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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.

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