Genetic Characterization Among Carbapenem-resistant *Escherichia coli* Strains Using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) Fingerprinting in South Africa

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Research

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Abstract

Background Carbapenems belong to beta-lactam class of antibiotics usually considered as the last line of defense because they can be effective against severe infections caused by prevalent multidrug-resistant (MDR) pathogens. However, carbapenems can be deactivated by bacteria that produce carbapenemase (beta-lactamase). This study was conducted to screen for carbapenem-resistance genes (CRGs) harbored by pathogenic strains of *Escherichia coli* recovered from different environmental samples. We also assessed the genetic relatedness among selected *E. coli* pathotypes using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR).

Method: Molecular identification and characterization of the presumptive isolates were performed using PCR and isolates that exhibited antimicrobial resistance (AMR) phenotypically were further screened for some relevant CRGs (*bla*_{NDM-1}, *bla*_{KPC} and *bla*_{OXA-48-like}). Furthermore, ERIC-PCR was used to determine the similarity and diversity of 31 *E. coli* strains which were randomly selected from the different sources analyzed in this study.

Result Our findings revealed a total of 238 presumptive *E. coli* isolates, out of which 192 were confirmed positive for *uidA* gene. Further screening revealed 77 (40%) isolates belong to six key *E. coli* pathotypes and 70 of them exhibited phenotypic AMR. Additionally, twenty-nine (41%) of the 70 MDR pathogenic *E. coli* strains harbored CRGs; with 24 strains harboring *bla*_{NDM-1}, 8 harboring *bla*_{KPC} and 2 harboring *bla*_{OXA-48-like} genes.

Conclusion Findings also suggest that the selected *E. coli* pathotypes belonged to different genomic clusters, while the cluster analysis showed a possible genetic diversity among aquatic and farm isolates. Proper treatment of final effluents before discharge as well as the development of more effective strategies to control and manage the use of antimicrobial agents were strongly recommended.

Background

Escherichia coli consist of a variety of strains widely disseminated in different ecological units across the world ranging from natural environmental niches to the digestive tracts in animals and humans [1]. Nonetheless, *E. coli* is frequently present in the normal microbiota of the human gut thereby playing an important role in human's livelihood [2], yet it is regarded as a suitable fecal indicator for food and water contamination [3]. There are diverse strains of *E. coli* amongst which consist of highly pathogenic strains responsible for serious infections such as Urinary Tract Infections (UTI), gastroenteritis and diarrhea. The pathogenicity is determined by strains that harbor virulence genes (VGs), and among which are the main causative agents of diarrhea such as Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Enterotoxigenic *E. coli* (ETEC) which harbor VGs comprising *bfp* (bundle-forming pili), *AafI* (aggregative adherence fimbriae I) and *lt* (heat-labile enterotoxin) respectively [4, 5]. Other relevant *E. coli* pathotypes and VGs they harbor include the following: diffusely adherent *E. coli*, DAEC (VG: *daae*); neonatal meningitis-causing *E. coli*, NMEC (VG: *ibeA*); enterohaemorrhagic *E. coli*, EHEC (VG: *stx/eae*); shiga toxin-producing *E. coli*, STEC (VG: *stx1/stx2*) [6]. For many years, beta-lactam class of antibiotics remained the key therapeutic option for stern infections, and carbapenems are often considered antimicrobials of last choice [7]. Antimicrobial-resistant bacteria (ARB) such as carbapenem-resistant *Escherichia coli* (CREC) can transfer antimicrobial resistance genes (ARGs) to other bacterial species [8]. Carbapenem-resistant Enterobacteriaceae (CRE) can acquire ARGs that produce β -lactamases (carbapenemases) encoding multiple antibiotic resistance (MAR) mechanisms which can eventually bring about the hydrolysis of other antimicrobial agents, thereby limiting treatment options [9, 10]. Moreover, many infections triggered by these multiple drug resistant (MDR) bacterial strains are often linked with higher fatality rates than those of infections instigated by microorganisms susceptible to carbapenems [11, 12]. ARGs such as *bla*_{NDM-1}, *bla*_{KPC} and *bla*_{OXA-48-like} are the most common CRGs widely reported in pathogenic *E. coli* strains [13].

The dissemination of ARGs among CREC is a matter of great concern considering that hospital wastewater effluents constitute a distinct type of waste that remains extremely hazardous since they comprise a myriad of drug deposits and infectious agents and are consequently a significant source of MDR bacterial strains [14]. Wastewater treatment plants (WWTPs) are also key hotspots affiliated with the fecology of pathogens and the dissemination of emerging superbugs. These niches are considered as reservoirs of nutrients and/or abundance of elevated bacterial numbers as well as sub-toxic levels of antibiotics favorable for the survival of ARB [15, 16]. Reports have revealed the implications of the reuse of treated wastewater final effluent for irrigation purposes in farms, likewise, a rise of soil microbial biomass as a result of wastewater irrigation was observed in many studies [17–19]. Agricultural practices involving reclaimed wastewater reuse serve as an avenue for pathogens (particularly *E. coli*) to be transferred to foods (e.g. raw vegetables and fruits), and available channels for the horizontal transfer of ARGs to other harmless bacteria [20, 21].

The knowledge of microbial diversities and understanding the role of environmental pathogens as well as the ecological relationships within species are vital to public health and epidemiological studies [22]. Due to vast variations in bacterial genes, the use of easy, rapid, effective and cheap techniques, such as molecular fingerprints and molecular diagnostic tools like ERIC-PCR may be considered as straightforward protocols for the evaluation of genetic relatedness of *E. coli* [23]. Among several more recent PCR-based techniques, such as Real-Time Whole-Genome Sequencing (WGS), randomly amplified polymorphic DNA (RAPD) and Multilocus Sequencing Typing (MLST), the Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR is a very affordable and swift method that has conserved and repeated sequences which can be observed in microbes (including bacteria and some fungi) [24, 25]. Unlike the other aforementioned PCR-based techniques, ERIC-PCR has been especially useful as a typing method for multi-resistant *Enterobacteriaceae* strains and allows for a straightforward identification of genome fragments useful as genome-specific markers for dynamic monitoring of bacterial populations in complex communities (e.g. human gut microflora) [26]. In this technique,

different strains of bacteria can be differentiated based on the amplification of random and rapid dispersal of inter-genome parts. Interestingly, bacterial strains vary in the regularity of repetitive sequences, hence, this procedure yields different arrays of specific primers which are designed to be able to fasten the recurrent sequences and to amplify the distances amid those attached primers [27]. Those incomplete palindrome sequences are frequently identified inside transcribed areas linked with intergenic consensus.

Remarkably, there is significant diversity of gene copy numbers which induces the evolution processes among specific bacterial strains within a certain species such as *E. coli* [28]. Different environmental niches such as surface waters are exposed to discharges from various sources, in receipt of microbial contaminants that originate from both agricultural and domestic origins, hence, there is a need for more studies investigating CRE isolates recovered from different samples in the environment of South Africa [29]. The aim of this present study was to investigate the antimicrobial resistance profiles and pathovars distribution of *Escherichia coli* recovered from some environmental niches in three District Municipalities (DMs) within the Eastern Cape Province (ECP), South Africa as well as to determine the genetic relatedness of selected *E. coli* strains using ERIC-PCR.

Method

Sample collection

A total of 243 different environmental samples ranging from surface water (47), WWTPs final effluents (29), hospital effluents (27) to irrigation water (45), soil (60) and vegetables (35) were collected from January to September 2018. Water samples (1.5L) was collected in duplicate using sterile glass bottles and transported in an ice box to the research laboratory for bacteriological analyses within 6 hours of sampling. Soil samples (20 g), irrigation water (1L) and vegetable samples (5–10 g) comprising spinach, broccoli and cabbage were collected randomly from selected farms in the study areas.

Processing of the collected water samples

Membrane filter technique was adopted for the isolation of the enteric bacteria from the water samples [30]. Briefly, one hundred millilitres of the water samples were filtered using a sterile membrane filter papers of 0.45-µm pore size. Afterwards, the filters were aseptically picked with sterilized forceps and carefully placed on plates of *E. coli*-coliforms chromogenic medium agar (Chromogenic media, Merck), then incubated at 37 °C for 18–24 h. Subsequently, presumptive *E. coli* isolates were streaked onto nutrient agar (NA) plates and incubated at 37 °C for 24 h. Afterwards, 25% glycerol stock was prepared from the cultured nutrient broths and stored at – 80 °C for further analyses.

Processing of the collected vegetable samples

For the processing of vegetable samples, the method of Du Plessis et al. [31] was adopted. Briefly, 10 g of each vegetable samples was placed in a Stomacher Bag and then 90 mL of sterile Trypticasein Soy Broth (TSB) was introduced and macerated with Mixer machine. Thereafter, the broth was incubated for 18–24 h at 37°C in sterilized bottles. Subsequently, a loopfull of TSB was streaked on the surface of *E. coli*-coliforms chromogenic media plates and then incubated at 37°C for 24 h. Single colonies from the overnight culture were streaked on NA plates, incubated at 37°C for 24 h and stored in glycerol stock for further analysis.

Processing of soil samples

We adopted the method of Da Silva et al. [32] for biological analysis of the soil samples. Sixty agricultural soil samples were randomly collected from some farms in the study area. Briefly, a total 10 g of each soil sample was introduced into about 90 mL of sterile TSB and afterwards incubated for 18–24 h at 37°C. Thereafter, a loopfull of TSB was streaked on *E. coli*-coliforms chromogenic medium agar petri dishes and incubated at 37°C for 24 h. Purification process was carried out on NA plates and incubated at 37 °C for 24 h. Afterwards, 25% glycerol stock was prepared from cultured nutrient broths and then stored at – 80 °C for further analyses.

Bacterial DNA extraction

Extraction of *Escherichia coli* DNA was achieved using the boiling technique as previously described by Jackson et al. [33]. In brief, 4 mL of nutrient broth (NB) was prepared, presumptive isolates were resuscitated by inoculating a loopfull of each isolate into the NB. After the incubation period of about 18–24 hours at 37 °C, 1 mL of the broth cultures was centrifuged at 12,800 rpm for 5 min, the supernatants discarded and the cells were suspended in 400 µL of sterile distilled water using sterile 1.5 mL Eppendorf tubes. The suspensions were boiled at 100 °C for 10 min in a heating block, allowed to cool and after which the suspensions were centrifuged at 12,800 rpm for 5 min and 3 microliters of supernatants per reaction were used as PCR templates.

Molecular identification of *E. coli*

Molecular identification of *E. coli* was carried out by PCR technique using the primer sets (F: CGC GTA CTA TAC GCC ATG AAC GTA; R: ACC GTT GAT CAC TTC GGT CAGG) and PCR conditions listed in Table S1 with some modifications in accordance with the protocols as reported elsewhere, [34] targeting *uidA* gene which encodes beta-Glucuronidase at 147 bp for the molecular confirmation of *E. coli*. The positive control (ATCC 25922) was used. The positively confirmed *E. coli* isolates were further screened for the presence of virulence genes representing the various pathotypes using

the primer sets reported by Ebomah et al. [6] as shown in Table S1. Verification of the PCR amplified products was determined by resolving them in 1.5% agarose gel at 135 volts for 30 min, identified under a short-wavelength UV light source (Alliance 4.7).

Antimicrobial Susceptibility test of the confirmed *E. coli* pathogenic strains

All confirmed *E. coli* pathotypes were subjected to antimicrobial susceptibility test (AST) against 4 panels of carbapenem following Kirby-Bauer disk diffusion procedure according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and results were thereby interpreted according to the CLSI [35] recommendations. The class of carbapenem antimicrobial agents includes doripenem (10 µg), imipenem (10 µg), meropenem (10 µg) and ertapenem (10 µg). Briefly, about 100–200 mL of the bacterial overnight broth was transferred into 5 mL normal saline solution, which was adjusted matching 0.5 McFarland standard. Thereafter 100 µL was spread on Muller-Hinton agar (MHA, Merck) plates through the use of sterilized glass spreader, and MHA plates were impregnated with 10 µg of the aforementioned carbapenem antimicrobials discs (ThermoFisher), incubated aerobically for 24 h at 37°C. After which diameters of the zone of inhibition were measured using a ruler then interpreted according to the recommended criteria by the CLSI [35].

Molecular characterization of the relevant carbapenem resistance genes

All strains of *E. coli* pathotypes that exhibited phenotypic resistance against one or more of the 4 test carbapenems were further screened for the relevant carbapenem-resistance genes using PCR technique and PCR products were observed via agarose (Separation, SA) gel electrophoresis (AGE). The list of primers that were employed for the PCR are listed in Table S1 [36].

ERIC-PCR and DNA amplification of *E. coli* strains

From the confirmed *E. coli* strains that harbored virulence genes, 31 strains were arbitrarily selected from different sources and were subjected to PCR using the ERIC primer sets listed in Table S1. The PCR reactions were verified by resolving them in 3% agarose gel in a 1 × TBE buffer (Merck), stained with ethidium bromide at 90 volts for 240 min and viewed as previously stated [37].

Dendrogram and clustering analysis

The pattern of bands in agarose gel electrophoresis regarding ERIC-PCR products was used as the principle structure for calculation of the dendrogram. The GelJ v.2.0 is a user-friendly software with computer-assisted pattern which can be used for dendrograms via gel electrophoresis images as supported by Heras et al. [37]. The dendrogram was designed to unweighted pair group method with arithmetic mean (UPGMA) which is categorized in clustering methodologies, and is based on clustering analysis.

Reproducibility

The ERIC-PCR analysis of the result can be directly correlated within a single PCR technique. In order to determine the reproducibility of this experiment, the clustered result of the 31 *E. coli* strains was repeated twice in further ERIC-PCR procedure.

Statistical analysis

Statistical analyses were carried out, charts and dendrograms were drawn using GelJ v.2.0 software [37].

Results

Molecular Identification and pathotyping of the obtained *E. coli* isolates

A total amount of 238 presumptive *E. coli* isolates was recovered from the different sample types (243 environmental samples in total). Results showed that 192 (81%) out of the 238 isolates were positive for *uidA* gene. The confirmed *E. coli* isolates were then screened for the selected VGs by PCR and categorized into various pathotypes utilizing particular primers as shown in Supplementary Table (Table S1). Out of 192 confirmed *E. coli* isolates, a total of 77 (40%) strains belonging to six pathotypes were identified (*lbeA*/NMEC 25, 13%; *eagg*/EAEC 15, 8%; *daae*/DAEC 11, 6%; *bfp*/EPEC 6, 3%; *lt*/ETEC 6, 3%; *stx1*/STEC 14, 7%). However, *stx2* was not detected and this could result in the low detection in STEC. Table 1 summarizes the total number of confirmed *E. coli* isolates obtained from the different sample types that were selected for the purpose of this present study. Supplementary Figure (S1) is a representation of an electrophoretic image of the amplification of *uidA* gene for confirmed *E. coli* isolates and Supplementary Figure (S2) represents the agarose gel electrophoresis (AGE) of the PCR products of NMEC (neonatal meningitis-causing *E. coli*) which harbors the VG *lbeA*. Based on the diverse sources, some of the *E. coli* strains were selected and used for the ERIC-PCR analysis to determine the similarity and diversity with regards to the different sample types. Majority of these strains (8: 25.81%) harbored *stx1* (STEC) which were isolated from mainly farm soil samples, and the remaining harbored *bfp* (EPEC) isolated from river water samples, final effluent, hospital effluent and irrigation water.

Table 1

Total number of confirmed *E. coli* pathotypes obtained from different sample types in 3 District Municipalities.

S/N	Sample types	Number of confirmed <i>E. coli</i> pathotypes (cfu / ml)					
		<i>lbeA</i> /NMEC	<i>AafI</i> /EAEC	<i>daae</i> /DAEC	<i>bfp</i> /EPEC	<i>lt</i> /ETEC	<i>stx1</i> /STEC
1	Hospital effluent	5	4	1	1	3	1
2	WWTP final effluent	8	6	7	0	2	1
3	Surface water	8	2	2	4	1	4
4	Irrigation water	2	2	1	0	0	1
5	Farm soil	0	1	0	1	0	3
6	Vegetables	2	0	0	0	0	4

Antimicrobial susceptibility patterns of the confirmed *E. coli* pathotypes

All pathogenic strains confirmed by PCR techniques were subjected to four carbapenems, and meropenem had the highest percentage resistance (70%). The percentage resistance against the other antimicrobials follow the order: imipenem (64%), doripenem (56%) and ertapenem (44%), and intermediate responses were considered as resistant.

Molecular characterization of the relevant carbapenem resistance genes

Of the 77 *E. coli* pathotypes, 70 *E. coli* strains exhibited resistance against the test carbapenems and were further screened for the relevant carbapenem-resistance genes (CRGs). Result showed 29 (41%) were positive for CRGs, out of which 24 strains (34%) harbored *bla*_{NDM-1}, 8 strains (11%) harbored *bla*_{KPC} and 2 strains (3%) harbored *bla*_{OXA-48-like}. Table 2 represents the distribution of multiple CRGs harbored by the *E. coli* strains.

Table 2

Distribution of single and multiple carbapenem-resistance genes among *E. coli* strains that were screened.

Patterns of multiple resistance genes	Number of <i>E. coli</i> pathotypes							Source	Total
	<i>lbeA</i> /NMEC (%)	<i>AafI</i> /EAEC (%)	<i>daae</i> /DAEC (%)	<i>bfp</i> /EPEC (%)	<i>lt</i> /ETEC (%)	<i>stx1</i> /STEC (%)	Unidentified <i>E. coli</i> pathotypes (%)		
<i>bla</i> _{NDM-1} , <i>bla</i> _{KPC} , <i>bla</i> _{OXA-48-like}	0	0	0	0	0	0	0	Hospital effluent, WWTP	0
<i>bla</i> _{NDM-1} , <i>bla</i> _{KPC}	2	0	0	0	1	0	1	Hospital effluent, WWTP	4
<i>bla</i> _{KPC} , <i>bla</i> _{OXA-48-like}	0	0	0	0	0	0	0	Hospital effluent, WWTP	0
<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48-like}	1	0	0	0	0	0	0	Hospital effluent	1
<i>bla</i> _{NDM-1}	3	3	1	0	2	3	6	Hospital effluent, River, Irrigation water, Vegetable	18
<i>bla</i> _{KPC}	2	0	1	0	0	1	0	WWTP, Soil	4
<i>bla</i> _{OXA-48-like}	0	0	0	0	0	1	0	Vegetable	1

Prevalence of the antimicrobial resistance genes (ARGs) in the *E. coli* pathotypes

After antibiotic susceptibility testing, all the *E. coli* strains that exhibited phenotypic resistance were further screened for the presence of carbapenem-resistance genes (CRGs). Results showed the isolates recovered from WWTPs final effluent (29%) had the highest prevalence of CRGs. Supplementary Figures S3 and S4 represent the PCR products of the amplification of the relevant CRGs. Figure 1 shows the proportions of the confirmed *E. coli* strains harboring ARGs in the different environmental samples collected. Results showed only NMEC strains recovered from hospital effluent and WWTP final effluent harbored multiple CRGs.

ERIC-PCR of amplified *E. coli* strains

Analysis of the genetic diversity of the 31 collected *E. coli* strains was carried out by ERIC-PCR fingerprint method using ERIC-I and ERIC-2 primers. The fingerprints obtained from the ERIC typing of the 31 CREC strains showed a DNA banding profile consisting of amplified bands ranging from 1 to 13 having size 100 bp to 5000 bp (Fig. 2). The gel image banding patterns of pathogenic strains of *E. coli* were diverse in relation to the distribution of the polymorphic bands. The most frequent band size was 1500 bp, which was observed with 23 strains, and the least frequent band size was 100 bp, which was found in one strain.

Dendrogram and clustering analysis

GelJ uses implemented clustering algorithms to generate dendrograms with the similarity-matrices. The software offers different methods to construct dendrograms based on hierarchical clustering, automatically selecting unweighted pair group method with arithmetic mean (UPGMA) which is the most used method. The dendrogram analysis showed that there were a total of 7 unique clusters (CL) of ERIC (CL-1 to CL-7) within the 31 *E. coli* strains (Figs. 3). Only two clusters (CL-4 and CL-6) represented three different pathotypes while the three clusters (CL-1, CL-2 and CL-7) contained two different pathotypes varying from strains harboring virulence genes to unidentified pathotypes. The dendrogram image obtained from GelJ v.2.0 clustering analysis showed that the highest ERIC-genotype cluster of *E. coli* strains produced was observed in CL-6 (comprised 8 strains each), followed by CL-4 and CL-7 (composed of 4 strains respectively), followed by CL-1, CL-2 and CL-5 (composed of 3 strains respectively) and lastly CL-3 (composed of 2 strains). Different *E. coli* strains isolated from different sources in the ERIC-PCR profile (two different strains from CL-2 and three different strains from CL-4 and CL-6) were found in the same clusters and this is an indication of clonal similarities between them. Results obtained from the dendrograms showed that strains in the CL-5 were recovered from irrigation water and farm soil, while those in CL-6 were recovered from three different sources which include river water, irrigation water and farm soil. Strains in CL-4 were also isolated from three different sources (hospital effluent, final effluent and farm soil). The similarity cut-off value of the different strains of *E. coli* detected was 95% for Fig. 3.

Discussion

The development of antimicrobial resistance genes (ARGs) in *Enterobacteriaceae* members has become a global health problem because antibiotic resistance (AR) leads to limitations in the treatment options thereby increasing the rate of morbidity and mortality [38]. Although carbapenem resistance (CR) among *E. coli* strains has been globally reported [39, 40, 41], there is a dearth of information on Carbapenem-resistant *Escherichia coli* (CREC) isolated from different environmental niches in South Africa. In the Eastern Cape Province, this is the first study that evaluates the genetic diversity among CREC strains using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) genotyping. The sources of contamination and AR patterns may generally be understood by using specific molecular marker to analyze the clonal similarities among bacterial isolates recovered from different environmental niches.

Among the total amount of presumptive *E. coli* isolates (238), the quantity of positively confirmed *E. coli* isolates (192/83%) was unsurprisingly very high and this was similar to other studies by Blaak et al. [42] and Maamar et al. [43] with both reporting high incidence of *E. coli* isolates from farm environments. In another study by Araújo et al. [44], *E. coli* was isolated from irrigation water as well as vegetable samples and this is in line with our findings. This study provides an evidence of the occurrence of pathogenic microorganisms in the environment linking to other niches and our results show the occurrence of CREC in farm samples (Fig. 1).

The natural environment plays a major part in the emergence of AR pathogens as a result of the discharge of WWTP final effluents into receiving surface waters which may be used for domestic and irrigation purposes [45, 46]. The reuse of wastewater in agricultural settings also contributes microbial flora to the natural environment [47], for instance during farm practices, there is high tendency of microbes being transferred to farm animals hence, some pathogens (such as bacteria, parasites, viruses) often emerge as zoonotic in origin. Previously established pathogens associated with raw farm produce (including fruits and vegetables) may re-emerge as more virulent pathogens (particularly *E. coli*) after the acquisition of new virulence factors, including AR determinants [48]. In this study, our findings show that at least 4 categories of diarrheagenic *E. coli* (DEC) are recognized, namely ETEC, EPEC, EAEC and STEC having a total of about 21% from 40% pathogenic *E. coli* strains that were recovered. Our results are in line with the results of Canizalez-Roman et al. [49] that reported the prevalence of some DEC strains isolated from food samples.

In this current study, meropenem had the highest percentage resistance (70%), next to imipenem (64%). Interestingly, our findings are in line with a report by Kagambega et al. [50] conducted in Burkina Faso and this is an evidence of the detection of multidrug resistant (MDR) *E. coli* strains in sub-Saharan Africa. Our results show that at least one out of the 6 detected *E. coli* pathotypes exhibited phenotypic resistance against one of the 4 test carbapenems (doripenem, imipenem, meropenem, ertapenem) while ertapenem had the highest antimicrobial activity as also reported in a study by Kuzucu et al. [51]. Although, little is known about the spread and clinical relevance of carbapenemase-producing genes (CRGs) in Africa, studies

of Manenzhe et al. [52] and Brink et al. [53] provided some reports that investigated various ARGs. In another study by Cakar et al. [54], it was also reported that CRGs were abundant in bacterial isolates of *Enterobacteriaceae* family. Moquet et al. [55] and Baroud et al. [56] both investigated the presence of *bla*_{OXA-48-like} harbored by *E. coli* strains and these findings are in accordance with our results. However, *bla*_{OXA-48-like} had the lowest percentage occurrence among the other selected CRGs that were screened for in our present study. Another study by Fischer et al. [57] revealed that *E. coli* harbored *bla*_{VIM-1} gene in their report, although this gene was not screened for in this present study.

In our study, farm samples (comprising soil, vegetables and irrigation water) had the lowest percentage occurrence (7%) of CRGs among the sample types which may be due to washing after harvesting, however our findings suggest a public health risk and probability of illness if raw vegetables are consumed without washing properly. Moreover, irrigation water had the lowest prevalence (4%) which may be due to the reduction in numbers as a result of environmental or climatic factors that can affect the survival of the microorganisms in the aquatic environments. Hospital effluents (32%) and WWTP final effluents (29%) are evidently proven hotspots of pathogenic microorganisms that may harbour ARGs. Furthermore, our study corroborates with one health strategies to address AR, through improving awareness and understanding of antimicrobial resistance (AMR) by effective communication, education as well as training. To determine the relative implications of AMR emergence and spread in food-animal production, there is a significant challenge due to the interconnectedness as well as interdependence of epidemiological pathways between humans, animals and the environment [58]. In our present study, hospital wastewater isolates cutting across the various *E. coli* pathotypes harboured all three carbapenem-resistance genes but *bla*_{NDM-1} occurred the most. Among the farm samples, only STEC strains harboured CRGs.

Some selected *E. coli* strains were investigated genetically with the use of ERIC-PCR. Osińska et al. [59] reported ERIC-PCR genomic fingerprinting technique as a vital tool for evaluating genetic relationships between bacterial strains isolated from environmental niches and some other studies reported ERIC-PCR genotypic diversity of multidrug resistant (MDR) bacterial strains isolated from different sources [60, 61]. However, in this study we analyzed selected *E. coli* strains comprising some CREC with the use of ERIC- fingerprinting in order to evaluate the links that exist between strains isolated from different sources. ERIC-PCR has been demonstrated to be an effective method in determining the genetic diversity or relatedness among bacterial species by grouping clusters according to band sizes [62]. Some strains showed equal clusters but were isolated from different sources and this indicates clonal similarities between these strains which may be as a result of a possible link between final effluent and receiving water sheds. In light of the various inter- and intra-genotypic background of *E. coli* pathotypes, it is of concern to comprehend if there is a connection between the ERIC-genotype and the existence of pathogenic *E. coli* strains in poorly treated WWTP final effluent which is being discharged into receiving surface waters.

In our study, 7 clusters of ERIC-PCR were generated from 31 pathogenic *E. coli* strains (Figs. 3) at 95 per cent similarity cut-off value and exhibited genetic heterogeneity, but Jonas et al. [63] reported isolates with similarities above 70–80% which were eventually assigned to the same genotypes. Another study by Pusparini *et al.* [64] reported several unique clusters of ERIC in *E. coli* strains isolated from ice cube production sites which showed genetic diversity with 50 per cent cut-off value which is in contrary to the similarity cut-off value generated in this present study. On the basis of ERIC-PCR fingerprint, some of the isolates included in our study were genetically diverse. This is the most expected outcome as the pathogenic strains were isolated after being randomly collected from different environmental sources during sample collection which demonstrates that, the transmission might have occurred from clones of different origins. These findings corroborated with other studies [65, 66, 67]. Some other studies that used ERIC-PCR in *E. coli* isolated from other sources include Zhang et al. [68] who reported genetic diversity analysis of *E. coli* serotypes isolated from retail foods in China and Oltramari et al. [69] who reported genetic heterogeneity of *E. coli* isolated from pasteurized milk in Brazil. In our present study, river water samples were also collected and this is corroborated by a study by Lyautey et al. [70] who reported distribution and diversity of *E. coli* in river water samples collected in Canada.

Our results showed ETEC which harbored *bla*_{NDM-1} and *bla*_{KPC} isolated from wastewater treatment plant (WWTP) final effluent having similar gene clusters as strains from river water samples. This may be as a result of discharge of final effluent into the river because both samples were collected from the same study area. A study by Shaikh et al. [71] reported genetic diversity in *E. coli* and another member of *Enterobacteriaceae* (*Klebsiella pneumoniae*) which were both isolated from clinical samples; however, in our present study, clinical samples were not collected. Our present study showed varied genetic diversity between three strains of neonatal meningitis *E. coli* (NMEC) isolated from WWTP final effluent and river water samples and this is in line with a study by Levert et al. [72] who reported genetic diversity of extraintestinal pathogenic strains of *E. coli*. In this study, STEC had the highest number of ERIC-PCR genotypes and our findings are in contrary with the report by Moredo et al. [73] where ETEC reportedly had the highest EPIC-PCR genotypes. Studies by Yasir et al. [74] and Sun et al. [75] reported the genetic diversities in multidrug resistant ESBL-producing *E. coli* although, our study did not screen for ESBL in the *E. coli* strains nevertheless provides an opportunity for further analysis. EPEC strains were predominantly isolated from river water samples and our study corroborates with another report by Khare et al. [76]. Our current study used ERIC-PCR to identify the various sources and this is supported by Ibekwe et al. [77] who reported *E. coli* isolated from swine wastewater samples in the United States.

This is the first study reporting the genetic diversity of carbapenem-resistant *E. coli* strains isolated from different environmental sources in the Eastern Cape Province, with other studies by Montso et al. [78] and Chukwu et al. [79] reporting on the genotypic diversity of *E. coli* isolates recovered from farms and water samples respectively both in the North West Province.

Conclusion

The detection of CREC in different environmental niches in the Eastern Cape Province, South Africa provides an evidence of high infection risk and this poses a public health concern. The occurrence of ARGs in isolates recovered from vegetables may be linked with the possibility of transmission of these genes as a result of the various farm practices and probably wastewater reuse for irrigation purposes and this is a public health worry. We suggest more surveillance studies to be carried out to screen for more ARGs harbored by various ARB especially the *Enterobacteriaceae* family and development of more effective strategies to control and manage the use of antimicrobial agents. More studies analyzing the genotypic diversity should be encouraged in the Eastern Cape Province and its surroundings. The results obtained by ERIC-PCR approaches reinforce the conclusion of the present study. Overall, the study concludes that ERIC-PCR had good differentiation power for molecular typing and genetic diversity of *E. coli* strains isolated from selected environmental niches and ERIC-PCR proved to be swift and cost-effective molecular-genomic tool for members of the *Enterobacteriaceae* group.

Description Of Supplementary Materials

The following are available as supplementary materials, Supplementary Figures: Figure S1. PCR products of the amplification of *uidA* gene (*E. coli*); Figure S2. PCR products of the amplification of *ibeA* gene (NMEC); Figure S3. PCR products of the amplification of *bla_{NDM-1}* gene; Figure S4. PCR products of the amplification of *bla_{KPC}* gene. Supplementary Table S1. List of primers for the PCR confirmation of *E. coli*, VG, ERIC-PCR and their corresponding amplicon sizes and PCR conditions.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' Contribution

K.E.E performed laboratory works, analyzed the data and drafted the paper. A.I.O designed the study, interpreted the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The proportions of occurrence of *E. coli* strains with ARGs recovered from three District Municipalities in the Eastern Cape Province, South Africa. The following is the order of the percentage occurrence of carbapenem-resistance genes in the different sample types; (a) hospital effluents (57%) and surface water (43%), (b) vegetables (100%), (c) final effluents (40%), hospital effluents (25%), surface water (15%), soil (10%), irrigation water (5%) and vegetables (5%).

Figure 2

The Electrophoresis result by the Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction system

Figure 3

Dendrogram of 31 *E. coli* strains fingerprint by cluster analysis based on ERIC-PCR (CL = Cluster; Scale unit = percentage similarity index; Red-line = cut-off value; strain number missing = unidentified pathotypes).

Supplementary Files

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- [SupplementarymaterialsEbomahOkoh2020ERICPCR.docx](#)