Research article

Treated wastewater used in fresh produce irrigation in Nsukka, Southeast Nigeria is a reservoir of enterotoxigenic and multidrug-resistant *Escherichia coli*

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A B S T R A C T

Background: Occurrences of pathogens in environmental and irrigation waters, as well as the use of inadequately treated sewage for fresh produce constitute potential public health threats worldwide.
Objective: To investigate the treated wastewater used in fresh produce irrigation in Nsuskka, Southeastern Nigeria, as a reservoir enterotoxigenic and multidrug-resistant *Escherichia coli*.
Methods: Treated wastewater (from the sewage treatment facility at Nsukka, Southeast Nigeria), soil and irrigated vegetable samples were collected and analyzed using standard procedures. *Escherichia coli* isolated from the samples were screened for the presence of enterotoxigenic *E. coli* strain encoding lt gene and profiled for antibiotic resistance using the conventional PCR and standardized agar disk diffusion assays respectively.
Results: Of the total presumptive 103 isolates, PCR detected *uid*A gene in 87 (84 %), of which 23 (26 %) harboured the *lt* encoding ETEC gene. Generally, imipenem, cefuroxime and norfloxacin proved to be most effective of all the antibiotics employed. Wastewater isolates were variously susceptible to ciprofloxacin (95 %), norfloxacin (95 %), cefuroxime (93 %), chloramphenicol (93 %), trimethoprim and tetracycline (88 %), soil isolates to streptomycin (75 %) and vegetable isolates to cefuroxime (90 %), norfloxacin (86 %), ciprofloxacin (81 %) and chloramphenicol. Contrariwise, high resistances observed to other antibiotics were in the order; ampicillin (95 %), penicillin (93 %), erythromycin (90 %) and clarithromycin (83 %) among wastewater isolates, ciprofloxacin and norfloxacin (75 %) in soil isolates; penicillin, vancomycin and erythromycin (98 %), rifampicin and clarithromycin (93 %), sulphamethoxazole (83 %), ampicillin (81 %), tetracycline and imipenem (76 %), trimethoprim (72 %) and amoxicillin (71 %) among vegetable isolates, with multidrug resistance patterns ranging from three to seventeen.
Conclusions: Our results reveal the treated wastewater as a reservoir of enterotoxigenic *E. coli* as well as multidrug resistance that may pose a health hazard for humans and animals when released to the natural environment. Hence, there is need to develop management strategies and ensure compliance in order to prevent water-borne diarrhoea caused by ETEC and reduce the menace of antibiotic resistance in the environment.

1. Introduction

Treated wastewater serves as an alternative source to scarce quality water, thus reducing excessive application of fertilizer drastically (Martinez et al., 2013). However, poorly operated plants and grossly inadequate disinfection processes can enable various pathogenic bacteria survive or even multiply in treated wastewater effluents discharged into aquatic and soil environments (Abbas et al., 2017; Christou et al., 2017; Mukatea et al., 2018). The application of such effluents or contaminated water bodies for fresh produce irrigation constitutes potential public health risks worldwide and the potential for producing contamination using untreated wastewater for irrigation of crops increases, especially in

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the developing world (United Nations Educational, Scientific and Cultural Organization, 2003; Thebo et al., 2017). Vegetables form an indispensable constituent of human diet and the consumption of contaminated vegetables has been attributed to disease outbreaks (Lynch et al., 2009; Berger et al., 2010), with concomitant heightening in the contamination of farm yield resulting in subsequent outbursts of food-borne illnesses (Chigor et al., 2010; Allende and Monaghan, 2015).

Enterobacteriaceae constitute integral part of the normal microflora of animals and humans. *Escherichia coli,* as one of the famous member species of this family, can disperse widely through faecal materials and wastewaters in diverse environments including soil, vegetables, among others (Bain et al., 2014). Globally, *E. coli* is a significant agent of food and water-borne illnesses in humans (Duffy, 2003). Diarrhoeagenic *E. coli* (DEC) strains are essential causes of diarrhoea among children in many developing nations, and have been established as emerging enteropathogens (Okeke et al., 2003; Nweze, 2009; Bielaszewska et al., 2011). They have been grouped into six which include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Others including cell-detaching *E. coli* (CDEC) and necrotoxic *E. coli* (NTEC) have been also documented (Hamelin et al., 2007; Guion et al., 2008). Of all, ETEC is the most frequently detected enteric bacterial pathogens among infants usually below 5 years (Ahmed et al., 2005; Walk et al., 2007; Nweze, 2009). Often times, it is the first enteric infection experienced by infants in low income economies, and in endemic regions almost all children will have had one ETEC diarrhoea episode in their first year of life, and one out of every six travellers to these areas has been observed to be infected with ETEC (Centres for Disease Control, 2004; Steffen et al., 2005).

The excessive and inappropriate usage of antimicrobials in preventing or treating human and veterinary bacterial infectious diseases has led to increased antimicrobial and multidrug resistance, yet no concomitant intervention (Freire-Moran et al., 2011; Garbisu et al., 2018). The extensive use of antimicrobials in animal and human therapy, subsequent release of treated or untreated wastewater into the environment, continuous application of recycled water in agricultural practices and agricultural runoff, may pose direct consequences for indigenous microbiomes, especially in freshwater milieus (Vaz-Moreira et al., 2014; Manaia et al., 2016). Prior investigations have reported the injurious effects of antibiotics on the environment, bacterial inhabitants, biogeochemical procedures and organic pollutants' degradation (Proia et al.,

2013; Roose-Amsaleg and Laverman, 2016; Zhang et al., 2019). Antimicrobial residues present in the environment have been found attributable to hospital use and subsequent release into effluents, then treated in WWTPs (Amos et al., 2014; Ben et al., 2019). Treated effluents, alongside any antimicrobial, is discharged into the environment and may encourage development of resistant bacterial strains (Korzeniewska et al., 2013; Pan and Chu, 2018; Ben et al., 2019).

In many developing nations, both low and middle income, including Nigeria, farmers in urban and semi-urban settlements usually obtain waters for irrigating their crops via different wastewater sources, and the associated dangers in agricultural practices is heightened by the proliferation and persistence of DEC in those waters. Its spread to vegetables and subsequent incorporation into the tissues of plants that shield the pathogens from the effects of sanitizers and disinfectants have been documented (Chigor et al., 2010; Titilawo et al., 2015a). For over 3 decades, the Nsukka wastewater treatment plant (WWTP) has been reliable and consistent in providing waters for irrigation purpose mainly for cultivation of vegetables. Recently, Echiegu et al. (2016) evaluated the physicochemical statuses of effluents from the wastewater treatment plant for possible irrigation suitability but excluded the microbial quality status of the treated sewage. Therefore, in this study for the first time, final effluent, irrigated soil and vegetable samples from Nsukka WWTP were assessed for the likely detection of enterotoxigenic and multidrug-resistant *Escherichia coli*.

2. Methods

2.1. Description of study area

The sewage treatment facility in Nsukka is situated at the northwest end, about 500m away from the Junior Staff residences, of the University of Nigeria, Nsukka (Figure 1). The WWTP consists of a screen, primary settling (Imhoff) tank, sludge drying beds and two oxidation ponds (Figure 2). The treatment procedures invlove channeling domestic sewage through pipes and screens directly into the primary treatment tank and from there enters the first oxidation pond for the production of intermediate effluents. These effluents undergo further treatment by allowing them pass through the second oxidation pond to yield the final effluents utilized for irrigation purpose by some members of the community (Echiegu et al., 2016). The major crops cultivated during the dry season include the green vegetable (*Amaranthus* spp) and fluted pumpkin (*Telfaria occidentalis*). The corrugated system of furrow irrigation is

Figure 1. Aerial view of University of Nigeria, Nsukka campus and its environment.
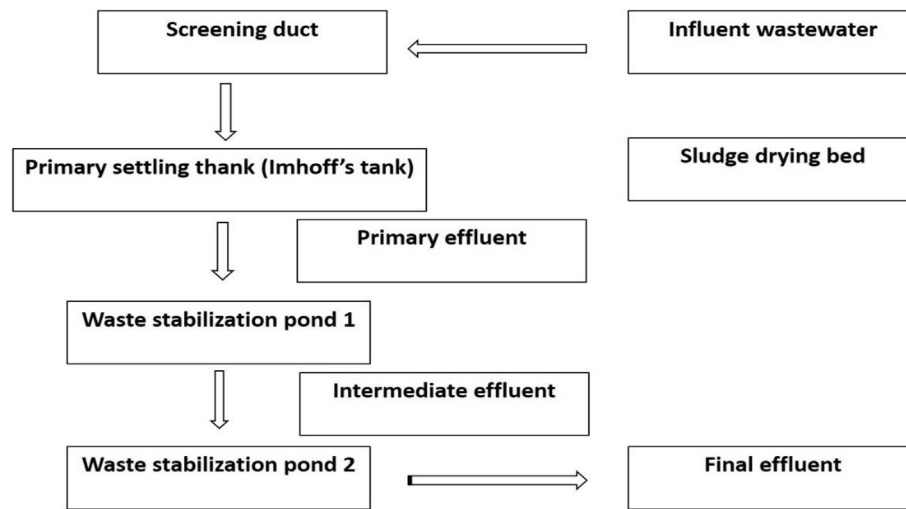
Figure 2. Schematic illustration of the Nsukka wastewater treatment plant.

widely practised whereby wastewater is transported from the treatment sites to the corrugation by gravity through diversions and by direct lifting of effluents from the source using metal and plastic buckets or pails (Echiegu et al., 2016).

2.2. Collection of wastewater effluent, soil and irrigated vegetable samples

Wastewater samples were collected using sterile wide-mouthed, screw-capped 250-ml bottles. In the same vein, composite surface soils (0–20 cm) were collected from each of the earthen pots on which the test crops were grown individually into sterile sampling bottles.

Amaranths grown in earthen pots in the Soil Science Departmental greenhouse and the vegetables were irrigated twice daily, using the sprinkler method. After six weeks of cultivation, vegetables were collected from all earthen pots into sterile beakers. All samples were conveyed on ice to the Microbiology Postgraduate laboratory of the institution and processed within 6 h of collection. All trials were performed in triplicates.

2.3. Isolation and presumptive identification of E. coli from wastewater, soil and irrigated vegetable samples

Water samples were analysed following Standard Methods (American Public Health Association, 2012). Exactly 100 ml aliquots of the water samples were filtered through a 90-mm diameter, 0.45-μm pore-sized membrane filters (Millipore, Ireland). The filters were incubated overnight at 44.5 °C on eosin methylene blue (EMB) agar (Oxoid, UK) plates. Similarly, soil samples were serially diluted (1/10) in a normal physiological saline (0.9 M NaCl) and 0.1 ml of the diluted suspension spread on to EMB agar plates using sterile glass rods. Also, 2.5 g each of vegetable samples was weighed, washed and homogenized in 45 ml of sterile distilled water using an electric blender. Aliquots of the extract were then streak-plated on EMB agar plates and incubated at 44 °C for 24 h. Characteristic metallic-sheen colonies were picked and purified on *E. coli* chromogenic agar (Conda Pronadisa, Spain) plates before preserving on 50 % glycerol for further studies.

2.4. DNA extraction and molecular identification of E. coli isolates and enterotoxigenic E. coli strain

Genomic DNA was isolated from all presumptive *E. coli* using boiling method as previously described (Chapman et al., 2006). Briefly, a single colony was inoculated onto 2 ml LB broth and incubated at 37 °C with moderate shaking (100 rpm) overnight. The culture was then centrifuged at 13,000 rpm, the supernatant was removed, and the pellet re-suspended in 200 μl of sterile distilled water, followed by heating at 100 °C for 10 min. After centrifugation, the supernatant which is the DNA was carefully transferred into a sterile 1.5 ml Eppendorf tube and stored at -20 °C for further analysis. The PCR reaction mixtures consisted of 25 μl of PCR Master Mix (Thermo Scientific, (EU) Lithuania), 0.5 μl each of oligonucleotide primers (Inqaba Biotech, Pretoria, South Africa), 10 μl of template DNA and 14 μl of nuclease free water constituting a total reaction volume of 50 μl. The PCR cycling conditions were in conformity with the protocols prescribed elsewhere (Bej et al., 1991; Lopez-Saucedo et al., 2003), with slight modifications though. The oligonucleotide primers used, target genes and expected amplification products are listed in Table 1.

2.5. Controls and product visualization

Both positive and negative controls were included in each PCR reaction. Reference strains ATCC 25922 (ATCC, USA) and DSM 10973 (DSMZ, Germany) were used for *E. coli* genus identification and enterotoxigenic *E. coli* strain detection respectively. Samples were labelled positive for a specific gene when the visible band had the same size of the positive control DNA. Exactly 5 μl aliquots of the amplicons were analyzed on a 1 % horizontal agarose gel (Merck, SA) for 45 min at 100 V in 0.5X TBE buffer and stained with ethidium bromide (Sigma-Aldrich, USA), using the gel documentation system (Alliance 4.7, France). Identification of the bands was established by comparing the band sizes with 100-bp molecular-weight size marker (Thermo Scientific, (EU) Lithuania).

Table 1. Primers used for the detection of *E. coli* and ETEC.

Genus/strain	Target gene	Oligonucleotide sequence (5′→3′)	Amplicon size (bp)	Reference
E. coli	*uid*A	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTAACAGTCTTGCG	147	Bej et al. (1991)
ETEC	*lt*	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	450	Lopez-Saucedo et al. (2003)

2.6. Antibiotic susceptibility testing of E. coli isolates

Susceptibility of *E. coli* isolates to selected antimicrobials was carried out by the standardized disk diffusion assay of Kirby-Bauer et al. (1966). Briefly, isolates grown on nutrient agar were suspended into normal saline with the aid of sterile wire loop until the turbidity equalled 0.5 MacFarland standard. Sterile non-toxic cotton swabs were used to streak the entire surface of Mueller-Hinton agar plates and antibiotic discs were applied aseptically on Muller-Hinton incubated for 18 h at 37 °C using antibiotic dispenser (Mast Diagnostics, UK). Selection of antibiotic discs was performed according to the guidelines previously recommended (Clinical and Laboratory Standards Institute, 2015). Exactly 18 different antibiotics belonging to 11 antimicrobial families together with their concentrations used are as follows: [Beta-lactams: amoxicillin (10 μg), ampicillin (5 μg), penicillin (10 U) and cloxacillin (5 μg)]; [Cephems: cefuroxime (30 μg)]; [Aminoglycosides: streptomycin (10 μg)]; [Ansamycins: rifampicin (5 μg)]; [Folate pathway inhibitors: metronidazole (50 μg), sulphamethoxazole (25 μg) and trimethoprim (5 μg)]; [Glycopeptides: vancomycin (30 μg) and erythromycin (15 μg)]; [Macrolides: clarithromycin (15 μg)]; [Phenicols: chloramphenicol (30 μg)]; [Fluoroquinolones: ciprofloxacin (5 μg) and norfloxacin (10 μg)]; [Tetracyclines: tetracycline (30 μg)]; [Carbapenems: imipenem (10 μg)] (Mast Diagnostics, UK). All plates were incubated at 35 °C for 18 h. Zones showing complete inhibitions around the discs were observed, recorded and interpreted as resistant (R), intermediate (I) and susceptible (S) accordingly (Clinical and Laboratory Standards Institute, 2015). *E. coli* ATCC 25922 (ATCC USA) was used as negative control.

2.7. Multi-antibiotic resistance phenotypes and indexing of E. coli isolates

Multi-antibiotic resistance phenotypes of *E. coli* isolates were evaluated and mapped out accordingly. The multiple antibiotic resistance index (MARI) of isolated was mathematically expressed as:

$$MAR_{index} = a/b;$$

"a" represents the number of antibiotics to which the isolates are resistant whereas "b" is the total number of antibiotics exposed (Titilawo et al., 2015a).

2.8. Data analysis

Data analysis was performed following the statistical package for the social sciences [IBM version 24]. One way ANOVA was performed to determine the variation in resistances and susceptibilities among the isolates with respect to sampling locations and a P value of 0.05 was used to declare significance.

3. Results

3.1. Presumptive identification, PCR confirmation of E. coli isolates and detection of ETEC strain

A total of 103 *E. coli* isolates, involving 55, 4 and 44 from wastewater, soil and irrigated vegetables respectively were obtained, of which 87 of them harboured the housekeeping *uid*A gene, ascertaining their identity. While 41 and 42 isolates were *uid*A positive for wastewaters and irrigated vegetables respectively, all 4 soil isolates had the gene (Table 2). Figure 3 shows the PCR products of *E. coli* confirmation by the *uid*A gene amplification. Likewise, the PCR-identified 87 *E. coli* isolates were further screened for probable detection of *lt* gene encoding ETEC, only 23 (26 %) isolates consisting of 12 (52 %) from wastewater, 1 (4 %) from soil and 10 (44 %) from irrigated vegetables harboured the gene (Table 2). Figure 4 shows the PCR products of the *lt* gene amplification.

Table 2. Presumptive identification, *PCR confirmation of E. coli and* detection of ETEC isolates.

Sample type	No. of presumptive *E. coli* isolates	No. of isolates positive for *uid*A gene	No. of isolates positive for *lt* gene
Wastewater	55	41	12
Soil	4	4	1
Irrigated vegetable	44	42	10
Total	103	87	23

3.2. Antimicrobial susceptibility profiles of E. coli isolates

All 87 *E. coli* isolates were elucidated for susceptibility to 18 antimicrobial agents with noticeable varying degrees of susceptibility towards antimicrobials tested. While all were susceptible to imipenem, only soil isolates were susceptible to cefuroxime and chloramphenicol (Figures 5 and 6). Furthermore, wastewater isolates were variously susceptible to ciprofloxacin and norfloxacin (95 %), cefuroxime and chloramphenicol (93 %), trimethoprim and tetracycline (88 %); soil isolates to streptomycin (75 %) and irrigated vegetable isolates to cefuroxime (90 %), norfloxacin (86 %), ciprofloxacin (81 %) and chloramphenicol (79 %) (Figures 5, 6, and 7).

On the contrary, all isolates from the three sources were resistant to cloxacillin and metronidazole. While all isolates from wastewater and vegetables were resistant to rifampicin and vancomycin treatments (Figures 5 and 7), all soil isolates displayed resistance to amoxicillin, ampicillin, penicillin, sulphamethoxazole, trimethoprim, erythromycin, clarithromycin and tetracycline (Figure 6). High resistances observed against other antibiotics were in the order; ampicillin (95 %), penicillin (93 %), erythromycin (90 %) and clarithromycin (83 %) among wastewater isolates; ciprofloxacin and norfloxacin (75 %) in soil isolates (Figure 4); penicillin, vancomycin and erythromycin 98 %, rifampicin and clarithromycin (93 %), sulphamethoxazole (83 %), ampicillin (81 %), tetracycline and imipenem (76 %), trimethoprim (72 %) and amoxicillin (71 %) among irrigated vegetable isolates (Figure 7).

3.3. Multi-antimicrobial resistance phenotypes of E. coli isolates

The percentage of resistant-*E. coli* isolates to five or more antimicrobials was noticed to be highest in soil (100 %), afterwards irrigated vegetables (90 %) and wastewater (83 %), with an overall MAR index of above 0.2. Table 3 depicts the resistance pattern to ≥ three antimicrobials, number of isolates and multiple antibiotic resistance indices with respect to sampling sites.

3.4. Statistical analysis

The one way ANOVA revealed that susceptibilities of cefuroxime, ciprofloxacin, norfloxacin and imipenem were significantly associated with ampicillin, penicillin, rifampicin, metronidazole, sulphamethoxazole, vancomycin, erythromycin and clarithromycin ($P < 0.05$), whereas amoxicillin, streptomycin, trimethoprim and tetracycline revealed no significant susceptibilities to other antimicrobials tested ($P >$
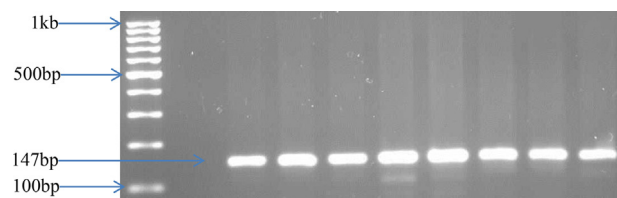
Figure 3. PCR products for *E. coli* confirmation by *uid*A gene amplification. Legend: MWM: Molecular-weight marker; NC: Negative control; PC: Positive control; Lanes 1–7: Positive isolates. See Figure S1 for full blot.

Figure 4. PCR products for ETEC strain confirmation by *lt* gene amplification. Legend: MWM: Molecular weight marker; PC: Positive control; NC: Negative control; Lanes 1–7: Positive isolates. See Figure S2 for full blot.

0.05). Likewise, it was observed that the resistances of amoxicillin, ampicillin, streptomycin, sulphamethoxazole, trimethoprim, clarithromycin, chloramphenicol, ciprofloxacin, norfloxacin and tetracycline did not significantly differ ($P > 0.05$), whereas cefuroxime and imipenem varied significantly in their resistances to other antimicrobials screened ($P < 0.05$).

4. Discussion

The occurrence of pathogens in environmental and irrigation waters has become an ongoing global concern (Anastasi et al., 2012; Titilawo et al., 2015a), and the incidence of gastroenteritis outbreaks caused by food-borne pathogens after ingestion of uncooked vegetables has greatly increased (Lynch et al., 2009; Berger et al., 2010). This study sought to

profile enterotoxigenic *E. coli* strains and multidrug *E. coli* isolates from wastewater, soil and irrigated vegetables. The *E. coli* isolates were PCR-confirmed using the housekeeping *uid*A gene. Recovery of *E. coli* in this study suggests that the samples have been subjected to faecal pollution due to poor sanitation, improper disposal of sewage, surface runoff and leakage from nearby defective sewage, contaminated ground and waste waters possibly (Igbinosa and Okoh, 2009; Odjadjare and Okoh, 2010; Titilawo et al., 2015a). The existence of *E. coli* in waters is commonly employed to signal faecal contamination and microbial water quality evaluation (World Health Organization, 2006; Sazakli et al., 2007).

Likewise, heat-labile (*lt*) toxin encoding ETEC pathogroup was variously detected among the samples. Of the 23 ETEC isolates harbouring the gene, 12 (52 %), 1 (4 %) and 10 (44 %) were from wastewater, soil and vegetables respectively (Table 2). It has been noted earlier that leakages from sewage lines and discharge of animal and human excreta discharged into open drains are responsible for the contamination sources of pathogenic *E. coli* in defective drinking water supplies (Ram et al., 2008b; Titilawo et al., 2015c). ETEC is the most important but under-recognized bacterial cause of diarrhoea or cholera-like disease in all age groups especially in areas with population pressure, poor sanitation and inadequate drinking water (Kaper et al., 2004). It induces watery diarrhoea in humans, distressing mostly infants and travelers (Turner et al., 2006). The strains, aside being extensively responsible for millions of infection cases, are significant pathogens linked to mortality from severe infantile diarrhea worldwide (Luo et al., 2014). The *lt* gene in
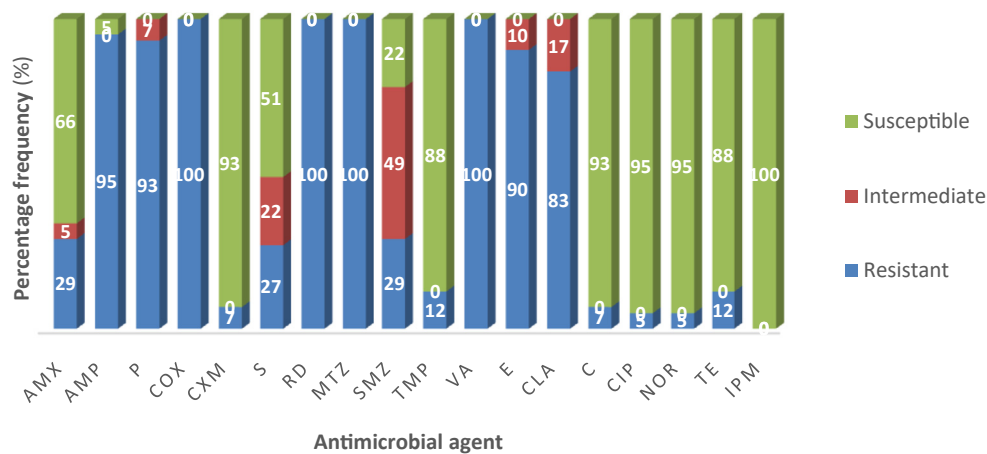

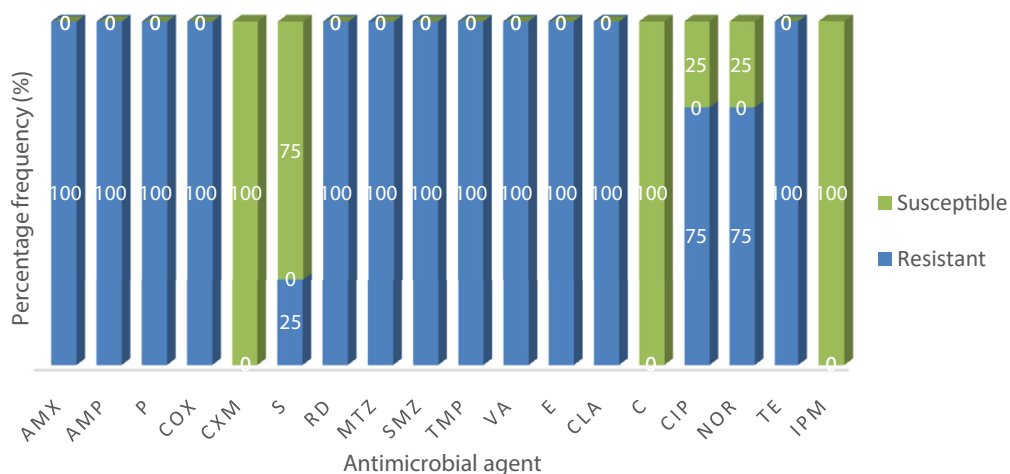**Figure 5.** Percentage frequency of antibiotic susceptibility patterns of wastewater isolates ($n = 41$).

Figure 6. Percentage frequency of antibiotic susceptibility patterns of soil isolates ($n = 4$).
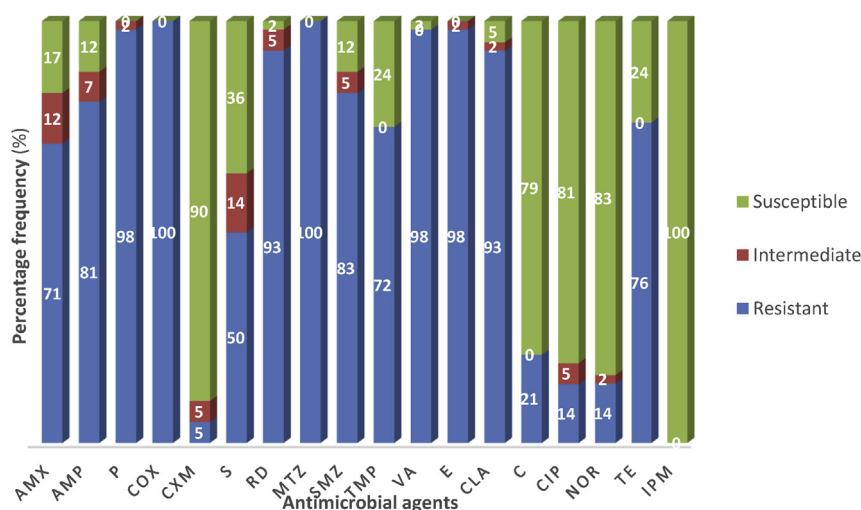
Figure 7. Percentage frequency of antibiotic susceptibility patterns of irrigated vegetable isolates ($n = 42$).

ETEC strains associated has been frequently detected among those recovered from surface waters of India and other South Asian countries polluted by human faeces (Begum et al., 2005; Ram et al., 2008c). Similarly, our findings on ETEC detection variously corroborates the findings of some previous studies on strains from sewage treatment plant's final effluents (Anastasi et al., 2012) and untreated surface waters used in potable water production (Begum et al., 2005; Ram et al., 2007; Lothigius et al., 2008; Singh et al., 2010).

Table 3. Multidrug resistance phenotypes of *E. coli* isolates from wastewater treatment plant, soils and vegetables.

No. of antimicrobials	Resistance pattern*	No. of isolates	MARI
Wastewater isolates ($n = 21$)			
4	COX, RD, MTZ, VA	5	0.3
5	P, COX, MTZ, VA, E	3	0.3
7	AMP, P, COX, RD, MTZ, VA, CLA	1	0.4
8	P, COX, RD, MTZ, SMZ, VA, E, CLA	1	0.4
10	AMX, AMP, P, COX, RD, MTZ, SMZ, VA, E, CLA	4	0.5
11	AMP, P, COX, RD, MTZ, SMZ, TMP, VA, E, CLA, TE	1	0.6
13	AMX, AMP, P, COX, S, RD, MTZ, SMZ, TMP, VA, E, CLA, TE	2	0.7
14	AMX, AMP, P, COX, RD, MTZ, SMZ, TMP, VA, E, CLA, CIP, NOR, TE	1	0.7
16	AMX, AMP, P, COX, CXM, S, RD, MTZ, SMZ, TMP, VA, E, CLA, CIP, NOR, TE	2	0.9
17	AMX, AMP, P, COX, CXM, S, RD, MTZ, SMZ, TMP, VA, C, E, CLA, CIP, NOR, TE	1	0.9
Soil isolates ($n=4$)			
12	AMX, AMP, P, COX, RD, MTZ, SMZ, TMP, VA, E, CLA, TE	2	0.7
15	AMX, AMP, P, COX, S, RD, MTZ, SMZ, TMP, VA, E, CLA, CIP, NOR, TE	2	0.8
Vegetable isolates ($n=29$)			
3	AMP, COX, MTZ	6	0.2
3	COX, MTZ, E	2	0.2
4	COX, MTZ, CIP, NOR	3	0.2
4	COX, CXM, MTZ, C	2	0.2
5	AMX, COX, CXM, MTZ, C	3	0.3
6	COX, CXM, S, RD, MTZ, TE	1	0.4
6	COX, CXM, MTZ, C, CIP, NOR	2	0.4
7	AMP, P, COX, RD, MTZ, VA, E	1	0.4
8	AMP, P, COX, S, RD, MTZ, VA, E	1	0.4
9	AMP, P, COX, MTZ, SMZ, VA, E, CLA, TE	2	0.5
10	P, COX, CXM, RD, MTZ, SMZ, VA, C, E, CLA	1	0.5
12	AMP, AMX, P, COX, RD, MTZ, SMZ, TMP, VA, E, CLA,TE	1	0.6
14	AMP, AMX, P, COX, S, RD, MTZ, SMZ, TMP, VA, C, E, CLA, TE	2	0.7
16	AMP, AMX, P, COX, S, RD, MTZ, SMZ, TMP, VA, C, E, CLA, CIP, NOR, TE	1	0.8
17	AMP, AMX, P, COX, S, RD, CXM, MTZ, SMZ, TMP, VA, C, E, CLA, CIP, NOR, TE	1	0.9

* Symbols: AMX: Amoxicillin; AMP: Ampicillin, P: Penicillin, COX Cloxacillin, CXM: Cefuroxime, S: Streptomycin, RD: Rifampicin, MTZ: Metronidazole, SMZ: Sulphamethoxazole, TMP: Trimethoprim, C: Chloramphenicol, E: Erythromycin, VA: Vancomycin, NOR: Norfloxacin, CIP: Ciprofloxacin, IPM: Imipenem, TE: Tetracycline, CLA: Clarithromycin.

The presence of pathogenic *E. coli* in environmental waters poses impending health dangers in animals and humans especially since water is used for drinking, irrigation and recreations (Koczura et al., 2012). Several studies on incidences of virulence gene properties of *E. coli* have dealt extensively with isolates of clinical and surface water origin, thereby making it difficult to compare and contrast between our findings and previous investigations, especially soils and vegetables. Frequent detections of potential *E. coli* pathotypes from clinical settings in Mexico (Estrada-Garcia et al., 2005), diarrhoeal stool isolates from Southeast Nigeria (Nweze, 2009), river water *E. coli* isolates of Osun State, Southwest Nigeria (Titilawo et al., 2015c), Kat river and Fort Beaufort abstraction water in South Africa (Nontongana et al., 2014), marine recreational water in the USA (Hamilton et al., 2010), Minjiang river in China (Chen et al., 2011), surface water in Brisbane, Australia (Sidhu et al., 2013) and Warta river in Poland (Koczura et al., 2013) have been properly documented.

The spread of antibiotic resistance phenomena has drawn increasing attention in recent years, because various infectious diseases that were once considered susceptible have steadily begun to be resistant to antimicrobial therapy, thus dashing man's hope in recovering from ailments (Titilawo et al., 2015b). In the present survey, antimicrobial resistance analysis reveals that all the strains were resistant to sulphamethoxazole and displayed a significantly high resistance to ampicillin, amoxycillin, gentamycin, cefuroxime, tetracycline and chloramphenicol (Figure 5). Our findings concur with previous reports on the high level of resistance among isolates from waters (Chigor et al., 2010; Titilawo et al., 2015a). This statement is premised on the definition of MDR that a strain can demonstrate resistance to three or more antimicrobials (Doyle et al., 2013). The occurrence of antimicrobial-resistant *E. coli* was equally noticed in some reports on animal and human faeces, wastewater treatment plants and surface waters (Mokracka et al., 2011; Sun et al., 2012).

Similarly, high resistances against β-lactams and cephalosporins were observed, though somewhat higher than those reported elsewhere (Schroeder et al., 2002; Mohanta and Goel, 2014). Recently, trimethoprim-sulfamethoxazole and quinolones have been alternatively prescribed in place of tetracycline as empirical therapy for community acquired UTIs, owing to the emergence of *E. coli* resistant strains (Karlowsky et al., 2002). Similarly, high resistances against ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin and tetracycline have been observed in *E. coli* strains from drinking waters in Jordan (Shehabi et al., 2006). The current study reveals multidrug resistance against several antimicrobials ranging from three to seventeen. Overall, the MARPs of all the *E. coli* isolates include three drugs (8), four drugs (12), five drugs (5), six drugs (3), seven drugs (2), eight drugs (2) and nine drugs (2), ten drugs (5), eleven drugs (1), twelve drugs (3), thirteen drugs (2), fourteen drugs (3), fifteen drugs (2), sixteen drugs (3) and seventeen drugs (2) resistances (Table 3). The differences in antimicrobial agent resistance patterns might have been resulted from exposure to different agents which can be used to differentiate water contamination sources (Graves et al., 2002; Guan et al., 2002; Titilawo et al., 2015a). Also, multidrug resistant *E. coli* strains have been frequently recovered from surface and ground waters in KwaZulu-Natal and North-West Provinces of South Africa respectively (Olaniran et al., 2009; Wose et al., 2010).

A MAR index of 0.2 is used to distinguish between low and high-risk contamination sources (Krumperman, 1983). The calculated MARI values obtained in this study reflect high exposure to antibiotics as they exceeded the threshold value of 0.2, suggesting contamination from high-risk origin (Table 3). MAR indices arbitrarily estimate the relative abundance of resistant *E. coli* stains in the environment. The major concern in our findings was that the isolates showed high resistance to ≥3 antibiotics with an overall MAR index >0.2. Multiple antibiotic resistance in bacteria is generally connected to plasmids containing one or more resistance determinants, each encoding a single phenotype of antibiotic resistance, and multiple bacterial resistance to drugs had earlier been reported (Daini et al., 2005; Baker et al., 2018). The

relatively high antimicrobial-resistant *E. coli* isolates in our study corresponds with reports previously documented (Rakic-Martinez et al., 2011; Puah et al., 2013). Multi-antimicrobial resistance has the tendency to breed newly emerging resistant bacteria, which may be spread to consumers, triggering infections that are very hard to cure. The observed high resistance frequency may not only result in the treatment letdown, but equally poses risk of infection with pathogens to all and sundry using the waters (Schmidt et al., 2001). Acquisition of resistance can also occur via uptake of resistance determinants through conjugation, transduction and transformation transference mechanisms of gene material (Zhu et al., 2017; Chen et al., 2018; Zhang et al., 2019). Anthropogenic-motivated selective burdens may be contributing to the distribution of antimicrobial resistant bacteria, and resistance genes in hospital settings (Titilawo et al., 2015b; Xu et al., 2015).

5. Conclusions

Antimicrobial resistance in *E. coli* is a key indicator of the emerging resistant microbes in diverse milieus. This study, the first of its kind, investigated the prevalence of ETEC strains and elucidated the multidrug resistance patterns among *E. coli* isolates from sewage treatment facility in Nsukka, Nigeria. The study concludes that the treated sewage, irrigated soils and vegetables harbour ETEC strains as detected by the conventional PCR assays, signifying that the waters are unsuitable for domestic and irrigation. Our demonstration clearly indicates that the percentage of resistance to antibiotics including cloxacillin, metronidazole, rifampicin, vancomycin, amoxicillin, ampicillin, penicillin sulfamethoxazole and clarithromycin was significantly high, suggesting wastewater treatment plant as a chief reservoir of multi-antimicrobial resistant *E. coli* strains, which could consequently pose a significant public health threat. Hence, there is need for regular monitoring of the sewage treatment plant for compliance in order to prevent water-borne diarrhoea caused by ETEC, effective intervention strategies, wide-ranging and confined antimicrobial resistance surveillance to lessen multidrug resistance in the environments.

Declarations

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e03780.

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