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Phenotypic expression and prevalence of ESBL-producing *Enterobacteriaceae* in samples collected from patients in various wards of Mulago Hospital, Uganda

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Abstract

Background: Resistance to extended-spectrum cephalosporins among Enterobacteriaceae has been reported yet they serve as the last line treatment for severe infections in Uganda and other countries. This resistance often leads to nosocomial infection outbreaks and therapeutic failures from multidrug resistant bacteria. The main objective of this study was to determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in clinical samples of patients in various wards of Mulago Hospital; Uganda's main national referral and teaching hospital.

Methods: This cross-sectional study was conducted between January-April, 2014. Purposive consecutive sampling was used to collect pus swab, urine, blood and CSF samples from patients in the various wards. A total of 245 consecutive, non-repetitive, clinical samples were obtained and tested for phenotypic ESBL production using the Double Disc Synergy Test using cefotaxime, ceftazidime, cefotaxime-clavulanic acid and ceftazidime-clavulanic acid.

Results: Results show that 47 % of the 245 samples had *Enterobacteriaceae* isolates. Of these isolates 62 % were ESBL producers while 38 % were of non-ESBL phenotype. *E. coli* was the most isolated organism (53.9 %), followed by *K. pneumoniae* (28.7 %). Majority of Enterobacteriaceae organisms were isolated from urine samples, followed by pus samples and of these 64.9 % and 47.4 % were ESBL-producers respectively. *Klebsiella pneumoniae* had the highest percentage of ESBL producers (72.7 %). There was a higher percentage of isolates showing resistance to ceftazidime (73 %) compared to cefotaxime (57.5 %). All *Enterobacter cloacae* isolates showed resistance to ceftazidime. There were no statistically significant association between phenotype (ESBL/non-ESBL) and patients' age or gender or Enterobacteriaceae spp.

Conclusions: This study reveals a high prevalence of ESBL producing organisms in Mulago Hospital and high levels of resistance to third generation cephalosporins. In addition to undertaking appropriate infection control measures, there is urgent need for formulation of an antibiotic policy in Uganda to prevent spread of these organisms. This also calls for continuous monitoring and reporting of the presence of such organisms in order to ensure rational and judicious use of antibiotics by clinicians.

Keywords: ESBL, Enterobacteriaceae, Cefotaxime, Ceftazidime, Clavulanic acid

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Table 1 Criteria for determining the potency of the test antibiotics

ESBLS	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 700603
Ceftazidime 30 μg	(25-32 mm)	(22-29 mm)
Cefotaxime 30 μg	(29-35 mm)	(18-22 mm)

The antibiotics had to show inhibition zone diameters in the above ranges in order to be used in the study

Background

Production of β-lactamase enzymes that hydrolyze the β-lactam ring is a predominant resistance mechanism for many Gram-negative bacteria including *Enterobacteriaceae* such as *E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter cloacae* and *Aeromonas spp.* [1]. Extended-Spectrum β-Lactamase (ESBL)-producing bacteria are capable of expressing these enzymes and this confers bacterial resistance to penicillins; first, second, and third-generation cephalosporins and aztreonam. ESBL-producing bacteria have been isolated in many parts of North America and Europe [2] and in Africa. In one South African hospital, 36 % of *K. pneumoniae* isolates were ESBL-producers and outbreaks of infections due to *Klebsiella* strains resistant to third-generation cephalosporins have also been reported in Nigeria and Kenya [3, 4]. In Uganda there has been lack of published information about ESBL-producers among organisms isolated from patients.

ESBL producers have a wide clinical significance and potential impact in healthcare settings especially in low income countries such as Uganda. The selection pressure and overuse of new antibiotics in the treatment of patients leads to selection for new variants of β-lactamase producers. ESBL producers are associated with various infections in virtually all body organs leading to meningitis, pneumonia, urinary tract infections, septicaemia and intra-abdominal infections [5, 6]. Other conditions include osteomyelitis, endophthalmitis, pyomyositis and wound infections [7].

The possible spread of ESBL-producing organisms in a clinical setting is real. ESBL-associated antibiotic resistance causes increased morbidity and mortality; and hampers the control of infectious diseases. This in turn leads to increase in durations of illness and hospital stay; increase in health-care costs and more economic burden to families. This study sought to determine the prevalence of ESBL-producing bacteria among isolates from samples in various wards of Mulago Hospital, the main national referral hospital in Uganda with a bed-capacity of 1,600.

Table 3 Enterobacteriaceae species isolated from patient samples

Isolates	Frequency	Percent
E. coli	62	53.9
Klebsiella pneumoniae	33	28.7
Proteus mirabilis	16	13.9
Enterobacter cloacae	4	3.5
Total	115	100.0

E. coli was the predominant isolate

Methods

Study design

This was a cross-sectional study conducted between January-April 2014 to determine the prevalence of ESBL-producing Enterobacteriaceae in clinical samples collected from in-patient and out-patient wards of Mulago Hospital. The samples collected included urine, swabs (oral, HVS, wound), blood and CSF. *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter cloacae* bacteria were isolated from the samples. The number of samples containing ESBL-producing *Enterobacteriaceae* was recorded. The prevalence of ESBL phenotypes among the isolates was determined.

Sample size

The sample size was determined using the formula advanced by Kish and Leslie [8]. Basing on results of a previous study in a similar setting, a prevalence of 20 % [9] and confidence interval of 95 % were used in the formula. The sample size was estimated to be about 245 samples.

Sample collection and bacterial isolation

Samples from 25 wards of Mulago Hospital were aseptically collected by purposive consecutive sampling from patients who gave informed written consent; properly

Table 2 Baseline characteristics of patients whose samples had Enterobacteriaceae isolates

	ESBL n = 71 (61.7 %)	Non-ESBL n = 44 (38.3 %)	Total	p value
Sex				
Female	37 (52.1 %)	30 (68.2 %)	67 (58.3 %)	0.089
Male	34 (47.9 %)	14 (31.8 %)	48 (41.7 %)	
Age			Overall mean	
Mean	42 (SD 22)	38 (SD 19)	40	0.355

The Chi-square and independent t-tests showed that sex and age were not significantly associated with ESBL phenotype
Chi-square and independent t-test

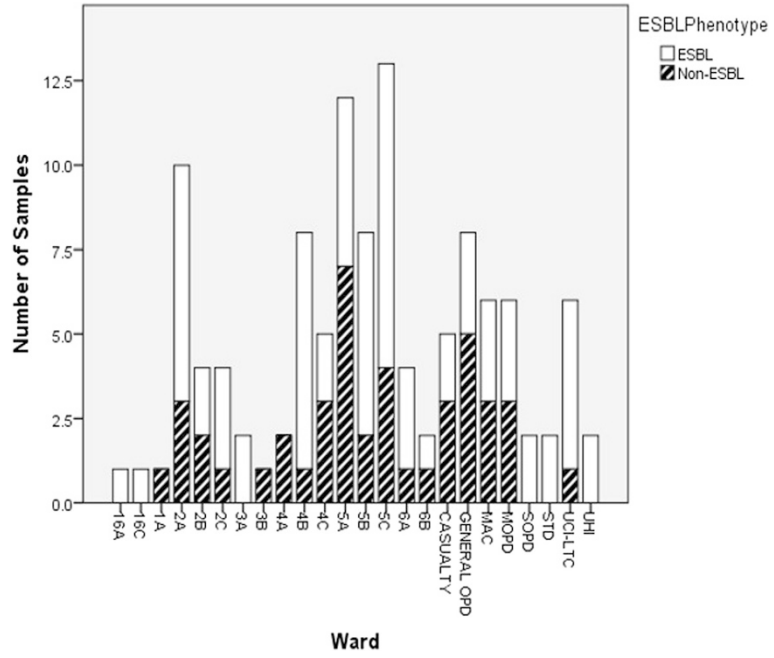

Fig. 1 Distribution of samples from the various wards. Surgical wards contributed the highest number of samples in the Microbiology laboratory during the study period. Surgical wards – 1A, 2A, 2B, 2C, 3A, 5A, 5B, 5C and SOPD; Medical wards – 3B, 4A, 4B, 4C, 6A, 6B, MOPD, MAC, UCI-LTC and UHI; Pediatric wards – 16A and 16C; General - OPD, MOPD, STD. Key: SOPD = Surgical Outpatients Dept; UCI-LTC = Uganda Cancer Institute-Liquid Tumor Cancer, MAC = Medical assessment Centre; MOPD = Medical outpatients Dept; OPD = Outpatients Dept; STD = Sexually Transmitted Diseases Dept; UHI = Uganda Heart Institute

labelled and taken to the Microbiology Laboratory for Enterobacteriaceae culture and isolation. The originating ward, patient's gender and age were recorded. The samples were inoculated by streaking on Blood agar (Oxoid, UK), MacConkey agar (Oxoid, UK) and CLED agar (Oxoid, UK) plates. The plates were incubated aerobically at 37 °C for 18–24 h to allow development of bacterial colonies. Preliminary identification of the isolates was done using phenotypic colonial characteristics. Confirmatory identification of the suspect colonies was carried out by conventional biochemical tests as described by Cheesbrough [10]. These were: indole, Methyl red, Voges-Proskauer, citrate utilization and urease production tests as well as triple sugar iron and oxidase tests.

Detection of ESBL-producing Enterobacteriaceae

ESBL detection was based on the Double Disc Synergy Test and interpretation of the results done using the CLSI M100-S20 (2010) [11]. Briefly; 3–5 colonies of each isolate were picked from the growth plates with sterile wire loop, and suspended in 1 ml of physiological saline. The resultant bacterial suspension was matched to the 0.5 McFarland turbidity standard so as to approximate the seeding density of the respective organisms. 100 μl the bacterial suspension/broth culture were then surface-spread on Muller Hinton Agar (Oxoid, UK) plates using a sterile spreader. Antibiotic discs containing ceftazidime (CTC), cefotaxime (CTZ), ceftazidime-clavulanic acid (CFC) and cefotaxime-clavulanic acid (CTX) were placed on the plates which were incubated overnight at 37 °C. The zones of clearance (mm) for the respective antibiotics were measured for each isolate using a divider and ruler. Organisms were considered to be ESBL-producers if the difference in zone of clearance between ceftazidime and ceftazidime-clavulanic acid or cefotaxime and cefotaxime-clavulanic acid was ≥5 mm. The prevalence of ESBL-producing bacteria was determined using the formula:

$$\text{Prevalence (P)} = \frac{\text{Number of ESBL producing organisms}}{245} \times 100$$

Quality control

Standard organisms (*E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603) were used to test for the potency of the antimicrobial discs (Table 1).

Data analysis

Clinical and socio-demographic data were entered into Epi Info™ v7 and exported to SPSSv21. Pearson Chi-square test was used to assess for any differences between the two ESBL phenotype categories with respect to clinical and demographic parameters. The means of the continuous variables, age and zones of clearance were

Table 4 Association between ESBL phenotype and Enterobacteriaceae isolated, sample type and susceptibility pattern

	ESBL (%)	Non-ESBL (%)	P value
Isolate			
E. coli	36 (58.1)	26 (41.9)	0.924
Klebsiella pneumoniae	24 (72.7)	9 (27.3)	0.908
Enterobacter cloacae	1 (25)	3 (75)	0.695
Proteus mirabilis	10 (62.5)	6 (37.5)	0.999
Sample type			
Urine	46 (63.9)	26 (36.1)	0.980
HVS	6 (54.5)	5 (45.5)	0.999
CSF	1 (100)	0 (0)	0.999
Urethral swab	1 (100)	0 (0)	1.000
Wound swab	2 (100)	0 (0)	1.000
Blood	2 (40)	3 (60)	1.000
Pus swab	9 (47.4)	10 (52.6)	0.999
Surgical wound swab	4 (100)	0 (0)	0.999
Ceftazidime Susceptibility			
Resistant	62 (73.8)	22 (26.2)	0.408
Intermediate	4 (36.4)	7 (63.6)	0.187
Susceptible	5 (25)	15 (75)	0.480
Cefotaxime Susceptibility			
Resistant	54 (81.8)	12 (18.2)	0.013
Intermediate	4 (30.8)	9 (69.2)	0.067
Susceptible	13 (36.1)	23 (63.9)	0.430
Binomial logistic regression			

When analysed using binomial logistic regression, ESBL phenotype was significantly associated with resistance to cefotaxime but not with resistance to ceftadizime, bacterial species or sample type

compared using the Independent *t*-test. Crude logistic regression analysis was used to explore clinical and laboratory features of the ESBL phenotype for comparison with non-ESBL *Enterobacteriaceae* phenotypes. The differences were considered significant at $p < 0.05$.

Ethical considerations

The study protocol was approved by the Ethics Review Committee of the School of Biomedical Sciences of Makerere University Medical School. Permission was sought from the hospital and laboratory authorities. The ethical principles of scientific research as well as related national laws and regulations were strictly adhered to.

Results

The mean age of the participants was 40 years as shown in Table 2. Results indicated that 115 of the 245 samples (47 %) had *Enterobacteriaceae* isolates. Of these isolates, 58.3 % were from female patients while 41.7 % were from males (Table 2). Statistical analysis of patient data using Chi-square and independent t-test indicated that gender and age were not significantly associated with ESBL phenotype. *E. coli* was the most isolated organism (53.9 %, n = 62), followed by *K. pneumoniae* (28.7 %) as shown in Table 3. Most samples with Enterobacteriaceae isolates were from Obstetrics and Gynaecology wards i.e. 5A (10.4 %, n = 12) and 5C (11.3 %, n = 13) (Fig. 1).

Results further showed that 62 % of Enterobacteriaceae isolates were of the ESBL phenotype while 38 % were of non-ESBL phenotype (Table 2). Most of the Enterobacteriaceae were isolated from the urine samples followed by pus samples as shown in Table 4 and Fig. 2. However, just 64.9 % and 47.4 % of urine and pus isolates respectively were ESBL-producers. On the other hand,

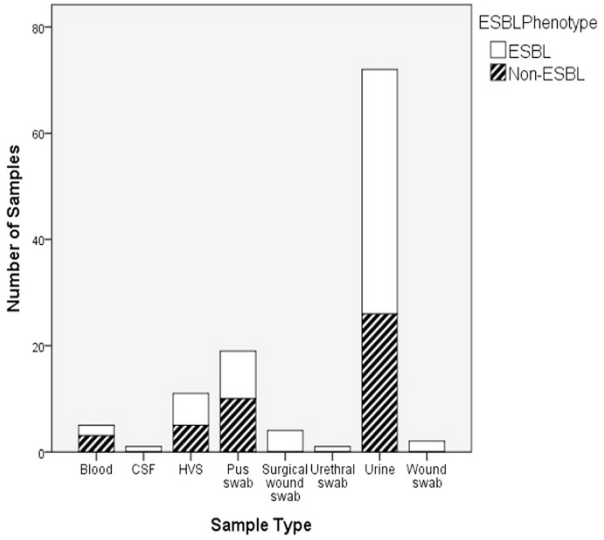

Fig. 2 Distribution of Enterobacteriaceae-positive samples. Most of the Enterobacteriaceae were isolated from urine samples

Table 5 Mean zone of clearance (± SD) of Enterobacteriaceae segregated by ESBL phenotype

Antibiotic	ESBL	Non-ESBL	P value
Ceftazidime	10.1 ± 6.2	18.1 ± 7	0.000
Cefotaxime	13.4 ± 8.3	21.6 ± 8.3	0.000
Ceftazidime-Clavulanic acid	22.3 ± 6	20.7 ± 6.8	0.198
Cefotaxime-Clavulanic acid	25.1 ± 6.6	22.3 ± 8.3	0.051

The zones of clearance of ceftazidime and cefotaxime were statistically significantly associated with ESBL phenotype (Independent samples t-test)

albeit their small number, all isolates from CSF, wound and urethral swabs were ESBL producers (Fig. 2).

The mean zones of clearance for ESBL and non-ESBL phenotypes were lowest for ceftazidime (10.1 ± 6.2 mm; 18.1 ± 7 mm respectively) and highest for cefotaxime-clavulanic acid (25.1 ± 6.6 mm; 22.3 ± 8.3 mm respectively) as shown in Table 5. The zones of clearance of ceftazidime and cefotaxime were significantly associated with ESBL phenotype (p = 0.000; 0.000 respectively) while those of ceftazidime-clavulanic acid and cefotaxime-clavulanic acid were not (p = 0.198, 0.051 respectively).

There was a higher percentage of isolates showing resistance to ceftazidime (73 %) compared to cefotaxime (57.5 %) as shown in Table 6. All *Enterobacter cloacae* isolates were resistant to both cefotaxime and ceftazidime. Table 4 shows that *Klebsiella pneumoniae* had the highest percentage of ESBL producers (72.7 %). There were no statistically significant association between phenotype (ESBL/non-ESBL) and patients' age or gender (Table 4). Similarly, there were no significant association between phenotype (ESBL/non-ESBL) and species of Enterobacteriaceae (*E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, Proteus mirabilis*) or sample type (Table 4).

Discussion

The 3rd and 4th generation cephalosporins are often reserved for severe infections [12] but resistance to these drugs has been strikingly rapid worldwide [4]. Consequently, therapeutic options for the infections caused by the ESBL producers are becoming increasingly limited and; if available, expensive for low and middle income countries. The study revealed a slight female preponderance for ESBL-producing Enterobacteriaceae among the patients though gender was not statistically significant (p = 0.089) as a factor. Kiratisin *et al*., [13] also revealed a female preponderance.

Urine samples constituted the greatest number of clinical samples in this study. According to Wilson and Gaido [14], urinary tract infections constitute the commonest bacterial infections and urine samples account for a significant percentage of samples in clinical microbiology laboratories worldwide. Most Enterobacteriaceae isolates were *E. coli*. Wilson and Gaido [14] indicates that *E. coli* is the most frequent cause of urinary tract infections and this could probably explain the high prevalence of *E. coli* isolates in our study. Similarly, studies in Tanzania [15] indicate that *E. coli* and *Klebsiella pneumoniae* are the most prevalent Enterobacteriaceae species in clinical samples. Furthermore, Maina *et al*., [16] in a study in Kenya reported higher prevalence for *E. coli* (53.8 %). Similar findings were reported in Bahrain [17].

In our study, the highest numbers of ESBL-producing isolates were from gynaecological and surgical wards. Many studies associate ESBL-producing Enterobacteriaceae with surgical wards. Studies by Seni *et al*., [9] show that most isolates from surgical wards are ESBL-producers. Prolonged hospital stay; inappropriate therapy; use of indwelling catheters, endotracheal/nasogastric tubes and severe illnesses are all possible drivers of their dissemination. Significantly, there is also movement of health workers between wards in the hospital and can migrate ESBL-producers from ward to ward leading to dissemination throughout the hospital.

The prevalence of ESBL producers among Enterobacteriaceae (62 %) was quite high compared to that reported by Moyo *et al*., [15] in Tanzania (45.2 %). This wide variation in prevalence is probably due to differences in types of samples analysed and the extent of antibiotic use in the various wards. The present study reveals that *K. pneumoniae* and *E. coli* are major ESBL producers. Moyo *et al*., [15] showed 51.5 % and 39.1 % ESBL positivity among *Klebsiella* spp and *E. coli* respectively. On the other hand,

Table 6 Susceptibility pattern of Enterobacteriaceae to ceftazidime and cefotaxime

	Ceftazidime (%)			Cefotaxime (%)		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
E. coli	41 (66.1)	7 (11.3)	14 (22.6)	32 (51.6)	9 (14.5)	21 (33.9)
E. cloacae	4 (100)	0 (0)	0 (0)	3 (75)	0 (0)	1 (25)
K. pneumoniae	27 (81.8)	1 (3)	5 (15.2)	21 (63.6)	3 (9.1)	9 (27.3)
Proteus mirabilis	12 (75)	3 (18.8)	1 (6.3)	10 (62.5)	1 (6.3)	5 (31.2)
%	73.0	9.6	17.4	57.5	11.3	31.3

There was a higher percentage of Enterobacteriaceae isolates showing resistance to ceftazidime than to cefotaxime. The interpretative criteria used was based on CLSI M100-S20 (2010) [11] where for ceftazidime (Resistant ≤17 mm; Intermediate 18-20 mm; Susceptible ≥ 21 mm) and for cefotaxime (Resistant ≤22 mm; Intermediate 23-25 mm; Susceptible ≥ 26 mm)

Seni *et al.*, [9] reported that 79.2 % and 92.3 % of *E. coli* and *K. pneumoniae* isolates are ESBL producers; further evidence that these two organisms account for most ESBL producers in the region. Our study showed a higher resistance to ceftazidime than to cefotaxime. On the other hand, Maina *et al.*, [16] reported 21.2 % resistance to ceftazidime and 65.4 % resistance to cefotaxime. The differences seen in this study could be due to regional differences and the type of samples collected.

Conclusions

This study has demonstrated high prevalence of ESBL-producing Enterobacteriaceae in Mulago Hospital. The spread of these organisms reduces the antibiotic alternatives for the treatment of infections by these pathogens to mainly carbapenems; which are often reserved for life-threatening infections. The study underscores the need for routine detection and reporting of ESBL-producers in Ugandan medical facilities so that measures are taken to avoid their uncontrolled spread and possible therapeutic failures. Clinicians need to be rational and judicious in use of antibiotics. An antibiotic use policy is also imperative to limit the dissemination of these organisms.

Abbreviations

ATCC: American type culture collection; CI: Confidence interval; CLED: Cystine Lactose-Electrolyte-Deficient (agar); CSF: Cerebro-spinal fluid; ESBL: Extended spectrum beta-lactamase; HVS: High vaginal swab.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JNK, MLN and RK conceptualized the project, performed most of the lab experiments and wrote the manuscript. MLN contributed specific knowledge in conduction of the microbiological assays. CA assisted in statistically analysing the data. JGN assisted in finalizing the manuscript. All authors read and approved the final manuscript.

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