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Prevalence and antimicrobial susceptibility of pathogenic *Escherichia coli* O157 in fresh produce obtained from irrigated fields

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HIGHLIGHTS

- Potential public health risks associated with irrigated vegetables in Nigeria.
- *E. coli* O157 showed marked phenotypic resistance to commonly used antibiotics.
- Genotypic characterization showed strains had antibiotic resistance genes.

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ABSTRACT

Escherichia coli O157 has been implicated in many outbreaks of gastroenteritis associated with the consumption of contaminated fresh vegetables, fruits and sprouts. In Nigeria, the use of untreated wastewater in irrigation is largely considered an inevitable option to compensate for water shortages. This study investigated the seasonal prevalence and antimicrobial susceptibility of potentially pathogenic *E. coli* O157 from fresh produce in two large vegetable producing areas in Nigeria (Kano and Plateau States). Four hundred and forty samples, comprising fresh produce (238), irrigation water (84), and soil/manure samples (118) were collected from May, 2010 to March 2011, and analyzed for the presence of potentially pathogenic *E. coli* O157. Overall, 7.3% (32/440) samples were identified as *E. coli* O157 with its highest detection from Kano State 18/230 (12.2%). *E. coli* O157 was 3 times higher in vegetables during wet season than dry season and 2.3 times higher in irrigation water in wet season than in dry season. *E. coli* O157 was tested for their susceptibility to eight commonly used antibiotics and by Polymerase Chain Reaction (PCR) for the presence of ; uid*A*, O157 and genes coding for the quinolone resistance-determining region (gyr*A*) and plasmid (pCT) coding for multidrug resistance. The confirmed isolates showed that 30/32 (93.8%) were resistant to two or more antibiotics distributed in seven different multidrug resistance patterns. Our results reflect occurrence of multidrug resistant *E. coli* O157 in these major produce regions. We recommend adequate treatment of wastewater before use to avoid possible public health hazards from consumption of these vegetables.

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1. Introduction

In 1982 in US, a hemorrhagic colitis outbreak caused by hamburger consumption resulted in *E. coli* O157 to be first recognized as an important human pathogens (Nataro and Kaper, 1998; Chai et al., 2012). It is one of the most significant foodborne pathogen affecting public health globally (Hodges and Kimball, 2005). The first reported outbreak of *E. coli* O157 infection in Africa occurred in South Africa in 1992, and was followed by outbreaks in Central African Republic in 1996 and Cameroun in 1997 (Chigor et al., 2010). *E. coli* O157 illness has been reported in Nigeria since 1994 (Chigor et al., 2010). Outbreaks associated with produce consumption have brought attention to contaminated compost manure and polluted irrigation water as potential sources of pathogens for the contamination of crops. Contaminated manure and polluted irrigation water have been reported to be probable vehicles for this pathogen (Oliveira et al., 2011).

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In Nigeria, as in many other developing countries, untreated wastewater reuse in irrigation is largely considered an inevitable option to compensate for water shortages (Sou et al., 2012). Urban vegetable farmers set up vegetable farms around surface waters and wastewater points along open channels used for the drainage of different domestic and industrial wastewaters. These water bodies are used to irrigate vegetables some of which are generally eaten raw. Health impacts of the use of contaminated waters for irrigation have been reported with close association with many food borne diseases like gastroenteritis, cholera and chemical toxicity (Sou et al., 2012). Most importantly, the occurrence of plasmid-mediated multidrug resistant *E. coli* O157 in surface waters used as sources of drinking, recreation and fresh produce irrigation has been reported (Chigor et al., 2010). Previous studies have also reported that, antibiotic resistance elements are embedded in promiscuous plasmids which facilitate their lateral transfer through manure into agro ecosystems from pathogen to pathogen (Chee-Sanford, 2009). In *Escherichia coli*, quinolone resistance has been linked mainly to mutations located in a region of *gyrA* known as the quinolone resistance-determining region (Vila et al., 1994). Fresh produce are vehicles of transmission of pathogens (*E. coli* O157) capable of causing human illness and transfer of these plasmids (Cooley et al., 2007).

This pathogen is identified by classical microbiological diagnostic procedures based on its inability to ferment sorbitol (Lee and Choi, 2006). Several methods from conventional culture methods such as MacConkey agar containing sorbitol instead of lactose (SMAC) to serological assays are used for isolation and identification of *E. coli* O157. Since the conventional methods have low sensitivity and specificity, many studies were designed based on molecular techniques such as polymerase chain reaction (PCR) that detect the presence or absence of specific genes (Bai et al., 2010).Many PCR assays have been developed using primers that target specific genes for more reliable determination of the presence *E. coli* O157.

This study was undertaken to determine the prevalence, seasonality and antimicrobial activities of potentially pathogenic *Escherichia coli* O157 obtained from irrigated vegetables, irrigation water and soil/manure in two large produce areas in Nigeria.

2. Materials and methods

2.1. Study area

Kano is one of the States in Nigeria with extensive irrigation farming. Farmers in Plateau State depend largely on rain fed agriculture, though they still practice irrigation farming. Plateau State has soil and climatic conditions that favor production of leafy vegetables such as lettuce, cabbage, spinach, carrots and other exotic crops for example grapes, chillies, broccoli etc. Sampling sites were selected after a survey of some irrigation sites in the two States. Five sites from each location were selected based on the availability of vegetables on the farms, the cooperation of the farmers, the source of irrigation water and its point source of contamination. The Global Positioning System (GPS) location shows the distance between the point sources of contamination and the vegetable fields (Table 1).

Kano State has an average rainfall of 1000 mm which lasts for between 3 and 5 months, hence farmers depend largely on irrigation. The State has more than 3 million hectares of cultivable land. Plateau State on the other hand, has an average rainfall of 1300–1500 mm which lasts for 6–8 months.

Four hundred and forty samples comprising 238 vegetable samples such as lettuce, cabbage, spinach, carrots and tomatoes and some environmental (84 irrigation water and 118 soil/manure) samples were collected and analyzed. Samples were collected for two seasons; in the wet (May–October, 2010) and dry (November–March, 2011) seasons.

Water samples were collected according to the procedure recommended by American Public Health Association (APHA, 1992) in sterile wide mouth, screw capped 250 ml bottles. Vegetables were collected in factory sterile polythene bags, while, representative soil/manure samples were collected aseptically using ethanol-sterilized spatula. All samples were packed on ice during the transportation to the laboratory and were analyzed within 6 h of collection.

2.2. Isolation and identification of E. coli O157

E. coli O157 was isolated from the samples using enrichment in *Escherichia coli* medium, streaked on sorbitol MacConkey (SMAC) agar (Oxoid) plates containing cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l) and incubated at 37 °C for 24 h (David et al., 2003). Three to four sorbitol negative colonies exhibiting typical *E. coli* O157 colony phenotype were selected, purified on freshly prepared SMAC media and stored on slants at 4 °C.

2.3. Biochemical characterization of E. coli O157

Biochemical characterization was based on standard techniques (Farmer, 1999). Isolates which gave indole positive, methyl red positive, Voges–Proskauer negative, citrate negative, oxidase negative, urease negative, did not produce sulfide and were catalase positive were selected and further subjected to more identification.

Microbact (Oxoid, UK) 12E Gram-negative bacillus (GNB) rapid identification system Microbact a miniaturized computer aided identification system for the identification of organisms belonging to the family Enterobacteriaceae with which organism identification is based on pH change and substrate utilization was used to confirm conventional biochemical characterization of isolates. *E. coli* O157 isolates characterized as above were further identified using the microbact kit and interpreted as recommended by the manufacturer. An 8 digit code was then obtained which was fed into the computer identification software which immediately gave the probable identity of the organism tested in percentage.

The Microbact software recommends a 75% cut-off point for a probable identification. All tests that gave less than 75% were not accepted as *E. coli* O157.

2.4. Determination of E. coli serogroup

Serological identification of *E. coli* O157 isolates was by using the *E. coli* O157 latex agglutination test kit (Oxoid) and slide agglutination method using specific antisera (Denka, Seiken Japan). About 3–5 discrete colonies of the bacterial isolate were suspended in a test tube

Table 1
Sampling locations and site description.

Locations	Sample sites (no tested)	Site description/land use	Geographic positioning system (GPS) location
Kano	1 (62)	Abattoir	N 1° 1.00 min; W 100° 2.11 min 100 m elevation
	2 (72)	Domestic refuse dump	N 12° 1.00 min; W 143° 4.0 min 100 m elevation
	3 (60)	Domestic sewage/waste	N 8° 0.31 min; W 100° 1.37 min 100 m elevation
	4 (13)	Industrial effluent	N 11° 0.43 min; W 100° 0.15 min 100 m elevation
	5 (22)	Control	N 8° 0.23 min; W 100° 1.14 min 100 m elevation
Plateau	1 (50)	Abattoir	N 9° 0.57 min; W 100° 2.00 min 100 m elevation
	2 (28)	Domestic refuse dump	N 8° 0.45 min; W 100° 1.37 min 100 m elevation
	3 (68)	Domestic sewage/waste	N 9° 0.14 min; W 100° 1.17 min 100 m elevation
	4 (57)	Industrial effluent	N 9° 0.50 min; W 100° 0.58 min 100 m
	5 (8)	Control	N 9° 2.6 min; W 100° 0.49 min 100 m elevation

Kano control site: Kadawa irrigation station.
Plateau control site: Rukuba.

containing physiological saline heated to 121 °C for 15 min. The heated suspension was then centrifuged at 900 g (1500 rpm) for 20 min, and the supernatant was discarded. The resulting precipitate was suspended with 0.5 ml physiological saline and was used as the antigenic suspension.

A drop of polyvalent antisera and 30 μl physiological saline were placed on a clean glass slide to serve as control. A 10 μl antigenic suspension was placed onto the serum and physiological saline on the glass slide. The reagents were mixed together by tilting the glass slide back and forth for 1 min and the agglutination pattern was observed. Strong agglutination observed within 1 min in the reaction with each serum was recorded as positive. While delayed or weak agglutination was regarded as negative. ATCC 25922 was used as control strain of *Escherichia coli.*

2.5. Detection of verotoxin (VTEC) VT1 and VT2 in E. coli O157

About nineteen representative isolates were assayed for verocytotoxin (VT1 and VT2) production by reverse passive latex agglutination (RPLA) using toxin detection kits (Oxoid). Toxin production and extraction were performed using the solid culture method. Isolated organism was inoculated onto Brain Heart infusion agar (Oxoid) slopes and incubated at 37 °C for 30 min for extraction, with occasional shaking. After extraction, the culture was centrifuged at 4000 rpm for 20 min at 4 °C. The filtrate was retained for the verocytotoxin assay. Assay method was according to the manufacturer's (Oxoid) instruction. Toxin controls in kit used provided reference as the positive patterns.

2.6. Antimicrobial susceptibility and resistant genes of the isolates

Antimicrobial susceptibility of confirmed isolates was performed by the discs diffusion method using eight antibiotics; amoxicillin/clavulanate (30 μg), sulfamethoxazole/trimethoprim (25 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), gentamicin (30 μg), tetracycline (30 μg), kanamycin (30 μg) and cephalothin (30 μg). Standard strain of *Escherichia coli* ATCC 25922 was used as quality control. Isolates were termed resistant or susceptible using the Clinical and Laboratory Standards Institute guidelines (CLSI, 2010).

2.7. Molecular characterization of Escherichia coli O157

In the present study, we further confirmed the presence of *E. coli* O157 in samples by using polymerase chain reaction (PCR) to detect; rfb_{O157} gene (encoding lipopolysaccharide O-antigen synthesis) and uid*A* (encoding beta-glucuronidase). Genes encoding antibiotic resistance were also assayed.

2.8. Template DNA preparation using extraction kit

A single colony of pure *E. coli* O157 was inoculated into 10 ml of Luria-Bertani (LB) broth medium and incubated at 37 °C overnight. One ml of the overnight culture was centrifuged for 2 min at 14, 000 × g. Pelleted bacterial cells were resuspended thoroughly in 480 μl of 50 mM EDTA, digested in lysozyme and incubated at 37 °C for 60 min before being subjected to DNA extraction using Wizard Genomic DNA Purification Kit (Promega, Madison, USA) by following the manufacturer's instructions.

2.9. DNA amplification and detection

Representative *E. coli* O157 isolated, identified and characterized in the study was further confirmed by detecting the presence of uid*A* gene encoding for beta-glucoronidase synthesis (Heijen and Medema, 2006) and rfb O157 which encodes for lipopolysaccharide O-antigen synthesis (Visetsripong et al., 2007). Isolates were also assayed for genes coding for multidrug resistance (Quinolone Resistance Determining Region QRDR and plasmid genes pCT) (Cottell et al., 2011).

The primers, PCR preparations and conditions used are presented in Table 2. Amplification was performed using thermal cycler (Bio-Rad Thermal cycler, California, USA). In all the PCR assays, a negative control (reaction tube with nuclease-free water only) was included.

Table 2
Primer sequences, PCR preparations and conditions used in molecular characterization of *E. coli* O157 in the study.

Target	Primer sequence	PCR preparation (25 μl)	PCR conditions	Reference (s)
Uid*A*	UAL-1939b 5′-ATGGAATTTCGCCGATTTTGC-3′ UAL-2105b 5′-ATTGTTTGCCTCCCTGCTGC-3′	12.5 μl Dream Taq mastermix, 8.5 μl nuclease free water, 3 μl template DNA and 0.5 μl of each primer mix.	3 min at 95 °C prior to 30 cycles of 30 s of 95 °C, Annealing temperature at 60 °C for 30 s, extension at 72 °C for 1 min and a final extension of 5 min at 72 °C.	Heijen and Medema, 2006.
$rfbO_{157}$	O157PF-8 5′-CGTGATGATGTTGAGTTG-3′ O157PR-8 5′-AGATTGGTTGGCATTACTG-3′	12.5 μl Dream Taq mastermix, 8.5 μl nuclease free water, 3 μl template DNA and 0.5 μl of each primer mix.	5 min at 94 °C prior to 35 cycles of 1 min at 94 °C, Annealing temperature at 60 °C for 90 min, extension at 72 °C for 1 min and a final extension of 10 min at 72 °C.	Visetsripong et al., 2007
QRDR of gyr*A*	gyrAF 5′-GCGCGTGAGATGACCCGCCGT-3′ gyrAR 5′-CTGGCGGTAGAAGAAGGTCAG-3′	12.5 μl Dream Taq mastermix, 8.5 μl nuclease free water, 3 μl template DNA and 0.5 μl of each primer mix.	5 min at 94 °C prior to 35 cycles of 1 min at 94 °C, Annealing temperature at 54 °C for 1 min, extension at 72 °C for 1 min and a final extension of 10 min at 72 °C.	Gomez et al., 2004.
pCT	pCT(008)F 5′-CATTGTATCTATCTTGTGGG-3′ Pct(009)R 5′-GCATTCCAGAAGATGACGTT-3′	12.5 μl Dream Taq mastermix, 8.5 μl nuclease free water, 3 μl template DNA and 0.5 μl of each primer mix.	5 min at 94 °C prior to 35 cycles of 1 min at 94 °C, Annealing temperature at 60 °C for 1 min, extension at 72 °C for 1 min and a final extension of 10 min at 72 °C.	Cottell et al., 2011.

Key: *uidA* = β-glucoronidase gene, $rfbO_{157}$ = O157 antigen gene, QRDR = Quinolone resistance determining region, pCT = Multidrug resistant plasmid.

Table 3
Distribution of potential pathogenic *Escherichia coli* O157 in samples analyzed from both study locations.

Sample type	State				Total	
	Kano		Plateau		No tested	No +ve (%)
	No tested	No +ve (%)	No tested	No +ve (%)		
Vegetables	94	18(19.1)	144	2(1.4)	238	20(8.4)
Water	57	7(13.0)	27	1(3.7)	84	8(9.5)
Soil/manure	79	3(3.8)	39	1(2.5)	118	4(3.9)
Total	230	28(12.2)	210	4(1.9)	440	32(7.3)

No (%) = Number (Percent).
+ve = Positive.

3. Statistical analysis

The resulting data were analyzed using the statistical software SPSS for windows 19.0. The association between season in the locations and the detection of *E. coli* O157 from samples was assessed by calculating Odds ratio (OR) and statistical significance was assessed by setting up 95% confidence interval.

4. Results

4.1. Demonstration of the occurrence of E. coli O157

Out of the four hundred and forty samples screened for the presence of *E. coli* O157, 32 were positive, giving a prevalence of 7.3% and the frequency of occurrence ranged from 3.9 to 9.5% for the two States (Table 3). *E. coli* O157 was most detected from vegetables samples of which 75% (15 of 20) were from leafy vegetables (66.7% from lettuce and 33.3% from cabbage respectively). The highest frequency of occurrence was from irrigation water (9.5%).

4.2. Seasonal distribution of E. coli O157 in the study

Association analysis by season revealed that *E. coli* O157 was 3 times more likely to be isolated from vegetables in the wet season than in the dry season (OR = 3.022, 95% CI; 1.0409, 8.719). It was 2.3 times more likely to be isolated in water used for irrigation in wet than in the dry season (OR = 2.308, 95% CI; 0.409, 13.302) in Kano State (Table 4). Although, the seasonal difference was not statistically significant the overall prevalence of *E. coli* O157 in this study showed a higher percent of 8.2% in the wet season and 6.5% in the dry. *E. coli* O157 was least detected from soil (3.9%).

4.3. Detection of verocytotoxins VT1 and VT2 from E. coli O157

Toxin production assay showed that of the nineteen isolates (twelve from vegetables, three from water and four from soil/manure) assayed for verocytotoxin (VT1 and VT2), four (21%) produced VT1 only, one (5.3%) produced VT2 only, while eleven (57.9%) produced both VT1 and VT2. Of the four isolates that produced VT1, two (16.7%) were isolates from vegetables, one (33.3%) from water and one (25.0%) from soil/manure samples. Only one (8.3%) produced VT2. Out of the eleven isolates that produced both VT1 and VT2, eight (66.7%) were from vegetables, two (66.7%) from water and one from soil/manure samples (Table 5).

Table 4
Seasonal distribution of potential pathogenic *E. coli* O157 in samples from the two states in the study area.

State	Dry season		Total	Wet season		Total	OR	95% CI
Sample	No +ve (%)	No (−ve)		No +ve (%)	No(−ve)			
Kano state								
Vegetable	7(12.3)	50	57	11(29.7)	26	37	3.022	1.047–8.719
Water	2(7.7)	24	26	5(16.1)	26	31	2.308	0.409–13.032
Soil/manure	1(4.0)	24	25	2(3.7)	52	54	0.923	0.080–13.032
Plateau state								
Vegetable	2(3.1)	63	65	0(0.0)	79	79		
Water	0(0.0)	8	8	1(5.3)	18	19		
Soil/manure	0(0.0)	15	15	1(4.2)	23	24		

Key: No = Number, +ve = positive, −ve = Negative.

Table 5
Detection of verocytotoxins VT1 and VT2 from *E. coli* O157 isolates.

Sample type	No of strains of *E. coli* O157	VT1 No (%)	VT2 No (%)	VT1/VT2 No (%)
Vegetables	12	2(16.7)	1(8.3)	8(66.7)
Water	3	1(33.3)	0(0.0)	2(66.7)
Soil/manure	4	1(25.0)	0(0.0)	1(25.0)
Total	19	4(21.1)	1(5.3)	11(57.9)

Key: VT1 = verocytotoxin 1, No (%) = Number (percent), VT2 = verocytotoxin 2.

Table 6
Multiple antibiotic resistant patterns of *Escherichia* coli O157 isolates obtained from vegetables and environmental samples from the two States.

No of antibiotics	Combination of antibiotics	Isolates			MARI (%)
		Vegetable	Water	Soil/manure	
1R	TE, KF	3	1	2	0.1
2R	AMC, TE; SXT, TE; KF, TE; KF, K; KF, CIP.	4	4	1	0.3
3R	SXT, CN; TE, CRO; CN, K; AMC, TE; KF, KF; TE, K.	7	1	–	0.4
4R	AMC, KF, TE, K; AMC, CN, TE, K; SXT, KF, TE, K; CN, CIP, KF, K; AMC, CN, KF, K; AMC, SXT, KF, TE.	2	4	2	0.5
5R	CN, CIP, KF, TE, K; AMC, CIP, KF, TE, K, CRO, CN, KF, TE, K, AMC, SXT, CN, KF, TE.	3	1	–	0.6
6R	CRO, CN, CIP, KF, TE, K	–	–	1	0.8
8R	AMC, SXT, CRO, CN, CIP, KF, TE, K.	–	–	1	1.0

Key: MARI = Multiple drug Resistance Index.
AMC = Amoxicillin, CRO = Ceftriaxone, CIP = Ciprofloxacin, CN = Gentamicin, K = Kanamycin, SXT = Sulphamethoxazole, TE = Tetracycline, KF = Cephalothin.

4.4. Evaluation of susceptibility of the isolates to antimicrobial agents

Antimicrobial susceptibility patterns of 32 *E. coli* O157 isolated from vegetables and environmental samples (water, soil and manure) using 8 commonly used antibiotics showed multidrug resistance. Isolates were most susceptible to the antimicrobial effects of ceftriaxone 28/32(87.5%). The highest number of isolates resistant to antimicrobial agents was observed against tetracycline 24(75%) and cephalothin 32(100%). Multidrug resistance (MDR) patterns of the 32 isolates tested for sensitivity to antibiotics showed that 30/34(93.8%) were resistant to two or more antibiotics; 9/32(28.1%) were resistant to 2 antibiotics, 8/32(25%) to three antibiotics, 7/32(21.9%) to four antibiotics, 4/32(12.5%) to 5 antibiotics, 1/32(3.1%) to 6 antibiotics and 1/32(3.1%) was resistant to all the 8 antibiotics used in the study. The remaining 2/32(6.3%) were resistant to only one antibiotic; cephalothin. Overall, higher numbers of resistant isolates were identified among the vegetables and soil/manure isolates. Three isolates from vegetables were resistant to 5 antibiotics, and the 2 obtained from soil/manure were each resistant to 6 and all 8 antibiotics tested (Table 6).

4.5. Molecular characterization of isolates by polymerase chain reaction

All 16 *E. coli* O157 isolates assayed for the Uid*A* gene (encoding β-glucuronidase synthesis) gave a 187bp DNA fragment (Fig. 1) while, 50% (6 of 12) gave 420bp (Fig. 2) for $rfbO_{157}$ genes (encoding lipopolysaccharide O-antigen synthesis) respectively. While, multidrug resistance was further confirmed with primer amplification of the fragments of the Quinolone resistance region and plasmids (pCT) 428bp and 448bp (not shown).

5. Discussion

The study revealed the presence of potential pathogenic *E. coli* O157 in two major vegetable producing regions in Nigeria with an isolation rate of 7.3%. The higher prevalence of this pathogen in vegetables from Kano State is not surprising. In Kano State, cattle rearing forms one of the major occupations in the area and these water bodies also serve for animal watering. Hence, high risk of contamination of water used for growing vegetables comes from these animals. Secondly, some of the sites from where samples were obtained also received effluents discharge from abattoir wastes and leachate from refuse dumps. This is probably why the detection rate (13.1%) from

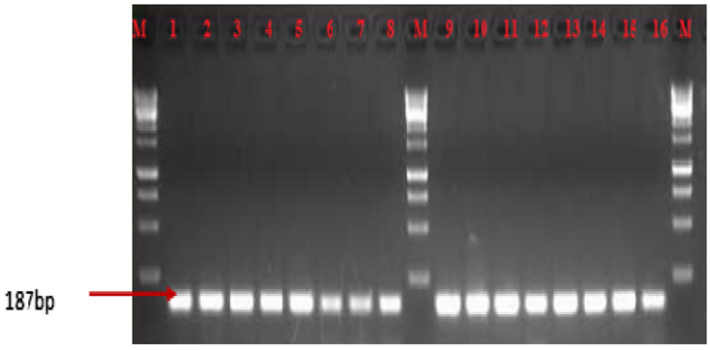

Fig. 1. Uid*A* gene in *E. coli* O157 isolates from the two study locations. M = DNA ladder, 1–8 = isolates from Kano State, 9–16 = isolates from Plateau State, Nigeria.

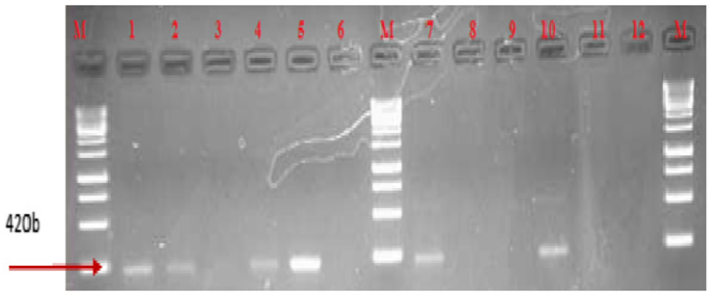

Fig. 2. O157 gene in *E. coli* O157 isolates. M = 1 kb ladder, Lane 1, 2, 4, 5 (O157) in isolates from Kano State while, 7 and 10 from Plateau State, Lane 12 = Negative control.

irrigation water in Kano State is also high. It has been well documented that cattle is the main reservoir of this pathogen (Chigor et al., 2010). Detection of this pathogen in samples from Plateau State was lower, the low detection rate could be due to less influence from cattle waste as poultry is the predominant livestock activity in the State. The detection of *E. coli* O157 in vegetables from Plateau State in the dry season may be related to total dependence on wastewater for irrigation of produce during the rain-free periods.

The finding of potentially pathogenic *E. coli* O157 in vegetables and from environmental samples in this study is of great epidemiological importance due to the growing importance of vegetables in daily consumption in Nigeria, poor public health systems, and the low infective dose of this pathogen (Chart, 2000). In most parts of the country, it is common practice to consume some of the fresh produce without proper washing. The sources of water studied are used for domestic and other purposes especially in the dry season, due to lack of potable water. The vegetables are often washed in the farm with the same irrigation water before being taken to the market for sale, hence more contamination. All these activities result in cross contamination. However, the detection from soil/manure was low and could be due to factors which had effect on its survival. It has been reported that the survival of *E. coli* O157 in soils is controlled by multiple factors (Ma et al., 2012).

The observed higher detection rate of *E. coli* O157 in the wet season than in the dry season confirms earlier reports of higher levels of this pathogen in fall conditions (Oliveira et al., 2011). Its higher prevalence in this season is not unrelated to the effect of wash off from rainfall, storm events, watering and grazing of animals and increased use of organic manure as fertilizer to improve yield. Other studies have also reported that the prevalence of *E. coli* O157 is seasonally modulated (Westphal et al., 2011). However, the detection of *E. coli* O157 in vegetables in Plateau State in the dry season may be indicative of total reliance on wastewater for irrigation of produce during the rain-free periods.

The production of verotoxin genes (VT1 and VT2) by *E. coli* O157 isolates assayed in this study represents potential risk to public health (Kruger et al., 2011). Previous studies have reported that among the toxins produced by strains of *E. coli*, verotoxins are considered to be the most virulent associated with human disease (Beutin et al., 2007). The production of VT2 toxin particularly is significant because VT2 positive VTEC strains have been found to be related to higher virulence and are significantly associated with hemolytic uremic syndrome (HUS) (Persson et al., 2007).

The detection of the housekeeping gene Uid*A* and the amplification of the O157 in *E. coli* O157 isolates in our study support reports of easier and faster methods of identification of *E. coli* O157 by PCR-based method to replace time-consuming plating and serotyping methods (Wang and Reeves, 1998). The failed PCR amplifications in some may be as a result of unidentified inhibitory residues in the DNA extract or sufficient sequence divergence at the primer-annealing sites (Wang and Reeves, 1998).

E. coli O157 showed high level of antimicrobial resistance in this study. This is of great public health significance as *E. coli* has been reported to be important in horizontal transfer of resistance among other pathogenic bacteria (Todd and Dundas, 2001). Johnson et al. (2007) stated that one suspected source of drug-resistant *E. coli* in humans is the use of antimicrobial drugs in agriculture. As humans are continuously exposed to bacteria in the environment, the accumulation of resistance genes in soil due to the spreading of manure is likely to contribute to the threat of antimicrobial resistance in the therapy of infectious diseases (Heuer et al., 2011). It is worthy of note that isolates from vegetables and environmental samples in this study did not only show resistance to most of the antibiotics used but they also possessed genes and plasmids encoding for resistance. Udo et al. (1994) also reported multiple antibiotic resistance in *E. coli* obtained from ready to eat vegetables in Calabar, Nigeria. Acquired resistance to first line antimicrobial agents may complicate the management of infections caused by *E. coli* O157. Hence, the multidrug resistance exhibited by *E. coli* O157 in this study is of major public health concern especially in Nigeria and other developing countries where access to good portable water is limited.

Bacterial plasmids are key vectors of horizontal gene transfer, mediating the mobilization of genetic material from bacteria to bacteria (Cottell et al., 2011). The detection of multidrug resistance plasmids and the QRDR in these isolates agrees with the findings of Cottell et al. (2011). This finding supports the suggestion that pCT persistence and dissemination have been driven by constant β-lactam exposure and that pCT can remain stable within a population. The ability and frequency with which antimicrobial resistance genes disseminate between bacteria in humans, the environment and in animals is still debated. The role of plasmids in the movement between ecosystems, including the food chain, is also still contested, despite mounting evidence that it occurs (Hunter et al., 2010). Interestingly, isolates in this study were obtained from vegetables, soil/manure and irrigation water. Representative isolates from these sources showed the presence of antibiotic resistance plasmids. Hence, this work supports earlier reports that pCT is disseminated broadly between bacteria in animal and human ecosystems (Cottell et al., 2011).

6. Conclusion

The study established the presence of verocytoxigenic strains of *E. coli* O157 in fresh produce in two major produce regions in Nigeria. High antimicrobial resistance was observed in the isolated organisms, indicating the role of the environment/irrigation water in emerging antibacterial resistance. The study showed possible public health hazard associated with the consumption of fresh produce affected by contaminated irrigation water from two of the major produce growing States in Nigeria. To our knowledge this is the first study that has exploited the role of wastewater irrigation in the epidemiology of *E. coli* O157 in these produce areas in the region. The need to control contamination of surface waters used for irrigation of fresh produce from these States, which are also major livestock production areas in Nigeria requires public health officials to work together with ranchers and growers to reduce or eliminate some of the contaminant sources.

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