Carbapenem resistance profiles of Pathogenic Escherichia coli in Uganda

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Abstract

Background: *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 CR profiles among pathogenic *E. coli*.

Methods: This was a retrospective cross-sectional study involving use of archived *E. coli* clinical isolates collected in 2019 from four Ugandan 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 virulent genes, ExPEC PAI and the *E. coli* phylogenetic groups.

Results:

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 *bla*OXA-48 with genotypic frequency of 33.0%, then *bla*VIM(21.0%), *bla*MP(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 virulent genes, where EPEC recorded significantly higher prevalence (10.8%) followed by STEC(3.1%), ET(2.9%), EIEC (2.0%) and L-ETE(2.0%). Genetic analysis characterized 46.1% of the isolates as ExPEC and only PAI IV536(33.0%) and PAI ICFT073(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.

Conclusion: 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 it promotes horizontal gene transfer.

Introduction

*Escherichia coli* is one of the most prevalent commensals of the human gastro-intestinal tract (GIT) microbiota. 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 chief etiology of both community and nosocomial acquired UTI worldwide.

Diarrheagenic *E. coli* are grouped into eight pathotypes basing on virulent factors responsible for their pathogenicity. These include Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (IEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETE) and 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 be associated with Crohn’s disease, but not with any diarrheagenic infections and a hybrid pathotype, enteroaggregative hemorrhagic *E. coli* (EAHEC) carrying STEC and EAE 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 virulent genes. These virulent genes include: eae for typing of EPEC; stx for STEC/EHEC; est/ for st-EUC; elt for II-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 septicaemia [9]. ExPEC pathogenicity is accounted for by presence of virulence factors encoding genes located either on plasmids or chromosomes. These virulent 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 chromosomes genes encoding virulent factors such as hemolysins (hlyA and hlyF), cytotoxic necrotizing factors (cnf1 and cnf 2), colicin V (cvaC), aerobactin (iutA), versiniabactin (fuyA), salmochelin (iromN), 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 (kpsI 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 TSP45.C2 DNA fragment [13]. The intestinal pathogenic *E. coli* strains belong to groups A, B1 and D, extraintestinal pathogenic *E. coli* strains generally follow under groups B2 and D, while commensal *E. coli* strains to groups A and B1 [13, 14].
High levels of antibiotic resistance in Enterobacteriaceae is of great concern to the healthcare system [15, 16]. *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 [17, 18]. 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).

**Materials And Methods**

**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 (KI-U). 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 each different isolate were made by adding 500 μl 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°C until further use.

**Biochemical assays to confirm the identity of *E. coli***

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

**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 [19]. Ampicillin (AMP) 25 μg, Amoxicillin/clavulanic acid (AMO) 20/10 μg, trimethoprim-sulphamethoxazole (TMP/SMX) 1.25/23.75 μg, Ciprofloxacin (CIP) 5 μg, Cefuroxime (CXM) 30 μg, Temocillin (TEM) 30 μg, Piperacillin-tazobactam (TPZ) 110 μg, Cefotaxim (FOX) 30 μg, Cefpieme (FEP) 30 μg, 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 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.

**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 shaker incubator at 37°C for 24hrs. 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°C until further use.

**Molecular characterization of virulent 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)

**Multiplex PCR amplification of carbapenem resistance genes**

The existence of carbapenem resistance genetic determinants was determined using primers targeting *blaVIM, blaIMP, blaKPC, blaOXA-48,* and *blaNDM* that carbapenemase encoding genes, Table 1. For co-amplification of target genes, multiplex PCR was conducted by adapting methods used by Dallenne et al., [20]. 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 MgCl2, 10 mM Tris–HCl, pH 8.3/50 mM KCl) (Biomatik USA) and 1.2 μl of 1U Tag 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, College of Health Science, Makerere University. 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 [21] were used as positive controls.

**Multiplex PCR components and conditions for *E. coli* pathotyping**
phenotypic CR to 97.3% (36/37). This study registered substantial variability between genotypic and phenotypic resistance. Among the carbapenemases genes encountered, the relationship between carbapenemase encoding genes and phenotypic resistance is highlighted (6.2%/14.8%), Tables 7 and 8. At a prevalence/genotypic frequency of 13.8%/33.0%, tailed by 18.7% (26/139) contained multiple genes. A total of 176 carbapenemases encoding genes was scored and the most predominant gene recorded was MBRRH (28/62=45.2%), MNRH (62/205=30.3%) and KIU-TH (21/102=20.6%). Out of the 139 Pentaplex PCR amplication revealed that 33.0% (139/421) of the 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 6.

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 S1. 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 6.

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 blaOXA-48 at a prevalence/genotypic frequency of 13.8%/33.0%, tailed by blaVIM (8.8%/21.0%), then blatMP (6.9%/16.5%), blakPC (6.2%/14.8%) and bla NDM (6.2/14.8%), Tables 7 and 8.

Relationship between carbapenemase encoding genes and phenotypic resistance

This study registered substantial variability between genotypic and phenotypic resistance. Among the carbapenemases genes encountered, blaVIM provided phenotypic CR to 97.3% (36/37) E. coli isolates that harbored it. This was trailed by blatMP (96.6%), blakPC (80.8%), blaNDM (65.4%) and blaOXA-48 (37.9%),

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Table 8. Four isolates were found to co-harbour more than one carbapenemase encoding genes, with \(blaOXA\) and \(blaNDM\) co-existing in two isolates, \(blaOXA-48\) and \(blaKPC\) in one isolate and \(blaOXA-48\), \(blaKPC\) and \(blaNDM\) in one isolate but exhibited no phenotypic resistance, Table S2. 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.

**Dispersion of in tertiary hospitals and clinical specimens**

*Diarrheagenic Escherichia coli (DEC)*

Out of 421 MDR *E. coli* isolates, 19.7% (83/421) harboured virulent genetic determinants. MBRRH had the highest prevalence of DEC (17/62=27.4%) followed by MBRHR (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 ESPEC 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/put swabs (1/83), Table 9.

*Extraintestinal Pathogenic Escherichia coli (ExPEC)*

Multiplex PCR amplification targeting the pathogenicity islands (PAIs) was used detect ExPEC. The overall prevalence of ExPEC was 46.1% (194/421). *E. coli* isolates possessing PAIs were predominantly obtained from MNRH (105/205=52.4%/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 prominent chromosomal region detected (33.0%) and then PAI ICFT073 (13.1%). Furthermore, 20 isolates had both PAI IV 536 and PAI ICFT073. PAI J96, PAI ICFT073, PAI I96 and PAI J96 were not amplified. 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/put 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/put swabs was substantially higher than ExPEC prevalence obtained from tracheal aspirate, sputum and anal swabs, Table 9.

**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. *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 10.

**Virulent 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 ICFT073 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 ICFT073) 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 ICFT073) and 2.1% (4) of the phylogenetic group D ExPEC were resistant. Table 11.

**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% [25], Nigeria 15.2%, 27.4% and 36.8% [26-28], India 31.77% [29], Contrary, this frequency is higher than carbapenem resistance levels reported in countries like Ghana (7.2%) [30], Morocco 5.99% [31], and Ethiopia 2.73%, [32] with similar healthcare settings but lower than the incidences above 50% reported in South Africa, Egypt and Tunisia [33-37].

Multiplex PCR screening identified carbapenem encoding genes in 33.0% of the isolates. This genotypic carbapenem resistance prevalence corroborates with earlier studies conducted in the East African region [18, 25, 38] and elsewhere [27, 39] 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%) [40], South Africa (68% and 86%) [33, 34], Egypt (89.6%) [37], Turkey (49.5%) [41]. KPC, VIM, NDM, OXA-48 and IMP are the commonest carbapenemases worldwide [42]. 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 [18, 25] but in agreement with recent studies in carried out in Africa [31, 33, 36, 40]. OXA-48 carbapenemase was first detected in Turkey and it became epidemic in the Middle East and Mediterranean countries [41]. This indicates that OXA-48 harbouring Enterobacteriaceae have spread widely in sub-Saharan Africa to become to most prevalent.

This study found considerable variation between phenotypic and genotypic resistance. Among the *E. coli* isolates that harboured *blaVIM* gene, 97.3% exhibited phenotypic resistance while for *blaOXA-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 [43-
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 [45, 46]. Thus, carbapenem resistance is not exclusively due to expression carbapenemases. This explains why not all the isolates that harbored carbapenemase genes were carbapenem susceptible 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 (OMP), (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) [47, 48] and (b) presence of other carbapenemase such as Guiana extended Spectrum enzyme / integron-borne cephalosporinase (GES/IBC), Seratia marcescens enzyme (SME-1), Not Metalloenzyme carbapenemase (NMC-A), Imipenem-hydrolyzing beta lactamase (IMI), Sao Paulo metal-lactamase (SPM), German imipenemase (GIM), Seoul imipenemase (SIM) and Kyorin University Hospital metallo-lactamase (KHM) [49].

Enterohemorrhagic 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 to be the main causes of diarrheal disease in several parts of Africa, predominantly among young children [50]. 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 harbored genetic virulence genetic determinant. Of the 83 DEC pathotypes, EPEC was the most encountered diarrheagenic pathotype (51.0%) followed by S-ETEC, STEC and EIEC and L-ETEC. This correlates well with studies conducted outside the African content [51, 52]. In contrast, similar studies carried out in sub-Saharan Africa (Tanzania, South Africa and Mozambique) reported EAEC as the most prevalent DEC pathotype [53-57] 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 [58, 59].

In this study, multiplex PCR was used to target Pathogenicity Islands (PAI). PAIs harbour virulent genes in ExPEC that are responsible for pathogenicity [60-62]. 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 [24, 60] 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. fimbiae as well as iron regulated proteins respectively [61, 63, 64]. A previous study in Uganda reported high prevalence of E. coli with P. fimbiae virulent factor encoded for by the pap gene in UPEC [65] 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 mainly 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 U96 E. coli have been reported to be both UPEC and NMNEC [66] but this study did not detect any PAI U96. Thus, all the E. coli isolates that possessed PAI IV536 and PAI IICFT073 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 [67-69]. However, contradicting results have been reported worldwide where A is the most abundantly isolated phylogroup [70-73]. 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 [74]. 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 [67, 75-77] 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 [72, 73, 78]. 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 [79]. 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 carbapenemase 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; [43] thus, infection with carbapenem resistant bacteria significantly prolong the period of stay in hospital and responsible for 10% mortality [80]. 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 scarifying 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 [44]. 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 [64, 81, 82]

**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.

**List Of Abbreviations**

MDR: Multidrug-resistant

PAI: Pathogenicity Island

UTI: Urinary tract infection

UPEC: Uropathogenic *coli*

MNRH: Mulago National Referral Hospital

MRRH: Mbale Regional Referral Hospital

MBRRH: Mbarara Regional Referral Hospital

KIU-TH: Kampala International University-Teaching Hospital

COVAB: College of Veterinary Medicine Animal Resources and Biosecurity

AMP: Ampicillin

AMO: Amoxicillin/clavulanic acid

CIP Ciprofloxacin

CXM: Cefuroxime

TEM: Temocillin

TPZ: Piperacillin-tazobactum

FOX: Cefoxitin

FEP: Cepime

CRO: Ceftriaxone

CAZ: Ceftazidime

CTX: Cefotaxime

ERT: Ertapenem

IMI: Imipenem

MEM: Meropenem

ATCC: American Type Cell Cultures

*bla*: beta lactamase

*KPC*: *Klebsiella pneumoniae* Carbapenemase

NDM: New Delhi Metallo-β-lactamase

VIM: Verona Integron-encoded Metallo-β-lactamase

IMP: Imipenemase Metallo-β-lactamase
OXA: Oxacillinase
DEC: Diarrheagenic coli
ExPEC: Extra-intestinal pathogenic coli
EPEC: Enteropathogenic E. coli
EAEC: Enteraggregative E. coli
EIEC: Enteroinvasive E. coli
DAEC: Diffusely adherent E. coli
EHEC: Enterohemorrhagic E. coli
STEC: Shiga Toxin producing E. coli
ETEC: Enterotoxigenic E. coli
CRE: Carbapanem resistant Enterobacteriaceae
NMEC: Neonatal meningitis coli
OMP: outer membrane protein
EAHEC: enteroaggregative hemorrhagic coli
VTEC: Verotoxigenic coli
PG: Phylogenetic groups
PSG: Phylogenetic sub groups
GES/IBC: Guiana extended Spectrum enzyme / integron-borne cephalosporinase
SME: *Serratia marcescens* enzyme
NMC: Not Metalloenzyme carbapenemase
IMI: Imipenem-hydrolyzing beta lactamase
SPM: Sao Paulo metallo-lactamase
GIM: German imipenemase (GIM),
SIM: Seoul imipenemase
KHM: Kyorin University Hospital metallo-lavtamase

**Declarations**

**Ethics and consent to participate:** 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.

**Consent for publication:** Not applicable

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**Availability of Data and Materials:** All data generated by this study have been submitted with this manuscript. Raw data and any other forms data generated by this project can be obtained from the authors on request by e-mail.

**Competing interests:** The authors declare that they have no competing interests

**Authors’ contributions**

This work was carried out in collaboration between all authors. Denis K. Byarugaba (BKB), Eddie Wampande (EW), Francis Ejobi (FE), Jesca L. Nakavuma (JLN), Robert Tweyongere (RT) and Charles Kato Drago (CKD) conceptualized and designed the format for this study. Kenneth Ssekatatawa (KS) carried out all
the Laboratory experiments. KS, CKD and EW conducted data analysis. All authors drafted and managed manuscript revisions. All authors read and approved the final manuscript.

Acknowledgements

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Table 1: Carbapenem resistance genes and respective primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5'-3')</th>
<th>Band size (Bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BlaKPC</td>
<td>F-ATG TCA CCG TAT CCG COT CT R-TTT TCA GAG CCT TAC TGC CC</td>
<td>538</td>
<td>[20]</td>
</tr>
<tr>
<td>BlaIMP</td>
<td>F-TGA GCA AGT TAT CTG TAT TC R-TTA GTT GCT TGG TTT TGA TG</td>
<td>139</td>
<td>[20]</td>
</tr>
<tr>
<td>BlaVIM</td>
<td>F-GAT GGT GTT TGG TCG CAT A R-CGA ATG CGC AGC ACC AG</td>
<td>390</td>
<td>[20]</td>
</tr>
<tr>
<td>BlaNDM</td>
<td>F-GGT TTT GCG ATC TGG TTT TC R-CGG ATG GCC TCA TCA CGA TG</td>
<td>822</td>
<td>[20]</td>
</tr>
<tr>
<td>BlaOXA-48</td>
<td>F-TTT GTG GCA TCG ATT ATC GG R- GAG CAC TTC TTT TGT GAT GGC</td>
<td>281</td>
<td>[20]</td>
</tr>
</tbody>
</table>

Table 2: Genes and their prime sequences for molecular typing of E. coli (adopted from Dias et al., 2012)
Table 3: Oligonucleotides used to amplify PAI markers harboring virulent genetic determinants

<table>
<thead>
<tr>
<th>n</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Virulent factors expressed by genes harbored by PAI markers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>TAA TGC CGG AGA TTC ATT GTC AGG ATT TGT CTC AGG GCT TT</td>
<td>1800</td>
<td>α-Haemolysin, CS12 fimbriae, and P17-like fimbrial adhesin</td>
<td>[24]</td>
</tr>
<tr>
<td>36</td>
<td>CAT GTC CAA AGC TCG AGC C CTA CGT CAG GCT GGC TTT T</td>
<td>1000</td>
<td>α-Haemolysin and P-related fimbriae</td>
<td>[24]</td>
</tr>
<tr>
<td>536</td>
<td>AAG GAT TCG TGG TAA CCG GAC TCG TCG GGC AGC AGC GTC TCT TCT GGA CAT CCT GTT ACA GGC GGC A</td>
<td>300</td>
<td>Yersiniabactin siderophore system</td>
<td>[24]</td>
</tr>
<tr>
<td>3</td>
<td>TCG CCA CCA ATC ACA GC GAA C ATG GAT GTT GTA TCG CG</td>
<td>400</td>
<td>P-fimbriae and iron-regulated genes</td>
<td>[24]</td>
</tr>
<tr>
<td>73</td>
<td>AAG GAT TCG TGG TAA CCG GAC TCG TCG GGC AGC AGC GTC TCT TCT GGA CAT CCT GTT ACA GGC GGC A</td>
<td>2300</td>
<td>α-Haemolysin, Prs-fimbriae, and cytotoxic necrotizing factor 1</td>
<td>[24]</td>
</tr>
</tbody>
</table>

Table 4: Primers used in phylogenetic analysis of E. coli

<table>
<thead>
<tr>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Protein expressed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAC GAA CCA AGG GTC AGG AT</td>
<td>279</td>
<td>Heterotransport in enterohemorrhagic O157:H7 E. coli</td>
<td>[13]</td>
</tr>
<tr>
<td>TGC CCG CAG TAC CAA AGA CA TGA GAT GTT AGG AGA CGG CGG TTT G</td>
<td>211</td>
<td>Protein function unknown</td>
<td>[13]</td>
</tr>
<tr>
<td>TGG CAT GCC ACA TTA TCG TGG CAT GCC ACA TTA TCG TGG CAT GCC ACA TTA TCG TGG CAT GCC ACA TTA TCG</td>
<td>152</td>
<td>Putative DNA fragment (TSEPC4.C2) in E. coli</td>
<td>[13]</td>
</tr>
</tbody>
</table>

Table 5: chuA, yjaA, and TspE4.C2 gene combinations for assigning of phylogenetic groups and subgroups of Escherichia coli

<table>
<thead>
<tr>
<th>TSEPC4.C2</th>
<th>yjaA</th>
<th>ChuA</th>
<th>Phylogenetic group</th>
<th>Phylogenetic subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>A</td>
<td>A0</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>A</td>
<td>A1</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>B1</td>
<td>B1</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>B2</td>
<td>B22</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>B2</td>
<td>B23</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>D</td>
<td>D1</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>D</td>
<td>D2</td>
</tr>
</tbody>
</table>

Table 6: Phenotypic carbapenem resistance profiles of E. coli samples isolated from several clinical specimens at different tertiary hospitals in Uganda

---

Page 12/14
Table 7: Distribution of carbapenem resistant genes in E. coli isolates obtained from different tertiary hospitals in Uganda

<table>
<thead>
<tr>
<th>Clinical Specimen</th>
<th>MNRH n</th>
<th>MRRH n</th>
<th>MBRRH n</th>
<th>KIU-TH n</th>
<th>Total n</th>
<th>CR Prevalence per clinical specimen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>16</td>
<td>5</td>
<td>12</td>
<td>5</td>
<td>41</td>
<td>24.1</td>
</tr>
<tr>
<td>Blood</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>14.3</td>
</tr>
<tr>
<td>Anal swab</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>32</td>
<td>31.1</td>
</tr>
<tr>
<td>Wound/pus swab</td>
<td>4</td>
<td>5</td>
<td>12</td>
<td>4</td>
<td>33</td>
<td>17.7</td>
</tr>
<tr>
<td>Sputum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>15.8</td>
</tr>
<tr>
<td>Tracheal Aspirate</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>12</td>
<td>16.7</td>
</tr>
<tr>
<td>Virginal Swab</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>27</td>
<td>11.1</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>45</td>
<td>52</td>
<td>18</td>
<td>62</td>
<td>22.8</td>
</tr>
</tbody>
</table>

CR Prevalence (%)      22.0  34.6  33.9  11.8  22.8

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

Table 8: Correlation between carbapenem resistance genes and phenotypic resistance

<table>
<thead>
<tr>
<th>CR Prevalence per clinical specimen (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>176</td>
</tr>
</tbody>
</table>

Table 11: Distribution of pathogenic genes among the Escherichia coli Phylogenetic groups and their carbapenem resistance profiles

<table>
<thead>
<tr>
<th>PG</th>
<th>PSG</th>
<th>eae</th>
<th>elt</th>
<th>ipaH</th>
<th>sxt</th>
<th>PAI IICPT073</th>
<th>PAI IV536</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>A1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>B1</td>
<td>B1</td>
<td>19</td>
<td>4</td>
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<td>3</td>
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</tr>
<tr>
<td>B2</td>
<td>B2</td>
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<td>0</td>
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<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>B2</td>
<td>B22</td>
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<td>0</td>
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<td>5</td>
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<tr>
<td>D</td>
<td>D2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>13</td>
<td>55</td>
<td>139</td>
</tr>
</tbody>
</table>

R: Resistant, S: Sensitive

Table 7: Distribution of carbapenem resistant genes in E. coli isolates obtained from different tertiary hospitals in Uganda

<table>
<thead>
<tr>
<th>Referral Hospital</th>
<th>NDM</th>
<th>KPC</th>
<th>IMP</th>
<th>OXA-48</th>
<th>VIM</th>
<th>NDM</th>
<th>IMP</th>
<th>KPC</th>
<th>IMP</th>
<th>VIM</th>
<th>VIM, NDM</th>
<th>NDM, KPC &amp; OXA-48</th>
<th>IMP, KPC &amp; OXA-48</th>
<th>Total CR isolates</th>
<th>CR Prevalence (%)</th>
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</thead>
<tbody>
<tr>
<td>MNRH</td>
<td>205</td>
<td>07</td>
<td>07</td>
<td>06</td>
<td>11</td>
<td>19</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>62</td>
<td>30.3</td>
</tr>
<tr>
<td>MRRH</td>
<td>52</td>
<td>03</td>
<td>04</td>
<td>05</td>
<td>07</td>
<td>02</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>03</td>
<td>02</td>
<td>28</td>
<td>53.9</td>
</tr>
<tr>
<td>MBRRH</td>
<td>62</td>
<td>02</td>
<td>03</td>
<td>09</td>
<td>04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>05</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>45.2</td>
</tr>
<tr>
<td>KIU-TH</td>
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<td>01</td>
<td>04</td>
<td>03</td>
<td>07</td>
<td>06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>20.6</td>
</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
<td>139</td>
<td>33.0</td>
</tr>
</tbody>
</table>
Table 9: Distribution of pathogenic E. coli among the tertiary hospitals and clinical samples

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>Tertiary hospitals</th>
<th>Clinical specimens</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MMRH</td>
<td>MBBRH</td>
</tr>
<tr>
<td>eae/EPEC</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>elt/L-ETEC</td>
<td>3</td>
<td>2 (3.9%)</td>
</tr>
<tr>
<td>est/S-ETEC</td>
<td>3</td>
<td>3 (4.8%)</td>
</tr>
<tr>
<td>stx/STEC</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>DEC</td>
<td>2</td>
<td>3 (5.8%)</td>
</tr>
<tr>
<td>ipaH/EIEC</td>
<td>4</td>
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</tr>
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<tr>
<td>ExPEC</td>
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</tr>
</tbody>
</table>

Table 10: Distribution of the Escherichia coli Phylogenetic groups in the four tertiary hospitals and clinical specimens

<table>
<thead>
<tr>
<th>Phylogenetic group (PG)</th>
<th>Phylogenetic subgroup (PSG)</th>
<th>MNRH</th>
<th>MRRH</th>
<th>MBBRH</th>
<th>KIU-TH</th>
<th>Total</th>
<th>PSG (%)</th>
<th>PGP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A0</td>
<td>33</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td>54</td>
<td>12.8</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td>21</td>
<td>2 (10.2%)</td>
<td>34.6%</td>
<td>11</td>
<td>23</td>
<td>73</td>
<td>17.3</td>
</tr>
<tr>
<td>B1</td>
<td>N/A</td>
<td>38</td>
<td>14 (26.9%)</td>
<td>172</td>
<td>17</td>
<td>91</td>
<td>21.6</td>
<td>21.6</td>
</tr>
<tr>
<td>B2</td>
<td>B22</td>
<td>6</td>
<td>2 (1.0%)</td>
<td>3.9%</td>
<td>21</td>
<td>4</td>
<td>15</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>B23</td>
<td>92</td>
<td>18 (44.9%)</td>
<td>34.6%</td>
<td>218</td>
<td>158</td>
<td>37.5</td>
<td>19.0</td>
</tr>
<tr>
<td>D</td>
<td>D1</td>
<td>5</td>
<td>0 (2.4%)</td>
<td>0.0%</td>
<td>0</td>
<td>3</td>
<td>2 (20.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>10</td>
<td>0 (4.9%)</td>
<td>0.0%</td>
<td>3 (9.7%)</td>
<td>6</td>
<td>22</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppltTables.docx