Microbes and Infectious Diseases 2022; 3(2): 348-359

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Molecular identification of virulence and antibiotic-resistant genes in *Escherichia coli* O157 and non-O157 recovered from water samples

***Olowe BM* [1], *Arogbodo JO* [*2]**

1- *Department of Biological Sciences, Faculty of Basic Medicals and Applied Science, Lead City University, Ibadan, Oyo State, Nigeria.*
2- *Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State,Nigeria.*

ARTICLE INFO

Article history:
Received 30 April 2021
Received in revised form 31 May 2021
Accepted 5 June 2021

Keywords:
Escherichia coli O157:H7
Non-O157
Virulence genes
Pathogenic genes

ABSTRACT

Background: This research work focused on ascertaining the presence of virulence and antibiotic-resistant genes in *Escherichia coli* (*E. coli*) O157 and non-O157 recovered from drinking water sources. **Methods:** Identification of *E. coli* O157 and non-O157 was carried out using standard serological and PCR techniques. Virulence genes (*rfb* O157, *fliC* H7, *stx*1, *stx*2, *eae* and *hly* genes) and antibiotic-resistant gene (Bla_{TEM}) were detected using PCR method on selected isolates (n= 15) from different water sources which demonstrated multiple antibiotic-resistance in a previous study. **Results:** The serological identification result revealed that a total of 68 out of 382 *E. coli* isolates, recovered in a previous work, were identified as a presumptive *E. coli* O157. These included 19.1 %, 21.7 %, 33.3 %, 14.3 % and 9.1 % of *E. coli* isolates from wells, boreholes, sachets, streams and pipe-borne respectively. Statistical analysis revealed that there was no significant difference in the frequency of *E. coli* O157 from the different water sources ($p > 0.05$). Also, there was a statistically significant positive correlation between the *E. coli* isolates and *E. coli* O157 (Pearson's $r = 0.996$). Detection of virulence and antibiotic-resistant genes showed that only 46.7 %, 33.3 %, 33.3 %, 93.3 %, and 66.7 % carried *rfb* O157, *fliC* H7, *stx*1, *stx*2 and *rpoS* gene respectively. In contrast, all the isolates possessed *hly* and Bla_{TEM} genes but none had *eae* gene. **Conclusion:** The presence of one or combination of these genes in these isolates depicts their virulence and resistance nature.

Introduction

Escherichia coli (E.coli) O157 is a pathogenic bacterium implicated to be a causative agent of human haemorrhagic colitis and haemolytic uraemic syndrome [1]. According to the reports of **Rompre et al.** and **Juhna et al.** [2, 3], pathogenic *E. coli* had been known to inhabit water from different sources. **Food Safety Authority**, **Mull and Hill**, **Jokinen et al.** and **Mezule et al.** [4-7], also corroborated the above as of community health importance. According to **Ajayi et al**. [8], the generic *E.coli* is considered as an intestinal pathogen and naturally exists as a commensal in the gut of humans and warm-blooded animals where it enjoys synergistic association with other members of the bacterial family. It also plays a beneficial role in the prevention of potentially pathogenic organisms in the gut. Studies revealed that *E. coli* which forms part of the normal intestinal flora of humans and warm blooded animals is capable of inhibiting the growth of other toxigenic strains of *E. coli* [9-11].

However, some strains of *E.coli* occasionally emerge as pathogens due to the

DOI: 10.21608/MID.2021.74664.1148
* *Corresponding author:* Arogbodo JO
E-mail address: arogbodojo@futa.edu.ng

presence of certain pathogenic features and virulence genes which are located on transmissible genetic elements and this distinguishes them from ordinary commensal strains as reported by **Ronsengren et al.** [12].

Escherichia coli strains causing enteric infections have been classified into various pathotypes which include enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC) and diffusely adherent *E. coli* (DAEC). This classification is based on the presence of different combinations of virulence factors such as production of haemolysins, colicins, haemagglutinins, enterotoxins, proteases, colonization factors and cell surface hydrophobicity [13,14]. Among these pathotypes, Shiga toxin-producing *E. coli* (STEC) is a group of enterohemorrhagic *E. coli* (EHEC) which produces Shiga toxins [15-18].

Escherichia coli O157 strains have been reportedly found in animals like cattle, horses, dogs and flies [19]. Human infection as well as water contamination can ensue, following defaecation by infected animals [20]. Contamination of drinking water sources with pathogenic *E. coli* can result to waterborne diseases such as dysentery with fever, urinary tract infection, severe bloody and watery diarrhoea, abdominal cramps, nausea, vomiting and possibly haemolytic uraemic syndrome (HUS) that may lead to kidney failure and eventual death [21,22]. Contamination of water sources with *E. coli* has long been a water quality issue due to the potential for disease transmission and antibiotic resistance. Investigations have identified antibiotics themselves in water sources [23-27]. The increasing rate and heavy use of antibiotics in health care, agriculture and animal husbandry contributes to the emergence of antibiotics in the environment and in turn contributes to the growing problem of antibiotic-resistant bacteria [28]. Furthermore, the spread of antimicrobial resistance between different bacterial strains and or species in different habitats has been reported [29-36]. The presence of virulence genes and increase in the number of resistant strains of *E. coli* is a major concern of health officials. The study site, Ado-Ekiti, is well known with cattle rearing and siting of abattoir near streams and river. These activities contribute to health risk of water sources available for the residents. And among the feacal contaminants, *Escherichia coli* O157 and non-O157 are highly pathogenic in human even at low infectious dose of 10^1- 10^2 cfu/ml. Therefore, this study aimed to detect virulence and antibiotic resistance gene in *E.coli* O157 and non-O157 recovered from drinking water sources in Ado-Ekiti, Ekiti State, Nigeria

Materials and Methods

Bacterial isolates

A total of 382 identified *E. coli*, from different drinking water sources from a previous published work were used in this study. They included 267 (69.89%) from well water samples, 33 (8.64%) from pipe borne water samples, 23 (6.02%) from borehole water samples, 56 (14.66%) from stream water samples and 3 (0.79%) from sachet (packaged) water.

Serological identification of *E. coli* O157

Detection and serotyping of toxigenic *E. coli* among the *E. coli* isolates were carried out using the standard method described by **Khudor et al.** [37]. Latex agglutination kit (DR0620M, Oxoid, UK) was used for conformity identification of *E. coli* O157 to detect the somatic antigen O157. The latex reagent (DR0620M, Oxoid, UK) was brought to room temperature. A drop of the test latex was dispensed onto the edge of a circle on the reaction card. Some loop-full drop of normal saline was added to the circle and it was ensured that the latex and saline did not mix at this stage. A portion of the test *E. coli* isolate was carefully emulsified in the saline drop giving a smooth suspension. Then the test latex and suspension was mixed and spread to cover the reaction area. The reaction card was rocked in a circular motion for exactly one minute and observed for agglutination reaction. *Escherichia coli* isolates positive at this stage was further confirmed by testing with the positive control latex reagent to ensure that the isolate is not auto-agglutinating. Identified *E. coli* which were positive for O157 latex agglutination were considered presumptive *E. coli* O157 based on latex agglutination.

Detection of virulence and antibiotic resistance genes

From among the serologically identified *E. coli*, *E. coli* O157 (n= 8) and *E. coli* non-O157 (n= 7) from different water sources were randomly selected for this study due to financial implication. These isolates were then tested for the presence of these genes: Virulence genes (*rfb* O157, *fliC* H7, *stx*1, *stx*2, *eae* and *hly* genes) and antibiotic-resistant gene (Bla_{TEM}) using PCR technique described below.

Bacterial genomic DNA extraction and purification

Escherichia coli O157 (n= 8) and *E. coli* non-O157 (n= 7) from different water sources were selected and grown overnight at 37ºC. The bacterial cultures were transferred to eppendorf tubes and spun down at 14,000 rpm for 2 mins. The supernatant of each sample was discarded and 600 µL of 2X CTAB buffer was added to the pellet and it was incubated at 65ºC for 20 mins. Then, it was allowed to cool to room temperature before the addition of chloroform. The resulting solution was mixed by gently inversion of the tube several times. Thereafter, it was spun at 14,000 rpm for 15 mins and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA.Then, it was incubated at -20ºC in the freezer for 1hr and later spun at 14,000 rpm for 10 mins and the supernatant was discarded and the pellet was washed with 70 % ethanol. Thereafter, it was air dried for 30 mins on the bench. The pellet was re-suspended in 100 uL of sterile distilled water as described by **Akinyemi and Oyelakin** [38]. The DNA concentration of each of the samples was measured on spectrophotometer at 260 nm and 280 nm and their genomic purity were determined.

DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 45ºC and 10µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 5µL sterile distilled water and 2 µL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source.

Polymerase chain reaction analysis using SSR primers

Polymerase chain reaction analysis was run with the specific primers (purchased from Integrated DNA Technologies, Inc., Coralville, Iowa) **(Table 1)** by a GeneAmp PCR thermocycler (Model 2400, Perkin-Elmer, USA). The PCR mix comprises of 1µL of 10X buffer, 0.4 µL of 50 mM $MgCl_2$, 0.5 µL of 2.5 mMdNTPs, 0.5µL of 5 mM forward and reverse primers, 0.05 µL of 5units/µlTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix. The PCR profile used has an initial denaturation temperature of 94ºC for 3 mins, followed by 30 cycles of 94ºC for 30 sec, 60ºC for 40 sec, 72ºC for 60 sec and the final extension temperature of 72ºC for 5 mins and the 10ºC hold forever.

Gel electrophoresis

Polymerase chain reaction amplicon electrophoresis was carried out by size fractionation on 1.2 % agarose gels. Agarose gels were prepared by dissolving and boiling 2.4 g agarose in 200 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 50ºC and 10 µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100 V for 2 hours. The DNA was visualized and photographed on UV light source.

Results

The serological identification result revealed that a total of 68 out of 382 *E. coli* isolates were identified as a presumptive *E. coli* O157. These included 19.1 %, 21.7 %, 33.3 %, 14.3 % and 9.1 % of *E. coli* isolates from wells, boreholes, sachets, streams and pipe-borne respectively. Statistical analysis revealed that no significant difference in the frequency of *E. coli* O157 from the different water sources (p>0.05). Also, there is a statistically significant positive correlation between the *E. coli* isolates and *E. coli* O157 (Pearson's r = 0.996) **(Table 2)**.

The presence or absence of O157 antigen, Shiga-toxins, virulence, antibiotic-resistant and stress response genes is summarized in **table (3)**. The result in **plate (1)** confirmed the presence of O157 antigens (259 bp) in isolates in lane 10, 11, 12 and 15 which were identified as presumptive *E. coli* O157 serologically. Also isolates in lane 13 and 14 has O157 antigen of 1.3 kbp and 3 kbp respectively. However, isolate (S33 from borehole sample) in lane 9 which was serologically identified as *E. coli* O157 did not carry the gene for O157 antigens. While the presumptive *E. coli* O157 isolates in lane 1 to 8 were negative for O157 antigen except for isolates in lane 4 which was positive for O157antigens of 1.6 kbp. All the isolates in lane 1 to 8 were negative for *flic* H7 antigen while from

among those positive for O157 antigen, only isolates in lane 10 to 14 were positive for the presence of *flic* H7 antigen (> 625 bp). This shows that isolates in lane 10 to 14 were *E. coli* O157:H7 while isolates in lane 4 and 15 were *E. coli* O157: H- **(Plate 2)**.

The results shown on **plate (3)** revealed that only isolates in lane 4, 10, 12 to 14 were positive for *stx*1 (>348 bp) while **Plate 4** showed that all the isolates except isolate in lane 15 possess *stx*2 gene (246 bp). All the 15 isolates (*E. coli* O157 and non-O157) were positive for the virulence plasmid encoding the enterohaemolysin gene, *hly* A, (165 bp) **(Plate 5)** but were negative for the intimin-coding *eae* gene **(Plate 6)**. The amplification of antibiotic gene (Bla_{TEM}), encoding ampicillin resistance, was used to confirm that all the isolates possessed this resistance gene (1.8 kbp) coding for Bla_{TEM} **(Plate 7)**.

Table 1. Polymerase chain reaction (PCR) primers as used in this study.

Primer set	Target Gene	Sequence (5'→ 3 ')	Size of PCR Product (bp)	References
A	*stx*1	5′CAGTTAATGTCGTGGCGAAGG3′ 3′CACCAGACAATGTAACCGCTG5′	348	**Osek** [39]
	*stx*2	5′AGGCCCACTCTTTAAATACATCC3′ 3′CGTCATTCCTGTTAACTGTGCG5′	246	**Beutin et al.** [40]
B	*eaeA*	5′GGGATCGATTACCGTCAT3′ 3′TTTATCAGCCTTAATCTC5′	837	**Osek** [39]
	HlyA	5′ACGATGTGGTTTATTCTGGA3′ 3′CTTCACGTGACCATACATAT5′	165	**Selim et al.** [18]
C	*Rfb* O157	5′CGGACATCCATGTGATATGG3′ 3′TTGCCTATGTACAGCTAATCC5′	259	**Khudor et al.** [37]
	fliC H7	5′GCGCTGTCGAGTTCTATCGAGC3′ 3′CAACGGTGACTTTATCGCCATTCC5′	625	**Khudor et al.** [37]
D	*E. coli* O157 *RpoS*	5′GCGTTGCTGGACCTTATC3′ 3′GAATAGTACGGTTTGGTTCATAAT5′	250	**Parry-Hanson et al**. [41]

Table 2. Serological identification of *E. coli* O157.

Sources (n)	Serotype O157 (%)	Serotype non-O157 (%)
Wells (267)	51 (19.1)	216 (80.9)
Boreholes (23)	5 (21.7)	18 (78.3)
Sachets (3)	1 (33.3)	2 (66.6)
Streams (56)	8 (14.3)	48 (85.7)
Pipe-borne (33)	3 (9.1)	30 (90.1)
TOTAL (382)	**68 (17.8)**	**314 (82.2)**
p>0.05	**X^2 = 0.4675, df= 4, r = 0.996**	

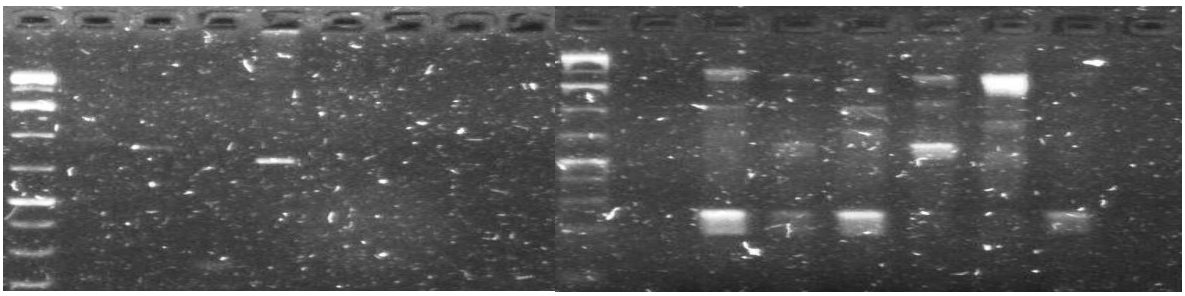
n = the total number of *E. coli* recovered from each water source.

Table 3. Detection of pathogenic, antibiotic resistant, and stress response genes with polymerase chain reaction (PCR) technique.

Lane	Isolates	Sources	Genes Detection						
			***rfb* O157**	***fliC* H7**	***Stx*1**	***Stx*2**	***EaeA***	***hlyA***	***Bla*$_{TEM}$**
1	S71	Well	-	-	-	+	-	+	+
2	S177	Stream	-	-	-	+	-	+	+
3	S31	Boreholes	-	-	-	+	-	+	+
4	S70	Well	+	-	+	+	-	+	+
5	S89	Well	-	-	-	+	-	+	+
6	S152	Well	-	-	-	+	-	+	+
7	S202	Pipe-borne	-	-	-	+	-	+	+
8	S34	Pipe-borne	-	-	-	+	-	+	+
9	S33	Borehole	-	-	-	+	-	+	+
10	S3B	Stream	+	+	+	+	-	+	+
11	S2	Well	+	+	-	+	-	+	+
12	S35	Well	+	+	+	+	-	+	+
13	S82R	Stream	+	+	+	+	-	+	+
14	S30	Well	+	+	+	+	-	+	+
15	S10	Well	+	-	-	-	-	+	+

Plate 1. Gel electrophoresis for *rfb* O157.

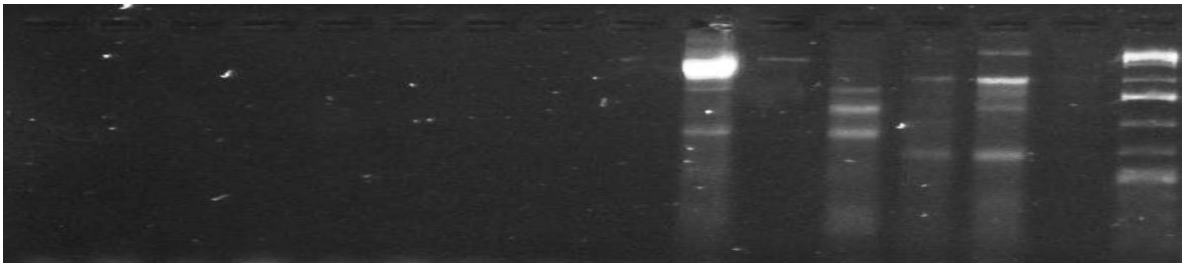
M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15



Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker)
Lane 9 (S33), Lane 10 (S3B), Lane 11 (S2), Lane 12 (S35), Lane 13 (S82R), Lane 14 (S30) and Lane 15 (S10) were positive for *rfb* O157 gene.

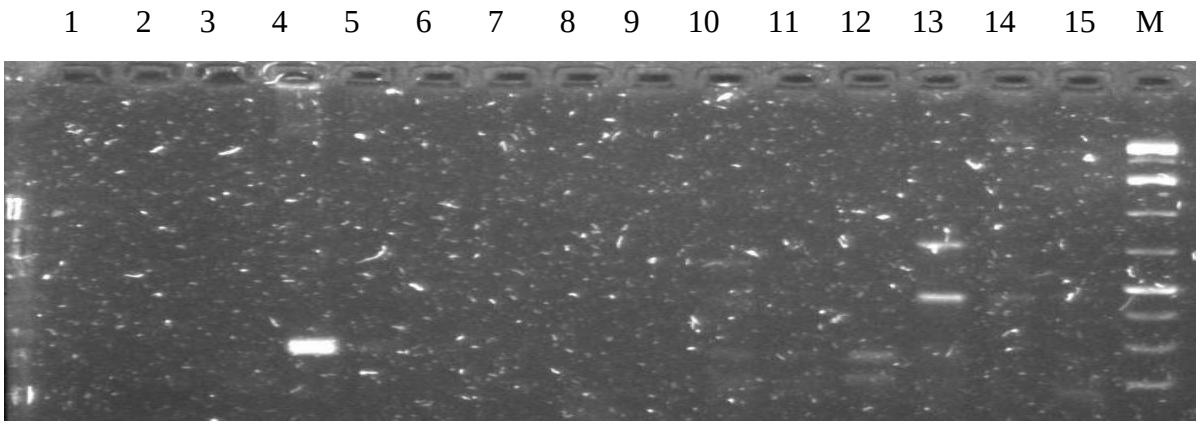
Plate 2. Gel electrophoresis for *flic* H7**.**

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 M



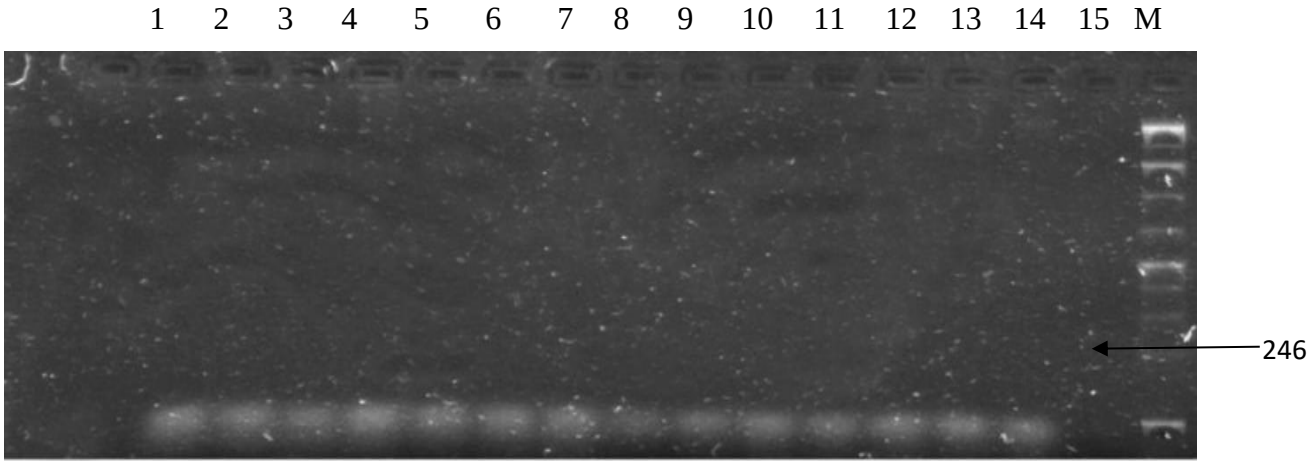
Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker)
Lane 10 (S3B), Lane 11 (S2), lane 12 (S35), Lane 13 (S82R) and lane 14 (S30) were positive for *flic* H7 gene.

Plate 3. Gel electrophoresis for *stx*1**.**



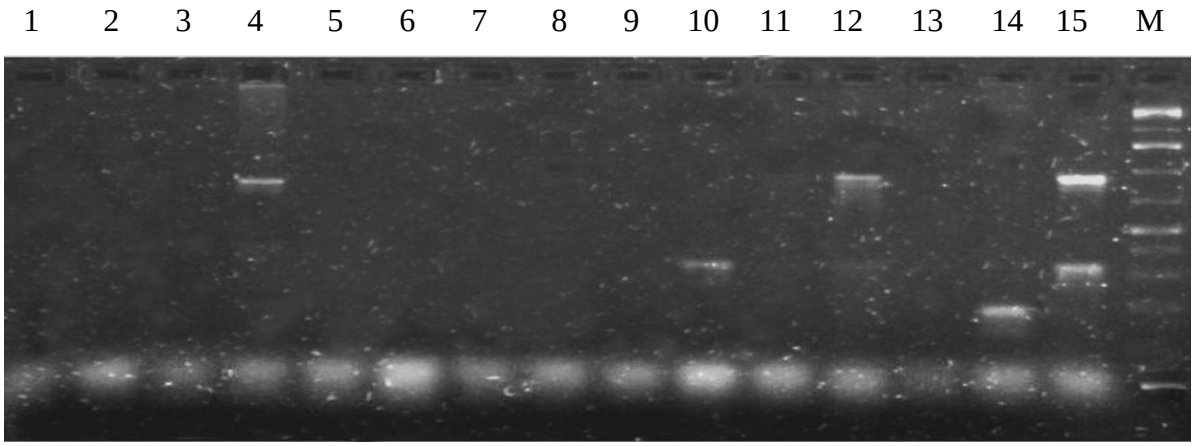
Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker)
Lane 4 – (S70), Lane 10-(S3B), Lane 12 (S35), Lane 13 (S82R), Lane 14 (S30) and Lane 15 (S10) were positive for *stx*1 gene.

Plate 4. Gel electrophoresis for *stx*2 gene.



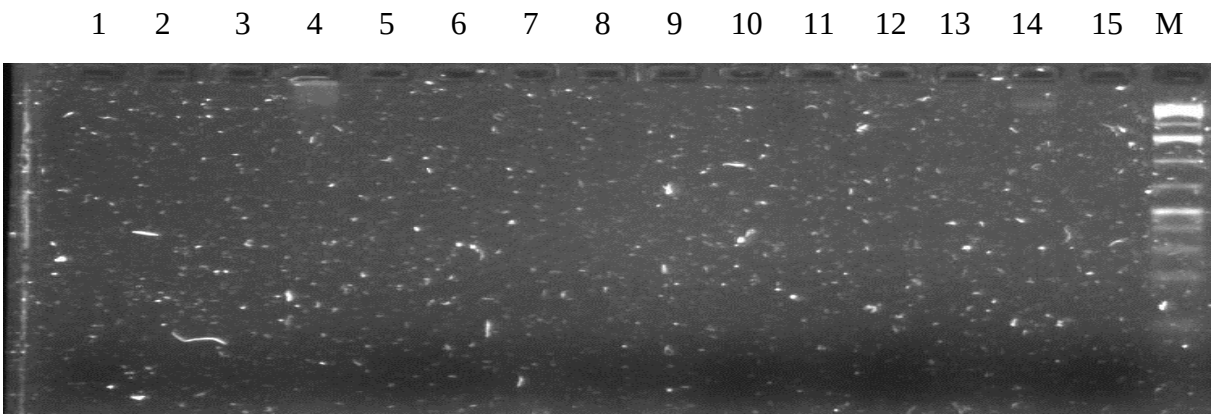
Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker) Lane 1 (S71), Lane 2 ((S177), Lane 3 (S31), Lane 4 (S70), Lane 5 (S89), Lane 6 (S152), Lane 7 (S202) from pipe-borne water sample), Lane 8 (S34), Lane 9 (S33), Lane 10(S3B), Lane 11 (S2), Lane 12 (S35), Lane 13 (S82R) and Lane 14 (S30) were positive for *Stx*2 gene.

Plate 5. Gel electrophoresis for *hlyA* gene.



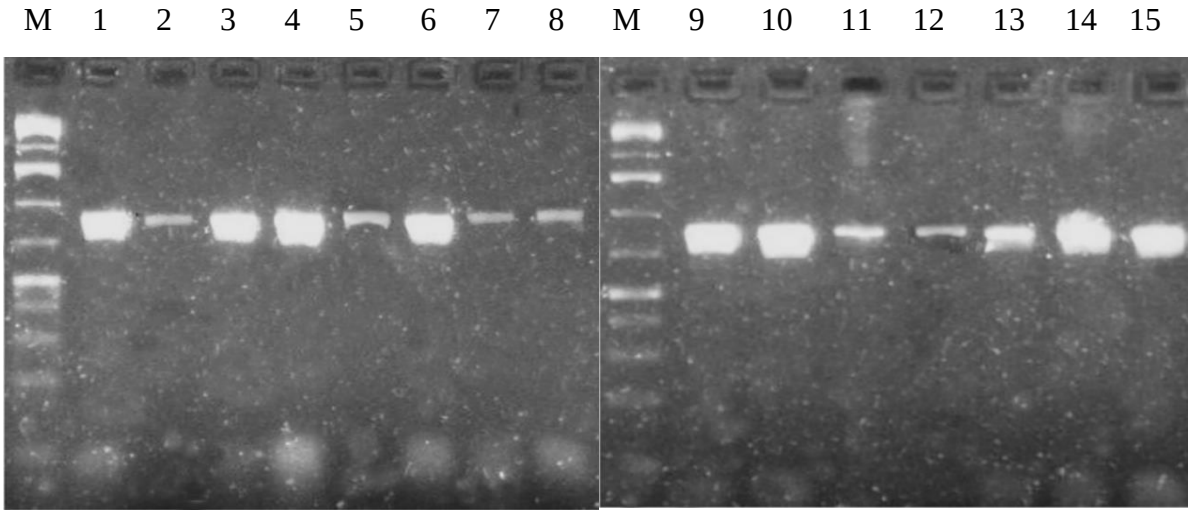
Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker). All isolates in all the lanes were positive for *hlyA* gene.

Plate 6. Gel electrophoresis for *eae* gene.



Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker). Isolates in Lane 1-15 were negative for *eae* gene

Plate 7. Gel electrophoresis for *Bla$_{TEM}$* gene.



Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker). Isolates in Lane 1-15 were positive for *Bla$_{TEM}$* gene.

Discussion

The serological identification of *E. coli* O157 which was carried out by latex agglutination assay for O157 antigens have greatly facilitated the screening of STEC O157. This latex kit is accurate and easy to use in clinical laboratory setting. This method was used to eliminate other serotypes of pathogenic *E. coli* which have the cultural and biochemical characteristics [42]. The recovery of *E. coli* O157 from the drinking water sources was contrary to the findings by **Hamieh et al.** [43]. They did not recover any *E. coli* O157 from the water samples in Lebanon using the latex agglutination kit. The serologically identified isolates were considered presumptive *E. coli* O157 and were further confirmed using PCR technique. The PCR is a rapid technique with high specificity and sensitivity [44]. The applications of singleplex and multiplex PCR for detection of STEC have been reported [45]. The detection of *rfb*O157 and *flic*H7 genes in the selected *E. coli* isolated from the water samples are characteristics of STEC O157: H7 and this has been associated with water related outbreaks. This study corroborates with the study by **Hamner et al.** [46] and **Thenmozhi** [47], who documented that *E. coli* O157: H7 was isolated and identified from drinking water sources. STEC O157:H7 is mainly transmitted to humans by the consumption of contaminated food and water. From among the identified *E. coli* O157 in this study, two were found to be negative for *flic*H7 and thus were non-motile type of the pathotype [10]. In a similar study, **Osek and Gallein** [48] also reported that H7 negative strains have been identified and they represent a distinct clone within *E. coli* O157 serogroup. However, *E. coli* O157: H- shares several

virulence characteristics with other STEC of the O157: H7 serotype.

In this study, majority of the isolates possessed either stx1 or stx2 singly while few possessed both. It has been shown that human isolates of *E. coli* O157: H7 that have stx1 and stx2 and those with only stx2 are very common but isolates having only stx1 are uncommon [49].

According to **Brooks et al.** [50], human illnesses have been aggravated due to combinations of virulence factors in genes. It was found that all the isolates were negative for *eaeA* gene but positive for *hlyA*. This finding is similar to the study by **Sajida** [51] who also recorded that all the isolates in his study were negative for *eaeA* gene but possessed the *hlyA* gene. Although *eaeA* carrying STEC strains are frequently associated with severe infection, outbreaks of HUS by *eaeA* negative STEC strains have been reported by **Paton and Paton** [52]. All the isolates screened for the β-lactam (Bla_{TEM}) resistance gene were positive. This finding shows the distribution of antibiotic resistance genes which can possibly be carried on *E. coli* plasmid. Resistance genes are often associated with integrons or mobile DNA elements such as plasmids and transposons that facilitate the spread of resistance genes [53]. More often, there is a linkage between many of these resistance genes on mobile elements and the distribution of antibiotic resistant bacteria in the environment [54]. Reports have indicated that the resistance genes currently present in human or animal associated microbiota are found in environment without antibiotic pollution [55,56]. This strongly supports the fact that resistant genes can persist and spread in the environment via horizontal gene transfer, thus increasing the chances of pathogens acquiring resistance.

The presence of *rpoS* gene in majority of the isolates screened suggested their ability to withstand environmental stress. This is in concordance with the study by **Parry-Hanson et al.** [43] who reported rpoS gene expression in acid-adapted *E. coli* O157:H7 during lactoperoxidase and lactic acid challenge in tryptone soy broth. *RpoS* is the primary sigma factor for global regulation of genes associated with environmental stresses [57]. Bacterial cells exposed to a large variety of harmful agents respond by increasing the cellular concentration of a stress sigma factor which replaces the house keeping sigma factor on the RNA polymerase enzyme, thereby changing its regulatory properties [57]. Since these isolates with virulence and resistance genes are recovered from water environment, further studies can be carried out to know the effect of different water treatments on these genes in the isolates.

Conclusion

The molecular studies carried out on selected environmental isolates (*E. coli* O157 and non-O157) revealed the presence of one or more virulence and antibiotic resistance genes. This therefore signifies that water sources contaminated with substances laden with these organisms is of high health risk to the consumer. This is because the virulence and resistance genes have more significant relation to human diseases. Also, the long survival of *E. coli* O157 in the environment and its low infectious dose are contributing factors to occurrences of water and food borne diseases which occurs as a result of consumption of water contaminated with these organisms. Moreover, these genes could be transferred horizontally in the environment to non-pathogenic bacteria leading to growing problem of clinical therapy.

Acknowledgements

The Management of Science Laboratory Technology Department, Federal Polytechnic, Ado-Ekiti, Nigeria is appreciated for the permission granted to carry out this work in their research laboratory.

Funding: None declared

Competing interests: There are no competing interests to declare.

References

1-**Sanchez S, Sanchez DS, Martinez R, Lorente MT, Herrera-Leon S, Vidal D.** The new allelic variant of the subtilase cytotoxin (sub AB2) is common among Shiga toxin producing *Escherichia coli* strains from large animals and their meat and meat products. Veterinary Microbiology 2013; 166: 645-649.

2-**Rompre A, Servais P, Baudart J, deRoubin M, Laurent P.** Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. Journal of Microbiology Methods 2002; 49: 31-54.

3-**Juhna T, Birzniece D, Larsson S, Zulenkovs D, Sharipo A, Azevedo NF, et al.** Detection of

Escherichia coli in biofilms from pipe samples and coupons in drinking water distribution networks. Applied Environmental Microbiology 2007; 73: 7456-7464.

4-**Food Safety Authority of Ireland.** *The consumption of bottled water containing certain bacteria or group of bacteria and the implications for public health* 2009. Report of the Scientific Committee of Food Safety Authority of Ireland.

5-**Mull B, Hill VR.** Recovery and detection of *E. coli* O157: H7 in surface water, using ultrafiltration and real time PCR. Applied and Environmental Microbiology 2009; 75 (11): 3593-3597.

6-**Jokinen CC, Schreier H, Mauro W, Taboada E, Isaac-Renton J L, Topp E. *et al.*** The occurrence and sources of *Campylobacter* spp., *Salmonella enterica*, and *Escherichia coli* O157:H7 in the Salmon River, British Columbia. Canadian Journal of Water Health 2010; 8: 374-386.

7-**Mezule L, Larsson S, Juhna T.** Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks. Drinking Water Engineering and Science 2013; 6: 25-31.

8-**Ajayi AO, Oluyege AO, Olowe OA, Famurewa O.** Antibiotic resistance among commensal *Escherichia coli* isolated from faeces of cattle in Ado-Ekiti, Nigeria. Journal of Animal and Veterinary Advances 2011; 10(2): 174–179.

9-**Eriksson E.** Verotoxinogenic *Escherichia coli* O157:H7 in Swedish cattle and pigs. Doctoral Thesis, 2010. Swedish University of Agricultural Science, Uppsala.

10-**Khan AB, Naim A.** Virulence traits of Shiga toxin producing *Escherichia coli*. The Health 2011; 2(4): 19-129.

11-**Azevedo M, Sousa A, Sousa JM, Thompson JA, Proenca JT, Gordo I.** Trade-off of *E. coli* adaptation to an intracellular lifestyle in macrophages. PLos ONE 2016; 11 (1): e0146123

12-**Ronsengren LB, Waldner, CL, Reid SRJ.** Association between antimicrobial resistance phenotypes, antimicrobial resistance genes and virulence genes of faecal E. coli isolates of healthy grown finish pigs. Applied Environmental Microbiology 2009; 75: 1373-1380.

13-**Glyes CL.** Shiga toxin-producing *Escherichia coli*: An overview. Journal of Animal Science 2007; M85: E45-62.

14-**Mody R, O'Reilly CE.** Infectious diseases related to travel. Centre for Disease Control and Prevention 2016; www.cdc.gov/e.coli

15-**Masoumeh N, Abdollah K, Mohammed R, Fatemeh F, Raheleh R A, Mohammed AM, et al.** Study on prevalence of verotoxigenic *E. coli* isolated from urinary tract infections in an Iranian children hospital. Open Microbiology Journal 2012; 6: 1-4.

16-**Law D.** Virulence factors of Escherichia coli O157 and other Shiga toxin-producing *E. coli.* J Appl Microbiol 2000; 88:729–745

17-**Maldonado Y, Fiser JC, Nakatsu CH, Bhunia AK.** Cytotoxicity Potential and Genotypic Characterization of *Escherichia coli* isolates from Environmental and Food Sources. Appl Environ Microbiol 2005*;* 71(4): 1890-1898.

18-**Selim SA, Ahmed SF, Aziz MHA, Zakaria AM, Klena JD, Pangallo D.** Prevalence and characterization of Shiga-toxin O157:H7 and Non-O157:H7 Enterohemorrhagic *Escherichia coli* isolated from different sources. Biotechnology and Biotechnological Equipment 2015; 27(3): 3834-3842.

19-**Saxena KL, Sewak R.** Livestock waste and its impact on human health. International Journal of Agricultural Sciences 2016; 6(7): 1084-1099.

20-**Harry HL.** Measuring *E. coli* gene expression during human urinary tract infections. MDPI Pathogens 2016; 5(7): 1-9.

21-**World Health Organization (WHO).** Guidelines for drinking water quality. Health criteria and other supporting information. International Prog. Chem. Safety 1996; 2: 973.

22-**Atif AP, Bushra BP, Vikram M.** High prevalence of multiple-drug resistant *Escherichia coli* in drinking water samples from Hyberabad. Gomal Journal of Medical Sources 2010; 8(1): 23-26.

23-**Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LBB, et al.** Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A National Reconnaissance. Environmental Sciences and Technology 2002; 36: 1202-1211.

24-**Calamari D, Zuccato E, Castiglioni S, Bagnati R, Fanelli R.** Strategic survey of therapeutic drugs in the rivers Po and Lambro in Northern Italy. Environmental Science and Technology 2003; 37: 1241-1248.

25-**Costanzo SD, Murby J, Bates J.** Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Bulletin 2005; 51: 218-223.

26-**Batt AL, Bruce IB, Aga DS**. Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environmental Pollution 2006; 142: 295-302.

27-**Manaina CM, Vaz-Moreira I, Nunes OC.** *Antibiotic resistance in waste water and surface water and human health implications.* The Handbook of Environmental Chemistry 2012; 173-212.

28-**Singh DV.** Emergence and dissemination of antibiotic resistance: A global problem. Indian Journal of Microbiology 2012; 30(4): 384-390.

29-**Turnidge J.** Antibiotic use in animals' prejudices, perceptions and realities, Journal of Antimicrobial Chemotherapy 2004; 53: 26-27.

30-**Sayah RS, Kaneene JB, Johnson Y, Miller R.** Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic and wild animal faecal samples, human septage, and surface water. Applied Environmental Microbiology 2005; 71: 1394 - 1404.

31-**Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM)**. The National Veterinary Institute (SVA) 2006, Uppsala, Sweden. pp. 23-24.

32-**Sandra G, Fiona B, Paul H, Dearbnaile V.** Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital and secondary treatment facility sources. American Society for Microbiology 2010; 76(4): 4772- 4779.

33-**Bodoczi FA.** Antimicrobial Susceptibility of *E. coli* isolated from Aries River (Romanian). Tom 2011; 18(1): 34-38.

34-**Kawane RS.** Studies on antibiotics and heavy metal resistance profiling of *Escherichia coli* from drinking water and clinical specimens, Bioscience Discovery 2012; 3(3): 292-295.

35-**Bahiru AA, Emire SA, Ayele AK.** The prevalence of antibiotic resistant *E. coli* isolates from faecal and water sources, Academia Journal of Microbiology Research 2013; 1(1):1-10.

36-**Sharma VK, Yu X, McDonald TJ, Jinadatha C, Dionysiou DD, Feng M.** Elimination of

antibiotic resistance genes and control of horizontal transfer risk by UV-based treatment of drinking water: A mini review. Frontiers of Environmental Science & Engineering 2019; 13:37

37-**Khudor MH, Issa AH, Jassim FL.** Detection of *rfbO157* and *fliCH7* Genes in *Escherichia coli* isolated from human and sheep in Basrah Province. Raf. Journal of Science 2012; 23(1): 19-33.

38-**Akinyemi AA, Oyelakin OO.** Molecular characterization of bacteria isolates from farm-raised catfish *Clarias gariepinus* (Burchell, 1822). British Microbiology Research Journal 2014; 4(12): 1345-1352.

39-**Osek.** Real-Time Application Development with OSEK. A review of the OSEK Standard by Peter, H. Feiler. Technical Note CMU/SEI-2003-TN-004. Copyright 2003 by Carnegie Mellon University. Pp 1 – 48.

40-**Beutin L, Miko A, Krause G, Pries K, Haby S, Steege K, et al.** Identification of human-pathogenic strains of shiga toxin-producing *Escherichia coli* from food by a combination of serotyping and molecular typing of shiga toxin genes. Appl Environ Microbiology 2010; 73(15): 4769 – 4775.

41-**Parry-Hansona AA, Joosteb P, Buysa EM.** Relative gene expression in acid-adapted *Escherichia coli* O157:H7 during lactoperoxidase and lactic acid challenge in tryptone soy broth. Microbiological Research 2010; 165: 546-556.

42-**Growther L, Andrew NS.** Diagnostic Methods for Shiga Toxin-Producing *E. coli.* Indian Journal of Fundamental and Applied Life Science 2011; 1(3): 337-342.

43-**Hamieh A, Olama Z, Khawaja G, Holail H.** Bacterial diversity and biofilm formation in drinking water distribution system in Lebanon. International Journal of Current Microbiology and Applied Science 2015; 4(5): 976-990.

44-**Agarwal M, Tomar SR, Jyoti, A.** Detection of waterborne pathogenic bacteria: where molecular methods rule. International Journal of Multidisciplinary and Current Research 2014; 2: 351-358.

45-**Lothigius A, Janzon A, Begum Y, Sjoling A, Qadri F, Svennerholm AM, et al.** Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area by real-time PCR. Journal of Applied Microbiology 2008; 104(4): 1121 – 1136.

46-**Hamner S, Broadaway SC, Mishra VB, Tripathi A, Mishra RK, Pulcini E, et al.** Isolation of potentially pathogenic *Escherichia coli* O157:H7 from the Ganges River. Applied Environmental Microbiology 2007; 73: 2369-2372.

47-**Thenmozhi M.** Isolation of potentially pathogenic *Escherichia coli* O157: H7 from drinking water sources. International Journal of Pharmaceutical and Bio-science 2010; 1(4): 84-86.

48-**Osek J, Gallein P.** Molecular analysis of *Escherichia coli* O157 strains isolated from cattle and pigs by the use of PCR and pulsed-field gel electrophoresis methods. Veterinary Med-Czech 2002; 47: 149– 158

49-**Wickham ME, Lupp C, Mascarenhas M, Vazquez A, Coombes BK, Brown NF, et al.** Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. Journal of Infectious Diseases 2006; 194(6): 819-27.

50-**Brooks JT, Sowers EG, Wells JG, Greene K D, Griffin PM, et al.** Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. Journal of Infectious Emerging Diseases 2005; 192 (8): 1422-1429.

51-**Sajida BP.** Detection and survival of *Escherichia coli* O157:H7 in cattle water troughs and the effects of cetylpyridinium chloride against *Escherichia coli* O157:H7 biofilms on the surface of stainless steel. Ph. D. Dissertation 2006, Louisiana State University.

52-**Paton JC, Paton AW.** Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. Clinical Microbiology Review 1998; 11: 450-479.

53-**Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J.** Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology 2010; 1-9.

54-**Ibekwe AM, Ma J.** Effects of fumigants on microbial diversity and persistence of *E. coli* O15:H7 in contrasting soil microcosms. Science and Total Environment 2011; 409: 3740 – 3748.

55-**Martinez JL.** Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. FEMS Microbiology Review 2012; 33: 44–65.

56-**Nontongana N, Sibanda T, Ngwenya E, Okoh AI.** Prevalence and antibiogram profiling of *Escherichia coli* pathotypes isolated from the Kat River and the Fort Beaufort Abstraction Water. International Journal of Environmental Research and Public Health 2014; 11: 8213-8227.

57-**Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL.** Microbial Responses to Microgravity and other Low-Shear Environments. Microbiology and Molecular Biology Review 2004; 68: 345–61.