Infection and Drug Resistance

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ORIGINAL RESEARCH

CTX-M, TEM, and SHV Genes in *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter spp* Isolated from Hematologic Cancer Patients with Bacteremia in Uganda

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Purpose: We determined the phenotypic resistance to third-generation cephalosporins, phenotypic extended spectrum beta-lactamase (ESBL) prevalence, and genotypic prevalence of ESBL-encoding genes bla_{CTX-M}, bla_{TEM}, and bla_{SHV} in *Enterobacteriaceae* isolated from hematologic cancer patients with febrile neutropenia and bacteremia at the Uganda Cancer Institute (UCI).

Patients and Methods: Blood cultures from hematologic cancer patients with febrile neutropenia were processed in BACTEC 9120. *E. coli, K. pneumoniae,* and *Enterobacter spp*. isolates were identified using conventional biochemical methods. Antimicrobial susceptibility tests, phenotypic ESBL characterization, and genotypic characterization of the ESBL-encoding genes bla_{CTX-M}, bla_{TEM}, and bla_{SHV} were determined for pure isolates of *E. coli, K. pneumoniae,* and *Enterobacter spp*.

Results: Two hundred and two patients were included in the study. Median age of patients was 19 years (IQR: 10–30 years). Majority (N=119, 59%) were male patients. Sixty (30%) of the participants had at least one febrile episode due to Enterobacteriaceae. Eighty-three organisms were isolated with *E. coli* being predominant (45, 54%). Seventy-nine (95%) Enterobacteriaceae were multidrug resistant. The ESBL phenotype was detected in 54/73 (74%) of Enterobacteriaceae that were resistant to third-generation cephalosporins. A higher proportion of Enterobacteriaceae with ESBL-positive phenotype were resistant to piperacillin-tazobactam (p=0.024), gentamicin (p=0.000), ciprofloxacin (p=0.000), and cotrimoxazole (p=0.000) compared to Enterobacteriaceae, which were sensitive to third-generation cephalosporins. The organisms were more susceptible to carbapenems and chloramphenicol than resistant. ESBL-encoding genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}) were detected in 55 (75%) of the 73 Enterobacteriaceae that were resistant to third-generation cephalosporins. Bla_{CTX-M}, was the most common ESBL-encoding gene identified with 50 (91%).

Conclusion: ESBL-producing Enterobacteriaceae are a predominant cause of bacteremia in hematologic cancer patients at UCI. The most common ESBL-encoding gene identified in the ESBL-PE was bla_{CTX-M}. Resistance to imipenem and meropenem was low.

Keywords: antimicrobial resistance, extended spectrum beta-lactamases, enterobacteriaceae, CTX-M, Uganda, cancer

Introduction

Bacteremia is a cause of significant morbidity and mortality in patients with hematologic malignancies. This has been worsened over the past decades by an increase in the number of infections caused by multidrug resistant (MDR) strains, which have been associated with increased morbidity, mortality, and hospital costs.[1] The emergence and spread of MDR bacteria make antibiotics inefficient and infectious diseases more difficult to treat. This threatens the ability to perform life-saving procedures, including providing chemotherapy to cancer patients.[2] Among the organisms listed as the main cause of bacteremia are the extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE), including

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E. coli, K. pneumoniae, Enterobacter spp., Citrobacter spp., and *Proteus spp*.[3–8] ESBL-PE are listed by the World Health Organization (WHO) in the critical group as one of the priority pathogens for research and development of new antibiotics.[9] The Centers for Disease Control and Prevention (CDC) has listed them as serious threats. According to the CDC, cases of ESBL-PE have increased since 2012, with 197,400 estimated cases in hospitalized patients in 2017 and 9100 estimated deaths in the same year. The attributable health-care costs at that time were 1.2 billion US dollars.[10] According to the WHO Global AMR and Use Surveillance System (GLASS) Report, low- and middle-income countries observed significantly higher rates of Enterobacteriaceae resistance to third-generation cephalosporins compared to high-income countries (58.3% vs 17.53%, respectively).[11]

Extended spectrum beta-lactamases (ESBLs) belong to the class A beta-lactamase Ambler classification or 2be functional classification scheme by Bush et al.[12] They confer resistance to beta-lactam antibiotics including expanded spectrum cephalosporins and monobactams (aztreonam), but not to carbapenems (imipenem, meropenem, and ertapenem) and cephamycins (cefoxitin and cefotetan), and are inhibited by beta-lactamase inhibitors such as clavulanic acid.[13] The main ESBL genes from which ESBLs are transcribed include bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$, which is the most common in recent years.[13]

The genetic environment of the ESBL-encoding genes indicates that they are harbored on mobile genetic elements such as plasmids, transposons, and insertion sequences.[13–15] This contributes to horizontal dissemination of enzymes within bacterial species and interspecies, thus spreading resistance. Additionally, in ESBL-producing Enterobacteriaceae (ESBL-PE), co-resistance has been observed between β-lactams and other antibiotics including fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole, leading further to multidrug resistance.[16] Furthermore, loss of the outer membrane porin and simultaneous production of CTX-M enzymes, and changes in amino acid substitutions in some CTX-M enzymes have been associated with carbapenem resistance.[17,18] Resistance to carbapenems is a major setback in management of ESBL-PE since carbapenems are the treatment of choice for ESBL-PE.

Patients in intensive care units including cancer centers are at an increased risk of infections and mortality caused by ESBL-PE due to their requirement for prolonged hospitalization and frequent exposure to antibiotics.[19–21] Moreover, among cancer patients, ESBL-PE infections are frequently observed in patients with hematologic malignancies.[19,22] High-income countries have reported ESBL-PE rates in their different cancer populations ranging from as low as 6% to 34%.[19,23,24] In Middle Eastern countries and Asia, the proportion of ESBL-PE isolates identified was 50.5% in India and 42.8% in Iran.[25,26] Studies in China and Iran further demonstrated the high prevalence of $bla_{CTX\text{-}M}$ genes.[26,27]

Only a few cancer centers in Africa have demonstrated the phenotypic presence of ESBL among the isolated Enterobacteriaceae. In Egypt, the proportion of ESBL-producing bacteria among hematopoietic stem cell transplant pediatric patients with bloodstream infections was 45%.[28] Another study in Egypt showed resistance to third-generation cephalosporins ranging from 67% to 94%, with a predominance of blaCTX-M genes.[29] In Ethiopia, one study surprisingly showed only one-third of *E. coli* were resistant to third-generation cephalosporins, while another showed no resistance to ceftriaxone.[30,31] In a study carried out at the Uganda Cancer Institute (UCI) in 2014, 41% of multidrug resistant Enterobacteriaceae isolated from febrile cancer patients were ESBL-PE; however, it did not determine the presence of ESBL-encoding genes.[32]

Assessing the local epidemiology of ESBL-PE especially in cancer units is necessary to not only inform hospital infection prevention and control strategies but also to guide prioritization of "last resort" antibiotics, which cover ESBL-PE infections. Therefore, given the limited data in the magnitude of ESBL-PE and ESBL genes responsible for resistance in cancer patients in Africa, the aim of this study was to determine the phenotypic resistance to third-generation cephalosporins, phenotypic extended spectrum beta-lactamase (ESBL) prevalence, and genotypic prevalence of ESBL-encoding genes $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} in Enterobacteriaceae isolated from febrile neutropenic hematologic cancer patients with bacteremia at the UCI.

Materials and Methods

Study Design, Site, and Setting

This was a laboratory-based cross-sectional study conducted from November 2017 to December 2021 at the Makerere University College of Health Sciences (MakCHS) Clinical Microbiology Laboratory of the Department of Medical

Microbiology (MUCML), and the Genomics and Molecular Biology Laboratory of the Department of Immunology & Molecular Biology, MakCHS. The Clinical Microbiology Laboratory is a College of Pathologists (CAP) accredited (No.7225593) laboratory, which processes human samples, identifies disease-causing organisms accurately and timely, and carries out antibiotic susceptibility testing, according to the Clinical and Laboratory Standards Institute (CLSI). The Genomics and Molecular Biology Laboratory of the Department of Immunology & Molecular Biology, MakCHS is facilitated to carry out basic and applied molecular studies in infectious diseases.

Study Population

We evaluated blood cultures collected as part of a prospective cohort study, which included hematologic cancer patients with febrile neutropenia and ESBL-PE bacteremia at UCI.

Sample Size

Using the Kish Leslie formula for cross-sectional studies, assuming a prevalence of ESBL-PE in the total population to be 5.3%[32] and a margin error of 5% and 95% confidence, a minimum sample size of 77 was calculated for determining the prevalence of ESBL-PE in the total population of hematologic cancer patients with febrile neutropenia. We, therefore, included all hematologic cancer patients with febrile neutropenia that were enrolled in the prospective cohort study during the study period. Participants were enrolled consecutively throughout the study period.

Laboratory Methods

Blood Culture Processing

Blood samples obtained from febrile hematologic cancer patients from November 2017 to December 2021 at the UCI were processed in MUCML. The samples were processed in the BACTEC 9120 blood culture system according to manufacturer's instructions and laboratory's standard operating procedures (SOPs). Positive cultures were Gram stained, subcultured onto chocolate, blood, and MacConkey agars (Becton-Dickinson, New Jersey, USA), and incubated at 35–37ºC for 18–24 hr.

Bacterial Identification

We included *E. coli, K. pneumoniae,* and *Enterobacter spp*. isolates in this study. Pure isolates were identified using conventional biochemical methods including Gram stain, colony morphology on agar plates, triple sugar iron (TSI), sulphur indole and motility (SIM), citrate, urease tests, and oxidase tests (Becton-Dickinson, New Jersey, USA).[33] The biochemical tests for each organism were as follows: *E. coli*: acid production in both slant and deep on triple sugar iron with gas production and no hydrogen sulfide, indole positive, citrate utilization negative, urease production negative, oxidase negative, and motile; *K. pneumoniae*: acid production in both slant and deep on triple sugar iron with gas production and no hydrogen sulfide, indole negative, citrate utilization positive, urease production positive, oxidase negative, and nonmotile; *Enterobacter spp*: acid production in both slant and deep on triple sugar iron with gas production and no hydrogen sulfide, indole negative, citrate utilization positive, urease production negative, oxidase negative, and motile.[33,34] Pure colonies were stored at −80ºC until characterization of ESBL genotypes was carried out.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests and ESBL phenotype test were carried out using the Kirby Bauer disc diffusion method, and the zone diameters of inhibition were measured and interpreted according to the Clinical & Laboratory Standards Institute (CLSI) guidelines.[35] Antibiotic discs (Becton-Dickinson, New Jersey, USA) used included amoxicillin-clavulanate (ANC) 20/10µg, piperacillin-tazobactam (TPZ) 100/10µg, ceftriaxone (CRO) 30µg, cefotaxime (CTX) 30µg, ceftazidime (CAZ) 30µg, cefepime (FEP) 30µg, aztreonam (AZT) 30µg, ertapenem (ERT) 10µg, imipenem (IMP) 10µg, meropenem (MER) 10µg, gentamycin (GM) 10µg, ciprofloxacin (CIP) 5µg, trimethoprim/sulfamethoxazole (SXT)1.25/23.75µg, and chloramphenicol (CAF) 30µg. The ESBL test was performed for isolates, which exhibited a zone of inhibition for at least one of the following: cefotaxime zone ≤27 mm, ceftriaxone ≤25 mm, ceftazidime ≤22 mm, and aztreonam ≤27 mm. The combination disc confirmatory method was used. Discs used in the test were ceftazidime (30µg), ceftazidime-clavulanate

(30/10µg), cefotaxime (30µg), and cefotaxime-clavulanate (30/10µg). Evidence for the presence of ESBL production (a positive test) was determined by a ≥5 mm increase in zone diameter for ceftazidime or cefotaxime in combination with clavulanate vs the zone of diameter of ceftazidime or cefotaxime alone.[35] Multidrug resistance (MDR) was defined as an isolate being non-susceptible to at least one agent in ≥3 antimicrobial categories. Extensively drug resistance (XDR) was defined as an isolate being non-susceptible to at least one agent in all but two or fewer antimicrobial categories. Pan drug resistance (PDR) was defined as an isolate being non-susceptible to all agents in all antimicrobial categories.[36]

Quality Control

Known quality control strains tested in parallel with the Enterobacteriaceae isolates included *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853. They were used to quality control the tests performed including Gram staining and quality and performance of culture media. Biochemical identification tests, and antimicrobial susceptibility tests.

Identification of ESBL Genotypes

The presence of the ESBL-encoding genes $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} was determined using conventional PCR. DNA extraction was performed using the previously described Cetyltrimethyl Ammonium Bromide (CTAB) method at the Genomics and Molecular Biology Laboratory of the Department of Immunology & Molecular Biology, MakCHS.[37] For each of the genes $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV}, Taq DNA Polymerase contained in 2X Taq master mix and respective primers were used for amplification in the respective amplification conditions (Table 1).

The PCR products were analyzed on 0.8% agarose gel electrophoresis with ethidium bromide staining (0.5 µg/mL) for 60 min at 130 V. The bands were visualized on the Bio imager screen. The product sizes included 600 bp for $bla_{CTX\text{-}M}$, 404 bp for bla_{TEM}, and 900 bp for bla_{SHV} (Figure 1). A negative extraction control (NEC), no template control (NC), and a positive control (PC) were used for quality control.

Statistical Analysis

Continuous data was described as median (IQR) and mean (SD). Categorical data were described as proportions. Comparison of the significance of difference in distribution was analyzed using chi-square test. P-values of ≤0.05 were considered to be statistically significant. Statistical analysis was performed using Stata Version 16.0

Table 1 Primer Sequences, PCR Conditions, and Product Sizes for the blaCTX-M, blaTEM, blaSHV ESBL Genes

Gene	Primer Sequences	PCR Conditions		Ref
blaCTX-M	Fw: ATGTGCAGYACCAGTAARGTKATGGC	Initial den.:	95°C for 5m	[38]
	Rev: TGGGTRAARTARGTSACCAGAAYCAGCGG	Den.:	94°C for 30s	
		Annealing:	56°C for 45s	
		Ext.:	72°C for 45s	
		Final Ext.:	72°C for 10m	
blaTEM	Fw: TTTCGTGTCGCCCTTATTCC	Initial den.:	95°C for 5m	[39]
	Rev: ATCGTTGTCAGAAGTAAGTTGG	Den.:	94°C for 30s	
		Annealing:	53°C for 45s	
		Ext.:	72°C for 45s	
		Final Ext.:	72°C for 10m	
blaSHV	Fw: ATGCGTTATATTCGCCTGTG	Initial den.:	95°C for 5m	[40]
	Rev: AGCGTTGCCAGTGCTCGATC	Den.:	94°C for 30s	
		Annealing:	66°C for 45s	
		Ext.:	72°C for 90s	
		Final Ext.:	72°C for 10m	

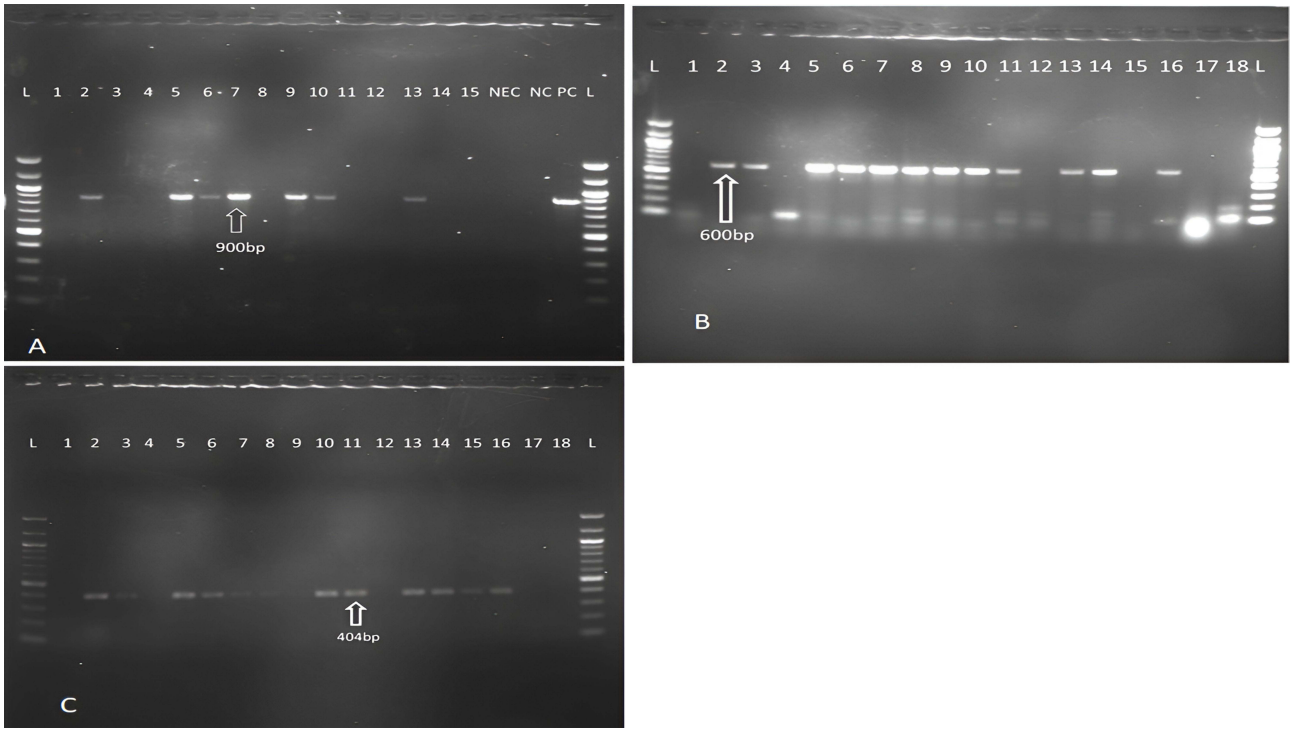

Figure 1 Gel electrophoresis picture showing the results for PCR amplification of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes. Picture (**A**) Analysis for bla_{SHV} (900bp); Lane L-100bp ladder; L2, L5, L6, L7, L9, L10 and L13 = samples positive for bla_{SHV}; L1, L3, L4, L8, L11, L12, L14, L15 = samples negative for bla_{SHV}. Picture (**B**) Analysis for bla_{CTX-M} (600bp); Lane L-100bp ladder; L2, L3, L5, L6, L7, L8, L9, L10, L11, L13, L14 and L16 = samples positive for bla_{CTX-M}; L1, L4, L12, L15, L17, L18 = samples negative for bla_{CTX-M}. Picture (**C**) Analysis for bla_{TEM} (404bp); Lane L-100bp ladder; L2, L3, L5, L6, L7, L8, L10, L11, L13, L14, L15, L16 = samples positive for bla_{TEM}; L1, L4, L9, L12, L17, L18 = samples negative for bla_{TEM}. NEC = negative extraction control; NC = negative control; PC = positive control.

Results

Demographics

Two hundred and two hematologic cancer patients with febrile neutropenia at the UCI were included in the study. Median and mean ages of patients were 19 years (IQR: 10–30 years) and 22 years (SD 16), respectively; 119 (59%) patients were male; 127 (63%) were in the adult ward; and 155 (77%) had leukemia (acute or chronic). Of the patients with acute leukemia, 77 (54%) had acute myeloid leukemia (AML), while 65 (46%) had acute lymphocytic leukemia. Sixty (30%) of the participants had at least one febrile episode due to Enterobacteriaceae. Nine participants (4%) had polymicrobial bacteremia caused by Enterobacteriaceae. There were no significant differences between positive and negative cultures for the categories sex, ward, and cancer type (Table 2).

Identification of Enterobacteriaceae Isolated

A total of 83 organisms were isolated. More than half (N=45, 54%) of the organisms were *E. coli* (Figure 2).

Antimicrobial Susceptibility Tests for Enterobacteriaceae Isolated

Overall, 79 (95%) of the Enterobacteriaceae isolated in this study were MDR, 47 (57%) were XDR and 3 (4%) were PDR. Specifically, 44/45 (98%) of *E. coli*, 30/32 (94%) of *K. pneumoniae*, and 5/6 (83%) of *Enterobacter spp*. were MDR, while 26/45 (58%) of *E. coli*, 18/32 (56%) of *K. pneumoniae*, and 3 (50%) of *Enterobacter spp*. were XDR. Only 1/45 (2%) of *E. coli* and 2/32 (6%) of *K. pneumoniae* were PDR. None of the *Enterobacter spp*. were PDR. Seventy-three of the 83 (88%) Enterobacteriaceae were resistant to third-generation cephalosporins. Forty (89%) of *E. coli*, 29 (91%) of *K. pneumoniae*, and 4 (67%) of *Enterobacter spp*. were resistant to third-generation cephalosporins. Overall, the ESBL phenotype was detected in 54/73 (74%) of Enterobacteriaceae that were resistant to third-generation cephalosporins. Among the organisms that were resistant to third-generation cephalosporins, 30/40 (75%) *E. coli* and 24/29 (83%) *K. pneumoniae* showed the ESBL

Table 2 Characteristics of Patients Showing Positive Cultures Caused by Enterobacteriaceae for the Total Population

Characteristics	Patients (N=202)		P value
	Positive Culture (N=60)	Negative Culture (N=142)	
	N(%)	N(%)	
Sex			
Male	32 (53)	87 (61)	0.295
Female	28 (47)	55 (39)	
Ward			
Adult	36 (60)	91 (64)	0.583
Pediatrics	24 (40)	51 (36)	
Cancer type			
Leukemia	51 (85)	104 (73)	0.071
Other	9 (15)	38 (27)	

phenotype. None of the *Enterobacter spp*. strains showed positive results for ESBL phenotypically (Table 3). Table 4 shows the comparison of antimicrobial susceptibility tests between Enterobacteriaceae sensitive to third-generation cephalosporins and resistant to third-generation cephalosporins with ESBL-positive phenotype. A significantly higher proportion of Enterobacteriaceae with ESBL-positive phenotype were resistant to piperacillin-tazobactam (p=0.024), gentamicin (p=0.000), ciprofloxacin (p=0.000), and cotrimoxazole (p=0.000) compared to Enterobacteriaceae, which were sensitive to third-generation cephalosporins. In both groups, the Enterobacteriaceae were more susceptible to carbapenems and chloramphenicol than resistant. Nineteen (26%) of Enterobacteriaceae that were resistant to third-generation cephalosporins were ESBL-negative. Of the 19 ESBL-negative Enterobacteriaceae that were resistant to third-generation cephalosporins, 18 (95%), 16 (84%), and 17 (89%), were resistant to ertapenem, imipenem, and meropenem, respectively.

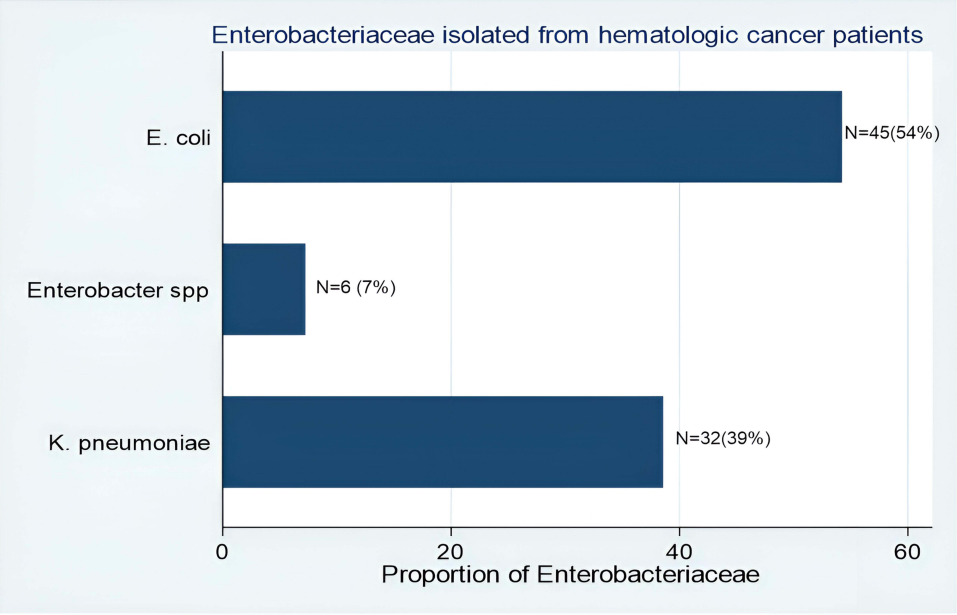

Figure 2 Proportion of Enterobacteriaceae isolated from hematologic cancer patients.

Table 3 Antimicrobial Susceptibility Tests for Enterobacteriaceae Isolates

Antibiotic	*E. coli* (N=45) N(%)			*K. pneumoniae* (N=32) N (%)			*Enterobacter spp.* (N=6) N (%)		
	S	I	R	S	I	R	S	I	R
Penicillin + β-lactamase inhibitors									
ANC	7(16)	7(16)	31(68)	4(12)	7(22)	21(66)	nt	nt	nt
Antipseudomonal penicillin + β-lactamase inhibitors									
TPZ	11(24)	6(13)	28(62)	9(28)	6(19)	17(53)	2(33)	0	4(67)
Extended spectrum cephalosporins									
CRO	6(13)	0	39(87)	5(16)	0	27(84)	2(33)	0	4(67)
CTX	5(11)	0	40(89)	4(13)	1(3)	27(84)	2(33)	0	4(67)
CAZ	5(11)	1(2)	39(87)	6(19)	1(3)	25(78)	2(33)	0	4(67)
FEP	6(13)	6(13)	33(73)	4(12)	4(12)	24(75)	2(33)	0	4(67)
Monobactams									
AZT	5(11)	2(4)	38(84)	5(16)	3(9)	24(75)	4(67)	0	2(33)
Carbapenems									
ERT	21(47)	3(7)	21(46)	21(66)	2(6)	9(28)	2(33)	0	4(67)
IMP	35(78)	1(2)	9(20)	27(84)	1(3)	4(13)	2(33)	0	4(67)
MER	35(78)	0	10(22)	27(84)	0	5(16)	2(33)	0	4(67)
Aminoglycosides									
GM	15(33)	1(2)	29(65)	8(25)	0	24(75)	4(67)	0	2(33)
Fluoroquinolones									
CIP	3(7)	2(4)	40(89)	6(19)	2(6)	24(75)	2(33)	2(33)	2(33)
Folate pathway inhibitors									
SXT	1(2)	0	44(98)	3(9)	1(3)	28(88)	1(17)	0	5(83)
Phenicol									
Chloramphenicol	34(76)	1(2)	10(22)	19(59)	2(6)	11(34)	3(50)	0	3(50)
Resistance pattern									
mdr	44 (98)			30 (94)			5 (83)		
xdr	26 (58)			18 (56)			3 (50)		
pdr	1 (2)			2 (6)			0		
ESBL phenotype*	30 (75)			24 (83)			0		

Note: *ESBL phenotype detected among isolates that were resistant to 3rd generation cephalosporins.
Abbreviation: nt, not tested.

ESBL-Encoding Genes

Overall, ESBL-encoding genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}) were detected in 55 (75%) of the 73 Enterobacteriaceae that were resistant to third-generation cephalosporins. Twenty-six out of 40 (65%) *E. coli*, 26/29 (90%) *K. pneumoniae*,

Table 4 Comparison of Antimicrobial Susceptibility Tests Between Enterobacteriaceae Sensitive to Third-Generation Cephalosporins and Resistant to Third-Generation Cephalosporins with ESBL-Positive Phenotype

Antibiotic	Sensitive to 3rd Gen Cephalosporin N=10 N (%)			ESBL-Positive N=54 N (%)			P value
	S	I	R	S	I	R	
TPZ	7 (70)	1 (10)	2 (20)	14 (26)	11 (20)	29 (54)	0.024
CTX	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)	54 (100)	0.000
CRO	10 (100)	0 (0)	0 (0)	1 (2)	0 (0)	53 (98)	0.000
CAZ	10 (100)	0 (0)	0 (0)	2 (4)	2 (4)	50 (92)	0.000
FEP	10 (100)	0 (0)	0 (0)	1 (2)	9 (17)	44 (81)	0.000
AZT	10 (100)	0 (0)	0 (0)	1 (2)	4 (7)	49 (91)	0.000
ERT	10 (100)	0 (0)	0 (0)	34 (63)	4 (7)	16 (30)	0.068
IMP	10 (100)	0 (0)	0 (0)	51 (94)	2 (4)	1 (2)	0.747
MER	10 (100)	0 (0)	0 (0)	52 (96)	0 (0)	2 (4)	0.536
GEN	9 (90)	0 (0)	1 (10)	13(24)	1 (2)	40 (74)	0.000
CIP	5 (50)	2 (20)	3 (30)	5 (9)	2 (4)	47 (87)	0.000
SXT	5 (50)	0 (0)	5 (50)	0 (0)	1 (2)	53 (98)	0.000
CAF	8 (80)	0 (0)	2 (20)	35 (65)	2 (4)	17 (31)	0.596

and 3/4 (75%) *Enterobacter spp* that were resistant to third-generation cephalosporins had ESBL-encoding genes. All three ESBL-encoding genes assessed were found in all three species. Most of the *E. coli* (15/26, 58%) and *K. pneumoniae* (21/26, 81%) had more than one ESBL-encoding gene. Only 1/3 (33%) of *Enterobacter spp*. had more than one ESBL-encoding gene (Table 5).

Overall, 50 (91%), 38 (69%), and 23 (42%) of the 55 organisms with ESBL-encoding genes had $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} respectively. Twenty-four out of 26 (92%) *E. coli* had $bla_{CTX\text{-}M}$. Twenty-five out of 26 (96%) *K. pneumoniae* had $bla_{CTX\text{-}M}$. Only 1 (25%) of the three *Enterobacter spp* had $bla_{CTX\text{-}M}$. (Figure 3).

Discussion

Bacteremia is a cause of significant morbidity and mortality in patients with hematologic malignancies, especially when caused by MDR strains. This has been worsened over the past decades by an increase in the number of infections caused

Table 5 ESBL-Encoding Genes Detected in Isolated Enterobacteriaceae

Genes	*E. coli* (N=26) N (%)	*K. pneumoniae* (N=26) N (%)	*Enterobacter spp.* (N=3) N (%)
$bla_{CTX\text{-}M}$ only	9 (35)	4 (15)	0
$bla_{CTX\text{-}M}$ + bla_{TEM}	11 (42)	4 (15)	1 (33)
$bla_{CTX\text{-}M}$ + bla_{SHV}	0	2 (8)	0
$bla_{CTX\text{-}M}$ + bla_{TEM} + bla_{SHV}	4 (15)	15 (58)	0
bla_{TEM} only	2 (8)	0	1 (33)
bla_{SHV} only	0	1 (4)	1 (33)

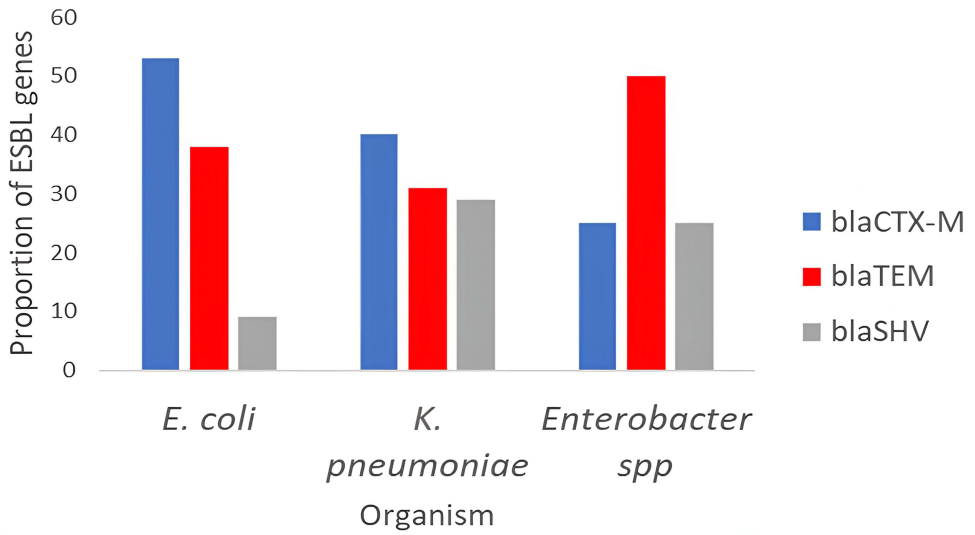

Figure 3 Proportion of blaCTX-M, blaTEM and blaSHV in isolated Enterobacteriaceae.

by MDR strains, which have been associated with increased morbidity, mortality, and hospital costs.[1] Among the organisms listed as the main causes of bacteremia are the ESBL-PE, which are listed as priority pathogens by the WHO. This study aimed to determine the phenotypic and genotypic prevalence of ESBL-determining genes found in Enterobacteriaceae isolated from hematologic cancer patients with bacteremia at UCI. Ninety-five percent of *Enterobacteriaceae* were MDR, the most common being *E. coli*. This was a slight increase from 85%, which was observed in a previous study carried out in the same institute.[32] Similarly, high rates of resistance have been reported in other health facilities in Uganda and surrounding regions.[34]

In this study, the overall prevalence of ESBL-PE phenotype was 74%. We previously reported a prevalence of 41%; however, at that time the number of organisms were fewer than in the present study.[32] The proportion of ESBL-PE in our study is higher than that found in intensive care unit (ICU) patients at Mulago Hospital, which observed the ESBL phenotype in 31% *E. coli* and *K. pneumoniae*.[41] The number of organisms isolated in that study was few. Similarly, it was higher than 62% observed in ESBL-PE isolates obtained from samples collected from patients in various wards of Mulago Hospital.[42] Moreover, that study only had 2 out of 3 (40%) blood samples that were ESBL phenotype positive. However, our ESBL proportion was lower than 81%, which was observed in patient population with surgical site infections at Mulago Hospital.[34] In cancer centers in other LMICs, the proportion of ESBL was shown to range from 23% to 64.1% while in HIC, rates as high as 34% have been reported.[19,23–26,28–31] MDR has been reported to be higher in LMIC compared to HIC.[43] Among the drivers of ESBL production are overuse and misuse of third-generation cephalosporins in LMICs.[44] Unlike HIC which have robust antimicrobial stewardship programs, LMICs lack adequate antimicrobial surveillance mechanisms.[45] Dissemination of MDR strains including ESBL-PE negatively impacts patient outcomes by limiting therapeutic options available. Moreover, this demonstrates the need for robust infection prevention and control practices in cancer centers where majority of patients are immunocompromised.

More than half of the ESBL-PE in our study had resistance to gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole. Different mechanisms of antimicrobial resistance have been demonstrated in Enterobacteriaceae including modifying enzymes, mutations, and presence of efflux pumps.[46] However, studies have shown an association between ESBL phenotypes/genotypes and resistance to fluoroquinolones and aminoglycosides.[16] In Ethiopia, a study showed that 63% of ESBL-PE were non-susceptible to gentamicin, 89.8% to trimethoprim-sulfamethoxazole, and 69% to ciprofloxacin.[4] Similar findings have been observed in studies in Burkina Faso, Ghana, and Israel.[47–49] This could be due to being encoded on a single plasmid that favors co-transmission. Co-resistance limits the number of available antibiotics, which work against ESBL-PE.

We also noted non-susceptibility of Enterobacteriaceae to carbapenems (ertapenem, imipenem, and meropenem), albeit the proportions of non-susceptibility were lower when compared to other antibiotics. Resistance to carbapenems was higher among isolates that were resistant to third-generation cephalosporins with ESBL phenotype negative compared to those that were resistant to third-generation cephalosporins with ESBL phenotype positive. In our previous study carried out at UCI, we found 36.4% *E. coli* and 57.1% *K. pneumoniae* to be resistant to a carbapenem.[32] Previous studies have only tested imipenem and included few organisms. Other cancer centers in Africa and worldwide have also reported resistance to carbapenems. The treatment of choice for infections caused by ESBL-producers is carbapenems.[50] Unfortunately, carbapenems are prohibitively expensive, especially for LMIC.[51] Furthermore, the increased spread of carbapenem-resistant *Enterobacteriaceae* strains complicates the choice of therapy for these infections, leading to poor patient outcomes.

Notably, 65% of ESBL-PE were susceptible to chloramphenicol. A study carried out in India showed 68% of the MDR Gram-negative bacilli isolates were found to be sensitive to chloramphenicol.[52] Chloramphenicol may play a role as a therapeutic option for MDR strains, especially in LMIC.[52] With no new antibiotics currently available, there has been a shift in focus to reevaluate older antibiotics, including chloramphenicol.[53,54] Chloramphenicol use was abandoned due to side effects including aplastic anemia and bone marrow suppression.[55] Perhaps, this warrants its cautious use in a hematologic cancer unit. However, not only does it have good oral bioavailability and tissue penetration, but it is also relatively cheap and affordable.[56,57]

The most common ESBL-encoding gene we identified in this study was $bla_{CTX\text{-}M}$ followed by bla_{TEM} and bla_{SHV} *which* was the least identified. Few studies in Uganda have characterized the ESBL genes in Enterobacteriaceae. In Western Uganda, one study carried out in various patient populations showed a predominance of $bla_{CTX\text{-}M}$ (70%) compared to bla_{TEM} (47%) and bla_{SHV} (34%), while another in a different hospital showed a predominance of bla_{SHV} (42%) compared to bla_{TEM} (27.3%) and $bla_{CTX\text{-}M}$ (22.4%).[58,59] Our findings are comparable with those from a cancer center in Egypt, which found a predominance of $bla_{CTX\text{-}M}$ (55.7%). However, unlike our study, bla$_{SHV}$ was more predominant than bla_{TEM} (44.3% vs 31.4%).[29] Furthermore, as seen in other studies, coproduction of all three genes was reported in our study.[60] Importantly, when combined with other resistance mechanisms including loss of outer membrane porins, presence of ESBL genes, most especially $bla_{CTX\text{-}M}$, has been associated with resistance to ertapenem.[17,18] This could explain the resistance to ertapenem observed in our study compared to imipenem and meropenem.

This is among the few studies in SSA that have described the magnitude of ESBL-PE in a cohort of hematologic cancer patients who are at risk for an ESBL infection because of their weakened immune systems. However, the sample size of the ESBL-PE evaluated is small warranting continuous surveillance of MDR, XDR, and PDR bacteria over time. Furthermore, our study was limited to the three most reported ESBL-encoding genes ($bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}). We did not investigate the presence of other ESBL genes. We also did not determine other possible resistance mechanisms responsible for resistance to third-generation cephalosporins, such as AmpC. While we demonstrated possible presence of co-resistance between ESBL-PE and aminoglycosides, fluoroquinolones, and sulfonamide, as well as resistance to carbapenems, it is important to determine the molecular resistance mechanisms responsible for the observed phenotype as a step towards genomic surveillance.

Conclusion

Our study shows the predominance of ESBL-PE as a cause of bacteremia in hematologic cancer patients at the UCI. The most common ESBL-encoding gene identified in the ESBL-PE was $bla_{CTX\text{-}M}$. Resistance to imipenem and meropenem was low, and therefore, carbapenems remain the recommended drug of choice for ESBL-PE at the UCI. We recommend continuous surveillance over time to assess the clinical features, risk factors, and outcomes of ESBL-PE bacteremia in immunosuppressed cancer patients. In addition, describing the genetic environment of ESBL-PE, including exploring associated resistance mechanisms, sequence types, and relatedness is important to inform infection prevention and control and antimicrobial stewardship protocols in cancer centers, which rely heavily on antibiotics for supportive management especially for patients who are on chemotherapy.

Data Sharing Statement

The data presented in this study are available on request from the corresponding author.

Ethics Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki. The study received ethical and scientific approval from the Institutional Review Board (IRB) of the School of Biomedical Sciences (SBS), College of Health Sciences, Makerere University (SBS 396); from the Uganda National Council of Science and Technology (HS 2217); and from Fred Hutchinson Cancer Research Center IRB (FHCC #: 8433). Informed consent was obtained from all subjects involved in the study. Parents of children under 18 years provided informed consent.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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