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Carbapenem Resistance Profiles of Pathogenic *Escherichia coli* in Uganda

Kenneth Ssekatawa, Denis K. Byarugaba, Jesca L. Nakavuma, Charles D. Kato, Francis Ejobi, Robert Tweyongyere, and Eddie M. Wampande

ABSTRACT

***Escherichia coli* has been implicated as one of the main etiological agents of diarrhea, urinary tract infections, meningitis and septicemia worldwide. The ability to cause diseases is potentiated by presence of virulence factors. The virulence factors influence the capacity of *E. coli* to infect and colonize different body systems. Thus, pathogenic *E. coli* are grouped into DEC strains that are mainly clustered in phylogenetic group B1 and A; ExPEC belonging to A, B2 and D. Coexistence of virulence and beta-lactamase encoding genes complicates treatment outcomes. Therefore, this study aimed at presenting the carbapenem resistance (CR) profiles among pathogenic E. coli. This was a retrospective cross-sectional study involving use of 421 archived *E. coli* clinical isolates collected in 2019 from four Uganda tertiary hospitals. The isolates were subjected to antibiotics sensitivity assays to determine phenotypic resistance. Four sets of multiplex PCR were performed to detect CR genes, DEC pathotypes virulence genes, ExPEC PAI and the E. coli phylogenetic groups. Antibiotic susceptibility revealed that all the 421 E. coli isolates used were MDR as they exhibited 100% resistance to more than one of the first-line antibiotics. The study registered phenotypic and genotypic CR prevalence of 22.8% and 33.0% respectively. The most predominant gene was blaOXA-48 with genotypic frequency of 33.0%, then bla_{VIM} (21.0%), bla_{IMP} (16.5%), bla_{KPC} (14.8%) and bla_{NDM} (14.8%). Spearman's correlation revealed that presence of CR genes was highly associated with phenotypic resistance. Furthermore, of 421 MDR E. coli isolates, 19.7% harboured DEC virulence genes, where EPEC recorded significantly higher prevalence (10.8%) followed by S-ETEC (3.1%), STEC (2.9%), EIEC (2.0%) and L-ETEC (2.0%). Genetic analysis characterized 46.1% of the isolates as ExPEC and only PAI IV536 (33.0%) and PAI IICFT073 (13.1%) were detected. Phylogenetic group B2 was predominantly detected (41.1%), followed by A (30.2%), B1(21.6%), and D (7.1%). Furthermore, 38.6% and 23.1% of the DEC and ExPEC respectively expressed phenotypic resistance. Our results exhibited significant level of CR carriage among the MDR DEC and ExPEC clinical isolates belonging to phylogenetic groups B1 and B2 respectively. Virulence and CR genetic factors are mainly located on mobile elements. Thus, constitutes a great threat to the healthcare system as this promotes horizontal gene transfer.**

Keywords: Carbapenem resistance, Virulence genes, Pathogenicity islands, Pathogenic E. coli, E. coli phylogenetic groups.

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I. Introduction

Escherichia coli is one of the most prevalent commensals of the human gastro-intestinal tract (GIT) microbiotas. However, some *E. coli* are pathogenic. Pathogenic *E. coli* comprises of diarrheagenic *E. coli* (DEC) [1] and Extra-intestinal pathogenic *E. coli (*ExPEC) pathotypes [2]. Diarrheagenic pathotypes are responsible for all gastrointestinal tract *E. coli* infections most importantly diarrhea. Diarrhea is one of the principal causes of illness and

death among children under 5 years in developing countries and DEC pathotypes account for the biggest percentage. Reaching protective immunity against DEC in children is hard as DEC is composed of a wide range of pathotypes, hence variant antigens. Extra-intestinal pathogenic *E. coli* is accountable for all *E. coli* associated infections outside the gastrointestinal tract, such as meningitis, urinary tract infections (UTI), pneumonia, septicemia, among others [3-5]. An alarming prevalence of bacterial UTI has been registered in primary healthcare. *Escherichia coli* has been implicated to be the main cause of both community and nosocomial acquired UTI worldwide.

Diarrheagenic *E. coli* are grouped into eight pathotypes basing on the virulence factors responsible for their pathogenicity. These include Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Diffusely Adherent *E. coli* (DAEC), Shiga toxin-producing *E. coli* (STEC) also commonly known as enterohemorrhagic *E. coli* (EHEC) or Verotoxigenic *E. coli* (VTEC), the newly identified adherent invasive *E. coli* (AIEC) which is alleged to been associated with Crohn's disease but not with any diarrheagenic infections and a hybrid pathotype known as enteroaggregative hemorrhagic *E. coli* (EAHEC) carrying STEC and EAEC virulence genetic determinants [3]. Thus, pathogenic DEC encompasses a genetically heterogeneous family of *E. coli* with a plastic genome. Several research articles suggest that each pathotype possesses and codes for distinctive virulence and colonization determinants harboured in their genomes distinguishing them from other pathotypes and non-virulent strains. These virulence factors for each pathotype are encoded for by conserved genes and are restricted within geographical boundaries [6, 7]. Therefore, molecular typing of *Escherichia coli* to identify the different DEC pathotypes can be achieved by targeting the virulence genes. These virulence genes include: *eae* for typing of EPEC; *stx* for STEC/EHEC; *est* for ts-ETEC; *elt* for tl-ETEC, *aggR* for EAEC; *ipaH* for EIEC. *eae* gene is translated into Intimin polypeptide which is the key factor for attaching and effacing lesions; *stx* gene encodes for the Shiga-like toxin; *elt* and *est* genes are translated into Thermolabile and Thermostable toxins respectively; *ipaH* gene accounts for invasion capacity and *aggR* gene is translated into a transcriptional activator protein of aggregative adherence fimbriae [8].

The most clinically significant pathotypes of ExPEC are uropathogenic *E. coli* (UPEC) responsible for UTIs and neonatal meningitis *E. coli*, (NMEC) causing meningitis and septicemia [9]. ExPEC pathogenicity is accounted for by presence of virulence factors encoding genes located either on plasmids or chromosomes. These virulence genes are characteristically positioned in particular regions known as pathogenicity islands (PAI) if found on the chromosome [10]. Therefore molecular typing of ExPEC pathotypes can be based on multiplex PCR amplification of PAI markers previously characterized in UPEC chromosomal genes encoding virulence factors such as hemolysins (hlyA and hlyF), cytotoxic necrotizing factors (cnf1 and cnf 2), colicin V (cvaC), aerobactin (iutA), yersiniabactin (fyuA), salmochelin (iroN), P-fimbriae (papC and papG), S-fimbrial adhesin (sfaA and sfaS), afimbrial adhesin (afa), serum resistance (iss and traT), brain microvascular endothelium invasion (ibe10), K1 capsule (kpsII and K1), and ompT outer membrane protein (ompT) [11, 12].

Furthermore, PCR analysis clusters *E. coli* strains into A, B1, B2, and D phylogenetic groups due to the presence of the chuA and yjaA genes as well as TSPE4.C2 DNA fragment [13]. The intestinal pathogenic *E. coli* strains belong to groups A, B1 and D, extraintestinal pathogenic *E. coli* strains are generally placed under groups B2 and D, while commensal *E. coli* strains belong to groups A and B1 [13, 14].

High levels antibiotic resistance in Enterobacteriaceae is of great concern to the healthcare system. *Escherichia coli* like other Enterobacteriaceae has evolved to acquire different mechanisms of antibiotic resistance which confer protection to lethal doses of different classes of antibiotics. Carbapenems are the most suitable antibiotics used in the treatment of multidrug resistant (MDR) gram-negative bacteria infections. Studies have documented high prevalence of carbapenem resistant Enterobacteriaceae (CRE) in Uganda [15, 16]. However, the carbapenem resistance profiles of DEC and ExPEC human isolates have not been investigated, yet for meaningful treatment outcomes and prescription decisions, knowledge about pathogen susceptibility patterns to antibiotics in question is very important. Thus, this study was aimed at profiling the carbapenem resistance profiles of intestinal and extraintestinal human pathogenic *E. coli* isolates for genetic markers allied with DEC and ExPEC strains. The study relied on the screening for DEC genetic markers, PAI associated sequences for ExPEC and determination of phylogenetic group and genetic determinants of carbapenem resistance (CR).

II. Materials and Methods

A. Study Design, Site and Source of Bacteria Isolates

This was a cross sectional-laboratory-based study conducted at the Microbiology Laboratory and Molecular Biology Laboratory, College of Veterinary Medicine Animal Resources and Biosecurity (CoVAB) Makerere University. The study involved use of archived MDR *Escherichia coli* samples isolated between January and December 2019 from clinical specimens in the Microbiology Laboratories of Mulago National Referral Hospital (MNRH), Mbale Regional Referral Hospital (MRRH), Mbarara Regional Referral Hospital (MBRRH) and Kampala International University Teaching Hospital (KIU-TH). The samples were transported in peptone water to the Microbiology Laboratory, CoVAB. Overnight cultures of *E. coli* were prepared by pipetting 1 ml of peptone water containing each isolate into 49 ml of Luria-Bertani (LB) broth. Glycerol stocks of the isolate were prepared by adding 500µl of the overnight LB culture to 500 µL of 50% glycerol in a 2 ml screw top tube and mixed gently mix. The screw tubes were stored at -80 ºC until further use.

B. Biochemical Assays to Confirm the Identity of E. coli

To confirm the identity of each isolate, Microgen (Microbiology International) kits for biochemical assays were employed using procedures described by the manufacturer (www.microgenbioproducts.com).

C. Screening for Carbapenem Susceptibility

This was achieved using the Kirby Bauer Disk Diffusion method and the results obtained were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. Ampicillin (AMP) 25 μg, Amoxicillin/clavulanic acid (AMO) 20/10 μg, trimethoprim-sulfamethoxazole (TMP/SMX) 1.25/23.75 μg, Ciprofloxacin (CIP) 5 μg, Cefuroxime (CXM) 30 µg, Temocillin (TEM) 30 µg, Piperacillin-tazobactum (TPZ) 110 µg, Cefoxitin (FOX) 30 µg, Cefipime (FEP) 30µg, Ceftriaxone (CRO) 30 µg, Ceftazidime (CAZ) 30 µg, Cefotaxime (CTX) 30 µg, Ertapenem (ERT) 10 µg, Meropenem (MEM) 10 µg and Imipenem (IMI)10 µg (Oxoid United Kingdom) carbapenem antibiotics disks were used. The turbidity of overnight *Escherichia coli* broth was adjusted using peptone water to a standard uniform concentration of 0.5 McFarland. Each *E. coli* isolate was inoculated on Mueller Hinton agar (Oxoid, United Kingdom) plates. Three antibiotic discs were placed about 2.0 cm apart and from the edge of plates, then incubated at 37 °C for 24 hours. The diameter of the zones of growth inhibition were scored in millimeters. For quality control, *E. coli* ATCC 25922 was used as a susceptible strain and *Klebsiella pneumoniae* ATCC BAA-1705 as a positive control.

D. DNA Extraction

Pure colonies of *E. coli* from different samples were selected and each sub-cultured in 5 ml of Luria-Bertani broth using sterile inoculating loop. The bacterial suspension was incubated in a shaker incubator at 37 ºC for 24 hrs. Then, 1 ml of bacterial suspension was transferred into a 1.5 ml Eppendorf tube, centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was re-suspended in 200 µl of Gram-negative bacteria lysis buffer provided in the Qiagen DNA extraction. Bacterial total genomic DNA was extracted following the Qiagen DNA extraction protocol and stored at -20 ºC until further use.

E. Molecular Characterization of Virulence Genes and Carbapenem Resistance Determinants

Molecular identification of carbapenem resistance determinants and virulent genes in *Escherichia coli* was carried out using multiplex PCR. Primers used for molecular characterization were obtained from Eurofins Genomics AT GmbH and PCR amplification was performed in a Bio-Rad PTC-200 Thermal Cycler (Bio-Rad, Hercules, CA, USA)

F. Multiplex PCR Amplification of Carbapenem Resistance Genes

The existence of carbapenem resistance genetic determinants was determined using primers targeting bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{NDM} that carbapenemase encoding genes, Table I. For co-amplification of the target genes, multiplex PCR was conducted by adapting methods used by Dallenne et al. [18]. Briefly, 2.5 µl of template DNA (100 ng/ µl) was added to 47.5 µl PCR mix containing 200 µM dNTPs (Biomatik, USA), 0.5 µM of each primer pair and 1X PCR Buffer (1.5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.3/50 mM KCl) (Biomatik USA) and 1.2 µl of 1U *Taq* DNA Polymerase. Amplification was performed as follows; preliminary denaturation at 95 °C for 5 minutes; then denaturation at 95 °C for 30 seconds; annealing at 56 °C for 30 seconds and elongation at 72°C for 1 minute; and a final elongation at 72 °C for 10 minutes. For quality assurance positive and negative control isolates were obtained as a kind donation from the Microbiology Laboratory, MNRH. Antibiotics susceptible DSMZ 9377 *Klebsiella pneumoniae* was used as a negative control for all genes. *Klebsiella pneumonia* Nr.8 for NDM-1, *Klebsiella pneumoniae* 714 for OXA-48, *Klebsiella pneumoniae* 211 (T) for KPC, *P. aeruginosa* for IMP (Positive control strains from the Institute of Microbiology, Giessen, Germany) and *E. coli* for the VIM gene, obtained from RESET research collaboration [19] were used as positive controls.

TABLE I: CARBAPENEM RESISTANCE GENES AND RESPECTIVE PRIMERS

Gene	Primer sequence (5^1-3^1)	Band size (Bp)	Reference
Bla_{KPC}	F-ATG TCA CTG TAT CGC CGT CT R-TTT TCA GAG CCT TAC TGC CC	538	[18]
Bla_{IMP}	F-TGA GCA AGT TAT CTG TAT TC R-TTA GTT GCT TGG TTT TGA TG	139	[18]
Bla_{VIM}	F-GAT GGT GTT TGG TCG CAT A R-CGA ATG CGC AGC ACC AG	390	[18]
Bla_{NDM}	F-GGT TTG GCG ATC TGG TTT TC R-CGG AAT GGC TCA TCA CGA TC	822	[20]
$Bla_{\text{OXA-48}}$	F-TTG GTG GCA TCG ATT ATC GG R- GAG CAC TTC TTT TGT GAT GGC	281	[18]

G. Multiplex PCR Components and Conditions for E. coli Pathotyping

Virulence genes *eae* for EPEC; *stx* for STEC/EHEC; *est* for ts-ETEC; *elt* for tl-ETEC; *aggR* for EAEC; *ipaH* for EIEC were amplified by multiplex PCR to characterize the different pathogenic bacteria using primers outlined in Table II [8]. Five *E. coli* strains INCQS 00181 (CDC 055 – EPEC), INCQS 00171 (CDC EDL – 933 – EHEC) and INCQS 00170 (CDC EDL – 1284 – EIEC) from Centre for disease control and prevention belonging to the five categories of pathogenic *E. coli* were used as control [20]. Multiplex PCR reaction was performed using [21] modified method to enable the concurrent amplification of all the target genes. A final PCR volume of 50 µl containing 5 µl of 100 ng DNA sample, 25 µl of 1X PCR Buffer mixed with $MgCl_2$ (1.5 mM), 1.2 µl of 1U *Taq* DNA Polymerase and dNTPs (200 µM) plus 0.5 µM each primer pair for DEC pathotypes. Sterile distilled deionized water was used to top up to 50 µl. The PCR was performed under the following condition. An initial denaturation at 95 °C for 5 minutes then 30 amplification cycles at 95 °C for 30 second, 50 °C for 30 second, 72 °C for 1 minute, and a final extension at 72 °C for 30 minutes.

H. PCR Amplification of PAI Markers

Seven different PAI markers designated as PAI I_{536}, PAI II_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{J96} and PAI

II_{J96} have been previously characterized in UPEC [22], Table III. Thus, the multiplex PCR used in the detection of PAI Markers, contained 2.5 µl of template DNA (100 ng/µl), 1U Taq DNA polymerase (Biomatik) in 1X PCR buffer (Biomatik), 200 µM of each dNTP, 2.5 mM $MgCl_2$, and 0.5μM of each primer, Table III. The program consisted of initial denaturation at 94 °C for 5min, followed by 30 cycles of 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1 minute, with a final extension step at 72 °C for 10 minutes [22]. The positive control used in the PCR was J96 O4:K6.

TABLE II: GENES AND THEIR PRIMER SEQUENCES FOR MOLECULAR TYPING OF *E. COLI* (ADOPTED FROM [23])

Gene	Primer Sequence (5'-3)	Size of amplicon (Bp)	Annealing Temp (ºC)	Reference
eae	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	881	50	[24]
stx	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518	50	[25]
est	ATTTTTMTTTCTGTATTRTCTT CACCCGGTACARGCAGGATT	190	50	[26]
elt	GGCGACAGATTATACCGTGC CGGTCTCTATATTCCCTGTT	450	50	[26]
paH	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	619	50	[27]
aggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	50	[28]

TABLE III: OLIGONUCLEOTIDES USED TO AMPLIFY PAI MARKERS HARBORING VIRULENCE GENETIC DETERMINANTS

PAI markers	Primer sequence (5'-3')	Amplicon size (bp)	Virulent factors expressed by genes harboured by PAI markers	Reference
PAI I_{536}	TAA TGC CGG AGA TTC ATT GTC AGG ATT TGT CTC AGG GCT TT	1800	α-Haemolysin, CS12 fimbriae, and F17-like fimbrial adhesin	[22]
PAI II_{536}	CAT GTC CAA AGC TCG AGC C CTA CGT CAG GCT GGC TTT G	1000	α-Haemolysin and P-related fimbriae	[22]
PAI IV_{536}	AAG GAT TCG CTG TTA CCG GAC TCG TCG GGC AGC GTT TCT TCT	300	Yersiniabactin siderophore system	[22]
PAI ICFT073	GGA CAT CCT GTT ACA GCG CGC A TCG CCA CCA ATC ACA GC GAA C	930	α-Haemolysin, P-fimbriae, and aerobactin	[22]
PAI IICFT073	ATG GAT GTT GTA TCG CG ACG AGC ATG TGG ATC TGC	400	P-fimbriae and iron-regulated genes	[22]
PAI IJ96	TCG TGC TCA GGT CCG GAA TTT TGG CAT CCC ACA TTA TCG	400	α-Haemolysin and P-fimbriae	[22]
PAI IIJ96	GGA TCC ATG AAA ACA TGG TTA ATG GG GAT ATT TTT GTT GCC ATT GGT TAC C	2300	α-Haemolysin, Prs-fimbriae, and cytotoxic necrotizing factor 1	[22]

TABLE IV: PRIMERS USED IN PHYLOGENETIC ANALYSIS OF *E. COLI*

Gene	Primer sequence (5'-3')	Amplicon size (bp)	Protein expressed	Reference
*chu*A	GAC GAA CCA ACG GTC AGG AT TGC CGC CAG TAC CAA AGA CA	279	Hemetransport in enterohemorrhagic O157:H7 *E. coli*	[13]
*yja*A	TGA AGT GTC AGG AGA CGC TG ATG GAG AAT GCG TTC CTC AAC	211	Protein function unknown	[13]
TSPE4.C2	GAG TAA TGT CGG GGC ATT CA CGC GCC AAC AAA GTA TTA CG	152	Putative DNA fragment (TSPE4.C2) in *E. coli*	[13]

I. Phylogenetic Classification

Phylogenetic classification exhibited 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 fragment (TSPE4.C2). Thus, a multiplex PCR was run to determine the phylogenetic classes of the *E. coli* strains using primers targeting chuA, yjaA and TSPE4.C2 DNA sequences, Table IV. The PCR amplification was conducted by adapting [13] methods. Briefly, the PCR contained 2.5 µl of template DNA, 1U Taq DNA polymerase (Biomatik, USA) in 1x PCR buffer (Biomatik), 200 µM dNTP, 2.5 mM $MgCl_2$, and 0.8 μM of each primer, Table 1. Amplification was conducted using the following PCR conditions; initial denaturation at 94 °C for 5 minutes, then 30 cycles performed at 94 °C for 5 seconds, 54 °C for 10 seconds, 72 °C for 30 second with a final extension step at 72 °C for 5 minutes. Phylogenic groups and subgroups were assigned depending on chuA, yjaA, and TspE4.C2 gene combinations [13, 14] (Table V).

TABLE V: CHUA, YJAA, AND TSPE4.C2 GENE COMBINATIONS FOR ASSIGNING OF PHYLOGENETIC GROUPS AND SUBGROUPS OF *ESCHERICHIA COLI*

TSECP4C2	yjaA	ChuA	Phylogenetic group	Phylogenetic subgroup
Negative	Negative	Negative	A	A0
Negative	Positive	Negative	A	A1
Positive	Negative	Negative	B1	B1
Negative	Positive	Positive	B2	B22
Positive	Positive	Positive	B2	B23
Negative	Negative	Positive	D	D1
Positive	Negative	Positive	D	D2

J. Ethical Consideration

Ethical Approval No: MHREC1611 was granted by the Research and Ethics Committee for ethical review and approval, Mulago National Referral Hospital. The Research Ethics Committee waived the need for informed consent to

use already coded archived samples in this study.

K. Data Analysis

Data analysis was done using the SPSS version 25 (SPSS Inc., Chicago, IL). Statistical differences were computed by chi-square and Spearman's correlation. A p value ≤ 0.05 indicated substantial statistical difference.

III. Results

A. Distribution of Escherichia coli Isolates in Clinical Specimen

We obtained a total of 618 MDR *E. coli* isolates whereby 300, 67, 142 and 109 isolates were from MNRH, MRRH, MBRRH and KIU-TH respectively. However, 206 isolates were neither viable (177) nor *E. coli* (29). Thus, this study used a total of 421 isolates, 205 were obtained from MNRH, 52 from MRRH, 62 from MBRRH and 102 from KIU-TH. The isolates were predominantly isolated from urine (170/40.4%), then anal swabs (103/24.5%), wound/pus swabs (62/14.7%), blood (28/6.7%), virginal swabs, (27/6.4), sputum (19/4.5%) and tracheal aspirate (12/2.9%), Table VI.

B. Phenotypic Carbapenem Resistance Profiles

The Kirby Bauer disk diffusion method was used to determine the susceptibility patterns of *the E. coli* clinical isolates according to CLSI interpretation. All the isolates demonstrated 100% resistance to Ampicillin and Amoxicillin/clavulanic acid hence were MDR, Table I. Out of the 421 *E. coli* clinical isolates obtained from the different tertiary hospitals, 96 were resistant to Ertapenem and out of the 96 Ertapenem resistant isolates, 43 (10.2%) were resistant to both Imipenem and Meropenem. Thus, this study registered an overall phenotypic carbapenem resistance prevalence of 22.8%. Furthermore, MRRH recorded the highest phenotypic carbapenem resistance prevalence of 34.6% followed by MBRRH (33.9%), MNRH (22.0%) and KIU-TH (11.8%). Carbapenem resistant *E. coli* were largely isolated from anal swabs (31.1%), followed by urine (24.1%), then wound/pus swabs (17.7%), tracheal aspirate (16.7%), Sputum (15.8%), blood (14.3%) and virginal swabs (11%), Table VI.

C. Distribution of Carbapenemase Encoding GENES

Pentaplex PCR amplification revealed that 33.0% (139/421) of the *E. coli* isolates obtained from different tertiary hospitals harbored one or more carbapenemases encoding genes. *E. coli* obtained from MRRH scored the highest genotypic prevalence of carbapenem resistance (28/52=53.9%) followed by MBRRH (28/62=45.2%), MNRH (62/205=30.3%) and KIU-TH (21/102=20.6%). Out of the 139 *E. coli* isolates that possessed carbapenem resistant genes, 18.7% (26/139) contained multiple genes. A total of 176 carbapenemases encoding genes was scored and the most predominant gene recorded was $bla_{\text{OXA-48}}$ at a prevalence/genotypic frequency of 13.8%/33.0%, tailed by bla_{VIM} (8.8%/21.0%), then bla_{IMP} (6.9%/16.5%), bla_{KPC} (6.2%/14.8%) and bla_{NDM} (6.2/14.8%), Tables VII and VIII.

D. Relationship between Carbapenemase Encoding Genes and Phenotypic Resistance

This study registered substantial variability between genotypic and phenotypic resistance. Among the carbapenemases genes encountered, bla_{VIM} provided phenotypic CR to 97.3% (36/37) *E. coli* isolates that harbored it. This was trailed by bla_{IMP} (96.6%), bla_{KPC} (80.8%), bla_{NDM} (65.4%) and $bla_{\text{OXA-48}}$ (37.9%), Table VIII. Four isolates were found to co-harbour more than one carbapenemase encoding genes, with bla_{OXA} and bla_{NDM} co-existing in two isolates, $bla_{\text{OXA-48}}$ and bla_{KPC} in one isolate and $bla_{\text{OXA-48}}$, bla_{KPC} and bla_{NDM} in one isolate but exhibited no phenotypic resistance, Table II. Despite of no carbapenemase encoding genes detected, a total of eight (8) isolates (MNRH=3, MBRRH=3 and KIU-TH=2) exhibited phenotypic carbapenem resistance.

E. Relationship between Carbapenemase Encoding Genes and Phenotypic Resistance

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TABLE VI: Phenotypic Carbapenem resistance Profiles of *E. coli* Samples Isolated from Several Clinical Specimens at Different Tertiary Hospitals in Uganda

Clinical Specimen	MNRH		MRRH		MBRRH		KIU-TH		Total		CR Prevalence per clinical specimen (%)
	n	CR	n	CR	n	CR	n	CR	n	CR	
Urine	86	19	16	5	30	12	38	5	170	41	24.1
Blood	19	3	3	1	0	0	6	0	28	4	14.3
Anal swab	41	17	14	6	20	5	28	4	103	32	31.1
Wound/pus swab	38	4	4	2	12	4	8	1	62	11	17.7
Sputum	0	0	14	3	0	0	5	0	19	3	15.8
Tracheal Aspirate	6	0	1	1	0	0	5	1	12	2	16.7
Virginal Swab	15	2	0	0	0	0	12	1	27	3	11.1
Total	205	45	52	18	62	21	102	12	421	96	22.8
CR Prevalence (%)	22.0		34.6		33.9		11.8		22.8		

n= population or number of samples and CR stands for carbapenem resistance.

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TABLE VII: DISTRIBUTION OF CARBAPENEM RESISTANT GENES IN *E. COLI* ISOLATES OBTAINED FROM DIFFERENT TERTIARY HOSPITALS IN UGANDA

Referral Hospital	n	Carbapenemase encoding genes															
		NDM	KPC	IMP	OXA-48	VIM	NDM & OXA-48	IMP & NDM	KPC & OXA-48	IMP & OXA-48	VIM & OXA-48	VIM, NDM & OXA-48	NDM, KPC & OXA-48	IMP, NDM & OXA-48	IMP, VIM OXA-48	Total CR isolates	Prevalence (%)
MNRH	205	07	07	06	11	19	02	-	04	-	-	02	04	-	-	62	30.3
MRRH	52	03	04	05	07	02	-	02	-			-	-	03	02	28	53.9
MBRRH	62	02	03	03	09	04	-	-	-	05	02	-	-	-	-	28	45.2
KIU-TH	102	01	04	03	07	06	-	-	-	-	-	-	-	-	-	21	20.6
Total	421															139	33.0

TABLE VIII: CORRELATION BETWEEN CARBAPENEM RESISTANCE GENES AND PHENOTYPIC RESISTANCE

Tertiary Hospital	Carbapenemase encoding genes										Total
	VIM		OXA-48		IMP		KPC		NDM		
	R	S	R	S	R	S	R	S	R	S	
MNRH	21	0	9	14	6	0	12	3	9	6	80
MRRH	4	0	5	7	12	0	4	0	6	2	40
MBRRH	6	0	8	8	7	1	3	0	2	0	35
KIU	5	1	0	7	3	0	2	2	0	1	21
Total	36	1	22	36	28	1	21	5	17	9	176
Prevalence (%)	8.8		13.8		6.9		6.2		6.2		
Phenotypic CR (%)	97.3		37.9		96.6		80.8		65.4		
Genotypic frequency (%)	21.0		33.0		16.5		14.8		14.8		

R: Resistant, S: Sensitive.

TABLE IX: DISTRIBUTION OF PATHOGENIC *E. COLI* AMONG THE TERTIARY HOSPITALS AND CLINICAL SAMPLES

		Tertiary hospitals					Clinical specimens							
	Pathotype	MNRH	MRRH	MBRRH	KIU-TH	Total/ prevalence	Urine	Blood	Anal Swabs	Wound/ pus swabs	Sputum	Tracheal aspirate	Virginal swabs	Total/ Prevalence
	eae/EPEC	21 (10.2%)	2 (3.9%)	8 (12.9%)	11 (10.8%)	42 (10.0%)	3 (1.8%)	1 (3.6%)	37 (35.9%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	42 (10.0%)
	elt/L-ETEC	3 (1.5%)	2 (3.9%)	1 (1.6%)	2 (2.0%)	8 (2.0%)	1 (0.6%)	0 (0.0%)	6 (5.8%)	0 (0.0%)	1 ((5.3%)	0 (0.0%)	0 (0.0%)	8 (2.0%)
	est/S-ETEC	3 (1.5%)	3 (5.8%)	4 (6.5%)	3 (2.9%)	13 (3.1%)	0 (0.0%)	0 (0.0%)	13 (12.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (3.1%)
	stx/STEC	4 (2.0%)	2 (3.9%)	3 (4.8%)	3 (2.9%)	12 (2.9%)	0 (0.0%)	0 (0.0%)	11 (10.7%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	12 (2.9%)
DEC	ipaH/EIEC	2 (1.0%)	3 (5.8%)	1 (1.6%)	2 (2.0%)	8 (2.0%)	0 (0.0%)	0 (0.0%)	8 (7.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (2.0%)
	PAI IICFT073	21 (10,2%)	4 (7.7%)	23 (37.1%)	7 (6.9%)	55/13.1%	34 (20%)	9 (32.1%)	0 (0.0%)	9 (14.5%)	0 (0.0%)	0 (0.0%)	4 (14.8%)	55/13.1%
ExPEC	PAI IV536	84 (41.0%)	16 (30.8%)	8 (13.0%)	31 (30.4%)	139/33.0%	89 (52.5%)	8 (28.6)	2 (1.9%)	21 (33.9%)	2 (10.5%)	4 (33.3%)	13 (48.1%)	139/33.0%

TABLE X: DISTRIBUTION OF THE *ESCHERICHIA COLI* PHYLOGENETIC GROUPS IN THE FOUR TERTIARY HOSPITALS AND CLINICAL SPECIMENS

Phylogenetic group (PG)	Phylogenetic subgroup (PSG)	Tertiary hospital							Clinical Specimen						
		MNRH	MRRH	MBRRH	KIU-TH	Total	PSGP (%)	PGP (%)	Urine	Blood	Anal swabs	Wound/ pus swabs	Sputum	Tracheal aspirate	Virginal swabs
A	A0	33 (16.1%	0 (0.0%)	3 (4.8%)	18 (17.7%)	54	12.8	30.2	19 (15%)	3 (2.4%)	12 (9.5%)	9 (7.1%)	2 (1.6%)	6 (4.7%)	3 (2.4%)
	A1	21 (10.2%)	18 (34.6%)	11 (17.7%)	23 (22.6%)	73	17.3		17 (13.4%)	8 (6.3%)	11(8.7%)	20 (15.8%)	12 (9.5%)	0 (0.0%)	5 (3.9%)
B1	N/A	38 (18.5%)	14 (26.9%)	17 (27.4%)	22 (21.6%)	91	21.6	21.6	7 (7.7%)	1 (1.1)	74 (81.3%)	3 (3.3%)	3 (3.3%)	1 (1.1%)	2 (2.2%)
B2	B22	6 (1.0%)	2 (3.9%)	4 (6.5%)	3 (2.9%)	15	3.7	41.1	11 (6.4%)	0 (0.0%)	1 (0.6%)	3 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	B23	92 (44.9%)	18 (34.6%)	21 (33.9%)	27 (26.5%)	158	37.5		94 (54.3%)	13 (7.5%)	3 (1.7%)	24 (13.9%)	2 (1.2%)	5 (2.9%)	17 ((.8%)
D	D1	5 (2.4%)	0. (0.0%)	0 (0.0%)	3 (2.9%)	8	1.9	7.1	6 (20.0%)	0 (0.0%)	2 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	D2	10 (4.9%)	0 (0.0%)	6 (9.7%)	6 (5.9%)	22	5.2		16 (53.3%)	3 (10%)	0 (0.0%)	3 (10%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total		205	52	62	102	421	100	100	170	28	103	62	19	12	27

F. Dispersion of Pathogenic E. coli in Tertiary Hospitals and Clinical Specimens

1. Diarrheagenic Escherichia coli (DEC)

Out of 421 MDR *E. coli* isolates, 19.7% (83/421) harboured virulence genetic determinants. MBRRH had the highest prevalence of DEC (17/62=27.4%) followed by MBRH (12/52=23.1%), KIU-TH (21/102=20.6%) and MNRH (35/205=17.1%). However, the DEC prevalence was not statistically different among hospitals. Among the DEC detected, EPEC pathotype recorded significantly higher prevalence of 10.8% followed by S-ETEC (3.1%), STEC (2.9%), EIEC (2.0%) and L-ETEC (2.0%). Pathotypes EAEC and ESHEC were not detected in this study, thus scored a prevalence of 0%. Among clinical specimens, DEC were predominantly isolated from anal swabs (75/83=90.4%) followed by urine (4/83=4.8%), sputum (2/83) blood (1/83) and wound/pus swabs (1/83) (Table IX).

2. Extraintestinal Pathogenic Escherichia coli (ExPEC)

Multiplex PCR amplification targeting the pathogenicity islands (PAIs) was used detect ExPEC, Fig. 1. The overall prevalence of ExPEC was 46.1% (194/421). *E. coli* isolates possessing PAIs were predominantly obtained from MNRH (105/205=54.2%/105), trailed by MBRRH (31/62=50.0%), MRRH (20/52=38.5%) and then KIU-TH (38/102=37.3%). PAI IV_{536} was the most predominant chromosomal region detected (33.0%) and then PAI II_{CFT073} (13.1%). Furthermore, 20 isolates had both PAI IV_{536} and PAI II_{CFT073}. PAI I_{536}, PAI II_{536}, PAI-$_{ICFT073}$, PAI I_{J96} and PAI II_{J96} were not detected. Urine samples registered the highest prevalence of ExPEC. Of the 170 isolates obtained from urine, 72.5% (123) were ExPEC followed by virginal swabs (17/27=62.9%), blood (17/28=60.7%), wound/pus swabs (30/62=48.4%), Tracheal aspirate (4/12=33.3%), sputum (2/19=10.5%) and anal swabs (2/103=1.9%). The prevalence of ExPEC in urine, virginal swabs, blood, wound/pus swabs was substantially higher than ExPEC prevalence obtained from tracheal aspirate, sputum and anal swabs (Table IX).

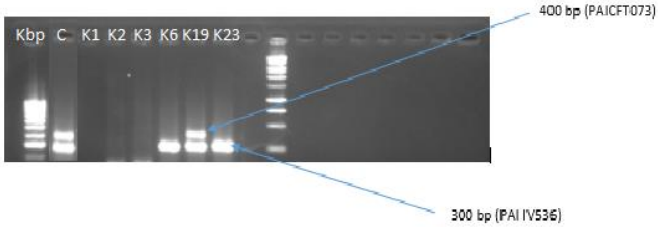

Fig. 1. PCR amplification of pathogenic islands; Kbp: 2 Kilo base pair ladder, C: Control, K: Kampala International University-Teaching hospital isolates.

G. Distribution of the Escherichia coli Phylogenetic Groups

E. coli (421) isolated from several clinical specimens were characterized into four phylogenetic groups (PG) and six phylogenetic subgroups based on the triplex PCR, Fig. 2. *E. coli* belonging to phylogenetic group B2 was predominantly detected and scored a prevalence of 41.1%. This was trailed by phylogenetic group A (30.2%), phylogenetic group B1 (21.6%) and phylogenetic group D (7.1%). E. coli belonging to Phylogenetic group A, B2 and D were majorly isolated from urine samples whereas phylogenetic group B1 isolates were mainly obtained from anal swabs (Table X).

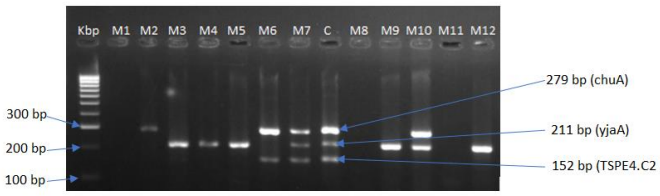

Fig. 2. Triplex amplification of the chuA, yjaA and TSPE4.C2 genes; Kbp: Kilo base pair DNA ladder, M: Mulago National Referral Hospital isolates and C: control.

H. Virulence Genes and Carbapenem Resistance Profiles among the Phylogenetic Groups

EIEC (*ipaH* gene) was detected in one carbapenem susceptible *E. coli* isolate belonging to phylogenetic group A. PAI II_{CFT073} and PAI IV_{536} were also detected in one carbapenem susceptible and two carbapenem resistant phylogenetic group A isolates respectively. Out of the 83 DEC, 98.8% (82) resided within phylogenetic group B1 and 38.6% (32) expressed phenotypic carbapenem resistance. Furthermore, 86.1% (120 PAI IV_{536} and 47 PAI II_{CFT073}) of the ExPEC were characterized as phylogenetic group B2 of which 21% (41) were resistant to carbapenems. Phylogenetic group D contained 12.4% ExPEC (17 PAI IV_{536} and 07 PAI II_{CFT073}) and 2.1% (4) of the phylogenetic group D ExPEC were resistant (Table XI).

TABLE XI: DISTRIBUTION OF PATHOGENIC GENES AMONG THE *ESCHERICHIA COLI* PHYLOGENETIC GROUPS AND THEIR CARBAPENEM RESISTANCE PROFILES

PG	PSG	Virulent genes found in DEC pathotypes										Pathogenicity Islands that harbor virulent genes in ExPEC			
		eae		elt		ipaH		sxt		est		PAI IICFT073		PAI IV536	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S
A	A0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	A1	0	0	0	0	0	0	0	0	0	0	0	1	2	0
B1	B1	19	23	4	4	3	4	3	9	3	10	0	0	0	0
B2	B22	0	0	0	0	0	0	0	0	0	0	2	1	4	6
	B23	0	0	0	0	0	0	0	0	0	0	15	29	20	90
D	D1	0	0	0	0	0	0	0	0	0	0	1	5	0	2
	D2	0	0	0	0	0	0	0	0	0	0	0	1	3	12
Total		42		8		8		12		13		55		139	

PG: Phylogenetic group, PSG: Phylogenetic subgroup.

IV. Discussion

Despite the fact that *E. coli* is the leading cause of urinary tract infections and diarrheal infection worldwide, to the best of our knowledge, this is the first study from the East African region to investigate the carbapenem resistance profile, virulence pattern and phylogenetic groups among MDR *E. coli* clinical isolates. Knowledge of the prevalence of pathogenic E. coli and their antimicrobial resistance pattern is vital in the designing of strategies to control the spread of such superbugs.

Findings of this study revealed that the overall phenotypic carbapenem resistance prevalence stood at 22.8%. Comparable results were achieved by previous studies in Low Middle Income countries (LMIC) with similar healthcare systems. For example, prevalence of carbapenem resistance in Tanzania was 24% [29], Nigeria 15.2%, 27.4% and 36.8%) [30-32], India 31.77% [33]. Contrary, this frequency is higher than carbapenem resistance levels reported in countries like Ghana (7.2%) [34], Morocco 5.99% [35], and Ethiopia 2.73%, [36] with similar healthcare settings but lower than the incidences above 50% reported in South Africa, Egypt and Tunisia [37-41].

Multiplex PCR screening identified carbapenemase encoding genes in 33.0% of the isolates. This genotypic carbapenem resistance prevalence corroborates with earlier studies conducted in the East African region [29, 42, 43] and elsewhere [31, 44] that reported levels ranging from 25% to 40%. Contrary, this frequency is significantly lower than carbapenem genotypic levels reported by studies in Tunisia (76.7%) [45], South Africa (68% and 86%) [37, 38] , Egypt (89.6%) [41], Turkey (49.5%) [46]. KPC, VIM, NDM, OXA-48 and IMP are the commonest carbapenemases worldwide [47]. Findings of this study revealed the existence of all those carbapenemases encoding genes in Uganda and OXA-48 was the most predominant gene in contrast with previous studies in the region [29, 42] but in agreement with recent studies in carried out in Africa [35, 37, 40, 45]. OXA-48 carbapenemase was first detected in Turkey and it became epidemic in the Middle East and Mediterranean countries [46]. This indicates that OXA-48 habouring Enterobacteriaceae have spread widely in sub-Saharan Africa to become too most prevalent.

This study found considerable variation between phenotypic and genotypic resistance. Among the *E. coli* isolates that harboured *bla*VIM gene, 97.3% exhibited phenotypic resistance while for *bla*OXA-48, only 37.9% expressed phenotypic resistance. It is important noting that four isolates coharboured more than one gene each but susceptible to carbapenems. Carbapenemases expressed by OXA-48 and its variant genes possess low carbapenems hydrolyzing activity [48-50]. This provided an insight into why 62.1% of the isolates which possessed OXA-48-like genes did not exhibit phenotypic resistance. Alteration and reduced expression of the outer membrane proteins that act as drug channels complement enzymes expressed by the resistant genes and this mechanism is highly effective against Ertapenem [50, 51]. Thus, carbapenem resistance is not exclusively due to expression carbapenemases. This explains why not all the isolates that harbored carbapenemase genes were carbapenem insusceptible and why resistance to ertapenem was significantly higher. Despite of absence of carbapenem resistance genes, a total of eight sample displayed phenotypic resistance. Thus, resistance in these isolates may be attributed to (a) a combination of loss of outer membrane proteins (OMPs), (OmpK35 and OmpK36) and overexpression of extended spectrum beta-lactamases-ESBLs (CTX-Ms or SHV-2) or plasmid-borne AmpC enzymes (ACT-1, CMY-2, CMY-4 or DHA-1) [52, 53] and (b) presence of other carbapenemase such as Guiana extended Spectrum enzyme / integron-borne cephalosporinase (GES/IBC), *Serratia marcescens* enzyme (SME_{1-3}), Not Metalloenzyme carbapenemase (NMC-A), Imipenem-hydrolyzing beta lactamase (IMI), Sao Paulo metallo-lactamase (SPM), German imipenemase (GIM), Seoul imipenemase (SIM) and Kyorin University Hospital metallo-lavtamase (KHM) [54].

Enterohemorrhaghic *E. coli* (EHEC), enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative E. coli (EAEC) and diffusely adherent *E. coli* (DAEC) have been reported as the main causes of diarrheal disease in several parts of Africa, predominantly among young children [55]. This study revealed high prevalence of diarrheagenic MDR *E. coli* clinical isolates obtained from different tertiary hospitals as 19.7% (83/421) of the isolates harboured virulence genetic determinant. Of the 83 DEC pathotypes, EPEC was the most encountered (51.0%) followed by S-ETEC, STEC and EIEC and L-ETCE. This correlates well with studies conducted outside the African content [56, 57]. In contrast, similar studies carried out in sub-Saharan Africa (Tanzania, South Africa and Mozambique) reported EAEC as the most prevalent DEC pathotype [58-62] yet it was not detected in this study. As expected, DEC were predominantly isolated from Anal/fecal swabs. However, 9.6% (8/83) were obtained from other clinical specimens. Indeed, several have isolated DEC pathotypes from other clinical specimens other than stool and have been implicated as some of the causes of hemolytic uremic syndrome [63, 64].

In this study, multiplex PCR was used to target pathogenicity Islands (PAI). PAIs harbour virulence genes in ExPEC that are responsible for pathogenicity [65-67]. The overall prevalence of ExPEC as revealed by molecular typing of PAI in our study was 46.1% (194/421). Of the two PAIs detected, PAI IV536 also known as high pathogenicity Island (HPI) was substantially dominant with a genotypic frequency of 71.7% and PAI IICFT073 had a frequency of 28.3%. This is in agreement with previous studies [22, 65] which reported PAI IV536 as the most prevalent PAI. The main virulence genes residing in the PAI IV536 and PAI IICFT073 are yersiniabactin siderophore iron-uptake system and P. fimbriae as well as iron regulated proteins respectively [66, 68, 69]. A previous study in Uganda reported high prevalence of *E. coli* with P. fimbriae virulent factor encoded for by the pap gene in UPEC [70]indicating high prevalence of PAI IICFT073 pathotypes. However, this study never attempted to detect genes encoding the yersiniabactin siderophore iron-uptake system in PAI IV536 UPEC; thus, there is no available data about the prevalence of PAI IV536 for comparison purposes. As anticipated, ExPEC that harboured PAIs were majorly isolated from urine and virginal swabs. However, a total of 55 isolates obtained from blood (17), wound/pus swabs (30), tracheal aspirate (4) anal swabs (2) and sputum

(2) harboured PAIs. PAI IJ96 *E. coli* have been reported to be both UPEC and NMNEC [71] but this study did not detect any PAI IJ96. Thus, all the *E. coli* isolates that possessed PAI IV_{536} and PAI II_{CFT073} were deemed to be UPEC.

Phylogenetic analysis revealed that E. coli isolates obtained from the four tertiary hospitals located in the Central region (MNRH), Western region (MBRRH), South Western Region (KIU-TH) and Eastern Region (MRRH) belonged to the phylogroups A B1, B2 and D and phylogenetic sub groups A0, A1, B22, B23, D1 and D2. Pathogenic intestinal E. coli (DEC) mainly belong to Phylogenetic groups A, B1, and D, commensals to the groups A and B1, and strains usually belong to the groups B2 and D [13, 14]. In our study, phylogenetic analysis predominantly clustered *E. coli* clinical isolates into B2 followed by A, B1 and D and this corroborates with findings from previous studies [72, 73]. However, contradicting results have been reported worldwide where A is the most abundantly isolated phylogroup [74-77]. Distribution of *E. coli* phylogroups among different ecological zones is influenced by environment factor; thus, this accounts for variability in prevalence of the phylogenetic groups in different countries [78]. In this study, statistically similar (P value 0.9998) distribution of phylogenetic groups A, B1, B2 and D among regions was observed. This pattern of distributes indicates inter-region transmission of UPEC, DEC and commensals. It was observed that phylogenetic group A, B2 and D strains were majorly isolated from urine and this is in affirmative with all studies that conducted phylogenetic analysis of *E. coli* clinical isolates [79-81] whereas B1 strains were predominantly isolated from anal/fecal swabs, this does not corroborate with previous studies which found phylogroup A strains as the most dominant fecal isolates [76, 77, 82].

World over, an increase in pathogenic and commensal *E. coli* strains harbouring antibiotic resistance determinants has been observed. The situation has been complicated by acquisition of antibiotic resistance by other Enterobacteriaceae as several studies have reported that infections caused by resistant bacteria are hard to treat, lead to increase in treatment costs, morbidity and mortality [83]. Antibiotic resistance in Enterobacteriaceae is mainly mediated by beta-lactamase enzymes that inactivate beta-lactam antibiotics by hydrolyzing the peptide bond of the beta-lactam ring. Among the beta-lactamases, carbapenemases are the most important because acquisition of carbapenem resistance genes confer resistance to all beta lactam antibiotics. Furthermore, carbapenem are the most suitable choice antibiotics for treatment of MDR Gram-Negative bacterial infections; [48] thus, infection with carbapenem resistant bacteria significantly prolong the period of stay in hospital and responsible for 10% mortality [84]. Thus, in this study we assessed the carriage of carbapenem resistance and virulence genetic factors among *E. coli* phylogroups. We observed that among the 83 isolates that harboured virulence genetic determinants for DEC, 98.8% (82) and 1.2% (1) belonged to phylogenetic group B1 and A respectively and 38.6% (32) expressed phenotypic resistance. Whereas 86.1% (167), 12.4% (24) and 1.6% (3) of the isolates that had PAIs were characterized as phylogroups B2, D and A respectively and 24.1% (47) were carbapenem resistant. Coexistence of virulence factors and carbapenem resistance was observed in 18.8% (79/421) of the total isolates. Our findings show that carbapenemases production was significantly higher in B1 and B2 ($P<0.0001$). This is extremely scaring as DEC and ExPEC mainly fall under phylogenetic groups B1 and B2 respectively. Furthermore, existence of virulence genes and genetic determinants of resistance in phylogenetic groups A and D where commensal mainly fall should be treated as a major threat as they are considered to reservoirs of genetic determinants of virulence and antibiotic resistance and they donate these traits to the pathogenic strains of phylogroups B1 and B2 through horizontal gene transfer, arbitrated regularly by plasmids and transposons [49]. Indeed, previous studies observed that PAIs are mobile genetic elements (transposons) that are transferred from one *E. coli* strain to another through horizontal gene transfer mediated by bacteriophages, conjugative plasmids, conjugation and homologous DNA recombination [69, 85, 86].

V. Conclusion

Our data indicate high level of carriage of carbapenem resistance among the DEC and ExPEC clinical isolates belonging to phylogenetic group B1 and B2 respectively. DEC and ExPEC pathogenicity and antimicrobial resistance are mediated by genetic factors such as chromosomal/plasmid borne virulence and antibiotic resistance genes as well as chromosomal PAIs virulent genes. Plasmid and PAIs are mobile genetic elements that facilitate horizontal gene transfer contributing to plasticity of the genome. In light of this, routine genetic analysis of *E. coli* clinical and environment isolates is important to better understand the level of pathogenicity and antimicrobial as this will inform the possible burden such isolates are likely to pose to the healthcare system.

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