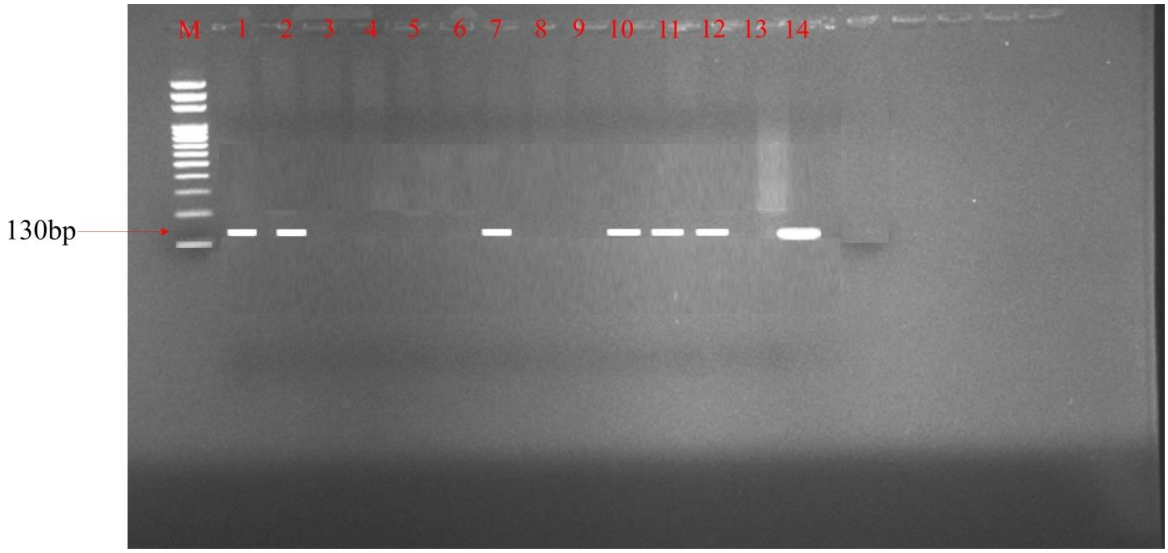

Figure 2. Electrophoretogram showing PCR detection of stx_1 gene in STEC O157:H7 isolates. Lane M is 130 bp DNA marker/ladder. Lanes 1-2, 7, 10-12 show the amplified product stx_1 gene in STEC O157:H7 isolates recovered in this study. Lanes 3-6, 8-9 show negative amplification for stx_1 gene. Lane 13 is negative control (nuclease free water). Lane 14 is positive control (EDL 933 primers set used to amplify stx_1 gene).

STEC O157:H7 harboured the *fliC* 7 (58.3) and *hlyA* 1 (8.3%) genes, respectively but lacked *eaeA* gene. The analysis of the results indicated that out of seven isolates that had *fliC* genes, 5 (71.4%) originated from faecal sources while 2 (40.0%) emanated from effluent samples. Also, the lone isolate that harboured *hlyA* gene was from effluent samples.

Distribution of virulence genes in faecal and effluent samples

Out of the twelve (12) isolates analysed for the presence of virulence genes, 7 (53.8%) were from faecal samples while 5 (38.5%) were from effluent samples. 10 (83.3%) had stx_1 gene comprising 6 (85.7%) from faecal samples

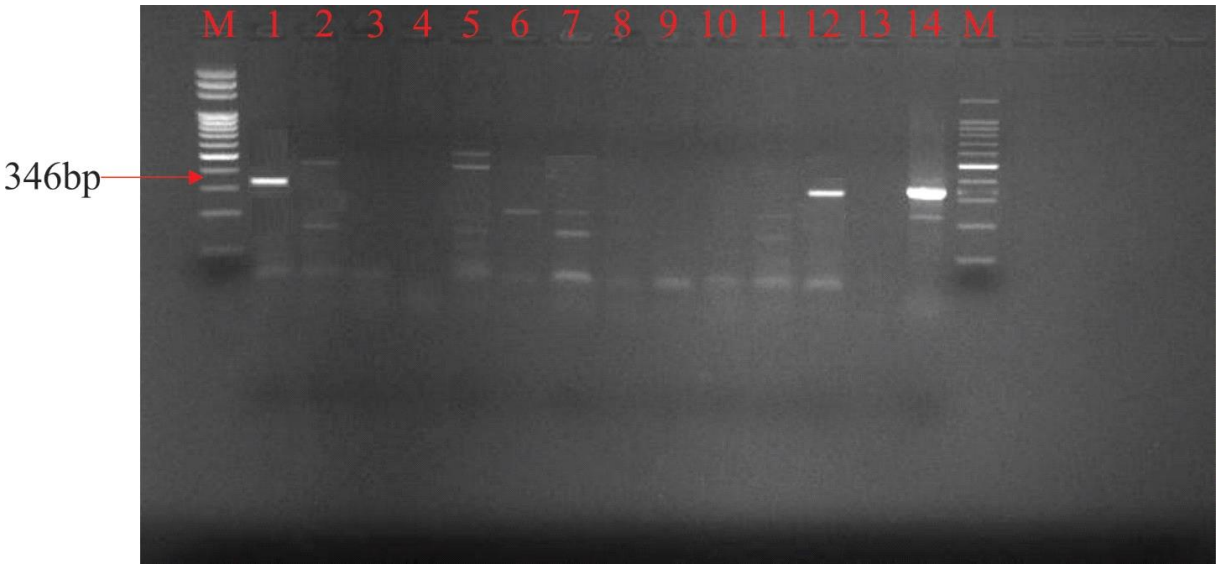

Figure 3. Electrophoretogram showing PCR detection of *stx*$_2$ gene in STEC O157:H7 isolates. Lane M is 346 bp DNA marker/ladder. Lanes 1&12 show the amplified product *stx*$_2$ gene in STEC O157:H7 isolates recovered in this study. Lanes 2-11 show amplification for *stx*$_2$ gene. Lane 13 is negative control (nuclease free water). Lane 14 is positive control (EDL 933 primers set used to amplify *stx*$_2$ gene).

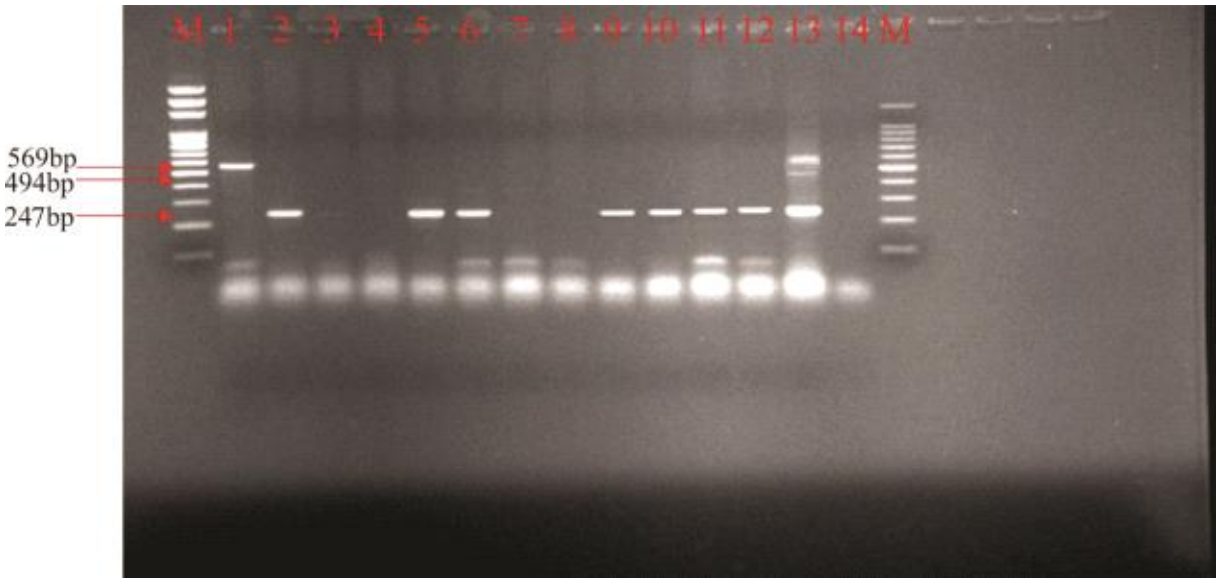

Figure 4. Electrophoretogram showing PCR detection of *fliC, hlyA* and *eaeA* genes in STEC O157:H7 isolates. Lane M contains 247bp, 494bp and 569bp DNA markers/ladders. Lane 2, 5-6, 9-12 and Lane 1 show the amplified products of *fliC* and *hlyA* genes respectively in STEC O157:H7 isolates recovered in this study. Lanes 1, 3-4, 7-8; 2-12 and 1-12 indicates negative amplification for *fliC, hlyA* and *eaeA* genes respectively. Lane 13 is the negative control (nuclease free water). Lane 14 is positive control (EDL 933 primers set used to amplify *fliC, hlyA* and *eaeA* genes).

and 4 (80.0%) from effluent samples. 2 (16.7%) lacked *stx*$_1$ gene. Also, 2 (16.7%) out of the twelve isolates were positive for *stx*$_2$ gene composing of 1 (14.3%) isolate from faecal and 1 (20.0%) from effluent samples. 2 (16.7%) harboured both *stx*$_1$ and *stx*$_2$ genes comprising 1 (14.3%) from faecal sample and 1 (20.0%) from effluent samples. 10 (83.3%) had *stx* genes which constituted of 6 (85.7%) faecal samples and 4 (80.0%) effluent samples while 2 (16.7%) lacked *stx* genes making up of 1 (14.3%) faecal sample and 1 (20.0%) effluent sample (Table 3).

Table 3. Percentage distribution of virulence genes in faecal and effluent samples.

Genes tested	No. tested faeces	No +ve faeces (%)	No. tested effluent	No +ve effluent (%)	Total no. tested	Total No +ve (%)
stx$_1$	7	6 (85.7)	5	4 (80.0)	12	10 (83.3)
stx$_2$	7	1 (14.3)	5	1 (20.0)	12	2 (16.7)
fliC	7	5 (71.4)	5	2 (40.0)	12	7 (58.3)
hlyA	7	0	5	1 (20.0)	12	1 (8.3)
eaeA	7	0	5	0	12	0

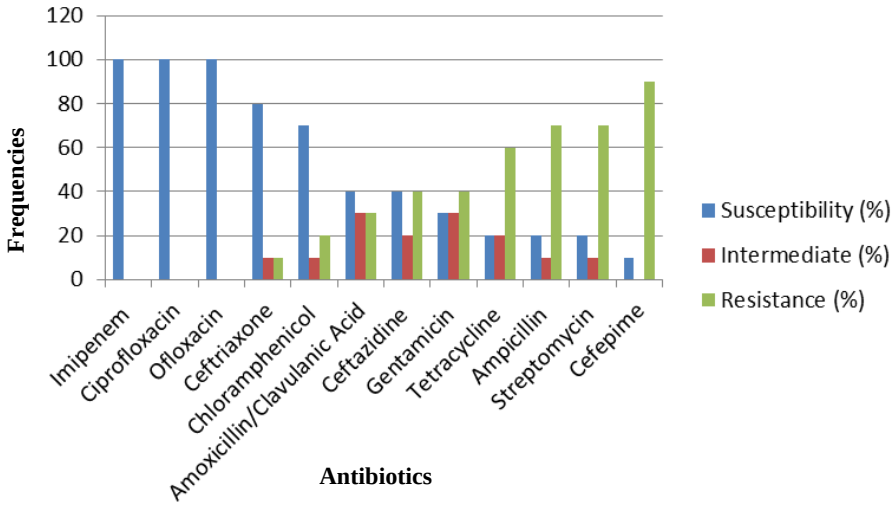

Figure 5. Comparison of sensitivity patterns of STEC O157:H7 isolates from faecal and effluent to different antibiotics.

Interestingly, 7 (58.3%) of the twelve STEC O157:H7 isolates investigated harboured *fliC* gene, comprising 5 (71.4%) from faecal samples and 2 (40.0%) from effluent samples. Furthermore, 1 (8.3%) of the twelve isolates possessed *hlyA* genes which was detected from effluent samples 1 (20.0%). No *hlyA* gene was detected from faecal samples. 11 (91.7%) were negative for *hlyA* gene. However, no *eaeA* gene was detected in all the STEC O157:H7 isolates investigated (Table 3).

Antimicrobial susceptibility profile of *E. coli* 0157:H7 isolates

The results of the antibiotic susceptibility studies (Figure 5) revealed that all the ten STEC O157:H7 isolates were highly susceptible to Imipenem (100.0%), Ciprofloxacin (100.0%) and Ofloxacin (100.0%). Further analysis of results showed that the isolates exhibited the following susceptibilities to other antimicrobial agents: Ceftriaxone (80.0%), Chloramphenicol (70.0%), Amoxicillin/Clavulanic acid (40.0%), Ceftazidime (40.0%), Gentamicin (30.0%), Tetracycline (20%), Ampicillin (20%), Streptomycin (20%), and Cefepime (10%). High level of resistance was observed against Cefepime (90.0%), Streptomycin (70.0%), Ampicillin (70.0%) and Tetracycline (60%). The level of resistance showed by other antibiotics was Gentamicin (40%), Ceftazidime (40%), Amoxicillin/ Clavulanic acid (30.0%), Chloramphenicol (20%) and Ceftriaxone (10.0%). Furthermore, intermediate susceptibilities were observed in Tetracyclic (40.0%), Amoxicillin/Clavulanic acid (30.0%), Gentamicin (30%), Ceftazidime (20.0%), Ampicillin (10%), Chloramphenicol (10%), Streptomycin (10%) and Ceftriaxone (10.0%).

Resistance patterns, MAR, and MDR of STEC O157:H7 isolates

The ten STEC O157:H7 isolates showed ten distinct susceptibility patterns to the twelve antibiotics (Table 4). Eight (80.0%) out of the ten STEC O157:H7 isolates exhibited MAR as they were resistant to at least two different antibiotics. Only 2 (20.0%) out of the ten STEC O157:H7 isolates did not exhibit MAR as they were resistant to only one antibiotics drug. The average MARI

Table 4. Resistance patterns, MARI and MDR of STEC O157:H7 isolates.

S/N	Isolate code	Resistance patterns	Mean diameter of growth inhibition (mm) ± S.E.M				
			NR (n=12)	MAR (%)	MARI	NACR (n=8)	MDR (%)
1	E1	FEP	1 ± 0.0	-	0.083	1 ± 0.0	-
2	E44	C AMC CAZ TE FEP S AMP GM	8 ± 2.0	+	0.667	6 ± 0.5	+
3	E68	CAZ TE CRO GM S AMP FEP	7 ± 1.5	+	0.583	3 ± 0.2	+
4	E212	AMP	1 ± 0.0	-	0.083	1 ± 0.0	-
5	F155	TE C S FEP AMP	5 ± 0.1	+	0.417	5 ± 0.1	+
6	F175	AMC CAZ GM TE S AMP FEP	7 ± 1.5	+	0.583	5 ± 0.1	+
7	F209	S FEP	2 ± 0.4	+	0.167	2 0.4±	-
8	F230	GM CAZ AMC TE S FEP AMP	7 ± 1.5	+	0.583	5 ± 0.1	+
9	F234	AMP S FEP	3 ± 0.2	+	0.250	3 ± 0.2	+
10	F279	TE FEP	2 ± 0.4	+	0.167	2 ± 0.4	-
				8 (80.0)	0.358		6 (60.0)

NR= Number resistance, NACR= number antibiotic category resistance, AMC=amoxicillin/clavulanic acid; AMP=ampicillin; C=chloramphenicol; CAZ=ceftazidine; CIP=ciprofloxacin; CRO=ceftriaxone; FEP=cefepime; GM=gentamicin; IPM=imipenem; OFX=ofloxacin; S=streptomycin; TE= tetracycline.

value of the STEC O157:H7 isolates was 0.358. The MARI of STEC O157:H7 isolates ranged from 0.083 to 0.667 (Table 4). 6 (60.0%) out of the ten STEC O157:H7 isolates exhibited multi-drug resistance traits as they were resistant to at least one antibiotic in three different antimicrobial categories. 4 (40.0%) out of the ten STEC O157:H7 isolates did not exhibit multi-drug resistance traits as they did not resist at least one antibiotics drug in three different antimicrobial categories (Table 4).

DISCUSSION

Bacterial identification is commonly performed in environmental samples using culturing methods. Recently, PCR amplification of 16S rRNA gene has been recognized as an effective method of bacterial identification. The use of 16S rRNA gene sequences as a common genetic marker for bacterial phylogenic and taxonomic studies is gaining momentum. The reasons may be the presence of 16S rRNA gene in almost all bacteria, often existing as a multigene family, or operons; the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution), and the 16S rRNA gene (1,500 bp) is large enough for informatics purposes (Manaka et al., 2017).

The present study also revealed an overall prevalence rate of 92.3% for identification of STEC O157:H7 using PCR amplification of 16SrRNA genes. These findings suggest that 92.3% of isolates were members of the genus *Escherichia*. This observation collaborates well with the reports of some previous studies (Manaka et al., 2017). Furthermore, the results of this study also revealed higher sensitivity (92.3%) to detect specific bacterial genus than usual cultural method. However, the results of this study did not distinguish closely related species. These findings are in accordance with Manaka et al. (2017) that reported several limitations associated with PCR amplification of the 16S rRNA. Obviously, closely related species might be difficult to distinguish using PCR amplification of 16S rRNA gene, and the identification of bacteria to a species level might be inaccurate. Furthermore, the antimicrobial susceptibility of bacterial and fungal strains could not be detected using PCR amplification of 16S rRNA gene.

The results of this study revealed the presence of both *stx₁* (83.3%) and *stx₂* (16.7%) genes in STEC O157:H7 isolates. The presence of either or both genes has been implicated in the ability of STEC strains to cause serious infections in humans. STEC strains producing *stx₂* only have been shown to be more commonly associated with serious human diseases such as HUS, than those producing *stx₁* alone or *stx₁* and *stx₂* possibly because the level of transcription of *stx₂ in vivo* is higher than that of *stx₁* (Bitrus et al., 2011). STEC cause infections such as gastroenteritis, haemorrhagic colitis (HC), HUS and renal failure in children by production of Shiga-toxins (Sharaf and Shabana, 2017).

In this study, *stx*$_1$ gene was detected more than *stx*$_2$ gene in the isolates (83.3% versus 16.7%, respectively). Similar observations were reported previously in Nigeria and Turkey (Bitrus et al., 2011; Ferreira et al., 2015). However, results of this study contradicted the reports of many other previous researchers where *stx*$_2$ gene was detected more than *stx*$_1$ (Wang et al., 2002). In contrast, this study did not corroborate well with the reports of many previous studies where no *stx*$_1$ was detected in any of the samples (Al-Ajmi et al., 2020). These discrepancies might be due to differences in samples, sources of collection, geographical locations and laboratory techniques used for detection of STEC O157:H7 isolates

(Pradel et al., 2000). Furthermore, the results of this study showed that the distribution of *stx*$_1$ gene detection was higher in cattle feces (85.7%) than in effluent water samples (80.0%), which was in accordance with the results of several previous studies (Kalender and Kilic, 2016).

Using multiplex PCR primer set conceived for detection of flagellin (*fliC*), hemolysin (*hlyA*) and intimin (*eaeA*) genes (Bitrus et al., 2011); two virulence genes, *fliC* (58.3%) and *hlyA* (8.3%) were detected in the STEC O157:H7 isolates studied. High distribution of H-7 specific flagellar protein, *fliC*$_{H7}$ gene observed in this study was in correlation with the results obtained by previous studies in China, Iraq, Greece and South Africa (Wang et al., 2002; Makhubalo et al., 2016; Alzubaidy, 2019). The presence of *fliC*$_{H7}$ gene in large proportion (58.3%) of the *E. coli* O157 isolates indicated that many of these isolates were genetically H7 with flagellum antigens as detected in serotyping tests. In contrast, the findings of this study were not consistent with the results of several earlier studies where no *fliC*$_{H7}$ gene was identified (Ferreira et al., 2015).

The low *hlyA* gene detection (8.3%) encountered in this study may be ascribed to different sources and pathogenesis of STEC O157:H7. These findings are in accordance with earlier studies performed in China (Wang et al., 2002). In the same vein, several earlier studies reported relatively high distribution of *hlyA* gene among STEC O157:H7 isolates. This may also be related to the variability of *hlyA* gene among *E. coli* strains. However, the result of this study is inconsistent with some previous investigations in Nigeria where no hemolysin (*hlyA*) gene was detected (Oloyede et al., 2016). The lone isolate that possessed *hlyA* genotype was isolated from effluent waste. This may suggest that STEC O157:H7 isolates without *hlyA* gene may possess reduced pathogenicity or may even be nonpathogenic in humans (Wang et al., 2002).

However, *eaeA* was not detected in the DNA of the twelve isolates. This report tallies with Kalin et al. (2012) in which all other virulence genes were detected except the *eaeA* gene. However, this result was not in agreement with reports of some previous studies in Turkey and Ethiopia which all the isolates harbored *eaeA* and other virulence genes (Kalender and Kilic, 2016). Intimin (eae gene product), has been recognized as the gene responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching and effacing lesions in the intestinal mucosas. However, a significant number of human STEC isolates, including those from patients with HC and HUS, lacked eaeA, showing that intimin is not important for human virulence (Bitrus et al., 2011). The differences observed in the distribution virulence genes in the DNA of the twelve isolates may be due to differences in geographical locations and laboratory techniques used for the detection of virulence genes (Adamu, 2014).

The antimicrobial susceptibility test results of STEC O157:H7 strains isolated from faecal and effluent sources showed the highest level of susceptibility for Imipenem, Ciprofloxacin and Ofloxacin (100% each). These were closely followed by Ceftriaxone and Chloramphenicol with susceptibility rates of 80.0 and 70.0%, respectively. This observation agrees with a previous study in Nigeria (Moses et al., 2018). However, these findings were not in conformity with previous studies conducted by Atnafie et al. (2017) and Ayandele et al. (2020). The differences observed may be due to differences in geographical locations and laboratory techniques used for the detection of antimicrobial susceptibility.

Interestingly, the highest level of resistance was observed against Cefepime (90%), Streptomycin (70%), Ampicillin (70%), Tetracycline (60%), and Amoxicillin (95.8%). The results of this study are in conformity with the findings of previous studies. The resistance rates recorded in this study are higher than the results of Moses et al. (2018) and lower than the results of Atnafie et al. (2017) and Tadesse et al. (2018). High level of resistance to Cefepime was not in consistence with the reports of previous studies in Nigerian (Moses et al., 2018; Ayandele et al., 2020); however, high level of resistance to Streptomycin agrees with a previous study in Nigeria (Atnafie et al., 2017).

The different trends of resistance patterns as observed in this study did not conform with reports of the previous studies conducted in Abakaliki, Southeast Nigeria and Lagos, Southwest, Nigeria where some isolates with more than one similar resistance patterns were observed in majority of the *E. coli* and *Salmonella* isolates evaluated, respectively (Akinyemi et al., 2018; Moses et al., 2018). Investigation on MAR showed that 80.0% of the ten STEC O157:H7 isolates exhibited multiple antibiotic resistances as they were resistant to at least two different antibiotics. The MARI of the STEC O157:H7 isolates in this study ranged from 0.083 to 0.667. Data obtained in this study however showed that the highest MARI of STEC O157: H7 isolates was 0.667. The findings of this present study are similar to the results of previous studies in Nigeria (Moses et al., 2018; Ayandele et al., 2020). Moses et al. (2018) and Ayandele et al. (2020) reported highest MARI value of 0.335 and 1.00, respectively. The optimal multiple antibiotic resistance of STEC O157:H7 isolates in this study may be attributed to robust control of antibiotics usage in livestock production in the area covered by this study. 60% of STEC O157:H7 isolates exhibited multi-drug resistance traits as they were resistant to one or more antibiotics in more than three different antimicrobial categories and were regarded as multidrug resistance strains. Multiple drug resistance has become an important trait of many microorganisms especially the human pathogens (Adenaike et al., 2016). Results of this study are similar to those of Subramani and Vignesh (2012) of which 50.0% of the isolates tested in their study exhibited

multidrug resistance attribute. Data obtained in this study however is much higher than those of Moses et al. (2018) of which 16.0 and 28.6%, respectively of the isolates tested in their studies exhibited multidrug resistance traits, suggesting the existence of lower frequency of MDR strains in their study areas. The differences observed in the multi-drug resistance of the isolates investigated in this study may be due to differences in geographical locations, samples and laboratory techniques. The higher multidrug resistance of Shiga-toxin producing *E. coli* O157:H7 isolates in this study may be attributed to the existence of greater frequency of MDR strains in the area covered by this study.

Conclusion

This study suggests that slaughtered cattle faeces and abattoir effluents are sources of contamination with STEC O157:H7 and non-STEC O157:H7. Our study exposed the presence of virulence genes such as *stx*$_1$ (83.3%), *stx*$_2$ (16.7%), *hlyA* (58.3%) and *fliC* (8.3%) genes. It also revealed that the pathogens lacked *eaeA* genes. Our results suggest that Imipenem (100.0%), Ciprofloxacin (100.0%), Ofloxacin (100.0%), Ceftriaxone (80.0%) and Chloramphenicol (70.0%) demonstrated high antibiotic susceptibility to STEC O157:H7 pathogens; while Cefepime (10.0%) is no longer effective. STEC O157:H7 isolates exhibited relatively high MAR (80.0%) and MDR (60.0%) traits, respectively. The presence of MDR pathogens indicates serious public health concern and signals possible outbreak of foodborne infections. Therefore, coordinated efforts are needed to safeguard the populace against this pathogen and to ensure proper use of antibiotics both in veterinary and human treatment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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