AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

68 (2021) 3, 169–176

DOI:
10.1556/030.2021.01380

ORIGINAL RESEARCH PAPER

Molecular characterization of ESBL- producing uropathogenic *Escherichia coli* recovered from urine samples of patients attending a University Teaching hospital in Nigeria

ABIMBOLA OLUMIDE ADEKANMBI[1*], SANDRA USIDAMEN[2**] and ANTHONY ABIODUN ONILUDE[3]

[1] Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria

[2] Pathogenic Microbiology Laboratory, Department of Microbiology, University of Ibadan, Nigeria

[3] Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan, Nigeria

Received: December 25, 2020 • Accepted: February 9, 2021
Published online: May 7, 2021

ABSTRACT

Infection of the urinary tract ranks as one of the most common infections affecting people worldwide and its treatment is made complicated by the rising incidence of antibiotic resistance. This study aimed to detect extended spectrum beta-lactamase (ESBL) genes and antibiotic resistance profile of uropathogenic *Escherichia coli* (*E. coli*) recovered from patients attending a University Teaching hospital in Nigeria. Uropathogenic *E. coli* isolates were obtained from the culture collection of Department of Microbiology and Parasitology of the University Teaching hospital for a period of four months (October 2019–January, 2020). Antibiotic susceptibility testing was done using the disc diffusion method while phenotypic ESBL production was detected using double disc synergy test (DDST). Detection of β-lactamase genes was done using Real-Time PCR. Forty-nine *E. coli* isolates were recovered from 120 urine samples, with 24 (49%) being ESBL positive. The resistance to antibiotics in the ESBL producers was: ciprofloxacin (100%), cefotaxime (100%), cefpodoxime (100%), tetracycline (95.7%), ceftazidime (56.7%), amoxicillin-clavulanate (50%), gentamicin (33.3%), and imipenem (0%). All the ESBL producers carried bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$, 75% (18/24) carried bla_{SHV}, while $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$ and $bla_{CTX\text{-}M\text{-}25}$ groups were detected in 20.8% (5/24) of the isolates. There was co-occurrence of CTX-M, SHV and TEM β-lactamases in 79.2% (19/24) isolates, while five isolates (20.8%) co-harbored $bla_{CTX\text{-}M}$ and bla_{TEM}. This study showed a high level of multidrug resistance and ESBL gene carriage in uropathogenic *E. coli* obtained in this study, suggesting a likely review of therapeutic options in the treatment of UTI to clamp down on the rising cases of antibiotic resistance.

KEYWORDS

Real-time PCR, Uropathogenic *Escherichia coli*, Extended spectrum β-lactamase (ESBL), University Teaching hospital, Antibiotic resistance signatures, bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}.

*Corresponding author.
E-mail: ao.adekanmbi@ui.edu.ng; bimboleen@yahoo.com

**Corresponding author.
E-mail: sandrausidamen@gmail.com

INTRODUCTION

One of the most common bacterial infections worldwide is urinary tract infection (UTI). It constitutes an emerging challenge among patients of different ages, as the infection is characterized by high mortality rate and expensive medical costs [1]. In several literatures, it has been reported that the female gender is more affected than males with about 20% of women experiencing at least one episode of the infection in their life time and the

reoccurrence of this scenario cannot be ruled out [2, 3]. This therefore, necessitates effective diagnosis and a properly guided use of antibiotics for prevention and treatment, so as to reduce the burden and long-term consequences associated with such infections [4]. There are diverse agents involved in the etiology of urinary tract infections, but the most commonly encountered are bacteria of the family *Enterobacteriaceae* of which *Escherichia coli* predominates [5]. *E. coli*, which is a normal inhabitant of the human intestine can acquire specific virulence attributes which confer on them the ability to adapt to new location and then cause a wide range of infections of which UTI is one [6].

Studies have shown that uropathogenic *E. coli* (UPEC) group has the ability to cause about 90% and 50% community and hospital-acquired UTIs respectively [7, 8]. Over the past twenty years, the rate of resistance to frontline antibiotics by UPEC strains is increasing. Several epidemiological studies during this period have reported about 20–45% resistance to these antibiotics by UPEC isolates [9, 10] and this is also in addition to the ability of UPEC to produce extended spectrum ß-lactamase (ESBL) [11]. ESBLs have the ability to inactivate beta-lactam antibiotics, by hydrolyzing the beta-lactam rings in beta-lactam antibiotics. However, they do not hydrolyze cephamycins and the carbapenem antibiotics, and their action is halted by β-lactamase inhibitors [12].

ESBLs consist of many plasmid-mediated genes such as TEM, OXA, and SHV [13]. In the year 2000, a new group of ESBL, called CTX-M emerged and ever since their emergence, they have been the most predominant ESBL type worldwide [14, 15]. This group of ESBLs has been implicated in the antibiotic resistance of many bacterial genera, including resistance to cephalosporins, penicillins and monobactams which are all ß-lactam agents [16, 17]. In a study carried out by Rogers et al. [18], it was reported that *E. coli* strains carrying the CTX-M β-lactamase are often associated with co-resistance to other antibiotic classes. The ever-increasing rate of resistance to antibiotics in UPEC is worrisome, as this has a lot of negative implications on the therapeutic choices for UTIs, highlighting the growing threat of pan-drug resistance posed by UPEC. In Nigeria, most studies have focused on the antibiotic susceptibility profile of uropathogens, with few focusing on the occurrence of genes encoding resistance to these antibiotics. This study however, aimed at screening a population of uropathogenic *E. coli* recovered from patients attending a tertiary hospital in South-west Nigeria for ESBL production, with a view to determining their carriage of ESBL genes.

MATERIALS AND METHODS

Study location and collection of uropathogenic *E. coli*

A Tertiary hospital, University College Hospital (UCH) in Ibadan, Oyo state was the location of this study. The hospital is located in the South-western part of Nigeria and is one of the largest and foremost Teaching hospitals on the African continent. Forty-nine *E. coli* isolates were recovered from the urine samples of one hundred and twenty out- and in- patients of the University College Hospital (UCH), who were diagnosed to have UTI. These isolates were collected from the culture pool of the Department of Medical Microbiology and Parasitology of the hospital for a period of four months (October, 2019–January, 2020). The biodata of the patients, with the exception of the name, were obtained and properly documented. The isolates which were initially identified using Microbact^{TM} Gram Negative System Identification kit (Thermo Scientific-Oxoid, UK) were transported to the laboratory where further studies were carried out. The uropathogens were cultured on MacConkey agar (Oxoid, UK) and thereafter stored on nutrient agar (Oxoid, UK) slants stored at 4^{o}C. Documented access was granted by the Department of Medical Microbiology and Parasitology, UCH for the collection of the uropathogenic isolates from the Urine bench of the Department.

Susceptibility to antibiotics and phenotypic detection of ESBL production

The disc diffusion technique [19], was employed in the determination of the susceptibility of the isolates to selected antibiotics. The panel of antibiotics used included: amoxicillin-clavulanate (30µg), ceftazidime (30µg), cefpodoxime (30µg), cefotaxime (30µg), imipenem (10µg), gentamycin (15µg), ciprofloxacin (5µg), and tetracycline (30µg) (Oxoid, UK), and these were selected according to CLSI [20]. The protocols of CLSI [20] were followed in the inoculum standardization, choice of medium, incubation conditions and interpretation of diameter of inhibition zones. The multiple antibiotic resistance index (MARI) and the resistance signatures of each isolate were determined from the data obtained. Any isolate observed to show resistance to three or more classes of antibiotics was referred to as multidrug resistant (MDR) [21]. Isolates showing reduced susceptibility or resistance to any of the tested 3rd generation cephalosporins was tested for ESBL production using the Double Disc Synergy Test [20].

DNA extraction

The DNA of ESBL- producing *E. coli* was extracted using the Zymo Research Corporation, USA Quick-gDNA Miniprep Kit. The procedures of the manufacturer including cell lysis, genomic binding, purification, elution and storage were followed.

Molecular identification of the ESBL- producing uropathogenic *E. coli*

Confirmed ESBL- producing uropathogenic *E. coli* were further characterized using molecular methods (detection of *uidA* gene). The gene encodes the production of β-D glucuronidase in strains of *E. coli*. The sequences of the

Table 1. Sequences of primers and probes used in detecting *uidA* and ESBL genes

Primer/Probe	Forward (5′ - 3′)	Reverse (5′ -3′)	Reference
uidA	5′-AAAACGGCAAGAAAAAGCAG-3	5′-ACGCGTGGTTACAGTCTTGCG-3′	[22]
TEM Primers	GCATCTTACGGATGGCATGA	GTCCTCCGATCGTTGTCAGAA	[26]
TEM Probe	6-Fam-CAGTGCTGCCATAACCATGAGTGA-BHQ-1		
SHV Primers	TCCCATGATGAGCACCTTTAAA	TCCTGCTGGCGATAGTGGAT	[26]
SHV Probe	Cy5-TGCCGGTGACGAACAGCTGGAG-3BBQ-650		
#CTX-A Primers	CGGGCRATGGCGCARAC	GCRCCGGTSGTATTGCC	[26]
CTX-A Probe	Yakima Yellow-CCARCGGGCGCAGYTGGTGAC-BHQ1		
#CTX-B Primers*	ACCGAGCCSACGCTCAA	CCGCTGCCGGTTTTATC	[26]
CTX-B Probe	Yakima Yellow-CCCGCGYGATACCACCACGC-BHQ1		

Note: #CTX-A was designed for the detection $bla_{\text{CTX-M-1 and}}$ $bla_{\text{CTX-M-9}}$; #CTX-B was designed for the detection of $bla_{\text{CTX-M-2 and}}$ $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-25}}$.

primers are given in Table 1. The PCR amplification conditions for the *uidA* gene were as described by Janezic et al. [22]. The amplified products were separated on agarose gel electrophoresis. The expected amplicon size of the gene was 147 bp. *E. coli* AG1b carrying the *uidA* gene and isolated from surface water as reported by Adekanmbi et al. [23] was used as the positive control, with nuclease-free water used in place of the DNA template serving as the negative control.

ESBL genotyping

The detection of ESBL genes in the ESBL positive *E. coli* was carried out using Real-Time PCR. The Real-Time PCR equipment used was Applied Biosystems, model 7500 Fast DX. The primer sequences and probes used are outlined in Table 1. The reaction mixture (25 µL) for the PCR amplification contained: PCR buffer (1 ×), $MgCl_2$ (5.0 mM), dNTPs (300nM), nuclease -free water, primers (forward and reverse) (200 nM), Internal Amplification Control (IAC) primers (forward and reverse) (75nM), platinum polymerase of *Thermus aquaticus* (1.50 U), IAC DNA (2 µL), DNA probes (150 nM) and DNA template (2 µL). The PCR conditions were as reported by CDC [24]. *Klebsiella pneumoniae* BAA-2146 (ATCC strain) harboring bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$ was used as the positive control [25]. Nuclease-free H_2O used in place of the DNA template was used as the negative control. All the consumables used in the amplification process were ordered from Roche, Indianapolis, USA, Integrated DNA Technologies, USA and Invitrogen, USA.

RESULTS

Biodata (age group and gender) of patients from which isolates were recovered

Table 2 is showing the age group and gender distribution of patients diagnosed with UTI and from which samples were obtained during the duration of the study. The age distribution falls within five groups, with the sixth group being unspecified at the time of documentation. Most of the patients fell within the 61–80 years group (fifty-two), while the least number of patients which was nine was obtained from the 0–20 years group. Of the total 120 patients that were sampled, 75 (62.5%) were males, while the remaining 45 (37.5%) were females. Age group 21-40 had the highest number of females (12), while age group 61-80 had the highest number of males with 42 patients.

Frequency of *E. coli* and ESBL producers recovered from the age groups

The frequency and percentage of ESBL producers recovered from the patients according to age group are shown in Table 2. Forty-nine *E. coli* isolates were recovered in the course of the study. All the isolates harbored *uidA*, thereby confirming their identity as *E. coli*. Of this number 24 (49%) were ESBL producers. Patients within the age group 61–80 years had the highest number of *E. coli* isolates (23) with ESBL producers being 11 (47.8%). Six isolates were recovered from age groups 21–40 and 41–60 with percentage

Table 2. Biodata and frequency of ESBL- producing *E. coli* in this study

Age distribution	Male	Female	Total (%)	Total *E. coli* recovered	Number and Percentage of ESBL- producing *E. coli* obtained
0–20	6	3	9 (7.5)	3	2 (66.7%)
21–40	7	12	19 (15.8)	6	2 (33.3%)
41–60	11	10	21 (17.5)	6	3 (50%)
61–80	42	10	52 (43.3)	23	11 (47.8%)
81–100	7	3	10 (8.3)	5	3 (60%)
Unspecified	2	7	9 (7.5)	6	3 (50%)
Total (%)	75 (62.5)	45 (37.5)	120 (100)	49	24 (49%)

ESBL production of 33.3% and 50% respectively. The highest percentage of ESBL- producing *E. coli* (66.7%) was recovered from patients within the age group of 0–20 years followed closely by those within the 81–100 age group (60%). Six isolates were obtained from patients within the unspecified age group with three of them (50%) being positive for ESBL production.

Susceptibility to antibiotics

Figure 1 is showing the susceptibility of the ESBL- producing *E. coli* to antibiotics. All the isolates showed resistance to three antibiotics namely ciprofloxacin, cefotaxime and cefpodoxime, with 95.8% resistance observed to tetracycline. The resistance to the other antibiotics was ceftazidime (66.7%), amoxicillin-clavulanate (50%) and gentamicin (33.3%). None of the isolates was resistant to imipenem.

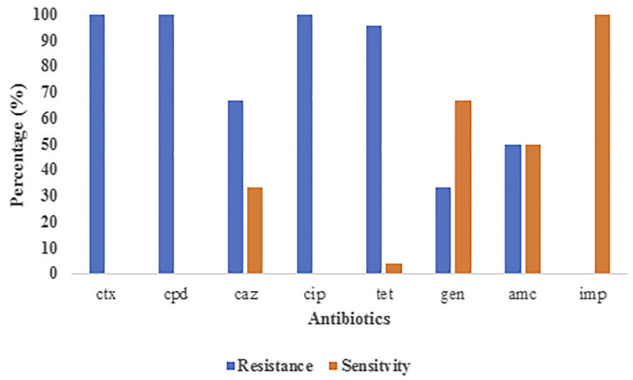

Fig. 1. Susceptibility of the ESBL- producing uropathogenic *E. coli* to selected antibiotics.
KEY: ctx: cefotaxime; cpd: cefpodoxime; caz: ceftazidime; cip: ciprofloxacin; tet: tetracycline; amc: amoxicillin-clavulanate; gen: gentamicin; imp: imipenem

Antibiotic resistance signatures and ESBL gene profile

Table 3 is showing the antibiotic resistance signatures and ESBL gene profile of each of the ESBL positive *E. coli*. All the isolates were resistant to more than two classes of antibiotics and are therefore multidrug resistant (MDR). The MARI for the isolates in this study ranged from 0.375 to 0.875. The ESBL genes profile shows that all the uropathogenic *E. coli* carried $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$ and bla_{TEM} genes, while only five isolates (20.8%) carried $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, and $bla_{\text{CTX-M-25}}$. The sulfhydryl reagent variable beta-lactamase (bla_{SHV}) was detected in 75% (18/24) of the isolates. Nineteen isolates (79.2%) carried a combination of $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}, while five isolates (20.8%) were detected to carry a combination of $bla_{\text{CTX-M}}$ and bla_{TEM}.

DISCUSSION

UTIs are one of the frequently encountered infections in hospitals [9]. They constitute a significant common clinical entity among patients in the outpatient section of most clinics and hospitals [2, 27]. The etiological agents of UTI are very diverse and the most implicated bacteria are gram

Table 3. Antibiotic resistance signatures and ESBL genes profile of the uropathogenic ESBL-producing *E. coli* obtained in this study

Isolate code	Antibiotic resistance signatures	MARI	ESBL genes profile
E. coli UP1	ctx, cpd, caz, cip, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1, -2, -8, -9 and -25)}}$, bla_{SHV}
E. coli UP2	amc, ctx, cip, cpd, gen, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP4	amc, ctx, cpd, caz, cip, gen, tet	0.875	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP7	ctx, cpd, cip, tet	0.500	bla_{TEM}, $bla_{\text{CTX-M (-1, -2, -8, -9 and -25)}}$, bla_{SHV}
E. coli UP12	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP19	ctx, cpd, cip, gen, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP32	ctx, cpd, cip	0.375	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP34	ctx, cpd, cip, tet	0.500	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$
E. coli UP37	ctx, cpd, caz, cip, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$
E. coli UP43	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP46	amc, ctx, cpd, caz, cip, gen, tet	0.875	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP60	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP66	ctx, cpd, cip, tet	0.500	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP67	ctx, cpd, caz, cip, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP75	ctx, cpd, caz, cip, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP79	ctx, cpd, cip, tet	0.500	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP82	ctx, cpd, caz, cip, gen, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1, -2, -8, -9 and -25)}}$, bla_{SHV}
E. coli UP88	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP90	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$, bla_{SHV}
E. coli UP95	ctx, cpd, cip, gen, tet	0.625	bla_{TEM}, $bla_{\text{CTX-M (-1, -2, -8, -9 and -25)}}$, bla_{SHV}
E. coli UP98	amc, ctx, cpd, caz, cip, gen, tet	0.875	bla_{TEM}, $bla_{\text{CTX-M (-1, -2, -8, -9 and -25)}}$, bla_{SHV}
E. coli UP99	amc, ctx, cpd, caz, cip, gen, tet	0.875	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$
E. coli UP104	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$
E. coli UP114	amc, ctx, cpd, caz, cip, tet	0.750	bla_{TEM}, $bla_{\text{CTX-M (-1 and -9)}}$

KEY: amc: amoxicillin-clavulanate; ctx: cefotaxime; cpd: cefpodoxime; caz: ceftazidime; cip: ciprofloxacin; gen: gentamycin; tet: tetracycline; -1, -2, -8, -9 and -25 represent the phylogenetic groups/variants of $bla_{\text{CTX-M}}$ detected.

negative bacteria belonging to the family Enterobacteriaceae of which *E. coli* is a major culprit [5]. The rapid emergence and evolution of multidrug resistance in *E. coli* coupled with the alarming rate of infections caused by the MDR and ESBL- producing strains of the organism have presented a serious challenge to the management of UTIs in patients. The organism accounts for about 74–90% of all uncomplicated cases of urinary tract infection worldwide [28, 29].

In this present study, forty-nine (49) *E. coli* isolates were obtained from the urine samples of 120 patients diagnosed with UTI and attending the University Teaching hospital over the four-month sampling period. Of the urine samples collected within the period, 75 (62.5%) were from male patients while the remaining 45 (37.5%) were obtained from the female patients. This observation is not in agreement with the study of Foxman [30], who reported a higher prevalence of UTI in females compared to their male counterpart, and also the reports of Kattel et al. [2] and Raza et al. [3], which agreed with the observation of Foxman [30]. However, it should be noted that several factors including the timing of the study could have contributed to the higher incidence of UTI cases in males than females in this present study.

In accordance with the report of Hu [31], who reported that UTI occurs in all age groups, the same trend was evident in this study as the six age groups to which the patients were grouped into had cases of UTIs. The highest incidence of UTI in this study was however observed in the elderly population within age groups 61–80 and 81–100 years with 62 (51.67%) patients; which is in line with the study of Cove and Almond [32] who reported that UTI cases are more predominant in the elderly population as against the younger generation. In contrast to this, Das et al. [29] reported a high occurrence of UTI patients (61.1%) in the 21–40 age group. However, it should be stressed that the patients in their study were all females.

In many countries of the world, the prevalence of ESBL-producing *E. coli* differs as evident in previous studies. In a two-year surveillance study carried out in Europe from 2001 to 2002, the percentage production of ESBL in *E. coli* was found to be around 5.4%, whereas it could be as low as 1.5% in countries like Denmark. The incidence of ESBL- producing *E. coli* can also be as high as 36.7% (Turkey) and 69% (India). The cases could be much higher in Africa, though accurate and reliable data are not available [33–37].

In our study, the percentage occurrence of ESBL production in uropathogenic *E. coli* was 49% and this is comparatively higher than the percentage occurrence of 33% in uropathogenic *E. coli* reported by Alqasim et al. [38] and also 40.3% reported by Pandit et al. [39]. Furthermore, Bakshi et al. [40] reported a 50% occurrence of ESBL producers in *E. coli* recovered from patients attending a Tertiary hospital in Rajindra in India, while Zorgani et al. [41], when carrying out a study on five Teaching hospitals in Libya reported a range of 4.2–67.6% ESBL production in *E. coli* isolated in their study. In their respective studies on uropathogens from hospitals in India and West of Iran, Das et al. [29] and Mohajeri et al. [42] reported a percentage occurrence of 36.11% and 24.5% respectively in the isolates obtained, while in Nigeria, Nwafia et al. [43] reported a percentage ESBL production of 35% in isolates recovered from the University of Nigeria, Nsukka Teaching hospital. Several factors such as self-medication and availability of 'over the counter drugs' and strain differences could be responsible for the observed discrepancies across countries. Furthermore, of all the age groups to which the patients were classified in this study, the highest percentage ESBL production was in the 0–20 age group (66.7%) while the least was observed in the 21–40 age group (33.3%).

The high occurrence of multidrug resistant bacteria in this study is similar to what was obtained by Das et al. [29] in their study on uropathogenic *E. coli* isolated from females in North India where they reported 100% multidrug resistance in the isolates obtained. Several authors have also reported the occurrence of MDR in uropathogens from several countries and hospitals. Notable among them is Zorgani et al. [41] and Bakshi et al. [40], who reported 22.2% and 28% MDR in uropathogenic *E. coli* from their respective studies.

In this study, there was 100% resistance to cefotaxime, cefpodoxime and ciprofloxacin and this agrees with the reports of Zorgani et al. [41] and Bakshi et al. [40], who observed a similar trend in their respective studies. Nwafia et al. [43] also reported a 100% resistance to tetracycline in ESBL- producing uropathogenic *E. coli* from a hospital in Nigeria, and this is slightly higher than the 95.8% resistance to tetracycline observed in this study. There was a high level of resistance to other antibiotic classes in this study and this agrees with the study of Gundoḡdu et al. [44], who reported a similar trend in *E. coli* recovered from the hospital settings. Apart from showing a high level of resistance to antibiotics with the β-lactam rings, ESBL- producing strains have also shown an elevated level of cross resistance to other classes of antibiotics owing to several resistance mechanisms as reported by Poirrel et al. [45].

In contrast to the reports of Adekanmbi et al. [46], where the resistance to ertapenem, (a carbapenem) was high in ESBL- producing *E. coli* obtained in their study, there was no resistance to imipenem, another carbapenem, by any of the ESBL producers in this present study. The observation in this study is also in concordance with the work of Alqasim et al. [38] and Pandit et al. [39], where imipenem was the most sensitive drug to the ESBL producers in their respective studies. The widest spectrum of the carbapenems and their stability to AmpC β-lactamases and other ESBLs could be responsible for this [47, 48].

CTX-M-Type β-lactamase (cefotaximases), with several variants or phylogenetic groups, are plasmid-mediated ESBL, with powerful activity against ceftriaxone and cefotaxime. The ecological migration of these genes has been a factor in the ESBL epidemiology, as these beta-lactamases are currently the most prevalent among the Enterobacteriaceae family [26]. Though they do not provide resistance against ceftazidime, some variants of the gene with enhanced ceftazidimase have been detected, and this has a lot of implication on the laboratory detection. In this

study all the isolates recovered harbored at least one of the five phylogenetic groups of CTX-M β-lactamase detected. This does not agree with other studies with less than 100% detection of the gene. Alqasim et al. [38], Nwafia et al. [43], Halaji et al. [49], and Pandit et al. [39], all reported a prevalence of 93.5%, 37.1%, 53.2% and 66.1% respectively in their studies. However, the trend in this study is in concordance with the study of Hussain et al. [50], where all the isolates in their study carried the CTX-M β-lactamase. Of the twenty-four isolates obtained in this study, 20.8% (5/24) carried $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, and $bla_{\text{CTX-M-9,}}$ while all of them carried $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ phylogenetic groups.

The TEM β-lactamases cause the hydrolysis of penicillins and some narrow spectrum cephalosporins, and are one of the predominant ESBLs in most ESBL- producing bacteria. In this study, all the uropathogenic *E. coli* harbored the gene, and this is contrary to the frequency of detection of the gene in other related studies on uropathogenic *E. coli*. The frequency of the gene as reported by Pandit et al. [39] in their study was 83.8%, with Alqasim et al. [38] reporting 12.1%, while Hussain et al. [50] in their study on multiresistant *E. coli* from a UTI endemic region in India reported a 50% (8/16) detection of the gene in the isolates obtained. In Nigeria, Nwafia et al. [43], in their study on ESBL- producing *E. coli* from a tertiary hospital in Enugu, reported the detection of bla_{TEM} in 66 (94%) of the total 70 isolates studied. However, it should be stressed that the sources of the samples used in their study, though clinical were not well specified.

In this study, bla_{SHV} (sulfhydryl reagent variable), which initially was detected in some pathogenic members of the Enterobacteriaceae in the 1970s according to Pouget et al. [51] and Maravic et al. [52], was detected in 75% (18) of the twenty-four uropathogenic *E. coli* obtained in this study. Several other studies have reported the detection of the gene in uropathogenic *E. coli* from different countries. In a study carried out in a Teaching hospital in Nepal, Pandit et al. [39] reported the detection of bla_{SHV} in 4.8% (3) of the entire 62 isolates obtained in their study, which is lower than what was obtained in this present study. The gene was not detected in all the thirty-three uropathogenic *E. coli* obtained by Alqasim et al. [38] in a study carried out in Saudi Arabia. This is in addition to the low occurrence of the gene (2.9%) in a study on ESBL- producing *E. coli* reported by Nwafia et al. [43]. Despite the ecological migration of bla_{SHV} from the hospital settings to several other epidemiological compartments e.g. human, animal and environment, its ability to hydrolyze the beta-lactam moiety in monobactams and carbapenems, and the change of status from narrow to broad spectrum beta-lactamase, its frequency in most studies is still very low compared to bla_{TEM} and $bla_{\text{CTX-M}}$ [53-55]. This trend was very evident in this study, as the frequency of bla_{SHV} (75%), was lower than that of bla_{TEM} (100%) and $bla_{\text{CTX-M}}$ (100%).

The limitations of this study are that the isolates obtained were from one healthcare facility and also the low number of isolates employed, which might not give an accurate and significant representation of the status of ESBL- producing bacteria in the region where this study was carried out and other regions in Nigeria. These limitations can be a motivation for other studies in this regard.

CONCLUSION

This study shows a high occurrence of ESBL genes in the ESBL- producing *E. coli* recovered in the urine samples of UTI patients attending the University Teaching hospital during the duration of this study. Of the ESBL genes detected, bla_{TEM} and $bla_{\text{CTX-M}}$ predominate and multidrug resistance was observed in all the ESBL producers. This calls for a reappraisal of existing therapeutic guidelines for uropathogens, with a view to ensuring an effective treatment of UTI cases.

Conflict of interest: None declared.

ACKNOWLEDGMENTS

The authors would like to appreciate the Head of Department, Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Nigeria, for granting us access to their culture collection.

REFERENCES

[1] Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta- lactamase (ESBL)- producing *E. coli* compared to non-ESBL- producing *E. coli*. J Infect 2007; 55: 254–9.

[2] Kattel HP, Acharya J, Mishra SK, Rijal BP, Pokhrel BM. Bacteriology of urinary tract infection among patients attending Tribhuvan University teaching hospital Kathmandu, Nepal. J Nepal Assoc Med Lab Sci 2008; 9: 25–9.

[3] Raza S, Pandey S, Bhatt CP. Microbiological analysis of isolates in Kathmandu medical college teaching hospital, Kathmandu, Nepal. Kathmandu Univ Med J (Kumj) 2012; 9: 295–7.

[4] Hicklinsg DR, Nitti VW. Management of recurrent urinary tract infections in healthy adult women. Rev Urol 2013; 15: 41–8.

[5] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015; 13: 269–84.

[6] Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. Nat Rev Microbiol 2004; 2: 123–40.

[7] Zhang L, Foxman B. Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. Front Biosci 2003; 8: 235–44.

[8] Kucheria R, Dasgupta P, Sacks S, Khan M, Sheerin N. Urinary tract infections: new insights into a common problem. Postgrad Med J 2005; 81: 83–6.

[9] Foxman B. The epidemiology of the urinary tract infection. Nat Rev Urol 2010; 7: 653–60.

[10] Croxall G, Weston V, Joseph S Manning G, Cheetham P, Mgenally A. Increased human pathogenic potential of *Escherichia coli* from

polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. J Med Microbiol 2011; 60: 102–9.

[11] Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum ß-lactamases (ESBLs) in the community. J Antimicrob Chemother 2005; 56: 52–9.

[12] Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L. Broad spectrum beta-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiol Rev 2010; 34: 295–316.

[13] Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V. Intercontinental emergence of *Escherichia coli* clone O25: H4-ST131 producing CTX-M-15. J Antimicrob Chemother 2008; 61: 273–81.

[14] Cantón R, Coque TM. CTX-M ß-lactamase pandemic. Curr Opin Microbiol 2006; 9: 466–75.

[15] Peirano G, Pitout JDD. Molecular epidemiology of *Escherichia coli* producing CTX-M [beta]-lactamases: the worldwide emergence of clone ST131 O25: H4. Int J Antimicrob Agents 2010; 3: 316–21.

[16] Accogli M, Giani T, Monaco M. Emergence of *Escherichia coli* ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy. J Antimicrob Chemother 2014; 69: 2293–6.

[17] Cai JC, Zhang R, Hu YY, Zhou HW, Chen GX. Emergence of *Escherichia coli* sequence type 131 isolates producing KPC-2 carbapenemase in China. Antimicrob Agents Chemother 2014; 58: 1146–52.

[18] Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community associated strain. J Antimicrob Chemother 2011; 66: 1–14.

[19] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45, 4, 493–6.

[20] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing 27th CLSI supplement M100 (Wayne: Clinical and Laboratory Standards Institute) 2018.

[21] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18: 268–81.

[22] Janezic JK, Ferry B, Hendricks EW, Janiga BA, Johnson T, Murphy S, et al. Phenotypic and genotypic characterization of *Escherichia coli* isolated from untreated surface waters. Open Microbiol J 2013; 7: 9–19.

[23] Adekanmbi AO, Adeleke OJ, Aremu OO, Olaposi AV. Molecular characterization, antibiogram and distribution of *zntA* gene in zinc-resistant *Escherichia coli* population recovered from anthropogenically-influenced surface water sources in Nigeria. Meta Gene 2020a; 26 (2020), 100789.

[24] Centre for Disease Control and Prevention (CDC). Multiplex real-time PCR detection of *Klebsiella pneumoniae* Carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1) 2011.

[25] Leski T, Vora GJ, Taitt CR. Multidrug resistance determinants from NDM-1-producing *Klebsiella pneumoniae* in the USA. Int J Antimicrob Agents 2012; 40: 282–4.

[26] Roschanski N, Fischer J, Guerra B., Roesler U. Development of a real-time PCR for the rapid detection of the predominant Beta-Lactamase genes CTX-M, TEM-and CIT-type AMPCs in Enterobacteriaceae. Plos One 2014; 9(7): e100956. https://doi.org/10.1371/journal. pone.0100956.

[27] Yadav KK, Adhikari N, Khadka R, Pant AD, Shah B. Multidrug resistant Enterobacteriaceae and extended-spectrum beta-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. Antimicrob Resist Infect Control 2015; 4: 1, 42.

[28] Paterson D. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Clin Microbiol Infect 2000; 6: 9, 460–3.

[29] Das B, Mittal N, Goswami R, Adhana D, Rathore N. Prevalence of multidrug resistance (MDR) and extended spectrum beta-lactamases (ESBLs) among uropathogenic *Escherichia coli* isolates from female patients in a tertiary care hospital in North. India 2018; 7(12): 5031–6.

[30] Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. The Am J Med 2002; 113: 1, 5–13.

[31] Hu X, Shi YN, Zhang P, Miao M, Zhang T, Jiang B. d-Mannose: properties, production, and applications: an overview. Comprehen Rev Food Sci Food Saf 2016; 15: 773–85.

[32] Cove SA, Almond M. Management of urinary tract infections in the elderly. Trends Urology, Gynaecol Sex Health 2007; 12: 4, 31–4.

[33] Bouchillon S, Johnson B, Hoban D, et al. Determining incidence of extended-spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int J Antimicrob Agents 2004; 24: 2, 119–24.

[34] Celik AD, Yulugkural Z, Kuloglu F, et al. CTX-M type extended-spectrum β-lactamases in *Escherichia coli* isolates from community acquired upper urinary tract infections at a university in the European part of Turkey. J Microbiol Immunol Infect 2010; 43, 2, 163–7.

[35] Simner PJ, Zhanel GG, Pitout J, et al. Prevalence and characterization of extended-spectrum β-lactamase–and AmpC β-lactamase– producing *Escherichia coli*: results of the CANWARD 2007–2009 study. Diagn Microbiol Infect Dis 2011; 69, 3, 326–34.

[36] Hansen DS, Schumacher H, Hansen F, et al. Extended-spectrum β-lactamase (ESBL) in Danish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence, β-lactamase distribution, phylogroups, and co-resistance. Scand J Infect Dis 2012; 44, 3, 174–81.

[37] Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R, Lundborg CS. High prevalence of extended-spectrum β-lactamase-producing pathogens: results of a surveillance study in two hospitals in Ujjain, India. Infect Drug Resist 2012; 5: 65–73.

[38] Alqasim A, Abu Jaffal A, Alyousef AA. Prevalence of multidrug resistance and extended-spectrum β-lactamase carriage of clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia. Int J Microbiol 2018; 1–9.

[39] Pandit R, Awal B, Shrestha SS, Joshi G, Rijal B, Parajuli NP. Extended-spectrum β-lactamase (ESBL) genotypes among multidrug-resistant uropathogenic *Escherichia coli* clinical isolates from a teaching hospital of Nepal. Interdiscip Perspect Infect Dis 2020; 8. https://doi.org/10.1155/2020/6525826.

[40] Bakshi R, Kumar V, Kansal P, Kaur S. Detection of extended-spectrum beta lactamases and AmpC beta lactamases producing uropathogenic *Escherichia coli* in a tertiary care hospital 2019.

[41] Zorgani A, Almagatef A, Sufya N, Bashein A, Tubbal A. Detection of CTX-M-15 among uropathogenic *Escherichia coli* isolated from five major hospitals in Tripoli, Libya. OMJ 2017; 32: 322.

[42] Mohajeri P., Darfarin G., Farahani A. Genotyping of ESBL producing uropathogenic *Escherichia coli* in West of Iran. Int J Microbiol 2014; 6. https://doi.org/10.1155/2014/276941.

[43] Nwafia IN, Ohanu ME, Ebede SO, et al Molecular detection and antibiotic resistance pattern of extended-spectrum beta-lactamase producing *Escherichia coli* in a Tertiary Hospital in Enugu, Nigeria. Ann Clin Microbiol Antimicrob 2019; 18: 41. https://doi.org/10.1186/s12941-019-0342-9.

[44] Gündoğdu A, Jennison AV, Smith HW, Stratton H, Katouli M. Extended-spectrum β-lactamase producing *Escherichia coli* in hospital wastewaters and sewage treatment plants in Queensland, Australia. Can J Microbiol 2013; 59: 737–45.

[45] Poirrel L, Leviandier C, Nordmann P. Prevalence and genetic analysis of plasmid-mediated quinolone resistance determinants QnrA and QnrS in Enterobacteriaceae isolates from a French University Hospital. Antimicrob Agents Chemother 2006; 50: 3992–7.

[46] Adekanmbi AO, Akinpelu MO, Olaposi AV, Oyelade AA. Diversity of extended-spectrum beta-lactamase (ESBL) genes in *Escherichia coli* isolated from wastewater generated by a sick bay located in a university health care facility. Gene Rep 2020b; 20(2020): 100738.

[47] Edwards JR, Betts MJ. Carbapenems: the pinnacle of the β-lactam antibiotics or room for improvement? J Antimicrob Chemother 2000; 45: 1–4.

[48] Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, et al. Comparative review of the carbapenems, Drugs 2007; 67: 1027–52.

[49] Halaji M, Shahidi S, Atapour A, Ataei B, Feizi A, Havaei SA. Characterization of extended-spectrum β-lactamase-producing uropathogenic *Escherichia coli* among Iranian kidney transplant patients. Infect Drug Resist 2020; 13: 1429–37.

[50] Hussain A, Ranjan A, Nandanwar N, Babbar A, Jadhav S, Ahmed N. Genotypic and phenotypic profiles of Escherichia coli isolates belonging to clinical sequence type 131 (ST131), clinical non-ST131, and fecal non-ST131 lineages from India. Antimicrob Agents Chemother 2014; 58: 7240–9.

[51] Pouget JG, Coutinho FJ, Reid-Smith RJ, Boerlin P. Characterization of blaSHV genes on plasmids from *Escherichia coli* and *Salmonella enterica* isolates from Canadian food animals (2006-2007). Appl Environ Microbiol 2013; 79: 3864–6.

[52] Maravic A, Skocibusic M, Cvjetan S, Samanic I, Fredotovic Z, Puizina J. Prevalence and diversity of extended-spectrum-beta-lactamase-producing Enterobacteriaceae from marine beach waters. Mar Pollut Bull. 2015; 90: 60–7.

[53] Sana T, Rami K, Racha B, Fouad D, Marcel A, Hassan M. Detection of genes TEM, OXA, SHV and CTX-M in 73 clinical isolates of Escherichia coli producers of extended-spectrum beta-lactamases and determination of their susceptibility to antibiotics. Int Arab J Antimicrob Agents 2011; 10: 3823.

[54] Liakopoulos A, Mevius DJ, Olsen B, Bonnedahl J. The colistin resistance mcr-1 gene is going wild. J Antimicrob Chemother 2016; 71(8): 2335–6.

[55] Gundran RS, Cardenio PA, Villanueva MA, Sison FB, Benigno CC, Kreausukon K. Prevalence and distribution of $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM} genes in extended-spectrum [beta] - lactamase-producing E. coli isolates from broiler farms in the Philippines. BMC Vet Res 2019; 15: 1.