Bacteriology and Parasitology

Fashina et al., J Bacteriol Parasitol 2018, 9:4
DOI: 10.4172/2155-9597.1000340

Research Article Open Access

Prevalence and Molecular Characterization of *E. coli* O157:H7 Isolated from Water Bodies in Ile-Ife and Environs

Christina Dunah Fashina[1], **Gbolahan Ola Babalola**[1] **and Michael Omofowa Osunde**[1,2*]

[1]*Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria*

[2]*Department of Soil Science Land Resource Management, Obafemi Awolowo University, Ile-Ife, Nigeria*

[*]**Corresponding author:** Michael Omofowa Osunde, Department of Soil Science Land Resource Management, Obafemi Awolowo University, Ile-Ife, Nigeria, Tel: +2348068997476; E-mail: mikeosund2016@yahoo.com

Received date: May 26, 2018; **Accepted date:** June 18, 2018; **Published date:** June 25, 2018

Abstract

Water is one of the crucial necessities of man since it is needed for almost all of his activities. With increase in population growth, quality water needed for consumption by man is on the decline because of environmental pollution. Thus the main aim of this study was to investigate the prevalence level of *E. coli* O157:H7 in water bodies in Ile-Ife and its environs which are the sources of water for man's consumption. Four hundred and fifty-one samples were investigated from five major sources of water namely; well, stream, borehole, bottled and sachet. The water samples were screened for *E. coli* and serologically characterized for *E. coli* O157:H7. The *E. coli* O157:H7 isolates obtained were further characterized for *stx 1, stx 2* and *eaeA* genes. The result revealed that well water and stream water had a prevalence of 8.74% and 4.59% respectively. Antibiotic resistance profile of *E. coli* isolates showed that both sources harboured isolates that are resistant to three classes of antibiotics. The virulence gene distribution shows that *stx 1* and *stx 2* were present in all the *E. coli* O157:H7 investigated. In conclusion, it appears that the gastroenteritis outbreaks experienced in the study area may be due to the presence of *E. coli* O157:H7 in water samples.

Keywords: *E. coli* O157:H7; Water quality; Virulence gene; Serology; Antibiotic resistance profile; Membrane filtration

Introduction

Water resources development around the world has taken many different forms and directions since the dawn of civilization. Humans have long sought ways of capturing, storing, cleaning, and redirecting freshwater resources. With the view of reducing their vulnerability to irregular river flows and unpredictable rainfall [1]. With the tremendous increase in both human population and activities, the quality of water has been on the decline. Groundwater stored beneath Earth's surface in soil and porous rock aquifers accounts for as much as 33% of total water withdrawals worldwide [2]. In developing nations like Nigeria, the access to quality water is fast becoming a mirage due to poor infrastructural facilities, inappropriate farm practices as well as indiscriminate disposal of wastes. The release of human and animal wastes into water bodies both at the surface and underground has been reported to be the source of *E. coli* in the above listed water bodies [3]. The need for safe water which can also guarantee better life in both the rural and urban areas of the world has become a necessity.

Escherichia coli is a remarkable versatile bacterium whose presence has been established in water bodies including streams, rivers, wells and other sources of water [4,5]. Enterohaemorrhagic *Escherichia coli* O157:H7 was identified in 1982 as an important human pathogen causing haemorrhagic colitis and haemolytic uremic syndrome (HUS) and has been reported with increased frequency during the past decade as a cause of human illness [6-8]. The number of serotypes of vero-toxin-producing *E. coli* causing human disease is increasing, but *E. coli* O157:H7 continues to be the dominant cause of haemorrhagic colitis and HUS [6,9]. Findings have shown that very little information is available on *E. coli* O157:H7 in the study area as it pertains to water hence this study. The aim of this study was to investigate the prevalence of *E. coli* O157:H7 in Ile-Ife and characterize the isolates using molecular and antibiotic susceptibility testing techniques.

Study area

The study area of Ile-Ife and environs consisted of Ife Central Local Government (N 7°33'20", E 4°30'15"), Ife South Local Government (N 07°15', E 04°30'13"), Ife East Local Government (N 07°30'09", E 04°37'28") and Ife North Local Government (N 7°27'33", E 04°26'20") areas of Osun State in southwestern Nigeria. The inhabitants of these communities are traders, farmers, artisans and civil servants. The area under study is characterized by two seasons, i.e. rain and dry seasons. The temperature of the study area exists in the range of 30°C-38°C with an optimum of 35°C for the rain season while the temperature for the dry season is 25°C or less.

Materials and Methods

Sample collection

Well water collection: A total of one hundred and twenty (120) well water samples were collected from Ile-Ife and environs. The samples were from Sabo (Area Elevation: 935_t^F, Location: Latitude 07°29.470' N, Longitude 024°33.341' E), Ajebandele (Area: Elevation 853_t^F Location: Latitude 07°29.800' N, Longitude 004°30.089' E), Opa (Area: Elevation 883_t^F, Location: Latitude 07°31.350' N, Longitude 004°34.482' E) and Fajuyi (Area: Elevation 898_t^F, Location: Latitude 07°29.803' N, Longitude 004°33.807' E) areas. Water sample was collected aseptically from the wells into sterile bottles, kept on ice packs and transported to Microbiology laboratory (Obafemi Awolowo

University, Ile-Ife) for processing using membrane filtration method [10].

Sachet water collection: A total of three hundred (300) sachet water samples were purchased randomly from fifteen brands commonly sold around Ile-Ife and environs (20 samples per brand) and transported to Microbiology laboratory (O.A.U., Ife) for processing using membrane filtration method [10].

Bottled water collection: A total of forty (40) bottled water samples from four (4) different brands were purchased at random and screened for *E. coli* O157:H7. They were bought at different locations around Ile- Ife and its environs and transported to Microbiology laboratory (O.A.U., Ife) for processing using membrane filtration method [10].

Borehole water collection: Borehole water samples were collected from 63 boreholes at seven different locations randomly chosen without any specific bias. Samples were collected at the taps directly connected to the water in the bore holes into sterile bottles, kept on ice packs and transported to Microbiology laboratory (O.A.U., Ife) for processing using membrane filtration method [10].

Culturing of water samples: Twenty millilitres (20 ml) each of the samples was withdrawn aseptically (using sterile 10 ml syringes) and passed through the membrane filter apparatus (pore size 0.22 µl) at room temperature (25°C-28°C) and under tap vacuum. After filtration, the membrane filters were then removed from the filter heads aseptically using sterilized forceps and carefully placed on moistened solid medium (SMAC) in Petri dishes. The plates were then incubated at 37°C for 24 h.

Biochemical characterization: The biochemical tests carried out include: Motility, Citrate utilization, Indole production, Methylred, Voges Proskeuer, Oxidase, Catalase, Hydrogen sulphide production, Urease production and growth on TSI agar slants. The results obtained from this biochemical characterization allowed for the identification of the isolates to species level.

Serological identification of isolates: Wellcolex *E. coli* O157:H7 kit (Remel Europe Ltd 30959601 ZC61 Danford) was used to determine the serological classification of the isolates.

Antibiotic susceptibility profile of isolates: The antibiotic susceptibility testing of isolates was determined by the disc diffusion method on Müller-Hinton agar (CM0337 oxoid, England) according to Bauer et al. [11] and interpreted in line [12]. The antibiotic disks (Mast Diagnostica Germany) and their concentrations (in µg) used include Ampicillin (10 µg), cephalothin (5 µg), colistin (25 µg), gentamicin (10 µg), streptomycin (10 µg), sulphatriad (200 µg), Tetracycline (250 µg), and cotrimoxazole (250 µg). The antibiotic disks were firmly placed on sterile Mueller-Hinton agar (MHA) plates previously seeded with a 24 h old culture of the isolate (106 CFU/ml of 0.5 McFarland Standard). The plates were incubated at 37°C for 24 h and diameters of zones of inhibition were compared [12]. Multiple antibiotic resistant (MAR) isolates were defined as resistance to greater than or equal to three (≥ 3) classes of the antibiotics tested. Plates were incubated at 37°C and the diameter of zone of growth inhibition was measured to the nearest millilitre and interpreted [12].

Molecular characterization of isolates: The DNA of the isolates were extracted by suspending 5-7 pure colonies of a 24 h bacterial culture on TSA agar plate in 500 µl of polymerase chain reaction (PCR) water and in Eppendorf tube. The bacterial suspension was boiled in a water bath at 100°C for 5 min and cooled to room temperature and mixed by gentle votexing. The tube was centrifuged at 12,000 rpm for 5 min. The DNA-containing supernatant were then transferred to another set of well labelled Eppendorf tubes and stored at -20°C until when needed.

Polymerase chain reaction (PCR) amplification of the genes: The PCR amplification of the genes was carried out using the primer sets (Table 1) specifically designed for the amplification of genes of interest. Amplification was carried out in a GeneAmp PCR system 9700 thermocycler (Applied Biosystem) following the traditional protocol of stepwise denaturation, followed by primer annealing step, extension step and a final holding at 4°C. The melting temperature (TM) of each primer set is specific. The PCR product was separated on a 1% agarose gel and 100 bp DNA ladder (Fermentas) was used as DNA molecular weight standard. Control positive and negative preparations were also included.

Target Gene	Primer	Sequence (5'-3')	PCR product size (bp)	Number of cycles	Denaturation	Annealing	Extension
	eaeA						
	Forward	TGTCAGAGTGATGAAGGAGGG					
Intimin 1	Backward	AACCAGTTCTTTGGCAGAGC	233	35	94°C :30 s	56°C:30 s	72°C
	EaeA						
	Forward	TTGCTTCTGTTGATAACTCGGG					
Intimin 2	Backward	GCTTGATACACCTGATGACTGTTC	334	35	94°C :30 s	57°C:30 s	72°C
	Stx1						
	Forward (a)	TCCAGAGGAAGGGCGGTTTA					
	Backward (a)	ATCAGGCAGGACACTACTCAA					
	Stx1		462	35	94°C :30 s	57°C:30 s	72°C
Shiga toxin 1	Forward (b)	GTTACGGGAAGGAATCAGGGT	228	35	94°C :30 s	57°C:30 s	72°C

	Backward (b)	AAACGCCACATAGACGAAGC					
Shiga toxin 2	*Stx2*		228	35	94°C :30 s	57°C:30 s	72°C
	Forward (a)	AAACGCCACATAGACGAAGC					
	Backward (a)	GTTACGGGAAGGAATCAGGGT					
	Stx2		286	40	94°C :30 s	61°C:30 s	72°C
	Forward (b)	TTCTGCCACGAACTGACGGT					
	Backward (b)	AGCAACAGCGACATCATCCG					
bfp gene	Forward	GGAAGTCAAATTCATGGGGGTAT	300	3	95°C : 5 min	55°C : 1 min	72°C
bfp gene	Backward	GGAATCAGACGCAGACTGGTAGT	300	3	95°C : 5 min	55°C : 1 min	72°C

Table 1: Primer sets used in the polymerase chain reaction (PCR) to detect shiga toxins (*Stx1* and *Stx2*) and Intimin genes in the *Escherichia coli* isolates, O157:H7 and the PCR protocol conditions.

Results

A total of five hundred and seventy-one (571) samples were collected and cultured on Sorbitol MacConkey agar (SMAC). The samples were from well water (120), bottled water (40), borehole water (63), sachet water (300) and stream water (48). A total of four hundred and eighty (480) water samples were SF positive on EMB and one hundred and three (103) samples were SF negative (Table 2).

Water sources	Number of Samples	SF on EMB	NSF on EMB
Well (4 areas)	120 (21.02%)	120 (25%)	57 (55.34%)
Sachet (15 brands)	300 (52.54%)	300 (62.5%)	0
Bottled (4 brands)	40 (7.01%)	5 (1.04%)	0
Borehole (7 locations)	63 (11.03%)	7 (1.46%)	0
Stream (3 location)	48 (8.4%)	48 (10%)	46 (44.66%)
Total	571	480	103

Table 2: Occurrence of SF and NSF bacterial isolates obtained from all the samples cultured from Ile-Ife and environs.

Water sources	Number and % of samples	Number of sero-positive *E. coli* O157 (%)	Number of PCR (%) confirmed *E. coli* O157:H7
Well	120 (21.02%)	57 (55.34%)	9 (8.74%)
Sachets	300 (52.54%)	0	0 (0.0%)
Bottled	40 (7.01%)	0	0 (0.0%)
Borehole	63 (11.03%)	0	0 (0.0%)
Stream	48 (8.4%)	46 (44.66%)	5 (4.59%)
Total	571	103	14

Table 3: Distribution of sero-positive *E. coli* O157: H7 Isolates from water samples obtained in Ile-Ife and environs.

SF-Sorbitol fermenter; NSF-Non-sorbitol Fermenter; EMB-Eosin Methylene Blue Fifty-seven (57% and 55.34%) non-fermenters from well water were "O157" positive while forty-six (46% and 44.66%) from stream water samples were also "O157" positive isolates. Other sources of water examined did not yield any O157 isolates. Serotyping with the "H7" anti-serum resulted in 9 sero-positive isolates from well water and 5 (1.04%) from stream water (Table 2).

Discussion

A total of 480 isolates were sorbitol fermenters while 103 isolates were non-sorbitol fermenters. The prevalence level of NSF obtained from the various water sources used in this study showed that well and stream waters had 55.34% and 44.66%, respectively. Other sources namely sachet water, bottled water and borehole water did not contain the pathogen as shown in Table 3.

Characterization with wellcolex for the different sero-groups showed that total of 103 sero-positive *E. coli* O157 investigated, 14 of the isolates were confirmed *E. coli* O157:H7 positive, indicating 8.74% prevalence for well water and 4.59% for stream water. The findings of 5.3% prevalence level in stream water but the 20% in well water in this study contrasted with that of Vieira et al. [13]. The higher level of prevalence of *E. coli* O157:H7 in well water may be due to the relative slow movement of water along the lateral plains in the soil substratum when compared with fast moving streams with even distribution. Studies on *E. coli* O157:H7 in water in southwestern Nigeria have been limited but in northern Nigeria [14], reported the presence of *E. coli* O157:H7 in river Kubanni suggesting that cattle excrement due to in situ herd watering and discharges from the abattoir were the major causes of its presence.

The antibiotic resistance pattern shows that the *E. coli* O157:H7 isolates obtained from well water were at least resistant to 2 different antibiotics and at most to 4 different types and stream water showed higher level of resistance with 4-5 different types of antibiotics resisted (Table 4). Overall, the total number of classes of antibiotics resisted were 4 (four), with well water and stream water being resistant to three classes of antibiotics (Table 5). The presence of these antibiotic resistant isolates in stream water may be due to fecal pollution from both man and animals and transformation, while well water may be

due to poor hygiene, transformation and presence of the isolate in seepage water (Table 6).

Antibiotics (µg)	Number and % of Resistant Isolates						
	Ajebandele (16)	Fajuyi (09)	Opa (13)	Sabo (18)	Stwa (15)	Stwb (16)	Stwc (15)
Cotrimoxazole (250 µg)	0	0	0	0	0	0	1 (6.7%)
Ampicillin (10 µg)	0	0	3 (23.08%)	0	15 (100%)	16 (100%)	15 (100%)
Cephalothin (5 µg)	16 (100%)	9 (100%)	13 (100%)	18 (100%)	15 (100%)	16 (100%)	15 (100%)
Colistin (25 µg)	0	0	0	0	15 (100%)	16 (100%)	15 (100%)
Streptomycin (10 µg)	2 (12.5%)	0	0	0	0	0	0
Sulphatriad (200 µg)	0	0	13 (100%)	0	0	0	0
Gentamicin (10 µg)	0	1 (11.11%)	4 (30.77%)	0	0	0	0
Tetracycline (250 µg)	4 (25%)	4 (44.44%)	0	17 (94.44%)	15 (100%)	16 (100%)	15 (100%)

Table 4: Antibiotic resistance profile of *Escherichia coli* isolates obtained from untreated well water and stream waters. *The number of isolates (in brackets).

Sample (Number tested)	Antibiotic resistant	Antibiotic classes (MDR)
Well water		
Well water (Sa 15)	CF, T	β-lact & Tet
Well water (Abd 16)	CF, S, T	β-lact, Am &Tet
Well water (Fa 09)	CF, GM, T	β-lact, Am & Tet
Well water (Op 13)	CF, AP, GM, S	β-lact, Am
Stream water		
Stream A (Stw_a 15)	CF, AP, CO, T	β-lact & Tet
Stream B (Stw_b 16)	CF, AP, CO, T	β-lact & Tet
Stream C (Stw_c 15)	CF, AP, CO, TS, T	β-lact, SP & Tet

Table 5: Multiple antibiotic resistant pattern of *E. coli* isolates from well and stream waters. Key: Stw_a-Stream A; Stw_b-Stream B; Stw_c-Stream C; Tet-Tetracyclines; β-lact-Beta lactamase; SP-Sulfonomides; ST-Sulphatriad (200 µg); TS-Cotrimoxazole (250 µg); AP-Ampicillin (10 µg); CF-Cephalothin (5 µg); CO-Colistin (25 µg); GM-Gentamycin (10 µg); S-Streptomycin (10 µg); T-Tetracycline (250 µg); Am-Aminoglycosides; MDR-Multi-Drug-Resistance.

Molecular characterization of the *E. coli* O157:H7 isolates for virulent genes indicated the presence of shiga toxin 1 (*stx 1*), shiga toxin 2 (*stx 2*) and intimin (*eaeA*) genes in both well and stream waters. *E. coli* O157:H7 can cause HUS mainly by secretion of Shiga toxins encoded by the genes *stx 1* and/or *stx 2* and their variants [9,15]. It has been proposed as reported by that *stx 2* has a lower receptor-binding affinity which enables it to stay longer in circulation and to reach the kidneys more easily than *stx 1*. Shiga toxins can be released by antibiotics that may cause bacterial lysis and liberate the free Shiga toxins in the intestinal tract [16] or enhance the expression of shiga toxin genes [17,18].

Sample	Frequency of O157:H7 Isolation	No. of Strain with *eaeA*	No. of Strain without *eaeA*	No. of Strain with *stx 1*	No. of Strain without *stx 1*	No. of Strain with *stx 2*	No. of Strain without *stx 2*
Ww	9 (64.29%)	8 (61.54%)	1	9 (64.29%)	0	9 (64.29%)	0
Stw	5(35.71%)	5(38.46%)	0	5(35.71%)	0	5(35.71%)	0
Bhw	0 (0%)	0 (0%)	0	0 (0%)	0	0 (0%)	0
Sw	0 (0%)	0 (0%)	0	0 (0%)	0	0 (0%)	0
Bw	0 (0%)	0 (0%)	0	0 (0%)	0	0 (0%)	0
Total	14 (100%)	13 (92.86%)	1 (7.14%)	14 (100%)	0 (0%)	14 (100%)	0 (100%)

Table 6: Distribution of *eaeA*, *stx 1* and *stx 2* genes among strains isolated in samples. * Bw-Bottled Water; Sw-Sachet Water; Ww-Well Water; Bhw-Borehole Water; Stw-Stream Water.

Conclusion

The recent epidemic of diarrheal incidence among the people living in the study area, especially young children which are often times attributed to cholera may as well be associated with *E. coli* O157:H7. Therefore, for the proper treatment of gastroenteritis in the study area, there is the need for correct laboratory diagnoses before medication is administered to patients. This is to avoid the development of superbug that will be resistant to antibiotic.

References

1. Gleick PH (2000) The changing water paradigm a look at twenty-first century water resources development. Water Int 25: 127-138.
2. Famiglietti JS (2014) The global groundwater crisis. Nat Clim Change 4: 945-948.
3. Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A (2013) Microbial source tracking markers for detection of faecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol Rev 38: 1-40.
4. Delgado-Gardea MC, Tamez-Guerra P, Gomez-Flores R, Zavala-Díaz de la Serna FJ, Eroza-de la Vega G, et al. (2016) Multidrug-Resistant Bacteria Isolated from Surface Water in Bassaseachic Falls National Park, Mexico. Int J Environ Res Public Health 597: 1-15.
5. Umaru GA, Adamu Z, Ishaya D, Abubakar YU, Hussaini A, et al. (2015) Prevalence and antimicrobial resistance pattern of Escherichia coli in drinking waters in Jalingo Metropolis, Taraba State, North-Eastern Nigeria. Microbiol Res Int 3: 8-13.
6. Thenmozhi M (2010) Isolation of Potentially Pathogenic Escherichia coli O157:H7 from the Water Sources. Int J Pharma Bio Sci 1: 84-88.
7. Doyle MP (1991) Escherichia coli O157:H7 and its significance in foods. Int J Food Microbiol 12: 289-302.
8. Griffin PM, Tauxe RV (1991) The epidemiology of infections caused by Escherichia coli O157:H7, other enterohaemorrhagic E. coli, and the associated haemolytic uremic syndrome. Epidemiol Rev 13: 60-98.
9. Karmali MA (1989) Infection by verocytotoxin- producing Escherichia coli. Clin Microbiol Rev 2: 15-38.
10. Taylor WE, Burman NP (1964) The application of membrane filtration techniques to the bacteriological examination of water. J Appl Bact 27: 294-303.
11. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.
12. Clinical and Laboratory Standards Institute (2013) Performance Standards for Antimicrobial Disk Susceptibility Tests.
13. Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, et al. (2007) High Prevalence of Enteroinvasive Escherichia coli in a remote Region of Northern Coastal Ecuador. Am J Trop Med Hyg 76: 528-533.
14. Chigor VN, Umoh VJ, Smith SI (2010) Occurrence of Escherichia coli O157 in a River Used for Fresh Produce in Irrigation in Nigeria. Afr J of Biotechnol 9: 178-182.
15. Pierard D, Muyldermans G, Moriau L, Stevens D, Lauwers S (1998) Identification of new verocytotoxin type 2 variant B-subunit genes in human and animal Escherichia coli isolates. J Clin Microbiol 36: 3317-3322.
16. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI (2000) The risk of the haemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. N Engl J Med 342: 1930-1936.
17. Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, et al. (2000) Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. J Infect Dis 181: 664-670.
18. Read RJ, Ling H, Pannu NS, Boodhoo A, Armstrong GD, et al. (2000) A Mutant Shiga-like Toxin IIe bound to its Receptor Gb3: Structure of a Group II Shiga-like Toxin with altered Binding Specificity. Structure 8: 253-264.