Prevalence of plasmid-mediated AmpC beta-lactamases in Enterobacteria isolated from urban and rural folks in Uganda

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Abstract

Background: AmpC beta-lactamases are associated with increased resistance to third-generation cephalosporins. Here, we describe plasmid-mediated AmpC beta-lactamase-producing enterobacteria isolated from urban and rural dwellers in Uganda.

Methods: Stool and urine from 1,448 individuals attending outpatient clinics in Kampala and two rural districts in central Uganda (Kayunga and Mpigi) were processed for isolation of Escherichia coli and Klebsiella. Following antibiotic susceptibility testing, cefoxitin resistant isolates, and amoxicillin/clavulanate resistant but cefoxitin susceptible isolates, were tested for AmpC beta-lactamase production using the cefoxitin-cloxacillin double-disc synergy test. Carriage of plasmid-mediated AmpC beta-lactamase-encoding genes (pAmpC) and extended spectrum beta-lactamase (ESBL) encoding genes was determined by PCR.

Results: Nine hundred and thirty E. coli and 55 Klebsiella were recovered from the cultured samples, yielding 985 isolates (one per participant) investigated. One hundred and twenty nine isolates (13.1%, 129/985) were AmpC beta-lactamase producers, of which 111 were molecularly characterized for pAmpC/ESBL gene carriage. pAmpC genes were detected in 60% (67/111) of the AmpC beta-lactamase producers; pAmpC genes were also detected in 18 AmpC beta-lactamase non-producers and in 13 isolates with reduced susceptibility to third-generation cephalosporins, yielding a total of 98 isolates that carried pAmpC genes. Overall, the prevalence of pAmpC genes in cefoxitin resistant and/or amoxicillin/clavulanate resistant E. coli and Klebsiella was 59% (93/157) and 26.1% (5/23), respectively. The overall prevalence of pAmpC-positive enterobacteria was 10% (98/985); 16.4% (45/274) in Kampala, 6.2% (25/406) Kayunga, and 9.2% (28/305) Mpigi. Ciprofloxacin use was associated with carriage of pAmpC-positive bacteria, while residing in rural districts was associated with protection from carriage of pAmpC-positive bacteria.

Conclusion: pAmpC beta-lactamase producing enterobacteria are prevalent in urban and rural dwellers in Uganda, therefore, cefoxitn should be included during routine susceptibility testing in this setting.

Background

Enterobacteriaceae, a family of Gram-negative bacteria that inhabit the mammalian gut, includes leading causes of community- and hospital-acquired infections [1]. Enterobacteria have become increasingly resistant to antibiotics especially the beta (β)-lactam agents, the mainstay of treatment for infections caused by them. One of the main mechanisms underlying resistance to β-lactam antibiotics among the enterobacteriaceae are the AmpC β-lactamases. These enzymes have become clinically relevant as they confer resistance to most β-lactam antibiotics except the fourth-generation cephalosporins and carbapenems [2–4].

AmpC β-lactamases are chromosomally encoded in most species of the enterobacteriaceae, particularly the Citrobacter freundii, Enterobacter, Morganella morganii, Hafnia alvei, Aeromonas and Serratia spp. [3].

However, Escherichia coli and other enterobacteria, notably Proteus mirabilis, Salmonella and Klebsiella spp., can acquire plasmid-encoded AmpC β-lactamases (pAmpC), which are highly transferable between species. Note that Proteus mirabilis, Salmonella and Klebsiella spp. lack chromosomally-encoded AmpC enzymes, while a chromosomally-encoded AmpC β-lactamase exists in E. coli but it is expressed at low basal levels due to presence of a weak promoter and attenuator, which makes E. coli to be susceptible to cephamycins (e.g. cefoxitin, cefotetan) [2, 5] [6]. Acquisition of pAmpC β-lactamases in species like E. coli and Klebsiella pneumoniae (K. pneumoniae) enables an efficient spread of extended resistance in bacteria and ultimately their spread in the community [3]. Additionally, the widespread use of cephamycins and β-lactamase inhibitor combinations (e.g. clavulanic acid/amoxicillin and tazobactam/piperacillin) has contributed to selection of pAmpC β-lactamase producing strains, worldwide [7, 8].

Once they have colonized the gut, pAmpC β-lactamase producing strains may initiate an infection at various anatomical sites [9]. In low-income countries where such infections are empirically treated with third-generation cephalosporins, failure to detect AmpC β-lactamase-related resistance may lead to treatment failure. Moreover, the cut-off / break points for the disc diffusion / minimum inhibitory concentrations (MICs) may not detect AmpC β-lactamase production and resistance to third-generation cephalosporins. Carbapenems are currently the only effective drugs against infections caused by AmpC β-lactamase producing bacteria, since these bacteria tend to be multidrug resistant [10]. However, carbapenamase-producing enterobacteria are now common in low-income countries including Uganda [11].

Although pAmpC β-lactamase producing bacteria have been reported throughout the world [3, 7, 12, 13], there is little information about them in Uganda, especially their frequency among enterobacteria. Therefore, the aim of this study was to estimate the prevalence of pAmpC β-lactamase producing bacteria among Enterobacteriacea isolated from individuals attending outpatient clinics in Kampala city and two rural districts in central Uganda. We show that pAmpC β-lactamase producing bacteria are prevalent in enterobacteria from urban and rural dwellers in Uganda, implying that ceftriaxone, an antibiotic commonly used to treat systemic infections in Uganda, could be associated with treatment failure.

Methods

Study setting

This cross-sectional study was conducted on flora from stool and urine of clients attending outpatients clinics in Kampala and two rural districts (Kayunga and Mpigi) in central Uganda [14]. The study sites were purposively selected with an assumption that urban areas are associated with high bacterial carriage and exposure to antibiotics compared to rural areas [15]. Kampala (urban) and Mpigi districts have a wet-tropical climate while Kayunga has a wet-dry tropical climate [16].

Sample size estimation and sampling

The sample size for each study subsite was proportional to the contribution of the facility to the total outpatient clinic attendance in the months of April, May and June (2006). All individuals attending the clinics were eligible to participate. By then, no data existed on antibiotic resistance among *E. coli* and/or *K. pneumoniae* isolates in well-defined community infections in Uganda. As such, the sample size was estimated based on an observed prevalence of 19.6% for *K. pneumoniae* carriage in clinical samples (urine, etc.) at Makerere University's Clinical Microbiology Laboratory (unpublished observations). In each of the three districts, multistage sampling was done based on the average clinic attendance for the district. Thirty clusters of 16-20 participants were selected from each district using probability proportion to size sampling. Two busy days of a week were purposively chosen to visit a selected health care facility. When the number of participants exceeded 20, systematic sampling was done. A standardized interviewer-administered questionnaire was used to collect clinical and demographic data. Participants were instructed to provide stool or urine (if unable to provide stool) in a sterile screw-cap container. Samples from the rural districts were stored at 4°C for up to 24 hours prior to transportation while those from Kampala were immediately transported to the laboratory at Makerere University College of Health Sciences for microbiological investigation.

Culturing and identification of *E. coli* and *K. pneumoniae*.

The procedure for culturing and isolate identification was described previously [14]. Briefly, samples were streaked on MacConkey agar medium on the third/fourth quadrant, and incubated at 37°C for 18-24 hours in ambient air. In case of stool, samples were first emulsified in sterile normal saline before inoculation onto MacConkey agar plates. Lactose fermenting isolates with colony morphology suggestive of *E. coli* and *Klebsiella* spp. were subjected to oxidase testing and when negative, they were cultured for 18-24 hours on triple sugar and iron (TSI) agar, Simmons citrate agar, urea and Sulphide Indole Motility (SIM) medium for identification. Inconclusive isolates were confirmed as *E. coli* or *Klebsiella* by using the API 20E system (BioMerieux Marcy 1'Etoile, France).

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed with the disc diffusion tests (DDT) on Mueller Hinton Agar (MHA) (Biolab, Hungary) as recommended by the Clinical Laboratory Standards Institute (CLSI) [17]. Bacterial suspensions equivalent to 0.5 McFarland standard were prepared. The DDT included antibiotic disks (Biolab, Hungary) of ampicillin (10 μg), amoxicillin/clavulanate (20/10 μg), cefuroxime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), meropenem (30 μg), sulfamethoxazole/trimethoprim (co-trimoxazole) (23.75/1.25 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), nitrofurantoin (300 μg), cefepime (30 μg), piperacillin/tazobactam(100/10 μg) and cefoxitin (30 μg). *E. coli* ATCC25992, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC27853 and *Enterococcus faecalis* ATCC29212 were used as quality controls.

Testing for AmpC β-lactamase production

Typically, AmpC β-lactamases confer resistance to cephamycins (e.g. cefoxitin), a characteristic widely used to distinguish them from the extended-spectrum β-lactamases (ESBLs), and to functionally screen for AmpC β-lactamase producing isolates [3, 4, 18-21]. As such, all cefoxitin resistant isolates in this study were screened by the cefoxitin/cloxacillin double-disc synergy test (CC-DDST) to detect AmpC β-lactamase production as previously described [22]. As AmpC β-lactamases are associated with clavulanate resistance [17, 23], isolates with reduced susceptibility to amoxicillin/clavulanate were also tested for AmpC β-lactamase production. Susceptibility to cefoxitin and to third-generation cephalosporins was determined based on the CLSI guidelines (2007) [24]. Furthermore, isolates that were positive on the CC-DDST (i.e. AmpC β-lactamase producers) were re-tested with E-test strips containing cefotetan and cefotetan/cloxacillin (CN/CNI, AB BIODISC, Solna, Sweden). E-test screening was considered positive for AmpC β-lactamase production when MIC ratio for cefotetan / cefotetan/cloxacillin was ≥8 [22]. Testing for ESBL production was carried out as described previously [25], on isolates with reduced zone diameters to third-generation cephalosporins.

Screening for ESBL and pAmpC β-lactamase genes

All isolates testing positive for AmpC β-lactamase production on the CC-DDST were tested by polymerase chain reaction (PCR) for pAmpC gene carriage. Further, as pAmpC β-lactamase genes have been detected in isolates with reduced susceptibility to third-generation cephalosporins, we also tested isolates with inhibition zone diameters of ≤27 mm, ≤25 mm and ≤22 mm for cefotaxime, ceftriaxone and ceftazidime respectively, for pAmpC gene carriage [17, 23]. Multiplex PCRs targeting AmpC β-lactamase genes bla_{CIT}, bla_{DHA}, bla_{MOX}, bla_{FOX}, bla_{EBC}, bla_{ACC} and bla_{CMY-2} were performed using published primers and conditions [6, 26], and the expected amplicon sizes were successfully generated. ESBL genes bla_{CTX-M}, bla_{TEM} and bla_{SHV} were detected as previously described [27]. Amplicons were sequenced (ACGT, Wheeling, IL, USA) and confirmed through BLAST-searching at NCBI (www.blst.ncbi.nln.nih.gov/Blast.cgi). Phylogenetic group typing of *E. coli* was done as described previously [28].

Data analysis

The data was double entered for validation using EPIDATA software, cleaned and exported to STATA (v10) for analysis. Data were compared across the districts using descriptive statistics, frequencies and bivariate analyses (cross-tabulations). Associations between outcome variables i.e. isolates with ESBL/pAmpC genes and categorical independent variables i.e. socio-demographics, use of antibiotics, history of hospital admission and medical procedures three months prior to visits were tested using Pearson's Chi-square. A significant level was set at $p<0.05$. Similarly, odds ratios (ORs) between the categorical independent variables and outcome variables were determined. Variables with $p<0.2$ at bivariate analysis were entered into multivariate logistic regression models with backward elimination. Independent variables used were gender (male vs. female), health center level, health sub-district and district, history of admission, history of medical procedures and antibiotic use recalled by client and from health record during the previous 3 months. To control for the effect of clustering, regression with robust standard errors was used.

Results

Demographic characteristics, bacterial isolates

Of the 1,448 participants we enrolled, females were the majority i.e. 63.3% (913/1,448). Thirty three percent of the participants (474/1,448) were from Kampala, 35% (508/1,448) from Kayunga and 32% (466/1,448) from Mpigi. The age range the participants 1 to 81 years, and mean of 25 years. Around 56% (802/1,448) of the participants were in the 15–44 year age group (Table S1). Of the 730 stool and 718 urine samples processed, 985 enterobacteria were isolated, of which 94.4% (930/985) were E. coli and 5.6% (55/985) were K. pneumoniae. Per district, the 985 enterobacteria were distributed as follows: 58% (274/474) Kampala, 80% (406/508) Kayunga, and 65.5% (305/466) Mpigi. The characteristics of the participants whose samples grew E. coli and K. pneumoniae are shown in Table S4. None of the urine samples grew bacteria at $\geq 10^4$ colony forming units (CFU) per milliliter implying that there was no infection-related growth. Overall, 37% (535/1,448) of the participants visited outpatient clinics for general conditions and bacteria grew in 68% (363/535) of these participants. Of the 731 participants who presented with infectious conditions, 67% (488/731) had bacterial growth in their samples. Of the 122 participants who visited HIV/AIDS clinics for routine checks, 72% (88/122) had growth (Tables S1 & S2). Furthermore, 1,093 participants reported to have taken antibiotics 3 months prior to the visit, of whom 69% (755/1,093) had growth. Of the 125 participants who reported to have been previously admitted to hospitals, 62% (78/125) had growth. Of the 130 participants who reported to have undergone medical procedures 3 months prior to the clinic visit, 55% (71/130) had growth (Tables S1 & S2).

Prevalence of AmpC β-lactamase producing isolates

Of the 985 bacterial isolates investigated, 21% (209/985) were cefoxitin resistant. However, 25 cefoxitin resistant isolates were not available at the time of analysis, leaving 184 isolates that were investigated, of which 70% (129/184) were AmpC β-lactamase producers while 30% (55/184) were non-producers (Fig. 1). Therefore, the prevalence of AmpC β-lactamase producers among cefoxitin resistant isolates was 70% (129/184), implying the overall prevalence of AmpC β-lactamase producing isolates among enterobacteria was 13.1% (129/985); 12.5% (116/930 E. coli and 23.6% (13/55) Klebsiella. Per district the prevalence of AmpC β-lactamase producing bacteria was 23.7% (65/274) Kampala, 12.1% (37/305) Mpigi, and 6.7% (27/406) Kayunga. Furthermore, given the association between AmpC β-lactamases and clavulanate resistance, 247 amoxicillin/clavulanate resistant isolates in this study (see below) comprising of 229 E. coli and 18 Klebsiella, were tested for cefoxitin resistance, majority of which i.e. 84.6% (209/247) were found to be cefoxitin resistant while only 15.4% (38/247) were cefoxitin susceptible. Of the 209 amoxicillin/clavulanate and cefoxitin resistant isolates, 61.7% (129/209) were AmpC producers (Fig. 1).

Prevalence of pAmpC β-lactamase genes

One hundred and eleven of the 129 cefoxitin resistant and AmpC β-lactamase producing isolates were tested for pAmpC β-lactamase gene carriage. Of these, 60% (67/111) carried pAmpC genes. Furthermore,

47 of the 55 AmpC β-lactamase non-producers (see above) were tested for pAmpC gene carriage as they were cefoxitin resistant (MIC ≥ 16 µg/ml). Of these, 38% (18/47) carried pAmpC genes. Therefore, 54% (85/158) of the cefoxitin resistant isolates in this study (111 AmpC β-lactamase producers plus 47 AmpC β-lactamase non-producers) carried pAmpC β-lactamase genes. Isolates with reduced susceptibility to third-generation cephalosporins are suspects for AmpC β-lactamase production; in this study, 33.8% (22/65) of such isolates were cefoxitin susceptible, of which 59% (13/22) carried pAmpC genes. Overall, a total of 180 isolates (158 cefoxitin resistant plus 22 cefoxitin susceptible with reduced susceptibility to third-generation cephalosporins), comprising 157 E. coli and 23 Klebsiella, were tested for pAmpC gene carriage (Fig. 1). Of these, 54% (cefoxitin resistant isolates, 85/158) were pAmpC positive while 59.1% (cefoxitin susceptible isolates with reduced susceptibility to third-generation cephalosporins, 13/22) were pAmpC positive, giving a total of 98 pAmpC positive isolates investigated.

The overall prevalence of pAmpC genes in enterobacteria was 10% (98/985); by district it was 16.4% (45/274) Kampala, 6.2% (25/406) Kayunga and 9.2% (28/305) Mpigi, hence, the urban district of Kampala had more pAmpC gene positive isolates, Table 2. Per species the prevalence of pAmpC genes among cefoxitin resistant and/or amoxicillin/clavulanate resistant isolates was 59% (93/157) in E. coli and 26.1% (5/23) in Klebsiella. pAmpC β-lactamase gene carriage correlated with AmpC β-lactamase production (χ^2 = 11.7, P-value 0.0003). The pAmpC β-lactamase producing E. coli belonged to phylogenetic groups A (n = 23), B1 (n = 10), B2 (n = 35) and D (n = 25). Overall, 39.6% (44/111) of AmpC β-lactamase producing isolates did not carry pAmpC genes, of which eight were Klebsiella that do not carry chromosomal AmpC genes. The AmpC β-lactamase producing isolates of E. coli that were pAmpC negative were assumed to be hyper-producers of chromosomal AmpC β-lactamases. Relatedly, the AmpC β-lactamase producing isolates of Klebsiella that were pAmpC negative likely carried genes we did not screen for.

The pAmpC genes detected were bla_{CIT} (n = 54), bla_{CMY-2} (n = 23), bla_{CMY-4} (n = 31), bla_{EBC} (n = 51, mainly bla_{ACT-1}) and bla_{DHA} (n = 20), Table 2. Forty four isolates carried ≥ 2 pAmpC genes and the most frequent combination was bla_{EBC} plus bla_{CIT}. Furthermore, in this study, 11 ESBL producing isolates carried $bla_{CTX-M-15}$ while three carried $bla_{CTX-M-28}$. Co-existence of pAmpC with betalactamase genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) occurred in 49% (48/98), 9.2% (9/98) and 34.7% (34/98) isolates, respectively. Figure 2 depicts the distribution of β-lactamase genotypes in the three districts.

Table 2

Prevalence of pAmpC β-lactamase genes among E. coli and Klebsiella across the three districts

pAmpC gene[a]	Specimen type (N = 98)		District			Total[b]
	Stool 55 (56%)	Urine 43 (44%)	Kampala 45/274 (16.4%)	Kayunga 25/406 (6.2%)	Mpigi 28/305 (9.2%)	
bla_{CIT}	30	24	25 (13/12)	12 (2/10)	17 (8/9)	54
bla_{DHA}	10	10	5	8	7	20
bla_{EBC}	28	23	28	11	12	51

[a]Based on a multiplex PCR by Perez-Perez and Hanson, 2002; [b]Numbers are derived from specimen type

Antibiotic resistance patterns

The antibiotic resistance profiles of isolates investigated are depicted below (Table 2). Generally, cefoxitin resistance varied across the three districts and the variation was statistically significant (p = 0.023), Table 2. Furthermore, 59% (58/98) of the pAmpC β-lactamase producing isolates were resistant to a β-lactam antibiotic and to two other classes of commonly used non-β-lactam antibiotics, implying they were multidrug resistant (MDR) [29]. Two of the isolates were resistant to co-trimoxazole, ciprofloxacin, gentamicin, nitrofurantoin and chloramphenicol, while 7 were co-resistant to these drugs excluding nitrofurantoin, and one was resistant to the same drugs except chloramphenicol. Resistance to ciprofloxacin, co-trimoxazole and gentamicin was noted in 9 isolates, co-trimoxazole and chloramphenicol in 22 and co-trimoxazole and gentamicin in 5. Resistance to individual antibiotics among the pAmpC β-lactamase gene positive isolates was as follows, co-trimoxazole 79% (77/98); chloramphenicol 34.5% (34/98); ciprofloxacin 28% (27/98); gentamicin 23.5% (23/98); nitrofurantoin 8% (8/98); piperacillin/tazobactam 30.6% (30/98). None of the isolates was resistant to carbapenems.

Table 2

Antibiotic resistance rates among E. coli and Klebsiella isolated from individuals attending outpatient clinics in Kampala, Kayunga and Mpigi districts (2007–2008)

Antibiotic	Resistant (%)				P-value
	Kampala[d] n/N	Kayunga[e] n/N	Mpigi[f] n/N	Total n/N	
Ampicillin[a]	224/259 (86.5)	218/396 (55.1)	174/275 (63.3)	616/930 (66.2)	0.420
Amoxicillin/ clavulanate	184/274 (67.2)	80/406 (19.7)	88/304 (29.0)	352[c]/984 (35.8)	< 0.001
Cefuroxime	43/273 (15.8)	77/405 (19.0)	22/304 (7.2)	142/982 (14.5)	< 0.001
Ceftriaxone	16/274 (5.8)	2/406 (0.5)	10/303 (3.3)	28/983 (2.9)	< 0.001
Cefotaxime	14/273 (5.1)	2/406 (0.5)	6/302 (2.0)	22/981 (2.2)	< 0.001
Ceftazidime	15/273 (5.5)	2/406 (0.5)	8/304 (2.6)	25/983 (2.5)	< 0.001
Cefepime[b]	93/178 (52.3)	26/76 (34.2)	29/91 (31.9)	148/345 (42.9)	0.001
Ciprofloxacin	73/274 (26.6)	15/405 (3.7)	17/304 (5.6)	105/983 (10.7)	< 0.001
Sulfamethoxazole/Trimethoprim	234/272 (86.0)	262/405 (64.7)	189/304 (62.2)	685/981 (69.8%)	< 0.001
Gentamicin	68/273 (24.9)	19/406 (4.7)	18/303 (5.9)	105/982 (10.7)	< 0.001
Nitrofurantoin	23/270 (8.5)	4/403 (1.0)	8/304 (2.6)	35/977 (3.6)	< 0.001
Chloramphenicol	83/273 (30.4)	54/405 (13.3)	56/301 (18.6)	193/979 (19.7)	< 0.001
Cefoxitin[b]	106/181 (58.6)	40/78 (51.3)	63/88 (71.6)	209/347 (60.2)	0.023
Piperacillin/ Tazobactum[b]	126/173 (72.8)	64/79 (81.0)	67/93 (72.0)	257/345 (74.5)	0.315

[a]Refers to E. coli; [b]Only Amoxicillin/clavulanate resistant isolates tested; [c]Refers to inhibition zone diameter of ≤ 17 mm; [d]Urban district with a wet tropical climate; [e]Rural district with a wet & dry tropical climate; [f]Rural district with a wet tropical climate.

Antibiotic	Resistant (%)				P-value
	Kampala[d] n/N	Kayunga[e] n/N	Mpigi[f] n/N	Total n/N	
Meropenem	0/273 (0.0)	0/403 (0.0)	0/300 (0.0)	0/976 (0.0)	-

[a]Refers to E. coli; [b]Only Amoxicillin/clavulanate resistant isolates tested; [c]Refers to inhibition zone diameter of ≤ 17 mm; [d]Urban district with a wet tropical climate; [e]Rural district with a wet & dry tropical climate; [f]Rural district with a wet tropical climate.

Factors associated with carriage of pAmpC β-lactamase producing bacteria

There was significant association between Health Centre level, district of residence, use of ciprofloxacin and sample type with carriage of pAmpC β-lactamase producing bacteria, Table 3. After adjusting for each of them, the district of residence remained an independent risk factor for carriage of pAmpC β-lactamase producing bacteria, Table 3. Overall, we found that residing in a rural district (Kayunga/Mpigi) was associated with low carriage of pAmpC β-lactamase producing bacteria (aOR 0.23 (95% CI:0.11, 0.47) and aOR 0.49 (95% CI:0.25, 0.99), respectively. Similarly, participants who were 45 years and above carried less pAmpC positive bacteria (aOR 0.17 (95% CI:0.05, 0.62). Ciprofloxacin use was an independent risk factor for carriage of pAmpC positive bacteria (aOR 2.61 (95% CI:1.28, 5.32), Table 3.

Table 3

Factors associated with carriage of pAmpC β-lactamase producing bacteria

Characteristic	pAmpC gene				
	Not present, n (%)	Present, n (%)	p-value	cOR (95% CI)	aOR (95% CI)
Age group					
0–14	260 (31.8)	36 (36.7)	0.131	1.0	1.0
15–44	440 (53.9)	55 (56.1)		0.90 (0.58, 1.41)	0.59 (0.32, 1.07)
45+	117 (14.3)	7 (7.2)		0.43 (0.19, 1.00)*	0.17 (0.05, 0.62)*
Gender					
Female	544 (66.5)	59 (60.2)	0.214	1.0	1.0
Male	274 (33.5)	39 (39.8)		1.31 (0.85, 2.02)	1.48 (085, 2.56)
Health center level					
National Referral	75 (9.1)	18 (18.4)	0.023	1.0	1.0
General Hospital	220 (26.8)	28 (28.6)		0.53 (0.28, 1.01)	1.04 (0.40, 2.70)
Health Center IV	134 (16.3)	11 (11.2)		0.34 (0.15, 0.76)*	1.53 (0.47, 5.00)
Health Center III	392 (47.8)	41 (41.8)		0.44 (0.24, 0.80)*	0.74 (0.28, 1.98)
District					
Kampala[a]	198 (24.1)	44 (44.9)	< 0.001	1.0	1.0
Kayunga[b]	363 (44.2)	25 (25.5)		0.31 (0.18, 0.52)	0.23 (0.11, 0.47)*
Mpigi[c]	260 (31.7)	29 (29.6)		0.50 (0.30, 0.83)*	0.49 (0.25, 0.99)*

cOR, crude odds ratio; aOR, adjusted odds ratio; ISS, immune suppression syndrome (HIV/AIDS); *Statistically significant association.

Characteristic	pAmpC gene				
	Not present, n (%)	Present, n (%)	p-value	cOR (95% CI)	aOR (95% CI)
Reason for visit					
ISS	76 (9.8)	5 (5.3)	0.350	1.0	1.0
Infection	398 (51.2)	50 (52.6)		1.91 (0.74, 4.94)	2.60 (0.73, 9.32)
General	303 (39.0)	40 (42.1)		2.01 (0.77, 5.26)	3.20 (0.86, 11.86)
History of admission					
No	748 (92.4)	86 (88.7)	0.207	1.0	1.0
Yes	62 (7.6)	11 (11.3)		1.54 (0.78, 3.04)	0.55 (0.21, 1.49)
History of medical procedures					
Contact	4 (7.3)	1 (14.2)	0.178		
Inoculation	40 (72.7)	3 (42.9			
Surgery	11 (20.0)	3 (42.9			
Antibiotic use (any)					
No	194 (23.6)	24 (24.5)	0.850		
Yes	627 (76.4)	74 (75.5)			
Use of penicillin					
No	427 (67.8)	53 (73.6)	0.313		
Yes	203 (32.2)	19 (26.4)			
Use of Ciprofloxacin					
No	566 (89.8)	59 (80.8)	0.020	1.0	1.0

cOR, crude odds ratio; aOR, adjusted odds ratio; ISS, immune suppression syndrome (HIV/AIDS); *Statistically significant association.

Characteristic	pAmpC gene				
	Not present, n (%)	Present, n (%)	p-value	cOR (95% CI)	aOR (95% CI)
Yes	64 (10.2)	14 (19.2)		2.10 (1.11, 3.97)	2.61 (1.28, 5.32)
Use of co-trimoxazole					
No	239 (34.3)	34 (40.0)	0.297		
Yes	458 (65.7)	51 (60.0)			

cOR, crude odds ratio; aOR, adjusted odds ratio; ISS, immune suppression syndrome (HIV/AIDS); *Statistically significant association.

Discussion

AmpC β-lactamases are clinically important in that community-acquired infections arising from strains producing these enzymes may not respond to empiric treatment with common antibiotics. The cefoxitin/cloxacillin double-disc synergy screening of cefoxitin and amoxicillin/clavulanate resistant isolates for AmpC β-lactamase production is a simple and efficient method of quickly detecting these resistance mechanisms in isolates [21, 22]. Using this approach, the prevalence of AmpC β-lactamase producing bacteria in this study (13.2%), and the prevalence of pAmpC β-lactamase gene carriage (26–59%), was high but comparable to the rate of 36.5% at Mbarara Regional Referral Hospital in South-western Uganda [20]. The prevalence in our study is higher than rates from other East African settings [30–32], however, the study populations were varied making direct comparison difficult. Furthermore, in this study, co-carriage rates of ESBL- and pAmpC genes was high, which is a cause for concern as individuals carrying strains producing these enzymes could be reservoirs of spread for MDR bacteria [33, 34]. A significant number of isolates that were susceptible to third-generation cepharosporins but cefoxitin resistant carried pAmpC genes, a discordance that has been reported before [3, 35]. The overall carriage rate (10%) for pAmpC genes in enterobacteriaceae in this study reflects extensive use of antibiotics, as found in Libya where a fecal carriage rate of 6.7% for pAmpC β-lactamase producing bacteria in the community was reported [36]. Importantly, ceftriaxone has been used in Uganda for the last two decades, mainly in empirical treatment of systemic bacterial infections and currently its prescription among in-patients is higher than that of other antibiotics [37]. Such extensive use of ceftriaxone could be a driving force behind the high pAmpC gene carriage rates. Coexistence between bla_{EBC} and bla_{CIT} genes in isolates has been reported before in Africa and Asia [35, 38]. bla_{CIT} reported in this study comprised of the bla_{CMY-2} and bla_{CMY-4} genes. In Africa, bla_{CMY-4} was first reported in North Africa. The detection of bla_{DHA} and bla_{EBC} genes is of concern as bla_{ACT-1}, a prototype gene for the bla_{EBC} and bla_{DHA} genes, is linked to a functional ampR regulator and is inducible [39, 40]. In Seoul,

Korea, four of the five deaths from blood stream infections due to bla_{DHA} producing K. pneumoniae were associated with treatment with extended spectrum cephalosporins [41].

In this study, about half of the isolates exhibiting reduced susceptibility to third-generation cephalosporins carried pAmpC β-lactamase genes, which contrasted findings from Northern European where 100% of isolates exhibiting reduced susceptibility to third-generation cephalosporins carried pAmpC genes [23]. Furthermore, 73% of cefoxitin resistant and pAmpC positive isolates were susceptible to third-generation cephalosporins, in contrast with 26% (10/38) reported from Switzerland, for pAmpC producing isolates that were susceptible to third-generation cephalosporins [42]. Overall, findings in this study suggest that antibiotic susceptibility testing of enterobacteria in Uganda may yield false results for third-generation cephalosporins e.g. ceftriaxone, cefotaxime and ceftazidime. Given that bacterial isolates are not routinely tested for AmpC β-lactamase production, region specific protocols guided by surveillance data are necessary.

In E. coli, phylogenetic group analysis has been used to differentiate virulent/extra-intestinal strains which predominantly belong to phylogenetic groups B2 and D, from commensal strains that belong to groups A and B1 [28]. In this study, the predominance of groups B2 and D (n = 60) compared to groups A and B1 (n = 33) in the community is cause for concern as they are associated with pathogenicity, implying that strains with potential to cause extra-intestinal disease are prevalent, supporting the notion that occurrence of pAmpC β-lactamase producing strains in the community is of public health concern [43, 44]. One limitation in this study was that we were not able to genotype isolates with internationally acceptable procedures like the multilocus sequence typing (MLST) to determine the sequence types.

Conclusions

AmpC β-lactamase production and pAmpC β-lactamase encoding genes are prevalent among E. coli and K. pneumoniae isolates from urban and rural dwellers in Uganda. As pAmpC genes are easily transferrable between species and have been associated with outbreaks of community- and hospital-acquired infections, pAmpC beta-lactamase producing bacteria may represent a threat in low-income settings. There is need for testing for cefoxitin resistance during routine antibiotic susceptibility testing, especially among isolates that are resistant to amoxicillin/clavulanate, as well as isolates that are susceptible to third-generation cephalosporins.

Abbreviations

AmpC
Ambler Molecular Class C beta-lactamases
CC-DDST
Cefoxitin/cloxacillin double disk synergy test
pAmpC
Plasmid-mediated AmpC

ESBLs
Extended spectrum beta-lactamases
MIC
Minimum inhibitory concentration
DST
Drug susceptibility testing
MDR
Multidrug resistant
OPD
Outpatient department

Declarations

Ethics approval and consent to participate

The study protocol and consent procedure were reviewed and approved by the Research Ethics Committee and the Higher Degrees committee of Makerere University Medical School (IRB #-2006-009) and the Uganda National Council for Science and Technology (HS246). All adult participants and guardians gave written informed consent before participation. The consent process included storage and use of the collected stool and urine samples for further studies. We obtained assent from participants below the age of 18 years in addition to informed consent from their parent/guardians/caregivers.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

David Kateete is an editorial board member for BMC Infectious Diseases

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Authors' contributions

CFN conceived the study, analyzed and interpreted the data, and drafted the manuscript. MLJ and SYE supervised the study and participated in its design. DPK assisted in manuscript drafts and reviews. All authors read and approved the final manuscript.

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Figures

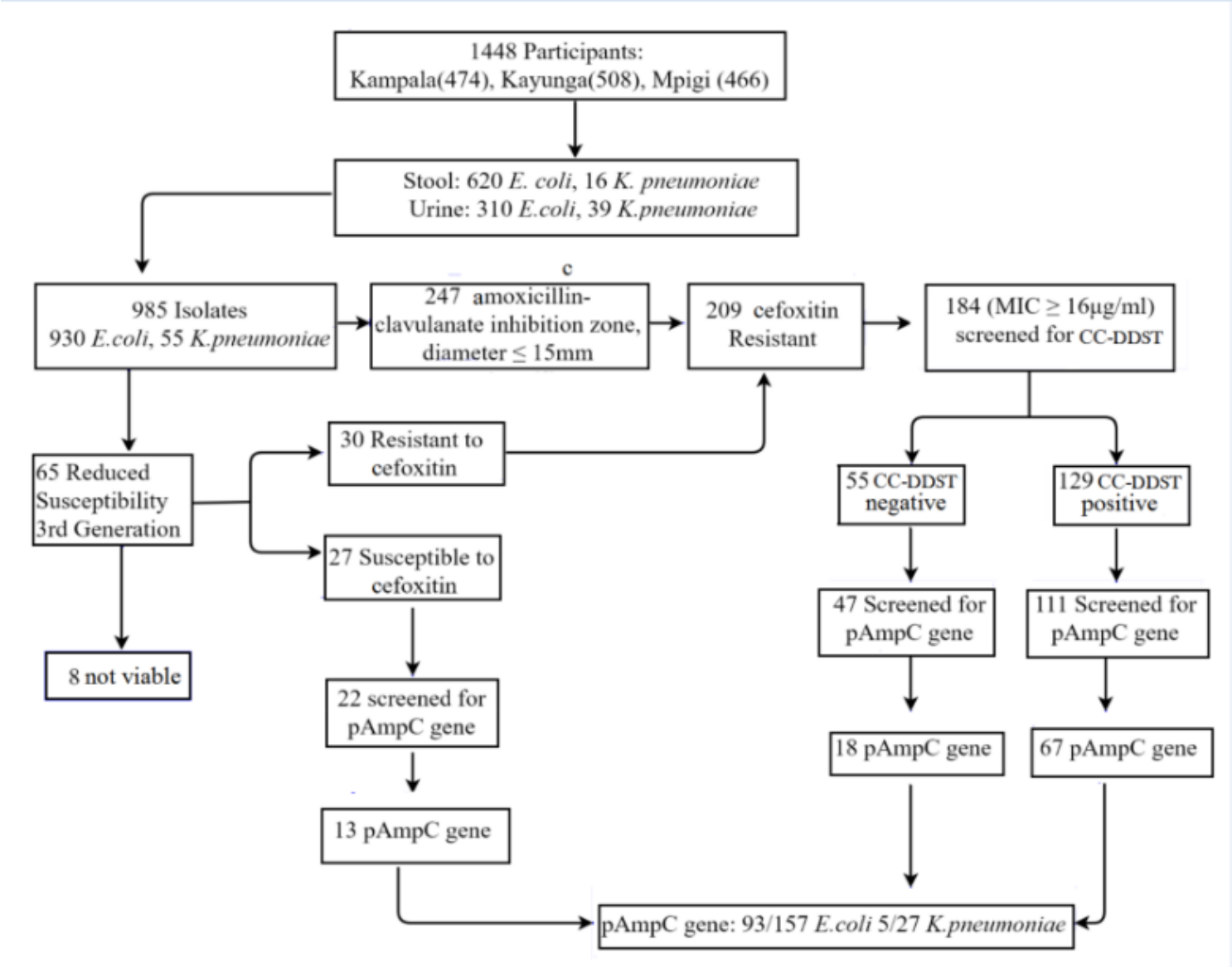

Figure 1

Study flow chart.

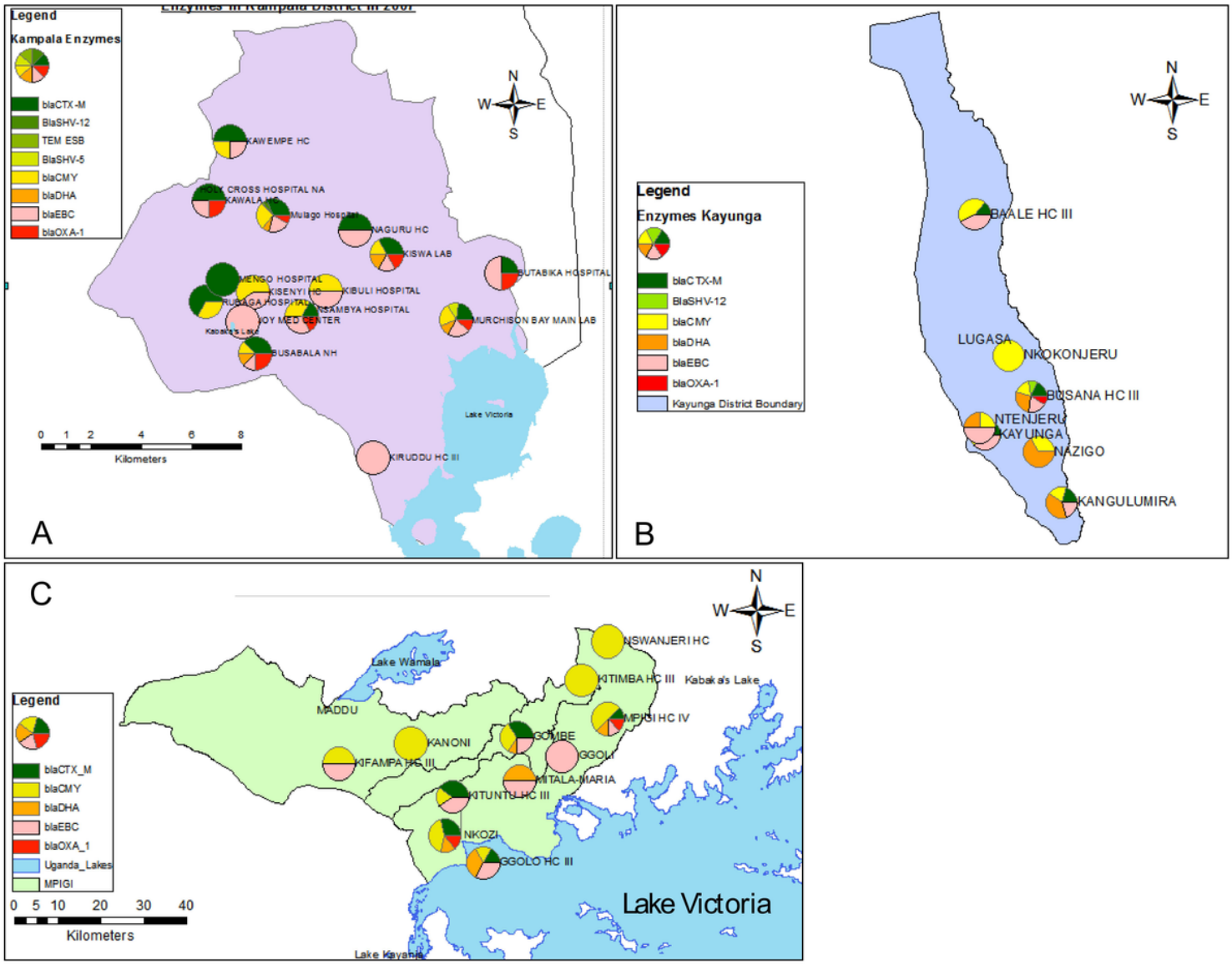

Figure 2

The distribution of β-lactamase genotypes in the three districts.