Bagaya *et al. Annals of Microbiology* (2023) 73:28
https://doi.org/10.1186/s13213-023-01732-9

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

Molecular characterization of Carbapenem-resistant *Escherichia coli* isolates from sewage at Mulago National Referral Hospital, Kampala: a cross-sectional study

Jamidah Bagaya[1], Kenneth Ssekatawa[2], Gloria Nakabiri[1], Julius Nsubuga[1], Anna Kitibwa[1], Charles Drago Kato[1] and Lawrence Fred Sembajwe[3*]

Abstract

Background *Escherichia coli (E. coli)* is one of the most frequent causes of fatal bacterial infections affecting both humans and animals. The resistance to Carbapenems is mainly associated with enzyme-mediated resistance mechanism, through the acquisition of Carbapenemase genes. In Uganda, no studies have been done to detect presence of Carbapenem-resistant *E. coli* in sewage. We therefore carried out a study to characterize Carbapenem-resistant *E. coli* from sewage from Mulago National Referral Hospital.

Methods and results In this cross-sectional study, a total of 104, sewage samples were aseptically collected, cultured on MacConkey agar supplemented with Meropenem 1 µg/ml with other standard microbiology methods to screen for Carbapenem-resistant *E. coli* (CREC). Antimicrobial susceptibility testing was performed on the CREC, using Imipenem (10 mg/disc) and Meropenem (10 mg/disc), Carbapenem drugs readily available on market. Multiplex PCR was performed on selected Carbapenem-resistant and susceptible isolates to detect Carbapenemase genes. Later the isolates were pathotyped for virulence genes that included pathogenicity islands (PAIs) and phylogenetic markers. The results showed that the Carbapenem-resistant *E. coli* isolates were more resistant to Meropenem (64%) than Imipenem (60%). KPC gene was the most predominant (75%), followed by NDM gene (30%) while no OXA-48, IMP-1, and IMP-2 genes were detected. Pathotyping of virulence genes showed presence of *eae* gene, as the most predominant (40%), followed by *elt* gene (25%) and negative for *stx* and *aggR* genes. For PAI markers, only the PAI IV_{536} gene was detected at 10%. Then, pathotyping of the phylogenetic markers was present in 85% of the typed isolates with *yjaA* gene the most abundant (60%) while both *chuA* and TSPE4.C2 were detected in 5% of the isolates.

Conclusion Both pathogenic and non-pathogenic Carbapenem-resistant *E. coli* strains are present in the sewage of Mulago National Referral Hospital in Uganda.

Keywords *Escherichia coli*, Carbapenem resistance, Sewage

*Correspondence:
Lawrence Fred Sembajwe
larryfeds@gmail.com
Full list of author information is available at the end of the article

Introduction

Escherichia coli (*E. coli*) is one of the most frequent causes of common bacterial infections including cholecystitis, bacteremia, cholangitis, urinary tract infections, traveller's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia (Torres et al. 2010). Between 1982 and 2002, the USA was reported to have 350 *E. coli* outbreaks, representing 8598 cases, 1493 (17%) hospitalizations, 354 (4%) hemolytic uremic syndrome cases, and 40 (0.5%) deaths (Rangel et al. 2005). The *E. coli* bacteremia of England increased by 76% between 2011 and 2015. When highly virulent *E. coli* pathotypes are involved, antibiotics are used for treatment of the infection (Steiner and Guerrant 2011). *E. coli* pathotypes are grouped based on the genes mediating the virulence factors; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), shiga toxin producing *E. coli* (STEC) or enterohemorrhagic *E. coli* (EHEC), and extra intestinal pathogenic *E. coli* (ExPEC) (Onanuga et al. 2014) reported to cause a mortality of up to 50% of infected patients (Okoche et al. 2015). The genetic determinants of virulence are carried either on plasmid or chromosomes. If they are found on chromosomes, they are located on DNA fragments termed as pathogenicity islands (PAI) (Sabaté et al. 2006). The different PAIs include PAI I536, PAI II536, PAI IV536, PAI IJ96, PAI IIJ96, PAI ICFT073, PAI IICFT073, haboring α-Hemolysin, CS12 fimbriae, α-Hemolysin and P-related fimbriae, Yersiniabactin siderophore system, α-Hemolysin, P-fimbriae, and aerobactin, P-fimbriae and iron-regulated genes, α-Hemolysin and P-fimbriae, α-Hemolysin, Prs-fimbriae, and cytotoxic necrotizing factor 1 and F17-like fimbrial adhesin virulence factors, respectively (Sabaté et al. 2006). In spite of the high mortality burden, *E. coli* infections have been associated with increased lengths of hospital admissions and difficulties with antibiotic treatment due to developing resistant strains (Bou-Antoun et al. 2016), hence a change in the prevalence of *E. coli* infections among patients (Wang et al. 2016).

Accumulation of multidrug resistance traits may correspond to the ultimate pan drug resistance of *Enterobacteriaceae* (Basak et al. 2016; Poirel et al. 2016) as an advent of untreatable infections. Beta-lactams are so far the most used antibiotics around the world and these include the Penicillins, Cephalosporins, Monobactams, and Carbapenems. They are distinct due to the common beta-lactam ring and act similarly by binding to and inactivating the Penicillin-binding proteins (PBPs), which are required in the formation of the bacterial cell wall (Khalil et al. 2017; Kong et al. 2010). Among the beta-lactams, Gram-positive and Gram-negative bacteria are most susceptible to Carbapenems; hence, it is a broad-spectrum antibiotic with a unique molecular structure that combine a Carbapenem and the Beta-lactam ring. The Carbapenems are regarded as the most reliable last-line treatment for bacterial infections; hence, the emergency and rapid spread of Carbapenem resistance among Gram negative bacteria is a major problem (Basak et al. 2016; Meletis 2016). Resistance to Carbapenem among Gram-negative bacteria in general can be acquired through several mechanisms: generation of new extended-spectrum beta-lactamases (ESBL) by amino acid substitution from available plasmid-mediated beta-lactamases, acquisition of genes encoding ESBL from the environmental bacteria, dissemination of plasmid mediated Carbapenemases, increased expression of chromosome-encoded beta-lactamases genes, and or ability to develop biofilm (Thakur et al. 2016). Infections due to these resistant strains are associated with higher morbidity and mortality rates. Therefore, rapid detection of these resistant *E. coli* strains is crucial for appropriate antimicrobial therapy and infection control measures (Birgy et al. 2012), especially with regard to the more recent characterization of Carbapenem-resistant *Enterobacteriaceae* from patients admitted at Mulago National Referral Hospital, Uganda (Okoche et al. 2015).

It was reported that Carbapenem-resistance prevalence is comparatively high in isolates obtained from the hospital rendering the hospital environment a potential source of infection for patients and health workers (Kateete et al. 2016). Therefore, the beta-lactam antibiotics which were the main treatment of *Enterobacteriaceae* infections, nonetheless have been rendered ineffective; for example, the percentage of *E. coli* ESBL producers have gradually increased from 18% in 2010 to 24% in 2014 and reached 31.7% in 2017 (January–June) as reported by (Eltai et al. 2018). Beta (β)-lactamases are divided into four functional groups: Penicillinases, ESBLs, Carbapenemases, and AmpC-type Cephalosporinases (Bush and Jacoby 2010). ESBLs are divided into three groups according to the encoding of TEM, SHV and CTX-M genes (Eltai et al. 2018; Lahlaoui et al. 2014). CTX-M enzymes are the most common and are further classified into five major phylogenetic groups based on gene sequences namely, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (Lahlaoui et al. 2014). ESBL-producing *E. coli* are the predominant organisms in human infections, and they pose significant threat to human health (Bakshi et al. 2013). Since the Carbapenem-resistant *Enterobacteriaceae* are resistant to all beta-lactams, they were also found to be resistant to Ciprofloxacin, Gentamycine, Co-trimoxazole, and Tetracycline (Cusack et al. 2019). Thus, it is important to monitor the use of Carbapenem combinations in treatment of infections and institute active surveillance

to identify Carbapenem-resistant *E. coli* pathotypes. Then, promote control practices that restrict its further dissemination. This study, therefore, was aimed at characterizing the Carbapenem-resistant *E. coli*, isolated from sewage within Mulago National Referral Hospital environment.

Materials and methods

Study design and study area

This was designed as a cross-sectional study to identify carbapenem-resistant *E. coli* isolated from different sewage sites within Mulago National Referral Hospital that serves as a general hospital for Kampala metropolitan, receives nationwide patients with different health complications and severities requiring antibiotic treatment as well as those presenting Carbapenem resistance. Six manholes were selected for the sampling and these included microbiology lab manhole, 1C ward manhole, Causality area manhole, hallway manhole, the hospital main manhole and mortuary manhole. Both effluent and swab-samples were collected on a 2-weekly basis from the different manholes and later transported under cool temperatures to the microbiology laboratory located at College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) for culture and isolation. After which the *E. coli* isolates were tested for susceptibility to two selected Carbapenems (Meropenem and Imipenem) that were readily available on the Ugandan market. Later on, selected *E. coli* isolates, were transferred to the Molecular laboratory at COVAB, where their DNA was extracted and amplified to detect for the presence of Carbapenemase genes; bla_{IMP-1}, bla_{IMP-2}, bla_{OXA-48}, bla_{VIM}, bla_{KPC}, and bla_{NDM}. Following Carbapenemase detection, pathotyping of *E. coli* was performed to detect for the presence of virulent genes: *eae*, *stx*, *est*, *elt paH*, and *aggR*; the presence of PAI markers: PAI I_{536}, PAI II_{536}, PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFTO73}, and PAI II_{CFT073}; as well as the presence of *chuA*, *yjaA*, and the DNA fragment TSPE4 C2.

Sample size calculation

Sample size was calculated using the Cochran formula to obtain an ideal sample size given a desired level of precision, desired confidence level, and the estimated proportion of the attribute present in the sample population.

The Cochran formula is $N_o = Z^2PQ/E^2$, where E is 0.08, the desired level of precision; P is 22.4% prevalence of Carbapenem-resistant *Enterobacteriaceae* at Mulago National Referral Hospital (Okoche et al. 2015); Q is $1 - p$, (1- 0.224) = 0.776; Z is 1.96; N_o is the number of samples to be collected; calculation; and $N_o = (1.96)^2 = 0.224 \times 0.776/(0.08)^2 = 104$ samples.

Sampling and sample collection

Sewage samples were collected purposively within and around Mulago National Referral Hospital; on a two-weekly basis, collecting from all the sites respective to the initial collection day. Both effluent and swab samples were collected from six manholes which include microbiology lab manhole, 1C ward manhole, causality area manhole, hallway manhole, hospital main manhole, and mortuary manhole. To collect effluent samples from the different manholes, sterile falcon tubes were filled allowing a small headspace of 1 ml as a provision for mixing the sample and recapped immediately. The samples were labeled with the correct identification, placed in a biohazard bag, and then placed into a cool box with ice packs. For collection of the swabs from the different manholes, a sterile swab was used to rub and roll firmly several times across the sampling area. Each swab was afterwards placed into a falcon tube containing Stuart's transport media (HiMedia, Uk) and capped tightly then labelled with its respective identification, placed in a biohazard bag, and then put in a cool box with ice packs. Samples were transported in a cool box with ice to maintain the integrity of the samples under a cold chain.

Laboratory analysis

Culture and isolation of E. coli

The samples were inoculated immediately on the pre-set sterile MacConkey agar (Condalab, Spain) plates supplemented with Meropenem 1 µg/ml (Oxoid™, UK) used as the selective media for screening Carbapenem-resistant *E. coli* (Kumar et al., 2015). Each effluent sample was first vortexed and using a sterile wire loop, a loopful of sample was obtained and inoculated onto an agar plate containing the MacConkey supplemented with Meropenem 1 µg/ml. For the swab samples, a primary inoculum was created on the agar plate and used a sterile wire loop to streak the sample onto the plate. After an anerobic incubation at 44 °C for 18 to 24 h, the culture was evaluated for growth. All morphologically distinct colonies were subcultured on standard MacConkey agar plate, and isolates were identified by conventional biochemical tests. Quality control procedures were done using *E. coli* BAA 1706 as the positive control and *E. coli* ATCC 25922 as the negative control.

Confirmatory tests of E. coli

Gram staining Gram staining was used to distinguish between gram negative and gram-positive bacteria based on their different cell wall constituents. A drop of normal saline was placed on a glass slide and a loop full of well-isolated bacteria colony added. A smear was made

gently and then air-dried. The dried smear was fixed by gently passing the slide on the flame at least three times immediately. The fixed smear was flooded with crystal violet stain for about 1 min, then washed slowly under running water and again flooded with Lugol's iodine for about 1 min, after which it was washed with tap water. Then, the smear was decolorized with 50% acetone-alcohol slowly until the purple color stopped running. The slide was rinsed with tap water and then flooded with 2% Safranin as a counter satin for 10–15 s. The slide was rinsed with slowly running tap water and allowed to air dry on a draining rack before examination. During examination, a drop of immersion oil was added on to the smear and examined under the light microscope with the 100× objective to visualize the morphology of the bacteria. The gram-negative bacteria stain pink and gram positive bacteria stain purple (Bartholomew and Mittwer 1952). *E. coli* is a gram-negative bacteria hence stained pink.

Biochemical confirmatory tests After phenotypic colony identification and cell identification of isolates by microscopic visualization through gram staining, conventional biochemical tests were performed to determine the isolated bacteria classification according to Gram-negative identification protocol (Cowan 1993). The IMViC biochemical tests (Benathen 1992), these include Indole test, Methyl red test, Voges Proskauer test, and citrate utilization test as well as Urease production test and Triple Sugar Iron (TSI) (Zinnah et al. 2007) were preformed to identify *E. coli.*

Indole test The indole test used to detect the ability of bacteria to split amino acid tryptophan to form indole. Sterilized tubes containing 4 ml of SIM broth media (Condalab, Spain) were prepared. From the pure colonies of the test organism, each tube was inoculated aseptically and incubated at 37 °C for 24–28 h in ambient air. Then, 0.5 ml of Kovac's reagent added to the broth culture and later observed for the presence or absence of a ring. The presence of a ring indicated positive results and absence indicated negative results (Miller and Wright 1982). *E. coli* is indole positive and formed a ring on addition of the Kovac's reagent.

Methyl red test The methyl red test used to detect the ability of an organism to produce stable acids as end products from supplied glucose. Using a sterile loop, the pure colonies of the organism were inoculated into fresh sterile MR-VP broth medium (Condalab, Spain). Another tube contained the medium and the control. Both tubes inoculated at 37 °C for 2–5 days. After incubation, 5 drops of methyl red reagent were added to both and observed for color change from yellow to red. A positive reaction is when the culture medium turns red and remains yellow for the negative reactions. *E. coli* is methyl red positive hence medium turned red.

Voges-Proskauer test Voges-Proskauer test used to determine if an organism produces *acetyl-methyl carbinol* from glucose fermentation. Using a sterile wire loop, pure colonies were inoculated into a fresh sterile MR-VP broth medium (Condalab, Spain). With the other tube containing the control and medium. Both tubes were incubated at 37 °C for 24–48 h. After incubation, at least 3 drops of Barrit's reagent A and 3 drops of Barrit's reagent B were added to both the tubes and well mixed. After 15–20 min, a red color change forms for a positive result and no color change for the negative result. *E. coli* is Voges-Proskauer negative, showed no color change.

Urease test Urease hydrolyses urea to yield two molecules of ammonia and one of CO_2. This reaction can be detected by increasing the medium pH caused by ammonia production. Urease-positive species vary in amount of enzyme produced; hence, the designations of positive, weakly positive, and negative. A heavy inoculum from an 18 to 24-h pure culture was streaked on the entire slant surface. The tubes were then incubated with loosened caps at 37 °C, and color change in the slant was observed from 6 h, 24 h, and everyday up to 6 days. Urease production was indicated by bright color while negative test was indicated by no color change. *E. coli* is urease negative, showed no color change.

TSI The triple sugar iron test employing Triple Sugar Iron agar is designed to differentiate among organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulphide production. A sterile wire inoculation needle was used to pick a well-isolated colony by just tapping. TSI was then inoculated first by stabbing through the center of the medium to the bottom of tube and then streaked the surface of the agar slant. The tube was loosely capped, and the incubated at 37 °C in ambient air for 18 to 24 h and then examined the color changes in the butt and slant and gas production (displacement of the media). *E. coli* showed a yellow butt and slant with or without displacement of the media.

Citrate utilization test The citrate utilization test was used in identification of bacteria that use citrate as the main source of carbon. During the test, bijou bottles were used to prepare slopes of Simmons citrate agar (Condalab, Spain). Using a sterile wire loop, the slope was streaked with a well isolated colony from a pure culture of the test organism and the butt was stabbed with a

straight sterile wire loop then cultured at 37 °C for 24 h. The positive test indicated by a bright blue color while negative test showed no color change. *Klebsiella pneumoniae* was used as a positive control; the test used bromothymol blue indictor, for the blue color (Elazhary et al. 1973). *E. coli* is citrate-negative and produced no change in color.

Antibiotic susceptibility testing Susceptibility testing was done using Kirby Bauer disc diffusion, on Muller Hinton agar (BiolabZrt Budapest, Hungary), and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2018 (Weinstein et al., 2018). Two antibiotic discs that were readily available on market, Imipenem (10 µg) and Meropenem (10 µg) (Oxoid™, UK), were used for testing. Three colonies of test isolate were emulsified into sterile saline and the resulting suspension adjusted to turbidity of 0.5 McFarland and then used a sterile swab to inoculate the suspension on the Muller Hinton agar plates to form a lawn, plates impregnated with the two antibiotic discs and incubated aerobically at 37 °C for 24 h. After which, the diameters of zone of inhibition were measured and interpreted according to the recommended criteria by the CLSI guidelines 2018 (Weinstein et al., 2018). Quality control procedures were done using *E. coli* BAA 1706 as the positive control and *E. coli* ATCC 25922 as the negative control.

Characterization for the different E. coli pathotypes

DNA extraction DNA extraction was done using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Briefly, 1 ml of bacteria suspension was transferred in an Eppendorf tube and later centrifuged at 4500 rpm for 5 min at 4 °C. The supernatant was then discarded, and the pellet obtained was resuspended in 200 µl buffer AL and 20 µl Proteinase K. The suspension was thoroughly mixed by vortexing at least 3 times for 5 s each time to obtain a homogenous mixture. Thereafter, the homogenous mixture was incubated at 56 °C in a water bath for 1 h. Following incubation, 200 µl of ethanol (96–100%) was added to the mixture and vortexed to obtain a homogenous mixture. Afterwards, the solution was carefully transferred into a mini column which was correctly capped and then centrifuged at 8000 rpm for 1 min. Following centrifugation, the filtrate was discarded and 500 µl of buffer AW1 was added to the mini column. The cap was then closed and centrifuged at 8000 rpm for 1 min. After which, the cap was carefully opened and 500 µl of buffer AW2 was added to the mini column. The cap was then closed and centrifuged at 14,000 rpm for 3 min. Thereafter, the mini column was transferred into a clean 1.5 ml Eppendorf tube and the collection tube containing the filtrate was then discarded. Afterwards, 60 µl of buffer AE was added to the mini column and the cap was closed. The mini column was then centrifuged at 8000 rpm for 1 min. After which, the mini column was discarded and the Eppendorf tube closed. The DNA was later stored at −20 °C.

Detection of Carbapenemase genes by multiplex PCR Carbapenemase genes were detected using specific primers targeting: bla_{IMP-1}, bla_{IMP-2}, bla_{OXA-48}, bla_{VIM}, bla_{KPC}, and bla_{NDM} genes (Table 1). The PCR reactions were performed in a total of 12.5 µl, "containing 1 × HS Taq PCR Master Mix" (Biolab, New England) and 6 oligonucleotide primer pairs (Eurofins, USA) each at 0.8 µM. One microliter of genomic DNA was added to each PCR reaction tube as template. The thermo cycling (Applied

Table 1 Gene-specific primer pairs targeting the different Carbapenemase genes and their corresponding sizes in base pairs

Gene	Primer sequence	Size (bp)	Reference
bla_{KPC}	F 5′ ATG TCA CTG TAT CGC CGT CT 3′ R 5′TTT TCA GAG CCT TAC TGC CC 3′	498	(Mushi et al. 2014)
bla_{IMP-1}	F 5′TGA GCA AGT TAT CTG TAT TC 3′ R 5′TTA GTT GCT TGG TTT TGA TG 3′	232	(Mushi et al. 2014)
bla_{IMP-2}	F 5′GGC AGT CGC CCT AAA ACA AA 3′ R 5′TAG TTA CTT GGC TGT GAT GG 3′	232	(Mushi et al. 2014)
bla_{VIM}	F 5′ GAT GGT GTT TGG TCG CAT A 3′ R 5′ CGA ATG CGC AGC ACC AG 3′	390	(Joji et al. 2019)
bla_{NDM}	F 5′ GGT TTG GCG ATC TGG TTT TC 3′ R 5′ CGG AAT GGC TCA TCA CGA TC 3′	621	(Zainol Abidin et al. 2015)
bla_{OXA-48}	F 5′TTG GTG GCA TCG ATT ATC GG 3′ R 5′ GAG CAC TTC TTT TGT GAT GGC 3′	238	(Mushi et al. 2014)

biosystems, USA) temperature and time profile was 95 °C for 5 min (initial denaturation) followed by 35 cycles of 95 °C for 30 s (denaturation), 56 °C for 30 s (annealing), 72 °C for 1 min (extension), and a final extension of 72 °C for 10 min. A negative control, double distilled (without template DNA) was included in the PCR amplification. Ten microliters of each amplicon were "mixed with 2 µl of a 6X loading dye" (Biolabs, New England) and subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. The amplified products were visualized using an ultra violet transilluminator (Waghtech international, UK), and the band sizes were estimated by comparison with a 100 bp standard DNA marker (HyperLadder IV [Bioline], Germany).

Pathotyping of E. coli

Detection of virulent genes Carbapenem-resistant isolates were pathotyped using specific primers to detect the presence of the virulent genes: *eae*, *stx*, *est*, *elt*, *paH*, and *aggR* as shown in Table 2. The PCR reactions were performed in a total volume of 12.5 µl, "containing 1 × HS Taq PCR master mix" (Biolab, New England) and 6 oligonucleotide primer pairs (Eurofins, USA) each at 0.8 µM. One microliter of genomic DNA was added to each PCR reaction as a template. The thermocycling (Applied biosystems, USA) temperature and time profile was 95 °C for 5 min (initial denaturation) followed by 30 cycles of 95 °C for 30 s (denaturation), 50 °C for 30 s (annealing), 72 °C for 1 min (extension), and a final extension of 72 °C for 30 min. A negative control, double distilled water (without DNA template added) was included in the PCR amplification. Ten microliters of each amplicon "mixed with 2 µl of a 6X loading dye" (Biolabs, New England) and were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. The amplified products were visualized using an ultra violet transilluminator (Waghtech international, UK), and the band sizes were estimated by comparison with a 1000 bp standard DNA marker (HyperLadder IV [Bioline], Germany).

Detection of PAI markers The presence of sequences associated with seven different PAI markers; PAI I_{536}, PAI II_{536}, PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073}, and PAI II_{CFT073} as shown in Table 3. The PCR reactions were performed in a total volume of 12.5 µl, "containing 1 × HS Taq PCR master mix" (Biolabs, New England) and seven oligonucleotide primer pairs (Eurofins, USA) each at 0.8 µM. One microliter of genomic DNA was added to each PCR reaction as a template. The thermocycling (Applied biosystems, USA) temperature and time profile was 94 °C for 5 min (initial denaturation) followed by 30 cycles of 94 °C for 1 min (denaturation), 55 °C for 1 min (annealing), 72 °C for 1 min (extension), and a final extension of 72 °C for 10 min. A negative control, double distilled water (without DNA template added) was included in the PCR amplification. Ten microliters of each amplicon "mixed with 2 µl of a 6X loading dye" (Biolabs, New England) and were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. The amplified products were visualized using an ultra violet transilluminator (Waghtech international, UK), and the band sizes were estimated by comparison with both 100 bp standard DNA marker (HyperLadder IV [Bioline], Germany) and Bench top 1 kb DNA ladder (Promega, UK).

Phylogenetic classification Showed that the *E. coli* strains belonged to four groups (A, B1, B2, or D) based on the presence of the *chuA* and *yjaA* genes and the DNA

Table 2 Genes and their primer sequences for molecular characterization of *E. coli* adopted from (Dias et al. 2012)

Gene	Primer	Sequence (5'–3')	Size of amplicon (Bp)	Reference
Eae	Sk1	CCCGAATTCGGCACAAGCATAAGC	881	(Madic et al. 2010)
	Sk2	CCCGGATCCGTCTCGCCAGTATTCG		
Stx	Vtcom-u	GAGCGAAATAATTTATATGTG	518	(Sato et al. 2003)
	Vtcom-d	TGATGATGGCAATTCAGTAT		
Est	EST-F	ATT TTT MTT TCT GTA TTR TCT T	190	(López-Saucedo et al. 2003)
	EST-R	CAC CCG GTA CAR GCA GGA TT		
Elt	ELT-F	GGC GAC AGA TTA TAC CGT GC	450	(López-Saucedo et al. 2003)
	ELT-R	CGG TCT CTA TAT TCC CTG TT		
paH	IpaIII	GTTCCTTGACCGCCTTTCCGATACCGTC	619	(Sethabutr et al. 1994)
	IpaIV	GCCGGTCAGCCACCCTCTGAGAGTC		
aggR	aggRKs1	GTATACACAAAAGAAGGAAGC	254	(Shin et al. 2015)
	aggRKas2	ACAGAATCGTCAGCATCAGC		

Table 3 Primers for detection of PAI markers

Gene	Primer sequence (5′-3′)	Size of product (bp)	Reference
PAI I_{536}	F: TAA TGC CGG AGA TTC ATT GTC	1800	(Sabaté et al. 2006)
	R: AGG ATT TGT CTC AGG GCT TT		
PAI II_{536}	F: CAT GTC CAA AGC TCG AGC C	1000	(Sabaté et al. 2006)
	R: CTA CGT CAG CGT GGC TTT		
PAI IV_{536}	F: AAG GAT TCG CTG TTA CCG GAC	300	(Sabaté et al. 2006)
	R: TCG TCG GGC AGC GTT TCT TCT		
PAI I_{J96}	F: TCG TGC TCA GGT CCG GAA TTT	400	(Sabaté et al. 2006)
	R: TGG CAT CCC ACA TTA TCG		
PAI II_{J96}	F: GGA TCC ATG AAA ACA TGG TTA ATG GG	2300	(Sabaté et al. 2006)
	R: GAT ATT TTT GTT GCC ATT GGT TAC C		
PAI I_{CFT073}	F: GGA CAT CCT GTT ACA GCG CGC	930	(Sabaté et al. 2006)
	R: TCG CAA CAA ATC ACA GCG AAC		
PAI II_{CFT073}	F: ATG GAT GTT GTA TCG CGC	400	(Sabaté et al. 2006)
	R: ACG AGC ATG TGG ATC TGC		

Table 4 Primers for phylogenetic classification of *E. coli*

Gene	Primer sequence (5′-3′)	Size of Product (bp)	References
chuA	F: GAC GAA CCA ACG GTC AGG AT	279	(Clermont et al. 2000)
	R: TGC CGC CAG TAC CAA AGA CA		
yjaA	F: TGA AGT GTC AGG AGA CGC TG	211	(Clermont et al. 2000)
	R: ATG GAG AAT GCG TTC CTC AAC		
TSECP4C2	F: GAG TAA TGT CGG GGC ATT CA	152	(Clermont et al. 2000)
	R: CGC GCC AAC AAA GTA TTA CG		

fragment (TSPE4.C2) using primers in Table 4. The PCR reactions were performed in a total of 12.5 µl, "containing 1×HS Taq PCR Master Mix" (Biolab, New England) and three oligonucleotide primer pairs (Eurofins, US) each at 0.8 µM. One microliter of genomic DNA was added to each PCR reaction tube as template. The thermo cycling (Applied biosystems, USA) temperature and time profile was 94 °C for 4 min (initial denaturation) followed by 30 cycles of 94 °C for 5 s (denaturation), 54 °C for 10 s (annealing), 72 °C for 30 s (extension), and a final extension of 72 °C for 5 min. A negative control, double distilled (without template DNA) was included in the PCR amplification. Ten microliters of each amplicon were subjected to electrophoresis on a 2% agarose gel containing ethidium bromide. The amplified products were visualized using an ultra violet transilluminator (Waghtech international, UK), and the band sizes were estimated by comparison with a 1000 bp standard DNA marker (HyperLadder IV [Bioline], Germany).

Data analysis

Data analysis was done using Microsoft Excel and then Statistical Package for the Social Sciences, SPSS version 21. Descriptive statistics namely frequency, percentage distributions, and means were used to present patterns in the data. Genotypes were analyzed using a chi-square or Fisher's exact test. A P value of ≤ 0.05 was considered as evidence of significant statistical difference.

Results

Carbapenem-resistant *E. coli* isolation

A total of 104 samples were collected from six manholes at Mulago National Referral Hospital every 2 weeks for up to 6 weeks. Of the 104 samples, 32 (30.8%) were collected from the microbiology laboratory manhole, 26 (25%) were collected from manholes around 1C ward, 16 (15.4%) were collected from the hallway manhole, 8 (7.7%) were collected from Causality area manhole, 10 (9.6%) were collected from the Hospital Main Manhole,

and 12 (11.5%) were collected from the mortuary manhole. Over the entire sampling period, a total of 25 (24%) were detected as being Carbapenem-resistant *E. coli* positive. Of the 25 Carbapenem-resistant *E. coli* strains, the majority were from the manholes around 1C ward, 13 (52%) followed by the hallway manhole, 9 (36%) and the mortuary manhole, 3 (12%) as shown in Table 5 and while the microbiology laboratory, causality area, and hospital main manholes lacked resistant *E. coli* strains.

Antibiotic susceptibility

Antibiotic susceptibility testing for 25 Carbapenem-resistant *E. coli* isolates was done using two Carbapenems (Imipenem and Meropenem) as they were readily available on the Ugandan market. The overall resistance to both Carbapenem drugs was with 16 (64%) resistant to Meropenem and 15 (60%) resistant to Imipenem according to CLSI interpretation. Furthermore, 15 (60%) isolates showed co-resistance to both Imipenem and Meropenem (Fig. 1). On comparison of Imipenem resistance between the different manholes, results showed that the majority of resistant isolates were from the manholes around 1C ward, 8 (53.33%) followed by the hallway manhole, 5 (33.3%) and mortuary, 2 (13.3%). While comparison of Meropenem resistance between the different manholes, results showed that the majority of resistant isolates were from the manholes around 1C ward, 9 (56.3%) followed by the hallway manhole, 5 (31.3%) and mortuary manhole, 2 (12.5%). The Carbapenem susceptibility of *E. coli* strains from the different manholes are shown in Table 6.

Detection of Carbapenem-resistant genes

In order to determine the mechanisms of Carbapenem resistance, five Carbapenemase genes were typed for the 20 amplified isolates from which 16 of these isolates were resistant and four isolates, susceptible to Carbapenem. Based on the multiplex PCR results, 16 (80%) of the 20 amplified isolates showed presence of Carbapenemase genes. Of the 16 Carbapenem-resistant *E. coli* majority were identified to have bla_{KPC} gene 15 (93.8%), followed by bla_{NDM} gene, 6 (37.5%), and bla_{VIM} gene with 1 (6.3%). On comparison of Carbapenemase genes identified, 10 (62.5%) of the 16 Carbapenem-resistant *E. coli* carried

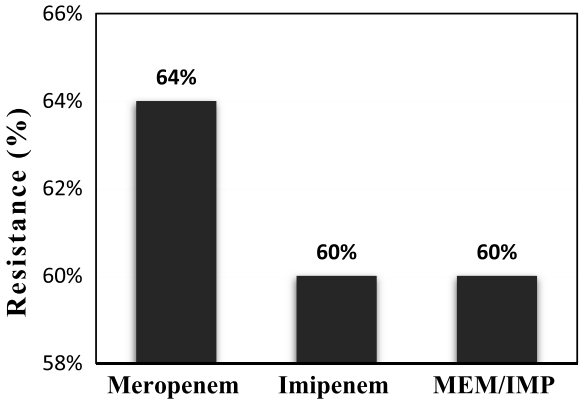

Fig. 1 Percentage resistance to Meropenem (MEM) and Imipenem (IMP)

one Carbapenem gene and 6 (37.5%) carried two Carbapenem genes, combination of bla_{KPC} and bla_{NDM} (5, 31.3%) and a combination of bla_{KPC} and bla_{VIM} (1, 6.3%) (Fig. 2). With regard to the manhole sites, 1C ward manholes had 9 (60%) isolates with bla_{KPC} gene, 3 (50%) isolates with bla_{NDM} gene, and 1 (100%) isolate with bla_{VIM} gene, hallway manhole had 4 (26.7%) isolates with bla_{KPC} gene and 2 (33.3%) isolates with bla_{NDM} gene while the mortuary manhole had 2 (13.3%) isolates with bla_{KPC} gene and 1 (16.7%) isolate with bla_{NDM} gene as shown in Table 7. All the susceptible isolates in this study did not harbor Carbapenemase genes. No significant differences were noted when Carbapenemase genes were compared between Imipenem and Meropenem-resistant isolates.

Pathotyping of *E. coli*

Detection of virulence genes

Using multiplex PCR, six virulence genes, *eae*, *stx*, *est*, *elt*, *paH*, and *aggR*, were pathotyped for 20 amplified isolates of which 16 isolates were resistant and 4 isolates were susceptible to Carbapenems. Of the 20 amplified isolates,10 (50%) showed presence of one or more virulence

Table 5 Number of confirmed Carbapenem-resistant *E. coli* strains

Manhole	Carbapenem-resistant *E. coli* (collected every 2 weeks)				
	1	2	3	4	Total
1C ward	2 (100%)	3 (100%)	4 (100%)	4 (100%)	13(52%)
Hallway	1 (33.3%)	2 (50%)	4 (100%)	2 (100%)	9 (36%)
Mortuary	1 (50%)	0	2(100%)	0	3(12%)

Table 6 Carbapenem susceptibility of *E. coli* strains from the different manholes

Carbapenem	Manhole	Susceptible	Intermediate	Resistance
Meropenem	1C Ward	4 (44.4%)	0	9 (56.3%)
	Hallway	4 (44.4%)	0	5 (31.3%)
	Mortuary	1 (11.1%)	0	2 (12.5%)
	Total	**9 (36%)**	**0**	**16 (64%)**
Imipenem	1C ward	4 (44.4%)	1 (100%)	8 (53.3%)
	Hallway	4 (44.4%)	0	5 (33.3%)
	Mortuary	1 (11.1%)	0	2 (13.3%)
	Total	**9 (36%)**	**1 (4%)**	**15 (60%)**

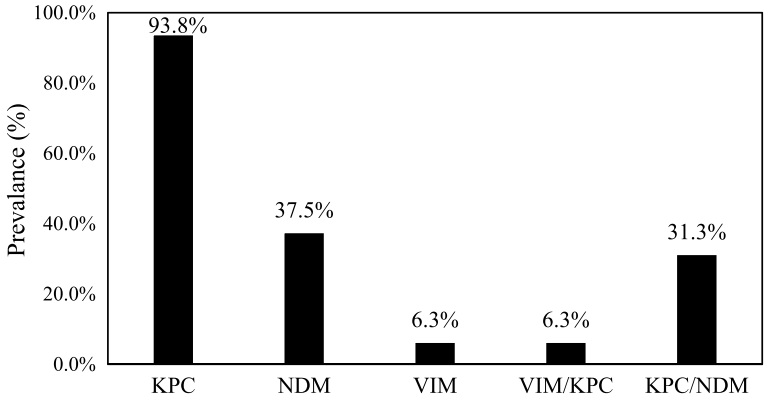

Fig. 2 Prevalence of the various Carbapenemase genes obtained

Table 7 Number of Carbapenem-resistant *E. coli* isolates positive for Carbapenemase genes

Manhole	Susceptibility	bla_{KPC}	bla_{OXA-48}	bla_{VIM}	bla_{NDM}	bla_{IMP-1}	bla_{IMP-2}	Total
1C Ward	Resistant	9 (60%)	0	1 (100%)	3 (50%)	0	0	9 (56.3%)
	Susceptible	0	0	0	0	0	0	0
Hallway	Resistant	4 (26.7%)	0	0	2 (33.3%)	0	0	6 (37.5%)
	Susceptible	0	0	0	0	0	0	0
Mortuary	Resistant	2 (13.3%)	0	0	1 (16.7%)	0	0	3 (18.8%)
	Susceptible	0	0	0	0	0	0	0
	Total	15 (75%)	0	1 (5%)	6 (30%)	0	0	

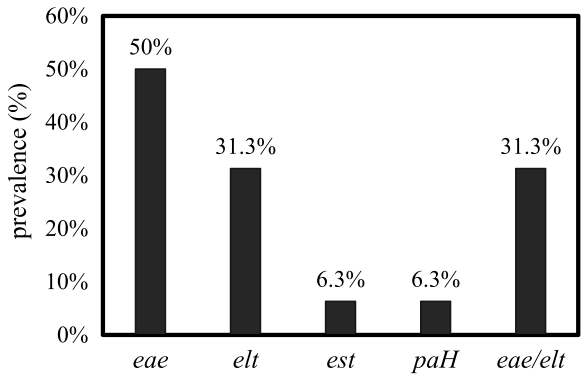

Fig. 3 Shows prevalence of virulence genes obtained

genes. Among the 16 Carbapenem-resistant *E. coli* isolates majority were identified to have *eae* gene 8 (50%), followed by *elt* gene with 5 (31.3%) and 1 (6.3%) for both e*st* and *paH* gene. Furthermore, 10 (62.5%) of the Carbapenem-resistant *E. coli* carried at least one Carbapenemase gene and 5 (31.3%) carried a combination of *eae* and *elt* gene (Fig. 3). On comparison between sampling sites, results showed that 1C ward manhole had 5 (62.5%) isolates with *eae* gene, 4 (80%) isolates with *elt* gene, and 1 (100%) isolate with *paH* gene, and hallway manhole had 1 (2.5%) isolate with *eae* gene, 1 (12.5%) isolate with *elt* gene, and 1 (100%) isolate with *paH* gene while mortuary had 2 (25%) isolates with *eae* gene (Table 8).

Table 8 Number of *E. coli* isolates identified with virulence genes

Manhole	Susceptibility	*Eae*	*Elt*	*est*	*paH*	Total
1C ward	Resistant	5 (62.5%)	4 (80%)	0	1 (100%)	6 (60%)
	Susceptible	0	0	0	0	0
Hallway	Resistant	1 (12.5%)	1 (25%)	1 (100%)	0	2 (20%)
	Susceptible	0	0	0	0	0
Mortuary	Resistant	2 (25%)	0	0	0	2 (20%)
	Susceptible	0	0	0	0	0
	Total	8 (50%)	5 (31.3%)	1 (6.3%)	1 (6.3%)	

Detection of PAI and phylogenetic markers

Using multiplex PCR, seven PAI and three phylogenetic markers were pathotyped for the 20 amplified *E. coli* isolates of which 16 isolates were resistant and 4 isolates were susceptible to Carbapenems. Of the 20 amplified isolates, 2 (10%) isolates showed presence of PAI markers while 17 (85%) isolates showed presence of phylogenetic markers. Results showed PAI IV_{536} (2 isolates, 10%) as the only PAI marker obtained and was identified in 1 (50%) resistant and 1 (50%) susceptible *E. coli* strain, both isolates were from 1C Ward manhole site (Table 9). Furthermore, among the 17 (85%) isolates positive for phylogenetic markers, 15 (60%) had *yjaA* gene, of which 14 (93.3%) were identified from *E. coli* strains resistant to Carbapenems and 1 (6.7%) isolate susceptible to Carbapenems, while 1 (100%) susceptible *E. coli* strain was identified for each gene: TSEP4.C2 and *chuA*. On comparison of the identified phylogenetic genes 17 (85%), 1C ward had majority of genes identified 9 (45%), where 8 (53.3%) isolates had *yjaA* gene and 1 (100%) isolate had TSEP4.C2, followed by hallway manhole with 6 (30%); 5 (33.3%) isolates had *yjaA* gene and 1 (100%) isolate had *chuA* gene while mortuary manhole had 2 (10%); the 2 (13.3%) isolates had *yjaA* gene (Table 9). However, the rest of the genes where not identified from neither the *E. coli* strains resistant nor those susceptible to Carbapenems.

Discussion

E. coli is a bacterial pathogen emerging as one of the most frequent cause of fatal bacterial infections affecting both humans and animals (Rojas-Lopez et al. 2018) with a rapidly growing multi-drug resistance and has been reported to cause a mortality of up to 50% of infected patients (Okoche et al. 2015). The current global emerging resistance of *E. coli* to Carbapenems, a class of antibiotic often considered as a last resort drug used in the management of multidrug-resistant gram-negative bacilli (Bharadwaj et al. 2018; Garcia 2013), is very worrying. *E. coli* has become intrinsically resistant to beta-lactamases due to the emergence of organisms carrying extended spectrum beta-lactamases (ESBLs) and plasmid mediated AmpC beta-lactamases (Nair and Vaz 2013). Carbapenem resistance is attributed to three main mechanisms: porin-mediated resistance, which reduces the uptake of Carbapenems, efflux pumps that expel the Carbapenems outside the cell and enzyme-mediated resistance effected through the acquisition of Carbapenemase genes (Codjoe and Donkor 2017; Elshamy and Aboshanab 2020; Nordmann et al. 2011). Therefore, the overall objective of this study was to characterize the Carbapenem-resistant *E. coli* from sewage using molecular techniques.

The prevalence of Carbapenem-resistant *E. coli* (CREC) in this study was 24%. This finding is comparable with other studies in Uganda and Africa that showed a 22.4% CREC prevalence isolated from clinical samples in Uganda (Okoche et al. 2015), 27.1% CREC prevalence isolated from the blood, urine, and wounds in Egypt (Kotb et al. 2020), and elsewhere in China indicating a 4/17 (23.5%) prevalence of CREC in effluent samples (Zhang et al. 2020). The high prevalence of 13/25 (52%) CREC from the 1C ward manhole site could be due to the many patients admitted with different health complications and severities requiring antibiotic treatment including those presenting Carbapenem resistance. Moreover, there is also a similar report showing presence of antibiotic resistance in fecal and body fluids from both healthy and non-healthy human beings (Gwenzi 2020). While the low prevalence of 3/25 (12%) CREC from the mortuary manhole site could be due to persistence of antibiotic-resistant bacteria and their resistant genes even after one's death (Gwenzi 2020) that run off with the waste water used in cleaning of the corpses.

In the present study, we found that 60% Imipenem-resistant isolates and 64% Meropenem-resistant isolates were positive for bla_{KPC}, bla_{NDM}, and bla_{VIM} Carbapenemase genes. Out of the 16 Carbapenem-resistant *E. coli* isolates, 15 (93.8%) were identified with bla_{KPC} (most abundant) gene, 6 (37.5%) had bla_{NDM} gene, and 1 (6.3%) had bla_{VIM} gene. The results were in agreement with another study by Okoche et al. 2015, in Uganda, in which bla_{VIM}, bla_{KPC}, and bla_{NDM} genes were obtained, although bla_{VIM} (10.7%) was the most prevalent gene, followed by bla_{OXA-48} (9.7%), bla_{IMP} (6.1%), bla_{KPC} (5.1%), and bla_{NDM-1} (2.6%) (Okoche et al. 2015). In contrast, our study findings were not in agreement with a study conducted in Khartoum-Sudan by Mahmoud et al. 2020, which showed that of the 86% of the Imipenem-resistant *E. coli* isolates, 15.5% harbored the bla_{OXA-48}, as the most abundant gene and no bla_{KPC} was detected (Mahmoud et al. 2020). Furthermore, a study conducted in the USA by Hoelle et al. 2019, which showed that about 55% of *E. coli* isolates were positive for bla_{VIM} gene and 1% were

Table 9 Number of *E. coli* isolates identified with PAI and phylogenetic markers

Manhole	Susceptibility	PAI marker	Phylogenetic markers		
		PAI IV_{536}	*yjaA*	TSEP4.C2	*chuA*
1C Ward	Resistant	1 (50%)	8 (53.3%)	1 (100%)	0
	Susceptible	1 (50%)	0	0	0
Hallway	Resistant	0	4 (26.7%)	0	0
	Susceptible	0	1 (6.7%)	0	1 (100%)
Mortuary	Resistant	0	2 (13.3%)	0	0
	Susceptible	0	0	0	0
	Total	2 (10%)	15 (60%)	1 (4%)	1 (4%)

positive for IMP gene (Hoelle et al. 2019). The variations may be due to the difference in the samples collected, site of collection, and their large sample sizes. On the other hand, the CREC isolates were negative for bla_{OXA-48}, bla_{IMP-1}, and bla_{IMP-2} genes as well as all susceptible isolates in this study did not harbor Carbapenemase genes. Failure to detect bla_{OXA-48} type producers could be due to their point mutant analogs with ESBLs, resulting into the most difficult Carbapenemase producers to be identified (Nordmann et al. 2011; Queenan and Bush 2007). Resistance to Carbapenems may be due to intrinsic and or acquired resistance mechanisms for both commensals and pathogenic bacteria (Codjoe and Donkor 2017).

Out of the twenty amplified samples, 16 CREC isolates and four susceptible isolates were pathotyped for six virulence genes, seven PAI markers, and three phylogenetic markers. Among the six virulence genes, only four genes were identified: *Eae* 8 (50%) as the most prevalent gene, followed by *elt* 5 (31.3%), *est* 1(6.3%), and *paH* with 1 (6.3%). These results deduce presence of enteropathogenic *E. coli* (EPEC) strains as most abundant pathogenic *E. coli* with *eae* genes, followed by enterotoxigenic *E. coli* (ETEC) strains with *elt* and *est* genes and then enteroinvasive *E. coli* (EIEC) with the *paH* gene. The four identified virulence genes were obtained from among the CREC isolates and were all negative for *stx* and *aggR* genes. It was not surprising that all susceptible isolates did not harbor any virulence genes and hence regarded as non-pathogenic *E. coli* strains.

Furthermore, *E. coli* associated infections outside the gastrointestinal tract for example meningitis, urinary tract infections (UTI), septicemia, and pneumonia, are caused by extra-intestinal pathogenic *E. coli* (Kaper et al. 2004; Russo and Johnson 2000). ExPEC pathogenicity is determined by the existence of virulence genes found either on the plasmids or chromosomes. If located on the chromosomes, the virulence factors are normally situated in specific regions termed as pathogenicity islands (PAI) (Johnson and Stell 2000; Sabaté et al. 2006; Sadat et al. 2022; Tangi et al. 2015). In this study, a multiplex PCR was employed to detect the pathogenicity islands. Of the seven PAI markers, only PAI IV_{536} (2/10%) sequence was identified, from 1 (6.3%) CREC isolate and 1 (25%) susceptible isolate. PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{536}, and PAI II_{536} genes were found negative from the isolates. Results show presence of PAI IV_{536} belonging to the uropathogenic *E. coli* (UPEC) pathogenicity islands (da Silva et al. 2017; Dobrindt et al. 2002) identified in the CREC isolate. This is in agreement with Ssekatawa et al. (2021b) who reported PAI IV_{536} as the most predominant pathogenicity island typed in *E. coli* archived isolates obtained from the Microbiology Laboratory of Mulago National Referral Hospital (Ssekatawa et al. 2021a). Several studies have found PAI IV536 to be of significantly high prevalence and hence named it high pathogenicity island (HPI) (Middendorf et al. 2001; Tangi et al. 2015). While the presence of the PAI IV_{536} gene in the susceptible isolate maybe explained as a result of horizontal gene transfer mechanisms mediated by mobile genetic elements (da Silva et al. 2017; Dobrindt et al. 2002). Of the three phylogenetic markers pathotyped, *yjaA* 15 (60%) was the most abundant gene, followed by TSPE4.C2 and *chuA* genes with 1 (4%) prevalence. We have also reported that *yjaA* gene had a 14 (93.3%) prevalence in CREC isolates and 1 (25%) prevalence in susceptible isolates while TSPE4.C2 and *chuA* had a 1 (25%) prevalence in susceptible isolates, both of which were not harbored in any of the CREC isolates. The presence of *yjaA* genes, belonging to phylogenetic group B2 is conclusive for virulent extra-intestinal pathogenic *E. coli* (ExPEC) (Clermont et al. 2000), identified in the CREC isolates.

In this study, we detected the Carbapenemase gene in *E. coli* with phenotypical resistance to two carbapenem drugs, Meropenem and Imipenem, but only screened for the Carbapenemases commonly produced (KPC, NDM, VIM, OXA-48, IMP-1, and IMP-2) as the resistance mechanisms using Carbapenemase primers. This might be due to the limited number of genes targeted in our study as well as to other mechanisms of resistance such as efflux pumps and porin loss/mutations (Codjoe and Donkor 2017).

An increase in the prevalence of antibiotic resistant pathogenic and commensal *E. coli* strains has been reported worldwide. Antibiotic resistance in *Enterobacteriaceae* is mainly due to the expression of beta-lactamase enzymes that breakdown the peptide bond of the beta-lactam ring rendering them inactive. Carbapenemases are the most clinically significant beta-lactamases because they can hydrolyze all beta-lactam antibiotics. Thus, Carbapenemase expressing *Enterobacteriaceae* are resistant to all beta-lactam antibiotics including Carbapenems yet Carbapenems are the most suitable antibiotics for the treatment gram-negative bacterial infections when other drugs have failed (Nordmann et al. 2012; Ssekatawa et al. 2021b). Therefore, this study evaluated the coexistence of Carbapenem resistance and virulence genetic factors among *E. coli* phylogroups. We observed that all the 16 (100%) and 1 (5%) CREC isolates had DEC virulence genes and PAI IV_{536}, while 93.3% (14/16) of the CREC isolates belonged to phylogenetic group B2 that mainly contains virulent (ExPEC) (Clermont et al. 2000). Similar results were documented by Ssekatawa et al. (2021a, b).

Co-existence of virulence genes, Carbapenem resistance genes, PAI, and yjaA markers that signifies

phylogenetic groups B2 in environmental *E. coli* isolates should be considered as an immense threat as they can lead to severe hard to treat hospital-acquire infections.

Limitations to our study include the few samples collected that did not represent a large population of the *E. coli* present in hospital sewage, the use of few primers that did not target all known Carbapenemase genes as well as the few pathotyping primers that did not target all known virulence genes due to the limited research budget. Despite the limitations, the study provided the prevalence of CREC in hospital sewage harboring virulence genes and the magnitude of the problem. Finally, the presence of CREC in hospital sewage calls for further investigations to identify all the possible CREC pathotypes that might pose a health-risk to the community living near the hospital sewage drainage system. Furthermore, PAI IV_{536} mainly harbors the Yersiniabactin siderophore iron-uptake. However, we never attempted to genotype the Yersiniabactin siderophore iron uptake system genes in PAI IV_{536}.

Conclusion

In this study, the prevalence of CREC isolated from sewage manholes within Mulago National Referral Hospital, Kampala, was 24%. The prevalence of the investigated virulence genes showed abundancy of various *E. coli* pathovars such as EPEC, ExPEC, ETEC, and EIEC in the sewage hence a potential major reservoir of pathogenic bacteria harboring virulence genes. Therefore, the presence of pathogenic and non-pathogenic Carbapenem-resistant *E. coli* in the sewage is a threat to management of bacterial infections in clinical settings in Uganda. Highlighting the importance of surveillance of hospital sewage treatment before its release into the common waste and close monitoring to eliminate potential health risks for humans and animals.

Abbreviations

CREC	Carbapenem-resistant *E. coli*
COVAB	College of veterinary Medicine, Animal Resources and Biosecurity
DAEC	Diffusely adherent *E. coli*
DNA	Deoxyribonucleic acid
EHEC	Entero-hemorrhagic *E. coli*
EIEC	Entero-invasive *E. coli*
EPEC	Entero-pathogenic *E. coli*
ETEC	Entero-toxigenic *E. coli*
ESBL	Extended-spectrum beta lactamases
EXPEC	Extra-intestinal pathogenic *E. coli*
IMP	Imipenem
MEM	Meropenem
PAI	Pathogenicity islands
PBP	Penicillin binding proteins
PCR	Polymerase chain reaction
STEC	Shiga toxin producing *Escherichia coli*
TSI	Triple sugar iron

Acknowledgements

We thank madam Monica Nambi for her technical contribution during the laboratory procedures.

Authors' contributions

BJ participated in the data collection and writing of the manuscript; SK participated in the data collection, data analysis, and coordination of laboratory activities; GN participated in the data collection; AK participated in the data collection, data analysis, and editing of the manuscript; JN participated in the data collection, data analysis, and manuscript review; KCD participated in securing the funding and manuscript writing; and SLF participated in the manuscript writing, securing funding, and manuscript submission.

Funding

This study was funded by the Government of Uganda, through the Makerere University Research and Innovation fund [MAK-RIF/IND-RD2/1730], which enabled us to conduct this research to its completion.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files attached).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]School of Biosecurity, Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P. O. Box 7072, Kampala, Uganda. [2]Department of Science, Technical and Vocational Education, Makerere University, P. O. Box 7062, Kampala, Uganda. [3]Department of Medical Physiology, College of Health Sciences, Makerere University, P. O. Box 7072, Kampala, Uganda.

Received: 18 April 2023 Accepted: 7 August 2023
Published online: 22 August 2023

References

Bakshi R, Walia G, Shikha J (2013) Prevalence of extended spectrum β-lactamases in multidrug resistant strains of gram negative Bacilli. J Acad Indus Res 1(9):558–560

Bartholomew JW, Mittwer T (1952) The Gram stain. Bacteriol Rev 16(1):1–29. https://doi.org/10.1128/br.16.1.1-29.1952

Basak S, Singh P, Rajurkar M (2016) Multidrug resistant and extensively drug resistant bacteria: a study. J Pathog 2016:4065603. https://doi.org/10.1155/2016/4065603

Benathen IA (1992) A simple alternative to the IMViC test in microbiology. Am Biol Teach 54(2):112–113

Bharadwaj R, Robinson ML, Balasubramanian U, Kulkarni V, Kagal A, Raichur P, Khadse S, Kadam D, Valvi C, Kinikar A, Kanade S, Suryavanshi N, Marbaniang I, Nelson G, Johnson J, Zenilman J, Sachs J, Gupta A, Mave V (2018) Drug-resistant *Enterobacteriaceae* colonization is associated with healthcare utilization and antimicrobial use among inpatients in Pune, India. BMC Infect Dis 18(1):504. https://doi.org/10.1186/s12879-018-3390-4

Birgy A, Bidet P, Genel N, Doit C, Decré D, Arlet G, Bingen E (2012) Phenotypic screening of carbapenemases and associated β-lactamases in carbapenem-resistant *Enterobacteriaceae*. J Clin Microbiol 50(4):1295–1302. https://doi.org/10.1128/jcm.06131-11

Bou-Antoun S, Davies J, Guy R, Johnson AP, Sheridan EA, Hope RJ (2016) Descriptive epidemiology of *E. coli* bacteraemia in England, April 2012 to March 2014. Euro Surveill 21(35):30329. https://doi.org/10.2807/1560-7917.Es.2016.21.35.30329

Bush K, Jacoby GA (2010) Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 54(3):969–976. https://doi.org/10.1128/aac.01009-09

Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the *E. coli* phylogenetic group. Appl Environ Microbiol 66(10):4555–4558. https://doi.org/10.1128/aem.66.10.4555-4558.2000

Codjoe FS, Donkor ES (2017) Carbapenem resistance: a review. Med Sci (Basel) 6(1):1. https://doi.org/10.3390/medsci6010001

Cowan ST (1993) Cowan and Steel's manual for the identification of medical bacteria. (G. I. Barrow & R. K. A. Feltham, Eds. 3 ed.). Cambridge University Press. https://doi.org/10.1017/CBO9780511527104

Cusack TP, Phimolsarnnousith V, Duangmala K, Phoumin P, Turton J, Hopkins KL, Woodford N, Shetty N, Stoesser N, Phan HTT, Dance DAB (2019) Molecular characterization of carbapenem-resistant *E. coli* and Acinetobacter baumannii in the Lao People's Democratic Republic. J Antimicrob Chemother 74(9):2810–2821. https://doi.org/10.1093/jac/dkz234

da Silva LC, de Mello Santos AC, Silva RM (2017) Uropathogenic *E. coli* pathogenicity islands and other ExPEC virulence genes may contribute to the genome variability of enteroinvasive *E. coli*. BMC Microbiol 17(1):68. https://doi.org/10.1186/s12866-017-0979-5

Dias MT, Bricio SML, Almeida DO, Oliveira LAT, de Filippis I, Marin VA (2012) Molecular characterization and evaluation of antimicrobial susceptibility of enteropathogenic *E. coli* (EPEC) isolated from minas soft cheese. Food Sci Technol 32:747–753

Dobrindt U, Blum-Oehler G, Nagy G, Schneider G, Johann A, Gottschalk G, Hacker J (2002) Genetic structure and distribution of four pathogenicity islands (PAI I(536) to PAI IV(536)) of uropathogenic *E. coli* strain 536. Infect Immun 70(11):6365–6372. https://doi.org/10.1128/iai.70.11.6365-6372.2002

Elazhary MA, Saheb SA, Roy RS, Lagacé A (1973) A simple procedure for the preliminary identification of aerobic gram negative intestinal bacteria with special reference to the *Enterobacteriaceae*. Can J Comp Med 37(1):43–46

Elshamy AA, Aboshanab KM (2020) A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. Future Sci OA 6(3):Fso438. https://doi.org/10.2144/fsoa-2019-0098

Eltai NO, Yassine HM, Al Thani AA, Abu Madi MA, Ismail A, Ibrahim E, Alali WQ (2018) Prevalence of antibiotic resistant *E. coli* isolates from fecal samples of food handlers in Qatar. Antimicrob Resist Infect Control 7:78. https://doi.org/10.1186/s13756-018-0369-2

Garcia M (2013) Carbapenemases: a real threat. APUA Newsl 31:4–6

Gwenzi W (2020) The 'thanato-resistome'- the funeral industry as a potential reservoir of antibiotic resistance: early insights and perspectives. Sci Total Environ 749:141120. https://doi.org/10.1016/j.scitotenv.2020.141120

Hoelle J, Johnson JR, Johnston BD, Kinkle B, Boczek L, Ryu H, Hayes S (2019) Survey of US wastewater for carbapenem-resistant *Enterobacteriaceae*. J Water Health 17(2):219–226. https://doi.org/10.2166/wh.2019.165

Johnson JR, Stell AL (2000) Extended virulence genotypes of *E. coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 181(1):261–272. https://doi.org/10.1086/315217

Joji RM, Al-Rashed N, Saeed NK, Bindayna KM (2019) Detection of VIM and NDM-1 metallo-beta-lactamase genes in carbapenem-resistant *Pseudomonas aeruginosa* clinical strains in Bahrain. J Lab Physicians 11(2):138–143. https://doi.org/10.4103/jlp.Jlp_118_18

Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic *E. coli*. Nat Rev Microbiol 2(2):123–140. https://doi.org/10.1038/nrmicro818

Kateete DP, Nakanjako R, Namugenyi J, Erume J, Joloba ML, Najjuka CF (2016) Carbapenem resistant *Pseudomonas aeruginosa* and Acinetobacter baumannii at Mulago Hospital in Kampala, Uganda (2007–2009). Springerplus 5(1):1308. https://doi.org/10.1186/s40064-016-2986-7

Khalil RAD, Ibrahim AM, Mohamed MBB (2017) Clinical microbiology: open access

Kong KF, Schneper L, Mathee K (2010) Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. Apmis 118(1):1–36. https://doi.org/10.1111/j.1600-0463.2009.02563.x

Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, Hafez S, El-Kholy J, Zaki MES, Rashed HG, Khalil GM, Sayyouh O, Talaat M (2020) Epidemiology of Carbapenem-resistant *Enterobacteriaceae* in Egyptian intensive care units using National Healthcare-associated Infections Surveillance Data, 2011–2017. Antimicrob Resist Infect Control 9(1):2. https://doi.org/10.1186/s13756-019-0639-7

Kumar S, Vyas A, Mehra S (2015) Utilization of MacConkey-Meropenem screening agar for the detection of carbapenem resistanant *Enterobacteriaceae* in a tertiary care hospital. SSRG Int J Med Sci (SSRG – IJMS) 2(4):1–5

Lahlaoui H, Ben Haj Khalifa A, Ben Moussa M (2014) Epidemiology of *Enterobacteriaceae* producing CTX-M type extended spectrum β-lactamase (ESBL). Med Mal Infect 44(9):400–404. https://doi.org/10.1016/j.medmal.2014.03.010

López-Saucedo C, Cerna JF, Villegas-Sepulveda N, Thompson R, Velazquez FR, Torres J, Tarr PI, Estrada-García T (2003) Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *E. coli*. Emerg Infect Dis 9(1):127–131. https://doi.org/10.3201/eid0901.010507

Madic J, Peytavin de Garam C, Vingadassalon N, Oswald E, Fach P, Jamet E, Auvray F (2010) Simplex and multiplex real-time PCR assays for the detection of flagellar (H-antigen) fliC alleles and intimin (eae) variants associated with enterohaemorrhagic *E. coli* (EHEC) serotypes O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7. J Appl Microbiol 109(5):1696–1705. https://doi.org/10.1111/j.1365-2672.2010.04798.x

Mahmoud NE, Altayb HN, Gurashi RM (2020) Detection of Carbapenem-resistant genes in *E. coli* isolated from drinking water in Khartoum, Sudan. J Environ Public Health 2020:2571293. https://doi.org/10.1155/2020/2571293

Meletis G (2016) Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis 3(1):15–21. https://doi.org/10.1177/2049936115621709

Middendorf B, Blum-Oehler G, Dobrindt U, Mühldorfer I, Salge S, Hacker J (2001) The pathogenicity islands (PAIs) of the uropathogenic *E. coli* strain 536: island probing of PAI II536. J Infect Dis 183 Suppl 1:S17-20. https://doi.org/10.1086/318843

Miller JM, Wright JW (1982) Spot indole test: evaluation of four reagents. J Clin Microbiol 15(4):589–592. https://doi.org/10.1128/jcm.15.4.589-592.1982

Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F (2014) Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed Res Int 2014:303104. https://doi.org/10.1155/2014/303104

Nair PK, Vaz MS (2013) Prevalence of carbapenem resistant *Enterobacteriaceae* from a tertiary care hospital in Mumbai, India. J Microbiol Infect Dis 3(04):207–210

Nordmann P, Naas T, Poirel L (2011) Global spread of Carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 17(10):1791–1798. https://doi.org/10.3201/eid1710.110655

Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 18(9):1503–1507. https://doi.org/10.3201/eid1809.120355

Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF (2015) Prevalence and characterization of Carbapenem-resistant *Enterobacteriaceae* isolated from Mulago National Referral Hospital, Uganda. PLoS One 10(8):e0135745. https://doi.org/10.1371/journal.pone.0135745

Onanuga A, Igbeneghu O, Lamikanra A (2014) A study of the prevalence of diarrhoeagenic *E. coli* in children from Gwagwalada, Federal Capital Territory, Nigeria. Pan Afr Med J 17:146. https://doi.org/10.11604/pamj.2014.17.146.3369

Poirel L, Kieffer N, Liassine N, Thanh D, Nordmann P (2016) Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of *E. coli*. Lancet Infect Dis 16(3):281. https://doi.org/10.1016/s1473-3099(16)00006-2

Queenan AM, Bush K (2007) Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 20(3):440–458, table of contents. https://doi.org/10.1128/cmr.00001-07

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL (2005) Epidemiology of *E. coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 11(4):603–609. https://doi.org/10.3201/eid1104.040739

Rojas-Lopez M, Monterio R, Pizza M, Desvaux M, Rosini R (2018) Intestinal pathogenic *E. coli*: insights for vaccine development. Front Microbiol 9:440. https://doi.org/10.3389/fmicb.2018.00440

Russo TA, Johnson JR (2000) Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *E. coli*: ExPEC. J Infect Dis 181(5):1753–1754. https://doi.org/10.1086/315418

Sabaté M, Moreno E, Pérez T, Andreu A, Prats G (2006) Pathogenicity island markers in commensal and uropathogenic *E. coli* isolates. Clin Microbiol Infect 12(9):880–886. https://doi.org/10.1111/j.1469-0691.2006.01461.x

Sadat A, Ramadan H, Elkady MA, Hammad AM, Soliman MM, Aboelenin SM, Al-Harthi HF, Abugomaa A, Elbadawy M, Awad A (2022) Phylotypic profiling, distribution of pathogenicity island markers, and antimicrobial

susceptibility of *E. coli* isolated from retail chicken meat and humans. Antibiotics (Basel) 11(9):1197. https://doi.org/10.3390/antibiotics11091197

Sato T, Shimizu T, Watarai M, Kobayashi M, Kano S, Hamabata T, Takeda Y, Yamasaki S (2003) Genome analysis of a novel Shiga toxin 1 (Stx1)-converting phage which is closely related to Stx2-converting phages but not to other Stx1-converting phages. J Bacteriol 185(13):3966–3971. https://doi.org/10.1128/jb.185.13.3966-3971.2003

Sethabutr O, Echeverria P, Hoge CW, Bodhidatta L, Pitarangsi C (1994) Detection of Shigella and enteroinvasive *E. coli* by PCR in the stools of patients with dysentery in Thailand. J Diarrhoeal Dis Res 12(4):265–269

Shin J, Oh SS, Oh KH, Park JH, Jang EJ, Chung GT, Yoo CK, Bae GR, Cho SH (2015) An outbreak of foodborne illness caused by enteroaggregative *E. coli* in a high school in South Korea. Jpn J Infect Dis 68(6):514–519. https://doi.org/10.7883/yoken.JJID.2014.460

Ssekatawa K, Byarugaba DK, Nakavuma JL, Kato CD, Ejobi F, Tweyongyere R, Wampande EM (2021a) Carbapenem resistance profiles of pathogenic *E. coli* in Uganda. Eur J Biol Biotechnol 2(2):63–73. https://doi.org/10.24018/ejbio.2021.2.2.171

Ssekatawa K, Byarugaba DK, Nakavuma JL, Kato CD, Ejobi F, Tweyongyere R, Eddie WM (2021b) Prevalence of pathogenic *Klebsiella pneumoniae* based on PCR capsular typing harbouring carbapenemases encoding genes in Uganda tertiary hospitals. Antimicrob Resist Infect Control 10(1):57. https://doi.org/10.1186/s13756-021-00923-w

Steiner TS, Guerrant RL (2011) *E. Coli* enteric infections. Vol. 2. https://doi.org/10.1016/B978-1-4377-1604-7.00312-2

Tangi SC, Tajbakhsh E, Soleimani NA, Shahraki MM (2015) Prevalence of pathogenicity island markers genes in uropathogenic *E. coli* isolated from patients with urinary tract infectious. Asian Pac J Trop Dis 5(8):662–666. https://doi.org/10.1016/S2222-1808(15)60909-4

Thakur P, Chawla R, Tanwar A, Chakotiya AS, Narula A, Goel R, Arora R, Sharma RK (2016) Attenuation of adhesion, quorum sensing and biofilm mediated virulence of carbapenem resistant *E. coli* by selected natural plant products. Microb Pathog 92:76–85. https://doi.org/10.1016/j.micpath.2016.01.001

Torres AG, Arenas-Hernández MMP, Martínez-Laguna Y (2010) Overview of *E coli*. In: Pathogenic *E. coli* in Latin America. pp 1–7. https://doi.org/10.2174/978160805192211001010001

Wang S, Zhao SY, Xiao SZ, Gu FF, Liu QZ, Tang J, Guo XK, Ni YX, Han LZ (2016) Antimicrobial resistance and molecular epidemiology of *E. coli* causing bloodstream infections in three hospitals in Shanghai, China. PLoS One 11(1):e0147740. https://doi.org/10.1371/journal.pone.0147740

Weinstein MP, Patel JB, Burnham C-A, Campeau S, Conville PS, Doern C, Eliopoulos GM, Galas MF, Satlin M, Humphries RM, Swenson JM, Jenkins SG, Wong A, Kircher SM, Wang WF, Lewis II JS, Zimmer BL (2018) Performance Standards for Antimicrobial Disk Susceptibility Tests. In C. a. L. S. Institute (Ed.), CLSI Microbiology documents (13 ed., pp. 1-13). Clinical and Laboratory Standards Institute. https://clsi.org/standards/products/microbiology/documents/m02/

Zainol Abidin NZ, Sulong A, Alfizah H, Muttaqillah NA, Ding CH (2015) Molecular detection of the New Delhi metallo-β-lactamase-1 gene in *Enterobacteriaceae* isolates in a tertiary medical centre. Malays J Pathol 37(3):227–232

Zhang L, Ma X, Luo L, Hu N, Duan J, Tang Z, Zhong R, Li Y (2020) The prevalence and characterization of extended-spectrum β-Lactamase- and Carbapenemase-producing bacteria from hospital sewage, treated effluents and receiving rivers. Int J Environ Res Public Health 17(4):1183. https://doi.org/10.3390/ijerph17041183

Zinnah M, Bari M, Islam M, Hossain M, Rahman M, Haque M, Babu S, Ruma R, Islam M (2007) Characterization of *Escherichia coli* isolated from samples of different biological and environmental sources. Bangladesh J Vet Med 5(1):25–32

Publisher's Note