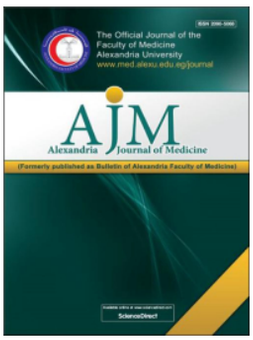

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Molecular characterization and distribution of cephalosporin resistance determinants in *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients attending Kampala International University Teaching Hospital in Bushenyi, Western Uganda

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ABSTRACT

Cephalosporins are the first-line therapy antibiotics used in the treatment of gram-negative bacterial infections. However, high prevalence of cephalosporins resistance in *Klebsiella pneumoniae* and *Escherichia coli* has been reported worldwide. Studies conducted in Uganda reported high incidences of cephalosporin resistance (CR). Successive studies at Mulago National Referral Hospital indicated a decline in the resistance levels pointing to the need for regular antibiotic resistance surveillance. Therefore, this study carried out molecular characterization of CR determinants in *E. coli* and *K. pneumoniae* isolated from patients attending Kampala International University Teaching Hospital (KIU-TH). A retrospective study using *E. coli* and *K. pnuemoniae* samples previously obtained from surgical wounds and urinary tract infections among patients treated at KIU-TH between September 2016 and August 2018 was conducted. Biochemical assays were used to confirm the identity of the samples. Combined disc and boronic acid assays were used to determine the cephalosporine resistance profile of the isolates. Multiplex PCR amplification was used to characterize the extended spectrum beta-lactmase (ESBL) encoding genes. The study revealed that *E. coli* (130/81.2%) isolates were more predominant than *K. pneumoniae* (30/18.8%) among the archived samples. *K. pneumoniae* showed the highest phenotypic resistance with a mean prevalence of 90.6% but comparable to that of *E. coli* (89.3%). Of the 160 isolates screened, 105 (65.6%) were ESBL producers. Multiplex PCR revealed that the most predominant ESBL encoding gene was bla_{SHV} at a prevalence of 42.0%, followed by bla_{TEM} at 27.3%, bla_{CTX-M} at 22.4% and $bla_{CTX-M-15}$ at 8.4%. The incidence of phenotypic resistance and distribution of ESBL genes were significantly higher in patients of Ishaka division. Our study reports a high prevalence of cephalosporin-resistant *E. coli* and *K. pnuemoniae* isolated from patients attending KIU-TH and highlights the need for routine screening of antimicrobial resistance in health-care facilities so as to guide clinicians on the rational prescription of antibiotics.

List of abbreviations: ATCC: American Type Culture Collection; ESBL: extended spectrum beta-lactmases; AmpC: aminopenicillin cephalosporinase; CTX-M: cephotaxime (M-first detected in Munich) hydrolyzing capabilities; CTX-M-U: cephotaxime hydrolyzing capabilities gene Universal primer; SHV: sulfhydryl variables (variant-2); CMY-2: cephamycins (variant-2); TEM: temoneira; ACT-1: AmpC type (variant-1); Bla: Beta lactam; DNA: deoxyribonucleic acid; PCR: Polymerase chain reaction; MNRH: Mulago National Referral Hospital; MRRH: Mbarara Regional Referral Hospital; KRRH: Kabale Regional Referral Hospital (KRRH); KIU-TH: Kampala International University Teaching Hospital; CDL: Central Diagnostic Laboratory; CoVAB: College of Veterinary Medicine, Animal Resources and Biosecurity; CR: cephalosporin resistance

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Escherichia coli; *Klebsiella pneumoniae*; cephalosporin resistance; extended-spectrum beta lactamases; ESBL encoding genes

1. Introduction

Cephalosporins form a relatively large group of antibiotics with a β-lactam ring. This class of antibiotics is bactericidal against gram-negative bacteria. Cephalosporins are classified into first, second, third, fourth, and fifth generations [1]. The third generation cephalosporins are the most widely used in the treatment of gram-negative bacterial infections. They include cefotaxime, ceftriaxone, ceftazidime, cefixime, ceftizoxime, and cefoperazone [2]. Cephalosporin resistance (CR) in Enterobacteriaceae, most importantly, *Escherichia coli* and *Klebsiella pneumoniae*, which are the leading causes of hospital- and

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community-acquired bacterial infections, has been reported worldwide [3–5]. Antibiotic resistance to beta-lactam antibiotics arises through a number of mechanisms, which include: (a) inactivation of antibiotics through hydrolyzing the beta-lactam ring by bacterial enzymes encoded by resistance genes located on chromosomes and plasmids, (b) modification and downregulation of porin expression that leads to the decrease in permeability of the outer membrane to cephalosporins; (c) flushing out of cephalosporins by efflux pumps; and (d) acquisition of a new penicillin-binding protein (PBP) or modification of the PBP, thereby reducing the affinity of PBP component to cephalosporins [6,7]. The mode of resistance exhibited by most gram-negative bacteria to cephalosporins is mainly attributed to bacterial enzymes called beta-lactamases, which are encoded by different genes that include TEM, SHV, CTX, ACT-1, CMY-2, CMY-4, and DHA-1 [6–8].

A study in Uganda reported CR prevalence of 79.2% and 92.3% in *E. coli* and *K. pneumoniae* isolates, respectively, at Mulago National Referral Hospital (MNRH) [9]. Another study at Mbarara Regional Referral Hospital (MRRH) revealed that the most common extended spectrum beta-lactmase (ESBL) producers were *E. coli* at 34% and *K. pneumonia* at 12%, where the ESBLs resistant determinants characterized included $bla_{\text{CTX-M}}$ (70%), bla_{SHV} (34%), mixed $bla_{\text{CTX-M}}/bla_{\text{SHV}}$ (7.4%), $bla_{\text{CTX-M}}/bla_{\text{TEM}}$ (5.8%), $bla_{\text{SHV}}/bla_{\text{TEM}}$ (0.8%), and $bla_{\text{CTX-M}}/bla_{\text{SHV}}/bla_{\text{TEM}}$ (3.8%) [10].

This variation in the CR prevalence and resistance genes reported in Uganda point to the need for antibiotic resistance surveillance at several localities. This is because drug resistance within the pathogens is an evolving process that requires routine surveillance and monitoring in order to provide physicians with the current status of antibiotic resistance for rational prescription of drugs [11]. Furthermore, hospital-based antibiotics resistance stewardship dictates antibiotics prescription in a given hospital and is determined by the prevailing resistance to a given class of antibiotics [11,12]. Thus, this study focused on prevalence and genotypic characterization of CR genes in *E. coli and K. pneumoniae* isolated from patients attending Kampala International University Teaching Hospital (KIU-TH) in Uganda.

2. Materials and methods

2.1. Study design and bacterial samples

We carried out a retrospective study using *E. coli* and *K. pneumoniae* samples previously obtained from surgical wounds and UTIs among patients of Ishaka, Bushenyi, and Nyakabirizi divisions treated at KIU-TH between September 2016 and August 2018. Archived resistant *E. coli* and *K. pneumoniae* isolates were collected and recovered from the Microbiology Laboratory, KIU-TH, and transferred to the Central Diagnostic Laboratory, Makerere University, for identity confirmation by biochemical assays. CR profile was determined using the disc diffusion method and, thereafter, the characterization of CR genes using multiplex PCR.

3. Inclusion and exclusion criteria

Archived samples previously isolated from urine and surgical wounds of in- and out-patients at KIU-TH were included. A total of 180 samples were included. Samples that were not isolated from patients of Bushenyi-Ishaka Municipality (Ishaka, Bushenyi, and Nyakabirizi divisions) and those that could not be confirmed as *E. coli* or *K. pnuemoniae* or those where no bacterial growth was observed were excluded from subsequent analysis.

4. Antimicrobial susceptibility test

E. coli and *K. pneumoniae* identity was confirmed using biochemical tests as previously described. Triple sugar iron test that detects glucose, lactose, and sucrose fermenters [13,14], indole test [14] and citrate utilization test [13]), urease test [14]), and methyl red and Voges Proskaue tests [14] were conducted. The susceptibility tests were performed using the disc diffusion method according to the standard guidelines [15]. The isolates were subjected to antibiotic susceptibility test on Muller–Hinton agar using Ampicillin 25 μg; amoxicillin/clavulanic acid 20/10 μg; ceftazidime 30 μg; cefuroxime (CXM) 30 µg; temocillin (TEM) (30 µg); piperacillin-tazobactum (TPZ) 110 µg; cefoxitin (FOX) 30 µg; ceftizoxime (CTX) 30 µg; ceftazidime (CAZ) 30 µg; ceftriaxone (CRO) 30 µg; and cefixime (CFM) 30 µg. *K. pneumoniae* American Type Culture Collection (ATCC) 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively [16,17].

5. Detection of ESBLs and aminopenicillin cephalosporinases (AmpCs)

To distinguish between ESBLs and AmpCs producers, the phenotypic confirmatory disc diffusion test was performed as follows. The Mueller Hinton Agar (MHA) was inoculated with standard inoculums (0.5 McFarland) of the test isolate. Antibiotic discs containing a mixture of ceftazidime and clavulanic acid at a concentration of 30 µg/10ug and ceftazidime alone was used to taste for the level of resistance. A larger inhibitory zone diameter (>5 mm) in the presence of clavulanic acid than with ceftazidime alone indicated the presence of ESBLs [18]. The phenotypic detection

Table 1. Cephalosporin resistance gene, gene primer sequences, amplicon size, and their prevalence in *E. coli* and *K. pneumoniae* isolated from patients of Ishaka-Bushenyi Municipality.

Primer Name	Primer Sequence (5′- 3′)	Amplicon size (bp)/Reference	E. coli	K. pneumoniae	Total
*bla*SHV	F- AGCCGCTTGAGCAAATTAAAC	230 (SHV-1) [24]	38 (26.6%)	12 (8.4%)	50 (35.0%)
	R- ATCCCGCAGATAAATCACCAC	713 (SHV-2) [24]	10 (7.0%)	00 (0%)	10 (7.0%)
*bla*CTX-M-15	F-CACACGTGGAATTTAGGGACT R-GCCGTCTAAGGCGATAAACA	995 [24]	12 (8.4%)	00 (0%)	12 (8.4%)
*bla*CTX-M	F-ATGTGCAGYACCAGTAARGT R-TGGGTRAARTARGTSACCAGA	596 [31]	22 (15.4%)	10 (7.0%)	32 (22.4%)
*bla*TEM	F-ATGAGTATTCAACATTTCCGC R-CAATGCTTAATCAGTGAGG	856 [24]	29 (20.3%)	10 (7.0%)	39 (27.3%)

of AmpC β-lactamases was achieved by using boronic acid disc test. Discs containing boronic acid were prepared as follows. One hundred twenty milligrams of phenyl boronic acid (benzeneboronic acid) were dissolved in 3 mL of dimethyl sulfoxide. Then, 3 mL of sterile distilled water was added to this solution. Twenty microliters of this solution was dispensed onto the discs containing 30 µg of cefotetan discs and allowed to dry for 30 minutes and used immediately. The boronic acid test was performed by inoculating MHA with standard inoculums of *E. coli* and *K. pneumoniae* followed by placing a disc containing 30 µg of cefotetan and a disc containing 30 µg of cefotetan and 400 µg of boronic acid solution onto the agar. The inoculated plates were incubated overnight at 35°C. Isolates that demonstrated zones of inhibition around the disc containing cefotetan and boronic acid ≥5 mm were considered AmpC producers. Isolates that were positive for phenotypic confirmatory disc diffusion test and boronic acid test were considered to express both ESBLs and AmpCs [18–20].

6. Molecular detection of ESBLs encoding genes

Genomic DNA was extracted using the Qiagen DNA extraction kit following the manufacturer's protocol. Multiplex PCR was performed as previously described by Voets et al. [21] and Dallenne et al. [22], with modifications to enable the concurrent amplification of all the target gene variants using bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and $bla_{CTX-M-15}$ general primers (Table 1). Briefly, a final PCR volume of 40 µL containing 5 µL of 0.5 µg DNA template, 1 µL of 1 U Taq polymerase, 1 µl of 200 µM dNTPs, 8 µL of all primer combinations (2 µl/primer pair), and 25 µL PCR buffer (2 mM $MgCl_2$,100 mM Tris-HCl,500 mM KCl pH 8.3) was constituted. The PCR program consisted of an initial denaturation at 94°C for 5 minutes followed by 35 cycles at 94°C for 1 minute, 60°C for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were run on a 1.5% agarose gel, stained using ethidium bromide, visualized and imaged under UV light using Image analyzer. The size of bands was used to identify the gene responsible for resistance (Table 1) [23]. *K. pneumoniae* 7881, *K. pneumoniae* ATCC 700603, and *E. coli* ATCC 35218 containing bla_{SHV}, bla_{CTX-M}, and bla_{TEM} genes were used as positive controls, whereas *E. coli* ATCC 25922 was used as the negative control.

7. Data analysis

Data analysis was done using OriginPro version 8.5. Comparisons of CR between *E. coli* and *K. pneumoniae*, distribution of phenotypic resistance, and genetic determinants of resistance among communities (division of the patients) were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A P-value of ≤0.05 indicated significant statistical variance.

8. Results

8.1. CR profile

Of the 180 isolates obtained from the archives, 130 (81.2%) were confirmed to be *E. coli*, 30 (18.8%) *K. pneumoniae*, and 13 (7.2%) isolates were neither *E. coli* nor *K. pneumoniae,* while 7 (3.9%) isolates exhibited no growth. Bacterial isolates were predominantly obtained from urine (96/60%), Tables 2 and 3. All the 160 isolates were at least resistant to one of the antibiotics. Resistance to Cefotaxime was the highest but not significantly different from the resistance registered by ceftriaxone, ceftazidime and cefixime. *K. pneumoniae* and *E. coli* exhibited statistically similar phenotypic resistance, Table 2.

8.2. Prevalence of ESBLs and AmpCs producers

Phenotypic confirmatory disc diffusion and boronic acid disc assays revealed that out of 160 isolates screened, 105 (65.6%) were ESBLs producers where 56.2% (69 *E. coli* and 21 *K. pneumoniae*) were positive for ESBLs, while 9.4% (12 *E. coli* and 3 *K. pneumoniae*) harbored both ESBLs and AmpCs. None of the isolates was positive for only AmpCs while 55 (34.4%) were negative for both ESBLs and AmpCs.

Table 2. Resistance profiles of *E. coli* and *K. pneumoniae* isolated from Ishaka, Bushenyi, and Nyakabirizi.

												CTX, CAZ, CRO & CFM phenotypic resistance (%)	
Division	Isolate	n	AMP	AMO	CXM	TEM	TPZ	FOX	CTX	CAZ	CRO	CFM	E. coli	K. pneumoniae
Ishaka	*E. coli*	65	65 (100%)	64 (99%)	64 (99%)	61 (94%)	49 (75%)	34 (52%)	61 (94%)	58 (89%)	64 (98%)	60 (92%)	93	
	K. pneumoniae	15	15 (100%)	15 (100%)	15 (100%)	14 (93%)	11 (73%)	8 (53%)	15 (100%)	13 (87%)	15 (100%)	15 (100%)		97
Bushenyi	*E. coli*	35	35 (100%)	33 (94%)	34 (97%)	33 (94%)	24 (69%)	21 (60%)	29 (83%)	27 (77%)	31 (89%)	31 (89%)	85	
	K. pneumoniae	10	10 (100%)	10 (100%)	10 (100)	10 (100%)	7 (70%)	5 (50%	9 (90%)	8 (80%)	10 (100%)	9 (90%)		90
Nyakabirizi	*E. coli*	30	30 (100%)	29 (97%)	29 (97%)	28 (93%)	24 (80%)	18 (60%)	26 (87%)	26 (87%)	28 (93%)	28 (93%)	90	
	K. pneumoniae	5	5 (100%)	5 (100)	5 (100%)	5 (100%)	4 (80%)	3 (60%)	5 (100%)	4 (80%)	4 (80%)	4 (80%)		85
Average			**100 ± 0.0^{A}**	**98 ± 2.2^{A}**	**98 ± 1.3^{A}**	**96 ± 3.1^{A}**	**76 ± 4.4^{B}**	**56 ± 4.3^{C}**	**92 ± 6.3^{A}**	**83 ± 4.5^{AB}**	**93 ± 7.2^{A}**	**91 ± 6.0^{A}**	**89.3% ± 5.51^{A}**	**90.6% ± 9.07^{A}**

Means in each column accompanied by the similar letter are not significantly different ($P > 0.05$) (Tukey Multiple Comparison) and values escorted by letter(s) that are not similar are significantly different ($P < 0.05$); n = number of isolates.

Table 3. Bacteria isolates obtained from different clinical specimens

Clinical specimen	Division	Bacteria isolates		Total number of isolates per clinical specimen/ percentage
		E. coli	*K. pneumoniae*	
Surgical and other wounds/ pus	Nyakabirizi Division	12	01	64(40%)
	Ishaka Division	28	06	
	Bushenyi Division	14	03	
Urine	Nyakabirizi Division	18	04	96 (60%)
	Ishaka Division	37	09	
	Bushenyi Division	21	07	

8.3. Distribution of ESBLs encoding genes

The 105 isolates that stood to be ESBL producers were typed by multiplex PCR and recorded a total of 143 genes (Figure 1). The *bla*SHV-1-like gene recorded the highest prevalence (35%), followed by bla_{TEM} (27.3%), bla_{CTX-M} (22.4%), $bla_{CTX-M-15}$ (8.4%), and then $bla_{SHV-2-like}$ (7.0%) (Table 1). Furthermore, multiple Tukey comparison revealed that the prevalence of CR genes in *E. coli* and *K. pneumoniae* was substantially dissimilar.

Of the 105 isolates, 35 (33.3%) contained more than one ESBL genes; eight (7.6%) *E. coli* and 3(2.8%) *K. pneumoniae* isolates coharboured $bla_{SHV-1-like}$ and bla_{TEM}, $bla_{SHV-1-like}$ and bla_{CTX-M} resided in 6 (5.7%) *E.coli* and 2 (1.9%) *K. pneumoniae,* bla_{CTX-M} and bla_{TEM} co-occurred in 2 (1.9%) *E. coli* and 3 (2.8%) *K. pneumoniae,* $bla_{CTX-M-15}$ and bla_{TEM} coexisted in 2 (1.9%) *E. coli,* $bla_{SHV-1-like}$ and $bla_{CTX-M-15}$ co-occurred in 3 (2.8%) *E. coli,* bla_{CTX-M} and $bla_{CTX-M-15}$ 3 (2.8%) were co-present in *E. coli*. Three (2.8%) *E. coli* isolates had $bla_{SHV-like}$, bla_{CTX-M}, and bla_{TEM} genes coexisting. However, the mean occurrence of ESBL encoding genes was substantially higher in *E. coli* (Table 4; Figure 2).

Table 4. Combinations of ESBL genes detected in Extended Spectrum β-lactmases producing *E. coli* and *K. pneumoniae* isolates.

ESBL encoding genes	E. coli	K. pneumoniae	Number of genes
bla_{SHV-1} + bla_{TEM}	8 (7.6%)	3 (2.8%)	22
bla_{SHV-1}+ $bla_{CTX-M-U}$	6 (5.7%)	2 (1.9%)	16
$bla_{CTX-M-U}$ + bla_{TEM}	2 (1.9%)	3 (2.8%)	10
$bla_{CTX-M-15}$+ bla_{TEM}	2 (1.9%)	0 (0%)	4
bla_{SHV-1} + $bla_{CTX-M-15}$	3 (2.8%)	0 (0%)	6
$bla_{CTX-M-U}$ + $bla_{CTX-M-15}$	3 (2.8%)	0 (0%)	6
bla_{SHV-1} + bla_{CTX-M} + bla_{TEM}	3 (2.8%)	0 (0%)	9
Mean ±STDEV	**3.6%± 0.022[A]**	**1.1% ± 0.014[B]**	

Means in each column accompanied by the similar letter are not significantly different (P > 0.05) (Tukey Multiple Comparison) and values escorted by letter(s) that are not similar are significantly different (P < 0.05)

Seventy isolates (66.7%) contained one ESBL gene. The incidence of $bla_{SHV-1-like}$ was greatest at 17.1% (18) and 6.6% (7) for *E. coli* and *K. pneumoniae* respectively, followed by bla_{TEM} (13.3%/14) for *E. coli* and (3.8%/4) for *K .pneumoniae,* $bla_{SHV-2-like}$ at 9.5% (10) for *E. coli,* bla_{CTX-M} at 7.6% (8) and 4.7% (5) for *E. coli* and *K. pneumoniae* respectively and then $bla_{CTX-M-15}$ at 3.8% (4) for *E. coli*, Table 5; Figure 2.

Table 5. One ESBL gene per isolate detected in ESBLs producing *E. coli* and *K. pneumoniae.*

Cephalosporin resistance determinant	E. coli	K. pneumoniae	Total
bla_{SHV-1}	18 (17.1%)	7 (6.6%)	25
bla_{SHV-2}	10 (9.5%)	0 (0%)	10
bla_{TEM}	14 (13.3%)	4 (3.8%)	18
$bla_{CTX-M-15}$	4 (3.8%)	0 (0.0%)	4
$bla_{CTX-M-U}$	8 (7.6%)	5 (4.7%)	13

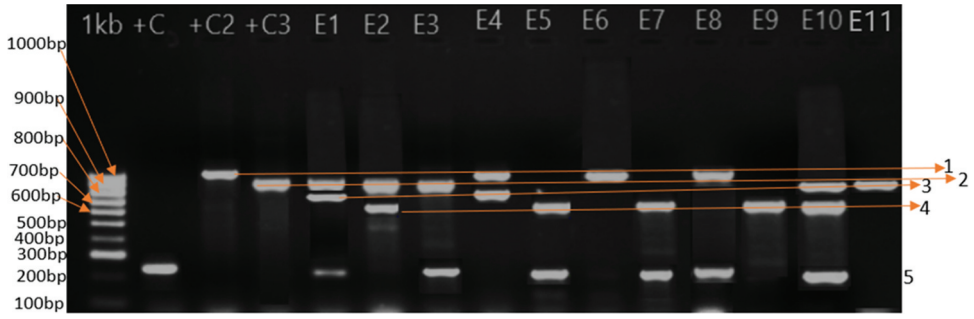

Figure 1. Multiplex PCR amplification of the ESBLs encoding genes. 1000 bp ladder (1Kb)-Eurofins genomics, Positive control 1 (+C1)-*E. coli* ATCC 35218, Positive control 2 (+C2)-*K. pneumoniae* 7881, Positive control 3 (+C3) *K. pneumoniae* ATCC 700603; Amplicon level 1 represents CTXM-15 gene (approx. 995bp), level 2: TEM gene (approx. 856bp), level 3:SHV-2 gene (approx. 713bp), level 4:CTXM-U gene (approx. 596bp, level 5:SHV-1 (approx. 230bp) and E1–E10 representative *E. coli* isolates.

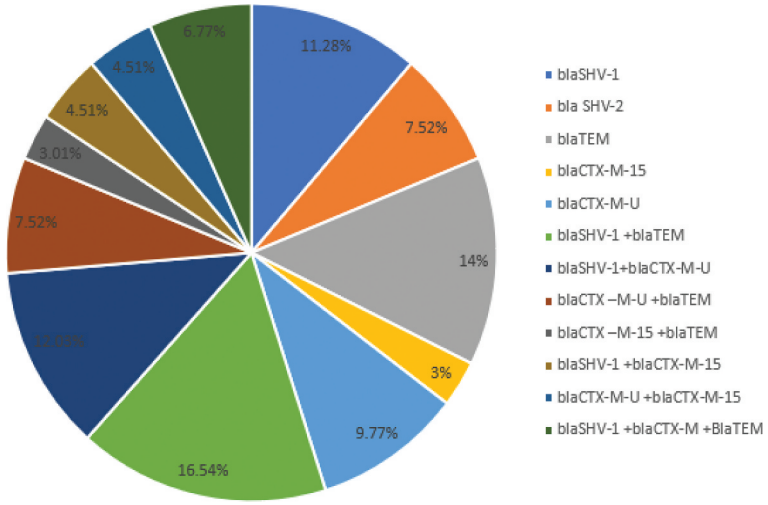

Figure 2. Prevalence of ESBLs encoding genes in *E. coli* and *K. pneumoniae*.

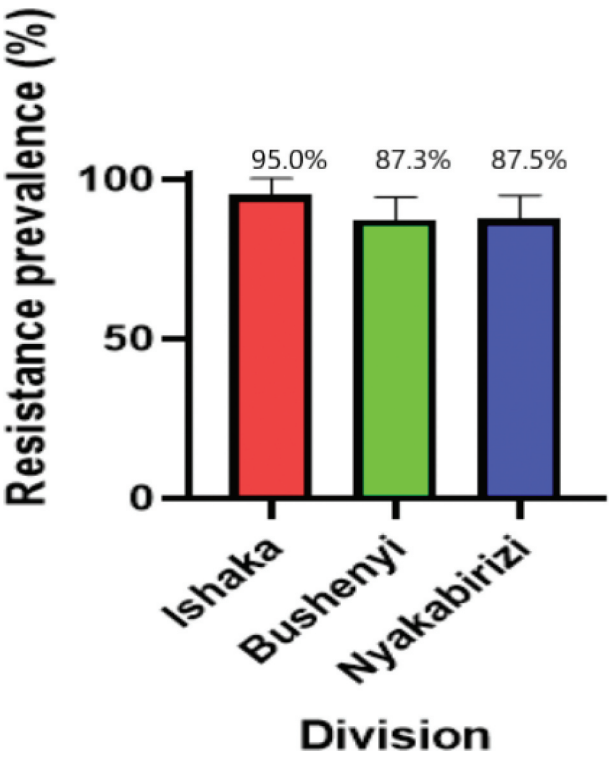

Figure 3. Mean phenotypic resistance prevalence among the divisions.

8.4. Comparison of phenotypic resistance prevalence among the divisions

One-way analysis of variance using Tukey's multiple comparisons test package was conducted to compare the mean phenotypic resistance prevalence among the different divisions of Bushenyi-Ishaka Municipality. The multiple comparison analysis registered *P* values >0.05 indicating statistically similar levels of phenotypic resistance among the different divisions (Figure 3).

8.5. Distribution of cephalosporin genetic resistance determinants among the divisions

ESBLs encoding genes were highly distributed among the Ishaka division population with a prevalence of 15.5% followed by Bushenyi division (7.3%) and Nyakabirizi division (2.3%). Comparison of the distribution of ESBLs encoding genes among the different divisions located in Bushenyi-Ishake Municipality was performed in one-way ANOVA. Tukey's multiple comparison analysis computed a *P* value >0.05 between Bushenyi and Nyakabirizi divisions indicating no statistical difference in the prevalence of ESBLs encoding genes among the population, whereas *P* values <0.05 were obtained between Ishaka and Bushenyi divisions, Ishaka and Nyakabirizi division signifying statistically higher prevalence of the ESBL genes among the Ishaka population. The distribution of the ESBL genes is illustrated by Figure 4.

9. Discussion

Phenotypic and molecular characterization revealed that 105 (65.6%) of the isolates expressed ESBLs. The phenotypic resistance in this study is comparable to that (62.0%) reported at MNRH in 2015 [25] but lower than resistance frequencies of 81.4% and 89% reported at MNRH in 2013 [9] and Kabale Regional Referral Hospital (KRRH) [26], respectively. Contrary to this, lower ESBLs phenotypic (29.9% and 13.4%) and genotypic (51%) frequencies were reported by studies carried out at MRRH [10] and MNRH in 2017 [27].

Nosocomial outbreaks in hospital settings with limited therapeutic options have been attributed to AmpC β-lactamase producing bacteria [28]. In this study, combined disc diffusion and boronic acid assays revealed that only 15 isolates co-expressed ESBLs

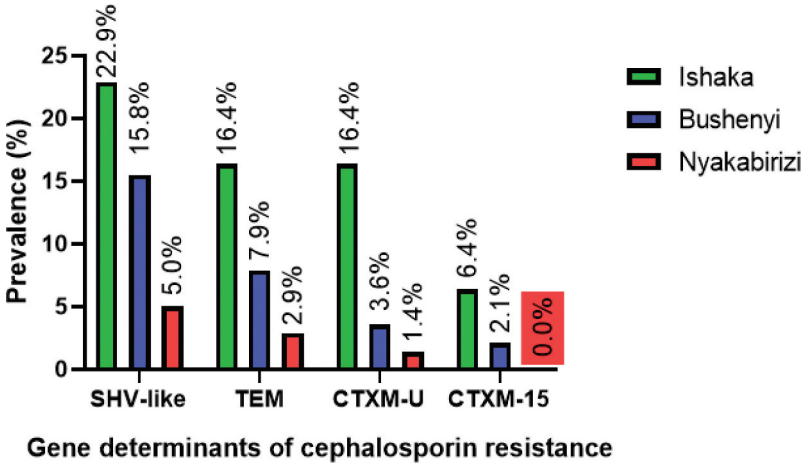

Figure 4. Distribution of ESBLs encoding genes within the different divisions of Bushenyi-Ishaka Municipality.

and AmpCs. Thus, a low prevalence (9.4%) of AmpC β-lactamase producers was registered by this study. These findings corroborate well with other studies that reported relatively low prevalence (0.5%–30%) of coproduction of ESBLs and AmpCs [29–32]

E. coli (81.2%) isolates were more predominant than *K. pneumoniae* (18.8%) among the archived samples. This is in agreement with other studies that attempted to establish the prevalence of ESBLs producing *Enterobacteriaceae* in Uganda [9,10,25,27]. The mean prevalence of CR was higher in *K. pneumoniae* (90.6% ± 9.07) but not significantly different from that registered by *E. coli* isolates (89.3% ± 5.51). This is in contrast with two studies where the prevalence of CR reported was 52% and 44% at KRRH [26] and 72.7% and 58.1% at MNRH in 2015 [25] but corroborates with other studies that reported CR incidence as 79.2% and 92.3% at MNRH in 2013 [9] and 85% and 88% globally [33] in *E. coli* and *K. pneumoniae,* respectively. However, studies by Moses et al. [10] at MRRH in 2013 and Ampaire et al. [27] at MNRH in 2017 reported *E. coli* as the most predominant ESBL producer. Antimicrobial resistance (AMR) stewardship strategies put forward to combat antibiotic resistance can only be assessed by routine quantification of AMR prevalence. Successive studies were carried out to determine the prevalence of ESBLs producers at MNRH. Indeed, the results are promising as the prevalence of ESBL producers reduced from >75% [9] to 13.4% [27] reported in 2013 and 2017, respectively.

For molecular characterization of ESBL genes, bla_{SHV} primer that amplified two genes, probably $bla_{SHV\text{-}1\text{-}like}$ and $bla_{SHV\text{-}2\text{-}like}$, with different amplicon sizes was used. The commonest ESBL gene identified was bla_{SHV} (SHV-1 and SHV-2) (42%) followed by bla_{TEM} (27.3%), $bla_{CTX\text{-}M}$ (22.4%), and $bla_{CTX\text{-}M\text{-}15}$ (8.4%). However, these findings are not in agreement with the ESBL genes prevalence registered at MRRH where the most prevalent ESBL gene was CTX-M (70%), SHV (34%), and TEM (0.8%) [10] but comparable to ESBL gene prevalence reported in other parts of the world. For example, studies conducted in Australia, Belgium, Turkey, South Africa, and USA reported bla_{SHV} (67.1%) as the most prevalent gene, trailed by CTX-M-type(23.3%) and TEM–type (16.4%) [34], and this incidence is similar in many other countries [35–37]. Furthermore, 35 out of the 105 ESBLs positive isolates expressed more than one ESBL gene. This collaborates with Moses et al.'s [10] study at MRRH where 87 of the 213 isolates harbored more than one ESBL gene and other studies done outside Uganda. For instance, in Turkey, 68 isolates had SHV/TEM coexisting, 61 had TEM/CTX-M, and 54 had SHV/CTX-M genes [38]. Moreover, seven (21.87%) isolates containing TEM/SHV were reported in Iran [39]. It is worth noting that this study registered a rare occurrence of three genes: $bla_{SHV\text{-}1}$/$bla_{CTX\text{-}M}$/bla_{TEM} coexisting in three of the *E. coli* isolates. Existence of three ESBL genes in one isolate is in line with Mohsen et al.'s [40] study who reported the co-occurrence of $bla_{CTX\text{-}M}$/bla_{TEM}/bla_{SHV} in 14 isolates.

Furthermore, 55 out of 160 isolates contained neither ESBLs nor AmpCs but exhibited excellent CR. This resistance may be attributed to the presence of resistance intrinsic factors, such as downregulation and mutation of the outer membrane proteins, through which antibiotics diffuse [41–44]. High prevalence of carbapenem resistance has been reported in Uganda [45–47]. Acquisition of carbapenemase encoding genes by *Enterobacteriaceae* confer resistance to all beta lactam antibiotics [41,45]. Thus, the most likely source of resistance in the 55 isolates is probably the presence of carbapenem resistance genes.

Among the three divisions, Ishaka has the highest population density (891.0/km^2), followed by Bushenyi division also known as Central division (490.4/km^2), and Nyakabirizi division (248.1/km^2) [48]. Statistical comparison of phenotypic resistance prevalence and distribution of cephalosporin-

resistance determinants within the divisions of

Bushenyi-Ishaka Municipality revealed higher phenotypic resistance and distribution of ESBLs encoding genes in the population of Ishaka division. The results corroborate well with Bruinsma et al.'s [49] findings. While studying the impact of population density on antibiotic resistance, Bruinsma et al. [49] observed that population density is a vital aspect that influences the development of antibiotic resistance. Similar findings were reported by Honda et al. [50].

10. Conclusions

High prevalence of CR in *E. coli* and *K. pneumoniae* isolated from patients treated at KIU-TH was revealed by this study. *K. pneumoniae* was encountered as the most prevalent resistant isolate and *bla*SHV as the most predominant gene. Thus, the third-generation cephalosporin endorsed for treatment of *Enterobacteriaceae* infections were not effective in vitro. Indeed, this study accentuates routine screening of AMR in different healthcare facilities. This will guide clinicians on the rational prescription of antibiotics so as to control the emergence and transmission of resistant strains. This study has provided an insight into the possible presence of determinants of CR other than ESBL and AmpCs; thus, further investigations are required to determine the other factors responsible for CR among *Enterobacteriaceae* isolated from patients attending KIU-TH.

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Availability of data and materials

Supplementary data or any other forms of data have been submitted. Raw data and any other forms data generated by this project can be obtained from the authors on request by e-mail.

Ethics and consent to participate

Ethical Approval No: NrUG-REC-023/201,838 was granted by the Research Ethics Committee for ethical review and approval, Kampala International University-Western Campus. The Research Ethics Committee waived the need for informed consent to use already coded archived samples in this study.

Disclosure statement

The authors declare that they have no competing interests.

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Limitations

This study did not look at carbapenem resistance, yet carbapenemase production confers resistance to all beta-lactams. Phenotypic resistance in isolates that neither harboured ESBLs nor AmpCs may be due to the presence of carbapenemase genes. Primers used to detect ESBLs encoding genes in this study targeted most gene variants. Thus, sequencing of the amplicons should be done to distinguish between the several gene variants.

Authors' contributions

This work was carried out in collaboration between all authors. Eddie Wampande and Kenneth Ssekatawa conceptualized and designed the format for this study. Kenneth Ssekatawa and Herbert Mbyemeire carried out all the laboratory experiments. Kenneth Ssekatawa conducted data analysis. All authors drafted and managed manuscript revisions. All authors read and approved the final manuscript.

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