

Original Research Article

Characterization of Extended Spectrum Beta-Lactamase Uropathogens isolated from refugees with urinary tract infections in Nakivale refugee settlement camp, South-western Uganda

ABSTRACT

The World Health Organization estimates one in four individuals have had at least one Urinary tract infections (UTI) episode requiring treatment with an antimicrobial agent. At Nakivale refugee camp, the overwhelming number of refugees often associated with poor living conditions do predispose the refugees to urinary tract infections.

This study determined the prevalence of urinary tract infections, antimicrobial susceptibility pattern of the isolated bacterial pathogens, the prevalence of Extended-Spectrum Beta-Lactamase (ESBL) bacteria, and molecular characterization of genes encoding for ESBLs among refugees in the Nakivale refugee settlement.

This was a cross-sectional study, involving 216 outpatients who visited Nakivale Health Centre III between July and September 2020. The urine samples were received and examined at the microbiology laboratory of Mbarara University of science and technology. The urine samples were cultured and identified. Antibiotic susceptibility was carried out following CLSI recommended guidelines while presence of genes encoding ESBL was detected using conventional PCR amplification

The prevalence of UTI was 24.1% (52/216). *Staphylococcus aureus* was the most prevalent causal agent, accounting for 22/52 (42.31%) of total isolates, followed by *Escherichia coli* 21/52(40.38%). Multidrug-resistant isolates accounted for 71.15% (37/52). A total of twenty-one isolates (70.0%) were extended spectrum beta lactamse producing bacteria. The most prevalent genes were TEM beta-lactamase (blaTEM) and CTX-M beta-lactamase (blaCTX-M).

The prevalence of UTI among refugees in Nakivale settlement is high. Therefore conducting continuous epidemiological surveys should be done to determine the existence and of ESBL producing organisms across refugee camps in Uganda.

Key words: Urinary tract infections, Uropathogens, Extended beta lactamase organisms, Nakivale Refugee Settlement camp.

UNDER PEER REVIEW

BACKGROUND

Urinary Tract Infections are infections of the urinary tract system that arise from bacterial migration within the genitourinary tract. They are usually transmitted through contaminated hands, normal flora migration, or through sexual intercourse [1]. Globally, the incidences of UTI have become endemic which has significantly increased the use of antimicrobial agent [2]. The widespread use of these antibiotics has caused the emergence and spread of resistant bacteria (extended-spectrum beta-lactamase -producing organisms) [3]. Extended Spectrum Beta-Lactamase producing uropathogens are widely spread around the world, and their severity is higher in poorer nations like Uganda [4]. ESBL-producing organisms frequently exhibit characteristics of multidrug resistance [5] to frequently used antibiotics because they frequently carry genes *bla*TEM, *bla*CTX-M, and *bla*SHV encoding resistance to aminoglycosides thus, ESBL producing organisms often possess multidrug resistance phenotypes [5].

Uganda is Africa's largest refugee-hosting country and the 8th in the world with a total population of about 1,381,122 of which 130,462 (9.45%) are found in Nakivale refugee camp [6]. The overwhelming number of refugees is often associated with poor living conditions such as communal bathrooms and toilets and multiple sex partners resulting in compromised health [7]. As a result, a high number of suspected urinary tract infection are reported at Nakivale refugee camp (at least 20 suspected UTI per day) according to the record from the health centre.

According to unpublished health center III records in Nakivale refugee settlement, the prevalence of clinically diagnosed UTI was seen to be 15%. Resistant UTI are among the challenges faced by health care providers in refugee population [8]. Accordingly, therapy is empiric and with the influx of refugees, new infections and uropathogenic gram negative bacterial resistant strains could be introduced as already reported by some studies [9]. Delayed recognition of severe UTI caused by ESBL producing organisms, and inappropriate treatment

with antibiotics has been associated with increased mortality and morbidity in resettlement camps a phenomenon that stresses the need for evaluation of UTI etiology.

MATERIALS AND METHODS

Study population and sample collection

The study was a descriptive cross-sectional study, conducted among 216 outpatients attending Nakivale Health Centre III in Nakivale Health Centre III refugee settlement in Isingiro district located 59km away from Mbarara district, South-western from July 2020 to September 2020. Participant with complaints suggestive of UTI, such as dysuria, frequency, urgency, suprapubic pain, cloudy urine as identified by the attending clinician, were considered for the study after receiving informed consent. Participants who did not provide a urine sample and females who were in their menstrual cycle were excluded. Participants were given oral and written instructions to collect midstream urine in sterile leak proof labelled urine containers.

Laboratory tests Urine culture and isolation of Organisms

The urine samples were collected from Nakivale settlement camp transported in a cold chain and received and examined at the microbiology laboratory of Mbarara University of science and technology. The color and turbidity of the urine samples were recorded. The urine was microscopically wet-prepared to look for significant pyuria (>10 cells/ μ l), casts, red cells, bacteria, and yeast. Urine dipstick biochemical testing and primary Gram staining were recorded. The urine samples were inoculated using a 2mm standard loop and plated on Cystine Lactose Electrolyte-Deficient (CLED) agar with bromotymol blue (HiMedia Laboratories supplies) and incubated aerobically at 37°C overnight for 24 hours. colonies were counted 50 and above colony forming units which were equivalent to 10000 (10^4) by ml (CFU/mL) were significant [10]. Purity plating was done on MacConkey Agar, Blood Agar, and Mannitol Salt Agar (Oxoid).

The pure isolated bacterial colonies were identified using through Gram staining, colony morphology [11], Biochemical testing that included Citrate utilization test, Urease test, Triple sugar iron agar (TSI) test, Indole test, Sulphur indole motility [12] and Analytical Profile Index (API).

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolated bacterial pathogens was performed using the disc diffusion method on Mueller-Hinton agar according to the guidelines of clinical and laboratory standards institute [13]. Antibiotic discs from Oxoid that were used for both Gram positive and Gram negative bacteria included: Nitrofurantoin (100 µg), Ampicillin (10µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Amoxicillin-clavulanic acid (20/10µg), Chloramphenicol (30µg) while Erythromycin (15µg), and Penicillin (10µg) were used for only Gram positive bacteria. Bacteria that showed resistance to at least three or more antibiotics of different classes were considered Multidrug resistant bacteria [14]

Screening for ESBLs

Gram-negative bacteria were confirmed for the presence of extended-spectrum β-lactamases by using the phenotypic detection method as per CLSI guidelines. The discs used were Ceftazidime (CAZ) disc containing 30µg of the antibiotic and ceftazidime- clavulanic acid (CZC) disc containing 30/10µg of the antibiotics and cefotaxime (CTX) disc containing 30µg of the antibiotic and a cefotaxime-clavulanic acid (CTC) disc containing 30/10µg of the antibiotics. The isolates were interpreted as ESBL producers if a clear extension of the edge of the inhibition zone of cephalosporin towards the CZC disc was observed [15].

Genotypic characterization of ESBLs

DNA extraction and genotyping

ESBL phenotypes were sub-cultured at 37°C overnight in Muller Hinton Agar (Oxoid, Wade Road, Basingstoke, UK). The bacterial DNA was extracted using the Zymo-Research quick-g-DNA Miniprep kit and protocol, v1.0.0.

The presence of genes encoding ESBL was detected using PCR amplification. This study detected *bla*-SHV, *bla*-CTX-M [16], and *bla*-TEM [17], genes. The PCR master mix reagents for each gene target were as follows: 12.5 µL master mix consisting of One Taq quick-load 2x master mix/w standard buffer, dNTPs & Taq polymerase (M0486S), 1.5 µL forward (100 µM), 1.5 µL primary reverse (100 µM), 5 µL DNA template and RNAase-free water up to 25 µL.

PCR Cycling: The PCR process was carried out in a thermocycler (Multigene Optimax) with a denaturation cycle of 95°C for 15 min, DNA amplification of 30 cycles (94°C for 1 min, 54.5-60°C for 1 min, and 72°C for 1 min) and final extension cycle of 72°C for 5 min (See table 1). DNA Amplicon was electrophoresed using 1.5% agarose gel, in Tris-Borate EDTA buffer (TBE) 1×concentration, Safe View Classic™ DNA stain (cat # G108), 6x loading dye (Thermo Scientific #R0611), and DNA ladder/marker 100 bp (NEB-Biolabs #N3231L). DNA Bands were visualized on a Dark reader Transilluminator (DR46-B).

Table 1: Primers sets used in the detection of ESBL encoding genes from the study isolates[18]

No.	Resistance gene	Primer	Annealing temperature	Amplicon bp
1	<i>bla</i> TEM	(F) 5'-ATTTCCGTGTCGCCCTTAT-3 (R) 5'-CTACGATACGGGAGGGCTTA-3'	54.5°C	759
2	<i>bla</i> CTX-M	(F) 5'-ATGATGAAAAAATCGTTATGC-3' (R) 5'-CAGCATCTCCCAGCCTAAT-3'	57°C	489
3	<i>bla</i> SHV	(F) 5'- CCTGTTAGCCACCCTGCC-3' (R) 5'- CCGCAGATAAATCACCAC-3'	60°C	768

Quality control

Reference strains of *Escherichia coli* ATCC25922 (ESBL negative), *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive), and *Staphylococcus aureus* ATCC 29213. *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 were used[19].

Data management and analysis

The obtained data were entered and cleaned in Microsoft Excel and, and was imported to STATA version 12.0 for analysis. Evaluations were carried out at 95 % confidence level (95% CI). Results were presented in form of tables, pie charts and graphs.

RESULTS

The demographic characteristics of study participants

There were 216 participants in the study, including 164 (75.93%) females and 52 (24.07%) males. Participant ages ranged from 18 to 76, with an average age of 35.86 (SD=11.218) and a median of 34. 86(39.81%) Democratic Republic of the Congo received the greatest number of refugees 86(39.81%) followed by those from Somalia 58(26.85%). Regarding education many 89(41.20%) were not educated, followed by those primary level accounting to 82(37.96%). The detailed findings are shown in table 2 below.

**Table 2: Demographic characteristics of the study participants
N=216**

Variable	Frequency (n)	Percentage (95% CI)
Sex		
Female	164	75.93 (69.66-81.47)
Males	52	24.07 (18.54 - 30.34)
Age group		
18-76 years	216	100 (98.31 - 100)
Country of origin		
Burundi	27	12.50 (8.40 - 17.66)
Democratic Republic of Congo	84	38.89 (32.35 - 45.74)
Ethiopia	01	0.46 (0.012 - 2.55)
Rwanda	35	16.20 (11.55 - 21.80)
South Sudan	11	5.09 (2.57 - 8.93)
Somalia	58	26.85 (21.07 - 33.29)
Education level		
None	89	41.20 (34.57 - 48.08)
Primary	82	37.96 (31.47 - 44.79)
Secondary	37	17.13 (12.37 - 22.83)
Tertiary	8	3.70 (1.61 - 7.17)
Marital status		
Divorced	15	6.94 (3.94 - 11.19)
Married	163	75.46 (69.17 - 81.04)
Single	27	12.50 (8.40 - 17.66)
Widowed	11	5.09 (2.57 - 8.93)

The prevalence of urinary tract infections among refugees in Nakivale refugee settlement

52 of the 216 samples taken and grown exhibited considerable bacterial growth, whereas the remaining 164 showed no growth. Thus, in this study, the overall prevalence of UTI was 24.1%.

Prevalence of UTI based demographic factors

The prevalence of UTI was 25.00% (41/164) among females and 21.15% (11/52) among males. With country-of-origin South Sudan had the highest prevalence of UTI which was 36.36% (4/11), followed by Democratic Republic of the Congo 29.76% (25/84). Refugees of primary level of education had the highest prevalence of UTI 29.27% (24/84), followed by uneducated 23.60% (21/89). Lastly, single refugees had the highest prevalence of UTI 29.63% (8/27), followed by married refugees 23.93% (39/163). (See details in table 3)

Table 3: Prevalence and Demographic distribution of UTI

N=216

Sex	Negative cultures for bacteria (%)	Positive cultures for bacteria (%)	Total	Prevalence of UTI % (95% CI)
Total enrolment	164	52	216	24.07 (18.53 - 30.34)
Positivity by Gender				
Female	123	41	164	25.00 (18.58 - 32.35)
Male	41	11	52	21.15 (11.06 - 34.70)
Country of origin				
Burundi	20	7	27	25.93 (11.11 - 46.28)
Democratic Republic of the Congo	59	25	84	29.76 (20.27 - 40.73)
Ethiopia	1	0	1	0.00 (0 - 97.5)
Rwanda	30	5	35	22.7 (4.81 - 30.26)
South Sudan	7	4	11	36.36 (10.93 - 69.21)
Somalia	47	11	58	18.96 (9.87 - 31.41)
Education level				
None	68	21	89	23.60 (15.24 - 33.78)
Primary	58	24	82	29.27 (19.73 - 40.35)
Secondary	31	6	37	16.22 (6.19 - 32.01)

Tertiary	7	1	8	12.50 (0.32 - 52.65)
Marital status				
Divorced	12	3	15	20.00 (16.58 - 40.46)
Married	124	39	163	23.93 (17.60 - 31.22)
Single	19	8	27	29.63 (13.75 - 50.18)
Widowed	9	2	11	18.18 (2.28 - 51.77)

The bacterial etiology of urinary tract infections among refugees in Nakivale settlement

Staphylococcus aureus was the most common organism accounting for 22/52 (42.31%) of total isolates, followed by *Escherichia coli* 21/52(40.38%), *Klebsiella pneumoniae* 4/52(7.69%), *Salmonella arizonae* 2/52(3.85%) while *Citrobacter freundii*, *Hafnia species*, and *Pseudomonas aeruginosa* accounted to 1/52(1.92%) each as illustrated in figure 1

The antimicrobial susceptibility pattern of the isolated bacterial pathogens among refugees in Nakivale settlement

According to table 4 below, the majority of the isolates showed resistance to at least one class of antimicrobials. Multidrug bacteria accounted for 37(71.15%) of the bacteria isolates were multi-resistant while 14 (26.92%) were mono-resistant to antibiotics, and 1 (1.92%) bacteria isolate was not resistant to any antibiotic.

Table 4: The antimicrobial susceptibility pattern of the isolated bacterial pathogens among refugees in Nakivale settlement

Resistance classification	Frequency	Percentage
None	1	1.92
Mono	14	26.92
MDR	37	71.15
Total	52	100.00

Antimicrobial susceptibility pattern of the individual isolated bacterial pathogens among refugees in Nakivale settlement

Multidrug-resistant isolates included *Klebsiella pneumoniae*, *Salmonella arizonae*, *Citrobacter freundii*, *Hafnia species*, and *Pseudomonas aeruginosa*. All *Escherichia coli* isolates were

resistant to at least one class of antimicrobials that are 33.33% being mono-drug-resistant and 66.67% being multidrug-resistant. Lastly of the 22 *Staphylococcus aureus* isolates only 1 (4.55%), the isolate was not resistant to any antimicrobial as in the table 5 below;

Table 5: Antimicrobial susceptibility pattern of the individual isolated bacterial pathogens among refugees in Nakivale settlement

Isolates	Non-drug-Resistance No. (%)	Mono-Drug-Resistance No. (%)	Multidrug-Resistance No. (%)	Total (%)
<i>Staphylococcus aureus</i>	1(4.55)	7(31.82)	14(63.63%)	22 (100%)
<i>Escherichia coli</i>	n.d	7(33.33)	14(66.67)	21 (100%)
<i>Klebsiella pneumoniae</i>	n.d	n.d	4(100%)	4 (100%)
<i>Salmonella arizonae</i>	n.d	n.d	2(100%)	2 (100%)
<i>Citrobacter freundii</i>	n.d	n.d	1(100%)	1 (100%)
<i>Hafnia species</i>	n.d	n.d	1(100%)	1 (100%)
<i>Pseudomonas aeruginosa</i>	n.d	n.d	1(100%)	1 (100%)

Phenotypic characterization of Extended Spectrum Beta-Lactamase organisms among refugees in Nakivale refugee settlement

Phenotypic characterization of ESBL production was performed on 30 Gram-negative organisms and 21 (70.00%) were ESBL producers for the different organisms while 9(30%) were non-ESBL producers as shown in table 6 below..

Table 6: ESBL production by the different organisms

ORGANISMS ISOLATED	ESBL PRODUCER		TOTAL
	NO (%)	YES (%)	
<i>Escherichia coli</i>	7(33.33)	14(66.67)	21

<i>Klebsiella pneumoniae</i>	1(25.00)	3(75.00)	4
<i>Salmonella arizonae</i>	1(50.00)	1(50.00)	2
<i>Citrobacter freundii</i>	n.d	1(100%)	1
<i>Hafnia species</i>	n.d	1(100)	1
<i>Pseudomonas aeruginosa</i>	n.d	1(100)	1
TOTAL	9(30.00)	21(70.00)	30

Genotypic characterization of Extended Spectrum Beta-Lactamase organisms among refugees in Nakivale refuge

The presence of any genes of interest were detected in 8 (26.67%) of 30 Extended Spectrum Beta-Lactamase organisms. The most prevalent genes were *bla*TEM and *bla*CTX-M, both of which were found in 5 (62.5%) of the organisms possessing genes while *bla*SHV was found in three (37.5%) of the organisms containing genes, as indicated in table 7

Table 7: Genotypic characterization of Extended Spectrum Beta-Lactamase organisms

ORGANISMS ISOLATED	ESBL PRODUCER			Positive for any gene (%)	Negative for any gene (%)	TOTAL (%)
	Genes Isolated					
	<i>bla</i> TEM	<i>bla</i> SHV	<i>bla</i> CTX-M			
<i>Escherichia coli</i>	2	2	4	5 (23.81)	16(76.19)	21(100)
<i>Klebsiella pneumonia</i>	2	1	0	2(50.00)	2(50.00)	4(100)
<i>Pseudomonas aeruginosa</i>	1	0	1	1(100.00)	n.d	1(100)
TOTAL	5	3	5	8(26.67)	22(73.33)	30 (100)

*bla*TEM,*bla*CTX-M,and *bla*SHV genes for ESBL producer by the different organisms

Bla-TEM

DISCUSSION

The prevalence of urinary tract infections

The prevalence of UTI was 24.1% in this study. This finding was consistent with the findings of Tibyangye [20] study in Bushenyi, where the prevalence of UTI was (22.5%) and Odongo [21] study in Gulu, where the prevalence was (24.2%). The rationale for the consistent results could be due to geographic locations as indicated by Tandogdu, [22].

When compared to Turktan's study of refugees [27], Aro's study of refugees in Finland [28], Gulati's study of Syrian refugees in Asia [29], and UNHCR's study of refugees in Tanzania [30], the finding was high according to the Uganda refugee health report 2018, UTI constituted 2% of all consultations made in all refugee settlements, a figure contradicts the findings of this study. Thus, the cause for disparity in UTI prevalence may be attributed to the procedures employed to diagnose UTI. We employed urine culture in our study, whereas they used conventional urinalysis in other studies.

However, the study prevalence was low when compared to 38.8% in a study conducted by Kabugo [23] at Mulago hospital in Uganda and (32.2%) in a study conducted among patients attending hospitals in Bushenyi [24]. Although our frequency is not much lower than of Mulago hospital and KIU teaching hospital, this is due to the fact that our study recruited largely outpatients who came to lesser health units rather than patients who sought care at a tertiary hospital.

The high prevalence of UTI could be attributed to the large number of refugees who frequently connected with poor living conditions such as communal restrooms and toilets as well as several sex partners, resulting in compromised health [25].

The bacterial etiology of urinary tract infections

Despite the fact that numerous studies have found *Escherichia coli* as the most prevalent bacteria causing UTIs [33, 34], the most common organism in our study was *Staphylococcus aureus*. *Staphylococcus aureus* was the most prevalent bacterial isolate in this investigation, accounting for 42.31% of total isolates, followed by *Escherichia coli* (40.38%). This was consistent with Ekwealor's [35] study in Nigeria, which discovered that the most prevalent isolate was *Staphylococcus aureus* (28%), followed by *Escherichia coli* (24.6%). A research conducted in Ethiopia by Regea [36] discovered that the most prevalent isolates were,

Staphylococcus aureus (24.2%), *CoN Staphylococcus spp* (24.2%), *E.coli*(12.1%), as well as *K. pneumonia* (12.1%).

The antimicrobial susceptibility pattern of the isolated bacterial pathogens

In this study, 37(71.15%) of the bacteria isolates were multi-resistant. This finding is low when compared to 89.5% (MDR) multi-drug resistant in the study done in Ghana by Agyepong [26], 78% of the isolates were multi-drug resistant (MDR) in 2014 in a study done in Netherlands[27] and 83% of the drugs that were multidrug-resistant to bacteria isolates according to Manikandan [28]

However, it was high when compared to 23% of the isolates were multi-drug resistant (MDR) in 2017 in a study done in Netherlands by Stalenhoef[27], 63% of the isolates that were multidrug-resistant (MDR) in a study done by Ramirez-Castillo [29], 54.2% of the bacterial isolates were multi-drug resistant in a study done in Bangladesh [30], 41.1% isolates that were multi-drug resistant in a study done in Nepal by Baral [31], 61.4% of the bacterial isolates that were multidrug-resistant in a study done in Egypt [32] and lastly 14.5% multidrug-resistance rate in the study done in the south and eastern Europe, Turkey and Israel by [33]. This high finding of multidrug resistance rate in this study could be because the most dominant bacteria isolate (*Escherichia coli*) has shown a substantial multidrug resistance to antibiotics normally given for the management of UTIs in studies by Odongo [34] and El-Wafa[35].

In this study 66.67% *Escherichia coli* and all the *Citrobacter freundii*, isolates were multidrug-resistant. This finding low when compared to results in a study done in Nepal [36] which found that *Escherichia coli* isolates were 38.2% MDR, and *Citrobacter spp.* was 72.7% MDR.

However, the results similar to those in a study done in Ghana [26] since both studies showed that all (100%) *Pseudomonas aeruginosa* were multidrug-resistant. Therefore, there is a high multidrug resistance rate of individual bacteria isolates to antibiotics used to treat UTIs.

Phenotypic characterization of Extended Spectrum Beta-Lactamase organisms ESBLs

Phenotypic characterization of ESBL production was performed on 30 Gram-negative organisms and 21 (70.00%) were ESBL producers for the different organisms while 9(30%) were non-

ESBL producers. This prevalence of ESBL was high when compared with the overall prevalence of ESBL in Kenya (37.4%), Rwanda (38.3), Algeria (19.9%) [37] and 6.4% in United states [38]. Another study by Mansouri in 2019 [39] determined increased ESBL production 69.1–78.6% in Europe. Increased prevalence (55.5%) of ESBL positive organisms has also been noted in Nairobi Kenya by Magale in 2015 [40] and between 27.8% to 64.9% in Uganda [41-43].

Therefore, this high prevalence of ESBL-producing *Enterobacteriaceae* reflects a more serious public health problem. Dissemination of ESBLs compromises the activity of broad-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcomes for patients [44]. Additionally, a study by Fatima [45] in Pakistan reported that 97% of ESBL producing bacteria isolates were resistant to Ampicillin, therefore, Ampicillin resistance in this study could have resulted from the high prevalence of ESBL producing *Enterobacteriaceae*.

Furthermore, according to results in a study by Sakina [46], the ESBL producing bacteria isolates were resistant to Ciprofloxacin (59%) and resistant to Ceftriaxone (50%). Therefore, the high prevalence of ESBL producing bacteria isolates could also be the reason for the high multi drug resistance in this study.

Genotypic characterization of Extended Spectrum Beta-Lactamase organisms ESBLs

In this study, the most common genes were *bla*TEM and *bla*CTX-M each detected in 5 (62.5%) of the organisms with genes while *bla*SHV was detected in 3 (37.5%) of the organisms with genes. This contradicts the finding in studies done by Jena [47, 48] wherein all these studies *bla*TEM the only predominant gene was followed by *bla*CTX-M. Other contradictions were reflected by studies conducted by Seyedjavadi [49] and Ahmed [50] were for their case *bla*CTX-M was the predominant gene was followed by *bla*TEM. The reason for such could be the difference in study areas and people.

Despite the difference on which gene is common the percentage of *bla*TEM and *bla*CTX-M in this study and others was above 60%. Thus, it can be conclusive that the most common genes are *bla*TEM and *bla*CTX-M.

CONCLUSION

The prevalence of UTI among refugees in Nakivale settlement is high. *Staphylococcus aureus* is the main cause of UTI followed by *Escherichia coli* and *Klebsiella pneumoniae*.

Multidrug resistance was 71.15% in tested isolates, whereas ESBL production in *Enterobacteriales* was 70%. The common ESBL genes detected were *bla*TEM and *bla*CTX-M.

Recommendations

Conducting continuous epidemiological surveys to determine the existence of ESBL producing organisms across refugee camps in Uganda.

Limitations

Sequencing of *bla*TEM and *bla*CTX-M was not performed as well as determining pandemic clones ST131 and ST405 of *E. coli* that would further determine the disease burden.

DECLARATION

Ethics approval and consent to participate

This study was approved by Research and ethics committee of Mbarara University of Science and Technology (Ref MUREC 1/7). Upon clearance, permission was sought from the Office of the Prime Minister to research a refugee population, and informed consent was sought from each participant.

Availability of data and material

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

A preprint <https://www.medrxiv.org/content/10.1101/2022.04.29.22274464v1.full.pdf>[51] was published and readily available online.

Consent for publication

Not applicable.

References

1. Flores-Mireles, A.L., et al., *Urinary tract infections: epidemiology, mechanisms of infection and treatment options*. 2015. **13**(5): p. 269-284.
2. Lee, D.S., S.-J. Lee, and H.-S.J.B.r.i. Choe, *Community-acquired urinary tract infection by Escherichia coli in the era of antibiotic resistance*. 2018. **2018**.
3. Teklu, D.S., et al., *Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia*. 2019. **8**(1): p. 1-12.
4. GASHAW, M., BERHANE, M., BEKELE, S., KIBRU, G., TESHAGER, L., YILMA, Y., AHMED, Y., FENTAHUN, N., ASSEFA, H. & WIESER, A. , *Emergence of high drug resistant bacterial isolates from patients with health care associated infections at Jimma University medical center: a cross sectional study*. *Antimicrobial Resistance & Infection Control*, , 2018. . **7**(138).
5. VENTOLA, C.L., *The antibiotic resistance crisis: part 1: causes and threats*. *Pharmacy and therapeutics*. P T., 2015. **40**(4): p. 277-83.
6. UNHCR, UNHCR, *Government of Uganda, Office of the Prime Minister, Operational portal refugee situations*. Kampala, Uganda. 2019.
7. Gebrehiwet, K., et al., *The social health impact of Eritrean refugees on the host communities: the case of May-ayni refugee camp, Northern Ethiopia*. *BMC Research Notes*, 2020. **13**: p. 1-5.
8. Sabih A, L.S.C.U.T.I.U.F., *Complicated urinary tract infections*. StatPearls Publishing; Treasure Island (FL) 2022.
9. URSEL, H., BERNHARD, K., KARATHANA, M., NIELS, K. & CHRISTIAN, Z. . . , *Multidrug-resistant bacteria in unaccompanied refugee minors arriving in Frankfurt am Main, Germany, October to November 2015*. . *Eurosurveillance*, 2016. **21**, (2).
10. Tille, P., *Bailey & Scott's Diagnostic Microbiology E-Book*. . Elsevier Health Sciences., 2015.
11. Carter, G., *Isolation and identification of bacteria from clinical specimens*, in *Diagnostic procedure in veterinary bacteriology and mycology*. 1990, Elsevier. p. 19-39.
12. Dimri, A.G., et al., *Morphological and biochemical characterization of food borne Gram-positive and Gram-negative bacteria*. *Science Archives*, 2020. **1**(1): p. 16-23.
13. ., C., *M100 Performance Standards for Antimicrobial Susceptibility Testing*. 2018.
14. Sahm, D.F., et al., *Multidrug-resistant urinary tract isolates of Escherichia coli: prevalence and patient demographics in the United States in 2000*. *Antimicrobial agents and chemotherapy*, 2001. **45**(5): p. 1402-1406.
15. Andrew B, K.A., Bazira J. Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, Southwestern Uganda. , *Prevalence of extended-spectrum beta-lactamases-producing microorganisms in patients admitted at KRRH, Southwestern Uganda*. *Int J Microbiol.*, 2017.
16. Momtaz, H., E. Rahimi, and S.J.V.M. Moshkelani, *Molecular detection of antimicrobial resistance genes in E. coli isolated from slaughtered commercial chickens in Iran*. 2012. **57**(4): p. 193-197.
17. Wu, H., et al., *Characterization of antimicrobial resistance in Klebsiella species isolated from chicken broilers*. 2016. **232**: p. 95-102.
18. Hayati, M., et al., *Molecular detection of extended-spectrum β -lactamase-producing Klebsiella pneumoniae isolates of chicken origin from East Java, Indonesia*. 2019. **12**(4): p. 578.
19. MUSINGUZI, B., KABAJULIZI, I., MPEIRWE, M., TURUGURWA, J. & KABANDA, T. J. A. I. I. D., *Incidence and Etiology of Catheter Associated Urinary Tract Infection among Admitted Patients at Kabale Regional Referral Hospital, South Western Uganda*. . *Advances in Infectious Diseases*, 2019. **9**(3).

20. TIBYANGYE, J., OKECH, M. A., NYABAYO, J. M. & NAKAVUMA, J. L. J. B. M. R. J., *In vitro antibacterial activity of Ocimum suave essential oils against uropathogens isolated from patients in selected hospitals in Bushenyi district, Uganda*. Br Microbiol Res J, 2015. **8**(3): p. 489-498.
21. ODONGO, C.O., et al., *Antibiograms from community-acquired uropathogens in Gulu, northern Uganda-a cross-sectional study*. 2013. **13**, : p. 1-8.
22. TANDOGDU, Z., et al., *Urinary tract infections in immunocompromised patients with diabetes, chronic kidney disease, and kidney transplant*. European urology focus., 2016. **2**, .
23. KABUGO, D., KIZITO, S., DAVE, D. A., KIWANUKA, A. G., NABIMBA, R., NAMUNANA, S., K., R., ACHAN, B. & C.N., F. . . . , *Factors associated with community-acquired urinary tract infections among adults attending assessment centre, Mulago Hospital Uganda*. Afr Health Sci, 2016. **16**(): p. 1131–1142.
24. MARTIN, O., ADAMU, A. A., TIBYANGYE, J., NYABAYO, J., MANIGA, E., WAMPANDE, C., K., D., EZERA, A. & , B., J. . . . *Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda*. International Journal of Microbiology, 2019. **Volume 2019**.
25. BIRAN, A., SCHMIDT, W. P., VARADHARAJAN, K. S., RAJARAMAN, D., KUMAR, R., GREENLAND, K., GOPALAN, B., AUNGER, R. & CURTIS, V. . I. , *Effect of a behaviour-change intervention on handwashing with soap in India (SuperAmma): a cluster-randomised trial*. Lancet Glob Health, 2014. **2**, (45-54).
26. Agyepong, N., Govinden, U., Owusu-Ofori, A. et al. *Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana.*, *Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana*. Antimicrob Resist Infect Control 2018. **7**(37).
27. STALENHOF, J.E., VAN NIEUWKOOP, C., MENKEN, P. H., BERNARDS, S. T., ELZEVIER, H. W. & VAN DISSEL, J. T. J. T. J. O. U. . *Intravesical gentamicin treatment for recurrent urinary tract infections caused by multidrug resistant bacteria*. 2019. **201**(3): p. 549-555.
28. MANIKANDAN, S., GANESAPANDIAN, S., SINGH, M. & KUMARAGURU, A. J. C. R. B., *Emerging of multidrug resistance human pathogens from urinary tract infections*. 2011. **4** (1): p. 9-15.
29. RAMÍREZ-CASTILLO, F.Y., MORENO-FLORES, A. C., AVELAR-GONZÁLEZ, F. J., MÁRQUEZ-DÍAZ, F., HAREL, J., GUERRERO-BARRERA, A. L. J. A. O. C. M. & ANTIMICROBIALS . . , *An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study*. 2018. **17**(1): p. 34.
30. ASMA, R., ALAM, M. J., RAHIMGIR, M., ASADUZZAMAN, M., ISLAM, A. M., UDDIN, N., KHAN, M. S. I., SIDDIKA, S. S., DATTA, S. J. A. J. O. C. , *Prevalence of Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Uropathogens Isolated From Urinary Tract Infection Cases in Dhaka, Bangladesh*. M. & INFECTION, 2019. **6** (44-48).
31. BARAL, P., NEUPANE, S., MARASINI, B. P., GHIMIRE, K. R., LEKHAK, B. & SHRESTHA, B. J. B. R. N. . . , Nepal. , *High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu*. BMC Res Notes, 2012. **5**: p. 1-9.
32. ALADEL, R.H., ABDALSAMEEA, S. A., BADWY, H. M., REFAT, S. A. & ELKHOLY, R. M. J. M. M. J., *Role of AdeB gene in multidrug-resistance Acinetobacte*. Menoufia Med J 2020. **33**(205).
33. GOMILA, A., SHAW, E., CARRATALÀ, J., LEIBOVICI, L., TEBÉ, C., WIEGAND, I., VALLEJO-TORRES, L., VIGO, J. M., MORRIS, S., STODDART, M. J. A. R. & CONTROL, I. , *Predictive factors for multidrug-resistant gram-negative bacteria among hospitalised patients with complicated urinary tract infections*. Antimicrobial Resistance and Infection Control, 2018. **7**, (1-11).
34. Odongo I, Ssemambo R, and K. JM., *Prevalence of Escherichia Coli and its antimicrobial susceptibility profiles among patients with UTI at Mulago Hospital, Kampala, Uganda*. Interdiscip Perspect Infect Dis., 2020. **1;2020:8042540**. .

35. EL-WAFA, W.M.A.I., Y. M. J. C. M., *In vitro activity of fosfomycin in double and triple combinations with imipenem, ciprofloxacin and tobramycin against multidrug-resistant Escherichia coli*. *Curr Microbiol*, 2020. **77**::(5): p. 755-761.
36. BARAL, P., NEUPANE, S., MARASINI, B. P., GHIMIRE, K. R., LEKHAK, B. & SHRESTHA, B. J. B. R. N. , *High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal*. 2012. **5**, .(1-9).
37. Storberg, V., *ESBL-producing Enterobacteriaceae in Africa—a non-systematic literature review of research published 2008–2012*. *Infection ecology & epidemiology*, 2014. **4**(1): p. 20342.
38. Kaye, K.S., et al., *Antimicrobial Resistance Trends in Urine Escherichia coli Isolates From Adult and Adolescent Females in the United States From 2011 to 2019: Rising ESBL Strains and Impact on Patient Management*. *Clinical Infectious Diseases*, 2021. **73**(11): p. 1992-1999.
39. HEMMATI, M., et al., *Molecular Investigation of Extended-Spectrum β -Lactamase and Patterns of Antibiotic Resistance in Enterobacter cloacae Isolates from Teaching Hospitals in Kermanshah, Iran*. *Journal of Clinical & Diagnostic Research*, 2019. **13**(9).
40. MAGALE, H., KASSIM, I., ODERA, S., OMOLO, M., JAOKO, W. & JOLLY, P. , *Antibiotic susceptibility of organisms causing urinary tract infection in patients presenting at Kenyatta national hospital, Nairobi*. *East African medical journal*, 2015. **92**, (7): p. 333-337.
41. KASANGO, S.D., LUTOTI, S., WEWEDRU, I., ABOCE, E. & ANGOL, D. C. , *Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta Lactamase Producers in Gram-negative Urine Isolates at MBN Clinical Laboratories, Kampala Uganda*. *Archives of Microbiology & Immunology*, 2018. **2**, : p. 042-052.
42. KATEREGGA, J.N., KANTUME, R., ATUHAIRE, C., LUBOWA, M. N. & NDUKUI, J. G. , *Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda*. . *BMC pharmacology and Toxicology*, 2015. **16**(14).
43. Abubakar, M., et al., *Prevalence of extended spectrum beta-lactamase producing bacteria (ESBL) in patients presenting with urinary tract infections (UTIs) at a peri-Urban hospital, Uganda*. 2020.
44. ADLER, A., KATZ, D. E. & MARCHAIM, D. , *The continuing plague of extended-spectrum β -lactamase-producing Enterobacteriaceae infections*. *Infectious Disease Clinics*, 2016. **30**(347-375.).
45. Fatima, S., et al., *Phenotypic expression and prevalence of multi drug resistant extended spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae in Karachi, Pakistan*. *Pak J Pharm Sci*, 2018. **31**(4): p. 1379-84.
46. Sakina FATIMA , et al., *Prevalence of CTX-M Variants in ESBL Producing Multidrug-Resistant Enterobacteriaceae from Outpatients in Karachi, Pakistan*. *LATIN AMERICAN JOURNAL OF PHARMACY*, 2019. **38**(8): p. 1516-21.
47. JENA, J., SAHOO, R. K., DEBATA, N. K. & SUBUDHI, E. J. B. , *Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β -lactamase-producing Escherichia coli strains isolated from urinary tract infections in adults*. *3 Biotech*, 2017. **7**(4): p. 244.
48. OGEFERE, H.O., IRIAH, S. E. & IBADIN, E. E. J. U. M. , *Detection of SHV and TEM-type Extended spectrum β -lactamase in bacterial isolates recovered from clinical samples of patients attending military hospitals*. . 2019. **38**,(3).
49. SEYEDJAVADI, S.S., GOUDARZI, M. & SABZEHALI, F. J. J. O. A. D. . . *Relation between blaTEM, blaSHV and blaCTX-M genes and acute urinary tract infections*. . *Journal of Acute Disease* 2016. **5**,(1): p. 71-76.
50. AHMED, Y.M.A., MOHAMMED EL IMAM, M., KHALID, K. E., MOHAMED, B. A. & ELHAG, K. M. J. B. R. J. O. M. , *Phenotype and genotype of extendedspectrum-beta-lactamases in Sudanese patients with UTI*. 2014. **3** (1-5).

51. Hussein, A.A., et al., *Prevalence and Genotypic Characterization of Extended Spectrum Beta-Lactamase Uropathogens Isolated from Refugees with Urinary Tract Infections in Nakivale Refugee Settlement camp, Southwestern Uganda*. medRxiv, 2022: p. 2022.04. 29.22274464.

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