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Original Article

Distribution of ESBLs among *Escherichia coli* isolates from outpatients with recurrent UTIs and their antimicrobial resistance

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Abstract

Introduction: Extended-spectrum β-lactamase (ESBL)-producing strains of Enterobacteriaceae are considered to be emerging pathogens. They are a major problem in hospitalized as well as community-based patients. Major outbreaks involving ESBL strains have been reported from all over the world. Recurrent urinary tract infection (UTI) is one of the risk factors for infection with ESBL-producing *E. coli* in hospitalized and non-hospitalized patients.

Methodology: Ninety-one *E. coli* isolates from outpatients with recurrent UTIs were surveyed phenotypically and genotypically for ESBL production and tested for antimicrobial susceptibility.

Results: Of 91 *E. coli* isolates, 75.8% were resistant to each of cefotaxime and ceftazidime and 74.7% produced ESBLs. CTX-M-type was the most frequent ESBL (accounting for 70.3%), with CTX-M-1 being the only subtype possessed by these isolates. The prevalence of OXA- and SHV-type was 32.9% and 10.9%, respectively. None of the isolates produced TEM β-lactamase. All OXA-type ESBL were produced concomitantly with CTX-M1. Both ESBL producers and non-producers had high resistance to ampicillin followed by trimethoprim-sulphamethoxazole, third-generation cephalosporins, and tetracycline. No isolate showed resistance to imipenem and meropenem. In total, resistance rates of ESBL producers were higher than those of ESBL non-producers, as was multidrug resistance (52.7% versus 8.7%, respectively).

Conclusions: Our study documented high distribution of ESBLs among *E. coli* isolates from outpatients with recurrent UTIs, with CTX-M as the predominant ESBL. In the current situation, it is important that antibiotic treatment is be started only after a proper sensitivity report is obtained from the laboratory.

Key words: *E. coli*; recurrent UTI; ESBL; antimicrobial susceptibility.

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Introduction

Recurrent urinary tract infections (UTIs) are symptomatic UTIs that follow resolution of an earlier episode, usually after appropriate treatment [1,2]. In the general population, 50%–80% of women have acute uncomplicated cystitis. Of these patients, 30%–44% will have a recurrence, often within three months. In all patient groups, *Escherichia coli* (*E. coli*) is the most common organism, causing 68%–77% of recurrences [2]. Treatment of an initial recurrence of UTI is the same as for other cases of uncomplicated cystitis. The drug of choice is trimethoprim/sulfamethoxazole [1,2]. Fluoroquinolones and nitrofurantoin become better options as suspicion for trimethoprim/sulfamethoxazole resistance increases [1,2]. A fluoroquinolone, oral amoxicillin/clavulanate, or an oral third-generation cephalosporin such as cefpodoxime, cefdinir, or ceftibuten can be useful in treating recurrent infections in outpatients [3]. Ampicillin should no longer be used for treatment of UTIs, since at least 30% of causative *E. coli* strains are ampicillin resistant [4]. Similarly increasing rates of trimethoprim-sufamethoxazole resistance have been reported among urinary *E. coli* isolates [3,4]. In addition, the emergence of *E. coli* with β-lactamase overproduction has decreased the usefulness of amoxicillin plus clavulanic acid combinations for the treatment of UTIs [4].

The main mechanism of bacterial resistance to the β-lactam class of antibiotics consists of the production of β-lactamases [5,6]. Emergence of the extended spectrum β-lactamases (ESBLs) results from mutations in β-lactamases, the major cause of which is believed to be the widespread use of third-generation cephalosporins and aztreonam. These enzymes mediate resistance to broad-spectrum cephalosporins and to monobactams, but have no detectable activity against cephamycins and imipenem. Because of the complexity

of the β-lactamase classifications and in order to be accessible to clinicians, infection control professionals, hospital management, and politicians, Giske *et al*. [7] introduced a revised comprehensible nomenclature scheme. The proposed new classification expands the definition of ESBL to other clinically important acquired β-lactamases with activity against extended-spectrum cephalosporins and/or carbapenems. Accordingly, the classical class-A ESBLs (which includes TEM, SHV, and CTX-M) have been designated $ESBL_A$ in this classification, whereas plasmid-mediated AmpC and OXA-ESBLs are classed as miscellaneous ESBLs ($ESBL_M$). Lastly, the carbapenemases, ESBLs with hydrolytic activity against carbapenems, have been designated $ESBL_{CARBA}$. Furthermore, it was increasingly reported that ESBL-producing clinical isolates are multidrug resistant (MDR), defined as concomitantly resistant to at least three different antibiotic classes in addition to their resistance to β-lactams [8]. ESBL-producing strains of Enterobacteriaceae are considered to be emerging pathogens, as they are a major problem in hospitalized as well as community-based patients. Major outbreaks involving ESBL strains have been reported from all over the world [5,8].

It has been shown that recurrent UTI is one of the risk factors for ESBL-producing *E. coli* (ESBL-EC) infections in hospitalized [9] and non-hospitalized patients [10,11]. This study was conducted to evaluate the distribution of ESBLs among *E. coli* isolates from Iraqi outpatients with recurrent UTIs living in Al-Kut, Wasit province, Iraq. Furthermore, we also aimed to determine the susceptibility patterns of these *E. coli* isolates in our setting and compare this with international data.

Methodology

Patients

This study included outpatients attending Al-Karama Educational Hospital in Al-Kut city, Wasit province, Iraq, clinically diagnosed as having recurrent UTI (diagnosed by three positive urine cultures within 12 months). Limited information was available concerning patients' previous treatment with antibiotics, previous hospitalization, or risk factors for UTIs. Patients' ages ranged from 3 months to 84 years (average mean age, 42.1 years). Sixty-nine percent of isolates were derived from female patients, and 30.7% were from male patients.

No formal ethical approval was obtained to use the clinical samples, because they were collected during routine bacteriologic analyses in a public hospital, and the data were anonymously analyzed. This work was approved by the Wasit Health Administration of Wasit province, Iraq. All bacterial isolates in this study were collected and analyzed anonymously; therefore, consent from the patients was not required.

Bacterial isolates

A total of 91 non-duplicate *E. coli* isolates from outpatients with symptomatic recurrent UTIs were included in the current study. The isolates were obtained from the laboratory of Bacteriology at Al-Karama Educational Hospital in Al-Kut, Wasit province, Iraq, during the period from 1 October 2013 to 1 April 2014. Before the experiments were performed, the isolates were re-identified biochemically as *E. coli* in the Microbiology Laboratory at the Department of Biology/College of Science, Wasit University [12].

Susceptibility testing

Disk-diffusion tests were carried out with antibiotic-containing disks (Bioanalyse, Ankara, Turkey) on Mueller-Hinton agar plates (Himedia Lab, Mumbai, India). The results were expressed as susceptible or resistant according to the criteria recommended by the Clinical Laboratory Standards Institute (CLSI) [13]. The following antimicrobial agents were tested: β-lactams (ampicillin, 10 μg; amoxicillin-clavulanic acid, 20/10 μg; cefoxitin, 30 μg; cefotaxime, 30 μg; ceftazidime, 30 μg; ceftriaxone, 30 μg; cefepime, 30 μg; aztreonam, 30 μg; imipenem, 10 μg; and meropenem, 10 μg), sulfonamides (trimethoprim-sulfamethoxazole, 1.25/23.75 μg and nitrofurantoin, 300 μg), quinolones (ciprofloxacin, 5 μg and nalidixic acid, 30 μg), aminoglycosides (gentamicin, 10 μg and amikacin, 30 μg), and tetracyclines (tetracycline 30 μg).

Phenotypic screening for ESBL

Screening of reduced susceptibility to third-generation cephalosporins was carried out using CTX, CAZ, CRO, and ATM disks. The double-disk synergy test (DDST) method was used to confirm the presence of ESBLs as recommended by CLSI [13].

Polymerase chain reaction (PCR) amplification for detection of β-lactamase genes

All isolates were screened for the resistance genes bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, and bla_{OXA} by a multiplex PCR assay using universal primers [14-16]. Before performing the PCR test for the isolates in this study, the activity of primers was checked using known

positive isolates used in a previous study [17]. Then, each isolate was subcultured on trypticase soy agar plates for 24 hours at 37°C. From the agar plate, five colonies were picked and suspended in 100 µL of sterile distilled water. Bacterial suspensions were run for 10 minutes at 94°C [18] in a DNA thermocycler (MultiGene, Labnet International, Inc., Edison, USA), and cell debris were removed by centrifugation (12,000 rpm for 1 minute). PCR amplification reactions were performed in a volume of 50 µL containing 25 µL of KapaTaq 2x Ready Mix (KAPA Biosystems, Wilmington, USA), 25 pmol concentrations of each primer, and 5 µL of DNA template. The cycling parameters were as follows: an initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 45°C for 1 minute, and 72°C for 1 minute, and with a final extension at 72°C for 10 minutes. The amplified PCR products were subjected to electrophoresis at a 1.5% agarose gel in 0.5X TBE buffer.

AmpC was detected by PCR in isolates that were ESBL producers by screening and PCR tests but were negative by confirmatory test and were resistant to cefoxitin. The cycling parameters were as follows: an initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes [14].

Multiplex PCR for CTX-M phylogrouping

CTX-M-positive isolates were further analyzed for CTX-M phylogroups (CTX-M-1, -2, -8, -9, and -25/26) by multiplex PCR [19]. Amplification conditions were as follows: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 25 seconds, 52°C for 40 seconds and 72°C for 50 seconds, and a final extension at 72°C for 6 minutes.

Results

Distribution of ESBLs among E. coli *isolates*

A total of 91 *E. coli* isolates from outpatients with recurrent UTIs were surveyed phenotypically and genotypically for ESBL production. Detection of resistance to third-generation cephalosporins and monobactams revealed that 75.8% of these isolates were resistant to each of cefotaxime and ceftazidime, and 74.7% were resistant to each of ceftriaxone and aztreonam. Phenotypically, 80.2% and 64.8% of the isolates were ESBL producers by screen and confirmatory tests, respectively (Table 1).

Genotypically, 74.7% of *E. coli* isolates possessed ESBL genes (Table 2). All isolates (n = 68) that were positive for ESBL genes by PCR technique were confirmed phenotypically as ESBL producers except for nine isolates. AmpC was found in all of these nine isolates which were also resistant to cefoxitin. Of these isolates, seven (77.7%) had CTX-M- in combination with OXA-type ESBL and two (22.2%) had CTX-M-type as a single.

Table 1. Phenotypic detection of ESBL production by *E. coli* isolates from outpatients with recurrent UTIs.

Characteristics		Positive *E. coli* isolates (n = 91)	
		Number	%
Resistance to	CTX	69	75.8
	CAZ	69: R = 57; I = 12	75.8
	CRO	68	74.7
	ATM	68	74.7
Screen test		73	80.2
Confirmatory test (DDST)		59	64.8

ESBL: extended spectrum β-lactamase; CTX: cefotaxime; CAZ: ceftazidime; CRO: ceftriaxone; ATM: aztreonam; R: resistant; I: intermediate resistant; DDST: double-disk synergy test.

Table 2. Breakup of 68 *bla* genotypes of ESBL-producing *E. coli* isolates from outpatients with recurrent UTIs.

bla genotype	Positive *E. coli* isolates (n = 91)	
	Number	%
bla_{TEM}	0	0
bla_{SHV}	4	4.3
bla_{SHV} + CTX- M-1	6	6.5
bla_{OXA}+ CTX-M1	30	32.9
$bla_{CTX\text{-}M\text{-}1}$	28	30.7
Total	68	74.7

ESBL: extended spectrum β-lactamase; total $bla_{CTX\text{-}M1}$: 64 (70.3%); total bla_{SHV}: 10 (10.9%).

CTX-M was the most prevalent ESBL type (70.3%), with CTX-M-1 being the only subtype possessed by these isolates, followed by OXA- (32.9%) and SHV-type (10.9%) isolates. TEM-type was not found in any isolate (Table 2). All OXA-type ESBLs detected in this study were produced concomitantly with CTX-M-1 (32.9% of the isolates).

OXA-type ESBLs, in addition to being resistant to third- and fourth-generation cephalosporins and monobactams (100% resistant to cefotaxime, ceftriaxone, ceftazidime, aztreonam and 96.6% to cefipeme), all were also resistant to amoxicillin-clavulanic acid, and 63.3%, 76.6%, 83.3%, 86.6%, and 93.3% were resistant to gentamicin, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, and tetracycline, respectively. They remained susceptible to cefoxitin (63.3%), nitrofurantoin (76.6%), amikacin (93.3%), and carbapenems (100%). Furthermore, 93.3% of them were MDR.

Antimicrobial susceptibility of E. coli *isolates*

In both ESBL producers and non-producers, the highest rate of resistance was to ampicillin (94.5%) followed by trimethoprim-sulphamethoxazole (78.0%), third-generation cephalosporins (~74.7%), and tetracycline (72.5%). On the other hand, no isolate showed resistance to imipenem or meropenem. For all drugs tested, resistance rates of ESBL-producing *E. coli* were much higher than those of ESBL-non-producing *E. coli*. (Table 3).

Fifty-six (61.5%) isolates were MDR, of which 48 (52.7%) were ESBL producers and 8 (8.7%) were ESBL non-producers. No isolates showed total resistance or total sensitivity to all antimicrobials included in the study. A total of 37 patterns were observed among the 56 isolates tested (Table 4). There was no obvious clustering, but pattern 10 with resistance to 12 drugs was most common, seen in five (10.4%) ESBL-producing and even in one non-ESBLs isolate, followed by patterns 6 and 18 at 8.3% each. Pattern 2 with resistance to as many as 14 drugs was seen in three isolates (6.2%), while one isolate was resistant to as many as 15 drugs. Non-ESBL producers were relatively much less resistant, though one isolate also showed pattern 10 and another pattern 26, both similar to ESBLs producers.

Discussion

A total of 91 uropathogenic *E. coli* (UPEC) isolates from outpatients with recurrent UTIs were evaluated for the production of ESBL enzymes. These isolates had high rates of resistance to most members of β-lactam antibiotics, especially third-generation cephalosporins and monobactams. ESBLs were produced by 74.7% of these isolates. Similarly, high percentages of ESBL-producing *E. coli* (ESBL-EC) from outpatients with recurrent UTIs were reported from Spain [11] and

Table 3. Antimicrobial susceptibility of ESBL-producing and non-producing *E. coli* isolates from outpatients with recurrent UTIs.

Antimicrobial	% of *E. coli* isolates								
	ESBL producers (n = 68)			ESBL non producers (n = 23)			Total (n = 91)		
	R	I	S	R	I	S	R	I	S
Cefotaxime	100	0	0	0	4.3	95.6	74.7	1.0	24.1
Ceftazidime	82.3	17.6	0	4.3	0	95.6	62.6	13.1	24.1
Ceftriaxone	100	0	0	0	0	100	74.7	0	25.2
Aztreonam	100	0	0	0	0	100	74.7	0	25.2
Ampicillin	98.5	0	1.4	82.6	4.3	13.0	94.5	1.0	4.3
Amoxicillin-clavulanic acid	76.4	20.5	2.9	26.0	8.6	65.2	63.7	17.5	18.6
Cefepime	86.7	5.8	7.3	4.3	0	95.6	65.9	4.3	29.6
Cefoxitin	20.5	4.4	75.0	0	0	100	15.3	3.2	81.3
Imipenem	0	0	100	0	0	100	0	0	100
Meropenem	0	0	100	0	0	100	0	0	100
Ciprofloxacin	50.0	1.4	48.5	13.0	4.3	82.6	40.6	2.1	57.1
Gentamicin	57.3	1.4	41.1	13.0	0	86.9	46.1	1.0	52.7
Amikacin	10.2	4.4	85.2	8.6	0	91.3	9.8	3.2	86.8
Tetracycline	76.4	1.4	22.0	52.1	4.3	43.4	70.3	2.1	27.4
Trimethoprim-Sulfamethoxazole	83.8	0	16.1	60.8	0	39.1	78.0	0	21.9
Nalidixic acid	70.5	2.9	26.4	60.8	0	39.1	68.1	2.1	29.6
Nitrofurantoin	23.5	2.9	73.5	21.7	17.3	60.8	23.0	6.5	70.3

ESBL: extended spectrum β-lactamase; UTI: urinary tract infection; R: resistant; I: intermediate resistant.

Table 4. Antimicrobial resistance patterns of multidrug-resistant *E. coli* isolates from outpatients with recurrent UTIs.

Serial number	Resistance pattern	Number (%) of *E. coli* isolates	
		ESBL producers (n = 48)	ESBL non-producers (n = 8)
1	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, CN, AK, TE, SXT, NA, NIT	1 (2.0)	0
2	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, CN, TE, SXT, NA, NIT	3 (6.2)	0
3	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CIP, CN, AK, TE, SXT, NA, NIT	1 (2.0)	0
4	CTX, CAZ, ATM, CRO, AM, FEP, FOX, CIP, CN, TE, SXT, NA, NIT	1 (2.0)	0
5	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CIP, CN, TE, SXT, NA, NIT	1 (2.0)	0
6	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, CN, TE, SXT, NA	4 (8.3)	0
7	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, TE, SXT, NA, NIT	1 (2.0)	0
8	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CN, TE, SXT, NA, NIT	1 (2.0)	0
9	CTX, ATM, CRO, AM, AMC, FEP, CIP, CN, TE, SXT, NA, NIT	1 (2.0)	0
10	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CIP, CN, TE, SXT, NA	5 (10.4)	1
11	CTX, CAZ, ATM, CRO, AM, FEP, CIP, CN, AK, TE, SXT, NA,	1 (2.0)	0
12	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, CN, TE, NA	1 (2.0)	0
13	CTX, CAZ, ATM, CRO, AM, AMC, FEP, FOX, CIP, CN, SXT, NA	1 (2.0)	0
14	CTX, ATM, CRO, AM, CIP, CN, AK, TE, SXT, NA, NIT	1 (2.0)	0
15	CTX, CAZ, ATM, CRO, AM, AMC, CIP, CN, TE, SXT, NA	2 (4.1)	0
16	CTX, ATM, CRO, AM, AMC, FEP, CIP, CN, TE, SXT, NA	2 (4.1)	0
17	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CN, TE, SXT, NA	2 (4.1)	0
18	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CIP, TE, SXT, NA	4 (8.3)	0
19	CTX, CAZ, ATM, CRO, AM, CN, TE, SXT, NA, NIT	2 (4.1)	0
20	CTX, ATM, CRO, AM, AMC, FEP, CIP, TE, SXT, NA	2 (4.1)	0
21	CTX, ATM, CRO, AM, AMC, FEP, FOX, SXT, NA, NIT	1 (2.0)	0
22	CTX, CAZ, ATM, CRO, AM, FEP, CIP, TE, SXT, NA	2 (4.1)	0
23	CTX, CAZ, ATM, CRO, AM, AMC, FEP, CN, TE, SXT	1 (2.0)	0
24	CTX, ATM, CRO, AM, AMC, FEP, CN, TE, SXT, NA	1 (2.0)	0
25	CTX, CAZ, ATM, CRO, AM, AMC, FEP, TE, SXT, NA	1 (2.0)	0
26	CTX, ATM, CRO, AM, AMC, TE, SXT, NA, NIT	1 (2.0)	0
27	CTX, CAZ, AM, AMC, AK, TE, SXT, NA, NIT	0	1 (12.5)
28	CTX, CAZ, ATM, CRO, AM, FEP, CN, SXT, NA	1 (2.0)	0
29	CTX, CAZ, ATM, CRO, AM, AMC, CN, TE, SXT	1 (2.0)	0
30	CTX, ATM, CRO, AM, FEP, CN, TE, SXT	1 (2.0)	0
31	CTX, CAZ, ATM, CRO, AMC, TE, SXT, NA	1 (2.0)	0
32	AM, CN, TE, SXT, NA	0	1 (12.5)
33	AM, TE, SXT, NA, NIT	0	1 (12.5)
34	AM, CIP, TE, SXT, NA	0	1 (12.5)
35	AM, AMC, TE, SXT, NA	0	1 (12.5)
36	AM, TE, SXT, NA	0	1 (12.5)
37	AM, SXT, NA, NIT	0	1 (12.5)

CTX: cefotaxime, CAZ: ceftazidime, ATM: aztreonam, CRO: ceftriaxone, AM: ampicillin, AMC: amoxicillin-clavulanic acid, FEP: cefepime, FOX: cefoxitin, CIP: ciprofloxacin, CN: gentamicin, AK: amikacin, TE: tetracycline, SXT: trimethoprim-sulfamethoxazole, NA: nalidixic acid, NIT: nitrofurantoin.

China [20], where 66.2% and 52% of *E. coli* isolates produced ESBLs, respectively. As few studies are available regarding ESBL production by *E. coli* isolates from outpatients with recurrent UTIs, especially in the Middle East area, comparisons included in this study were with reports that dealt with ESBL production by *E. coli* from cases of first-episode uncomplicated UTIs.

Our results demonstrated an increasing prevalence of ESBLs among *E. coli* isolates. In a previous local study [17] using vaginal *E. coli* isolates form pregnant and non-pregnant women living in Al-Kut, Wasit province, Iraq, the prevalence rate of ESBL-EC was 65.5%. In another study [21] that was performed in the northern provinces of Iraq (Duhok, Erbil, and Sulymania), ESBL production was found among 68% of *Klebsiella pneumoniae* isolates from different clinical cases (urine, wound swab, sputum, and blood). In Iraq, lack of control over antibiotic use and prescription and the extensive use of antibiotics in our community, especially β-lactams, explained these high rates of ESBL production by clinical isolates from Iraqi people. Other researchers [22,23] reported that antibiotic resistance has become a major problem worldwide as a result of the extensive use of antimicrobial agents. In the Middle East area, notable differences regarding ESBL-EC prevalence were apparent. For example, in a recent study in Saudi Arabia [24], 20.3% of UPEC isolates from hospitalized patients were ESBL producers. Reports from Egypt, Iran, and Turkey revealed that the distribution of ESBL-EC was 78.8% [25], 56% [26], and 36.7% [27], respectively. In a study that comprised 22 European countries for the period of 2004 to 2007, the rate of ESBL-EC was 9.8% [5], while in a more recent study, it varied from 2% to 8% [28]. Such geographical differences in the rates of ESBL production from country to country and even within countries from hospital-to-hospital were reviewed [8]. These geographical variations may be affected by local practices of antibiotic use in humans and animal husbandry [28]. Therefore, the prevalence of ESBLs differs among patient groups and clinical and geographic settings [8].

CTX-M enzymes were the dominant ESBLs (70.3%) among our isolates, and all of them were from subgroup CTX-M-1. These results are consistent with those of other researchers [10,23-25,28]. Also, our results are in accordance with Pitout *et al*. [29] who reported that repeat UTIs were more likely to be caused by CTX-M-producing strains than by non-CTX-M-producing strains. In most parts of the world, CTX-M-type replaced TEM- and SHV-type and became the predominant ESBL among Enterobacteriaceae, principally in community-acquired infections caused by *E. coli* [5,6]. This displacement might have occurred not only as a consequence of the extraordinary dissemination of the corresponding $bla_{\text{CTX-M}}$ genes in highly mobilizable genetic platforms, including plasmids and transposons, but also because of these platforms within successful clones. Another reason for this increase is the co-resistance phenomenon in CTX-M producing organisms, particularly to aminoglycosides and fluoroquinolones, which might facilitate co-selection processes [6]. Other factors that contribute to this worldwide spread of CTX-M ESBLs included antibiotic consumption and dissimilar risk factors in different geographic areas and groups of patients and particularities of different compartments [6]. Particular attention should be paid to the increasing prevalence of the CTX-M worldwide.

All OXA-type ESBLs detected in this study were produced concomitantly with CTX-M-1. Similar results were reported from Asia [24,30], Africa [23,25], and Europe [31,32]. In addition to their resistance to third- and fourth-generation cephalosporins and monobactams, a high percentage of the isolates were also resistant to amoxicillin-clavulanic acid, gentamicin, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, and tetracycline. Furthermore, 93.3% of them were MDR. Review of previous studies [6,30,31,33] revealed that these properties are characteristics of the *E. coli* ST131 clone producing CTX-M-15 (not detected in this work), which characterized by multidrug resistance and co-production of OXA-1 or TEM-1b β-lactamases as well as aminoglycoside resistance genes *aac*(3')-*IIa* and *aac*(6')-*Ib-cr* (which also deactivates ciprofloxacin) on transferable plasmids. This widespread nature of this clone all over the world urges us also to try, in the near future, to detect its prevalence among our isolates.

Genotypically, this study included 68 (74.7%) isolates that were ESBL producers, of which 59 (64.8%) were also confirmed phenotypically as ESBL producers. The remaining 9 isolates produced AmpC concomitantly with CTX-M-1. This coexistence of both enzyme types in the same strain results in false negative tests for the detection of ESBLs, as AmpC-type β-lactamases resist inhibition by clavulanate and hence obscure the synergistic effect of clavulanate and cephalosporins against ESBLs [34]. Therefore, negative ESBL confirmatory tests based on these inhibitors may provide indirect evidence of AmpC production. Also, resistance to cefoxitin as well as

oxyimino-β-lactams is suggestive of an AmpC enzyme [35].

As a whole, the isolates included in this study showed high resistance rates to most tested antimicrobials. In addition, much higher resistance rates were detected among ESBL producers compared to ESBL non-producers. Differences in resistance rates were also noted in comparison with previous studies [19,36,37]. Worldwide, this growing antimicrobial resistance may be due to irrational use of antibiotics and its transfer by various means, including antibiotic resistant plasmids, bacteriophages, transpons, and integrons [8,38]. Continuous exposure of patients with recurrent UTI to antimicrobials is the major cause of high resistance rates leading to persistence and in the prevalence of UPEC strains [39].

Worldwide, the drug of choice for treatment of recurrent UTIs was trimethoprim-sulfamethoxazole. In outpatients, a fluoroquinolone, oral amoxicillin/clavulanate, or an oral third-generation cephalosporin can also be useful in treating such infections [1-3]. Ciprofloxacin was the empirical antimicrobial therapy used for treatment of patients with recurrent UTIs in the study area. It was then stopped as a result of its clinical ineffectiveness and replaced by amoxicillin-clavulanic acid, as explained by urologists. Patients who did not respond to this empirical therapy were subjected to antimicrobial susceptibility testing to guide their treatment. For ESBL producers detected in this study, resistance to trimethoprim-sulfamethoxazole, ciprofloxacin, amoxicillin/clavulanate, and third-generation cephalosporins was 83.8%, 50.0%, 76.4%, and from 82.3% to 100%, respectively. Moreover, the pattern of drug resistance was alarming, with many isolates showing resistances to more than 12 drugs, almost indicating a move toward pan resistance. It is also a matter of concern that the most common pattern (No. 10), with resistance to 12 drugs seen in ESBL-positive isolates, was also there in non-ESBL isolates. Such patterns of resistance can act as epidemiological markers for identifying the circulating strains in the area. In light of this study's findings and the references quoted, first-line therapy is not likely to be effective for treatment of such patients in the study area. The only effective antimicrobials were imipenem and meropenem, to which all isolates were sensitive, and amikacin and cefoxitin, to which sensitivity was 88.4% and 86.5%, respectively. Multidrug resistance was high among these ESBL producers (48/68; 70.6%). These resistance results were expected, as ESBLs confer resistance against all β-lactam antibiotics except carbapenems and cephamycins. Also, ESBL-encoding plasmids frequently bear resistance genes for additional antibiotic classes, such as sulfonamides, aminoglycosides, and fluoroquinolones [8,40]. This frequent co-expression of resistance by these organisms to classes of antimicrobial agents other than those hydrolyzed by the ESBLs is an important factor that limits the array of active antibiotics against ESBL-producing Enterobacteriaceae such as fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole [5]. Thus, initial empirical therapy for infections due to these MDR organisms is often ineffective and associated with increased mortality [40]. Therefore, to guide empirical treatment, early recognition of patients who are at risk for infection with ESBL-producing bacteria is necessary. In addition, application of preventive measures is necessary to limit the dissemination of infection, as ignorance of this emerging public health threat may force the medical community, in the near future, to use carbapenems as the first choice for the empirical treatment of serious infections associated with UTIs originating from the community [30].

Conclusions

In the study area, this high ESBL prevalence among *E. coli* isolates from outpatients with recurrent UTIs is alarming, as these patients may be an important source of such isolates both in the community and in the hospital environment. <ore attention must be paid to these patients, and serious steps are urgently required to control the spread of these ESBL producers in our environment. Also, in the current situation, it is important that

antibiotic treatment is started only after a proper sensitivity report is obtained from the laboratory.

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Authors' contributions

SMG participated in the design of the study, carried out the antimicrobial susceptibility tests and PCR experiments, and drafted the manuscript. JHH participated in its design and coordination and collected patients' information from the hospital and brought the isolates from the hospital laboratory to the college laboratory. All authors read and approved the final manuscript

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