



Detection of O25b-ST131 clone, CTX-M-1 and CTX-M-15 genes via real-time PCR in *Escherichia coli* strains in patients with UTIs obtained from a university hospital in Istanbul

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ARTICLE INFO

Article history:

Received 12 October 2018

Received in revised form 6 December 2018

Accepted 13 February 2019

Keywords:

O25b-ST131

Escherichia coli

UTI

CTX-M-15

Real-time PCR

ABSTRACT

Background: *Escherichia coli* sequence type 131 is an important multidrug resistant clone responsible from more than half of ESBL-producing *E. coli* isolates. Aim of this study was to investigate the presence of O25b-ST131 clone, CTX-M-15 and CTX-M-1 genes in the *E. coli* strains isolated from both hospital and community acquired UTIs by real-time PCR and to reveal molecular epidemiological data.

Methods: Non-duplicate *E. coli* (n = 101) strains isolated from UTI patients were included. Bacterial identifications were performed with VITEK Compact. Antimicrobial susceptibility tests, phenotypic ESBL and E-tests were performed conventionally. Real-time PCR was utilized to detect presence of O25b-ST131 clone, blaCTX-M-15 and blaCTX-M-1.

Results: O25b-ST131 clone, CTX-M-1 and CTX-M-15 were detected in 22%, 73%, 37% in UTIs, respectively. Presence of O25b-ST131 clones and CTX-M-1 genes among *E. coli* strains isolated from inpatients were found statistically higher than outpatients. The most effective choice was found to be fosfomycin and nitrofurantoin in outpatients and inpatients, respectively. The MIC₉₀ values of Amikacin, Cefotaxime, Cefepime and Ciprofloxacin were higher in inpatients than in outpatients, whereas Cefotaxime and Ciprofloxacin MIC₅₀ values were found to be higher in inpatients than in outpatients. The highest increase of MIC₉₀ values was observed in O25b-ST131, CTX-M-1 and CTX-M-15 coexistence.

Conclusion: The presence of O25b-ST131 clone, CTX-M-1 and CTX-M-15 genes in *E. coli* strains in patients with UTI has been revealed. In the presence of the O25b-ST131 clone, a significant increase was observed in the ciprofloxacin MIC values indicating the importance of monitorization of the clone using molecular epidemiology.

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Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections affecting millions of people every year [1]. *Escherichia coli* (*E. coli*) is one of the most important causes of urinary tract infections (UTI), whether it is hospital-acquired or community-acquired [2]. Infections caused by Gram-negative multidrug-resistant (MDR) bacteria are a worldwide health problem [3]. In recent years, the production of extended-spectrum β -lactamase (ESBL) in the Enterobacteriaceae family, especially in *E. coli*, has increased significantly [4]. Among these, *E. coli* sequence type 131 (ST131)-O25b:H4 is an important multidrug resistant clone which is able to spread globally [3]. *E. coli* ST131 clone, dis-

covered in 2008, is a member of highly virulent phylogenetic group B2, also the strains in this clone often have serotype O25:H4 and the specific type of O25 is called O25b [5]. Besides being one of the main causes of hospital-acquired and community-acquired urinary tract infections in Europe, this clone is thought to be responsible for approximately 20% of all extraintestinal *E. coli* infections [6,7]. In addition, among the strains identified in many infections, the majority of fluoroquinolone-resistant isolates [8] and more than half of ESBL-producing isolates are associated with this clone [9]. CTX-M-15, the most widely spreading ESBL enzyme among CTX-Ms, is frequently detected in *E. coli* ST131 clones [10]. Thus, *E. coli* O25b-ST131 clone show a high resistance profile to many drugs and this leaves a few effective antibiotic options that can be used to treat patients [6]. Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTIs since oral and intravenous preparations are available [11]. Increased resistance to fluoroquinolones may have serious clinical consequences as they are one of the most effective

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therapeutic options in severe *Salmonella* spp. and *E. coli* infections [12]. In the light of all this information, we aimed to investigate the presence of O25b-ST131 clone as well as producing of CTX-M-15 and CTX-M-1 genes in the *E. coli* strains detected as a causative agents of both hospital and community acquired UTIs by real-time PCR and to reveal molecular epidemiological datas about our country and also present status against antimicrobials conventionally.

Material and methods

Non-duplicate *E. coli* ($n = 101$) strains isolated from urine samples of patients admitted to one of a private university hospital in Turkey, between April and August 2018. Isolates were deemed duplicates if the same organism from same patients with the same antibiogram is grown from the same sample type within 14 days (for inpatients) or 91 days (for outpatients). We considered patients who stayed for the clinical treatment in a given ward of the hospital to be inpatients, and patients who were not hospitalized but who visited an acute day ward or polyclinic were considered to be outpatients. The inclusion criteria for the outpatients were: not be pregnant, not have used antibiotics within one month prior to enrollment and wasn't recorded any hospital as within 72 h. Cobas 6500 (Roche Diagnostics, Mannheim, Germany) automated urine analyser system was used to detect white blood cells (WBC) count from urine samples and reports generated automatically the microscopic results as cells/high power field (HPF). Patients presenting with UTI, where CFU counts were $>1 \times 10^5$ mL were included in this study. Bacterial identifications were conducted with automatized test VITEK Compact (BioMerieux, Marcy L'Etoile, France). Antimicrobial susceptibility tests were performed with Kirby Bauers disc diffusion tests on Mueller-Hinton agar (BioMerieux, Marcy L'Etoile, France). The following antibiotic disks were used, amoxicillin-clavulanic acid (20/10 µg), ampicillin (30 µg), cefazolin (30 µg), cefixime (30 µg), cefotaxime (30 µg), cefepime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), fosfomycin (200 µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) (Oxoid, Basingstoke, UK) interpreted according to the EUCAST clinical breakpoints (EUCAST, 2016). To confirm ESBL phenotype, ESBL test was performed using the double-disc synergy procedure on Mueller-Hinton agar (BioMerieux, Marcy L'Etoile, France) [13]. MIC values of ciprofloxacin, amikacin, cefotaxime and cefepime were also determined with E-test (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar (BioMerieux, Marcy L'Etoile, France) and interpreted according to the EUCAST clinical breakpoints [14].

A single colony of each strain's overnight culture on eosin methylene blue (EMB) agar was suspended in 50 µL of ultrapure water. The suspension was heated at 95 °C for 10 min and centrifuged at 14,000 rpm for 10 min. Thirty microliters of the supernatant was used as a DNA template for real-time PCR [15]. All DNA was stored –80 °C until processing. Previously designed primers; ST131TF (5-GGT GCT CCA GCA GGT G-3), ST131TR (5-TGG GCG AAT GTC TGC-3), ST131AF (5-GGC AAT CCA ATA TGA CCC-3), ST131AR (5-ACC TGG CGA AAT TTT TCG-3), MC-3-15F (5-TGG GGG ATA AAA CCG GCA G-3), MC-3-15R (5-GCG ATA TCG TTG GTG GTG C-3), blaCTX-M-1F (5-AAC CGT CAC GCT GTT GTT AG-3) and blaCTX-M-1R (5-TTG AGG CTG GGT GAA GTA AG-3) were used to detect presence of O25b-ST131 clone, blaCTX-M-15 and blaCTX-M-1 respectively [15,16]. Real-time PCR amplification and melting curve analysis were performed using a LightCycler 480 II system with software version 1.5 (Roche Diagnostics, Mannheim, Germany). The real-time PCR mixture was prepared using the LightCycler 480 SYBR Green I Mastermix kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. Cycling conditions for the O25b-ST131 assays were: initial denatu-

Table 1

Demographic data of patients and distribution of Urine WBC/HPF, CTX-M-1, CTX-M-15 genes and O25b-ST131 clone.

	Inpatient (n:42)	Outpatient (n:59)	Total (n:101)
Age (Mean-SD)	35.40 ± 25.18	37.24 ± 25.23	37.23 ± 25.18
Sex (Male/female)	13/29	11/48	24/77
Urine WBC/HPF	O25b-ST131 positive (n) / total (n)		
≤10	1/6	2/6	3/12
11–20	2/5	0/4	2/9
20–50	2/4	0/3	2/7
>50	9/27	7/46	16/73
ESBL positivity	28 (66.67)	23 (38.98)	51 (50.49)
blaCTX-M-1	38 (90.48)	36 (61.02)	74 (73.27)
blaCTX-M-15	16 (38.10)	22 (37.29)	38 (37.62)
O25b-ST131 clone	14 (33.33)	9 (15.25)	23 (22.27)

Table 2

Distribution of CTX-M-1 and CTX-M-15 genes and O25b-ST131 clone by ESBL condition.

<i>E. coli</i>	ESBL positive (n:51)	ESBL negative (n:50)
blaCTX-M-1	46 (90.20)	28 (56.00)
blaCTX-M-15	18 (35.30)	20 (40.00)
O25b-ST131 clone	16 (31.37)	7 (14.00)

ration for 5 min at 95 °C and 40 cycles of 5 s at 95 °C and 10 s at 58 °C; those for the blaCTX-M-15 and blaCTX-M-1 assay were: 5 min at 95 °C and 50 cycles of 5 s at 95 °C and 10 s at 70 °C. The fluorescence signal was measured at the end of each annealing step. Following amplification, a melting curve was generated by heating the PCR product to 95 °C with a ramp rate of 0.05 °C /s. Statistical analysis were performed with chi-square test on SPSS vers. 20 software (IBM, USA).

Results

In this study we evaluated 101 *E. coli* strains, 42 were isolated from inpatients (mean age 35 years) and 59 isolated from outpatients (mean age 37 years). O25b-ST131 clone was detected in 22%, CTX-M-1 was detected in 73% and CTX-M-15 in 37% of all isolates (Table 1). Distribution of O25b-ST131 clone, according to WBC/HPF results showed in Table 1.

Inpatients were found to have significantly more *E. coli* O25b-ST131 clones compared to outpatients ($p < 0.05$). The CTX-M-1 gene responsible for ESBL production in strains isolated from inpatients was statistically significantly higher than in the strains isolated from outpatients ($p < 0.05$). On the otherhand, producing of CTX-M-15 gene in strains isolated from inpatients was not statistically higher than in the strains isolated from outpatients ($p > 0.05$). When presence of phenotypic ESBL was examined among the strains, it was found to be positive in 66.67% of inpatients and 38.98% of outpatients.

Among the phenotypically ESBL positive strains, it was found that producing of CTX-M-1 and CTX-M-15 gene and O25b-ST131 clone positivity were 90.2%, 35.3% and 31.37%, respectively (Table 2). CTX-M-1 gene and O25b-ST131 clone presence were significantly higher in the ESBL positive strains ($p < 0.05$).

Antimicrobial susceptibilities of the strains were given in Table 3. According to susceptibility results carbapenems were found to be the best therapeutic choice for all patients which was followed by nitrofurantoin, amikacin and fosfomycin. The susceptibility percentage for all antimicrobials, detected in inpatients were lower than in outpatients.

E. coli strains isolated from inpatients were found to be more resistant to antimicrobials than the strains isolated from outpatients. Although the most effective choice was found to be

Table 3
Antimicrobial susceptibilites of the strains.

Antimicrobials	Inpatient (n:42)	Outpatient (n:59)	Total (n:101)
Amoxicillin-clavulanic acid (20/10 µg)	14 (33.33)	33 (55.93)	47 (46.53)
Ampicillin (30 µg)	7 (16.67)	12 (20.34)	19 (18.81)
Cefazolin (30 µg)	9 (21.43)	18 (30.51)	27 (26.73)
Cefixime (30 µg)	13 (30.95)	33 (55.93)	46 (45.54)
Cefotaxime (30 µg)	14 (33.33)	34 (57.63)	48 (47.52)
Ceftriaxone (30 µg)	14 (33.33)	36 (61.02)	50 (49.50)
Cefuroxime (30 µg)	7 (16.67)	18 (30.51)	25 (24.75)
Cefepime (30 µg)	22 (52.38)	45 (76.27)	67 (66.34)
Fosfomycin (200 µg)	36 (85.71)	57 (96.61)	93 (92.08)
Amikacin (30 µg)	37 (88.10)	56 (94.92)	93 (92.08)
Gentamicin (10 µg)	26 (61.90)	47 (79.66)	73 (72.28)
Imipenem (10 µg)	41 (97.62)	59 (100.0)	100 (99.01)
Meropenem (10 µg)	42 (100.0)	58 (98.31)	100 (99.01)
Nitrofurantoin (300 µg)	41 (97.62)	56 (94.92)	97 (96.04)
Ciprofloxacin (5 µg)	11 (26.19)	33 (55.93)	44 (43.56)
Levofloxacin (5 µg)	11 (26.19)	34 (57.63)	45 (44.55)
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	19 (45.24)	34 (57.63)	53 (52.48)

Table 4
MIC values of the strains (µg/mL).

	Inpatients (n:42)			Outpatients (n:59)			Total (n:101)		
	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀
Amikasin	0.5–32	2	16	0.5–8	2	4	0.5–32	2	4
Cefotaxime	0.015–8	0.5	2	0.015–2	0.12	1	0.015–8	0.5	2
Cefepime	0.015–2	0.06	1	0.015–1	0.06	0.5	0.015–2	0.06	0.5
Ciprofloxacin	0.004–64	0.5	16	0.004–16	0.03	4	0.04–64	0.12	8

Table 5
Ciprofloxacin susceptibility according to CTX-M-1, CTX-M-15 and O25b-ST131 positivity.

<i>E. coli</i>	Ciprofloxacin				
	S	R	MIC range	MIC ₅₀	MIC ₉₀
CTX-M-1 positive (n:51)	25 (49.02)	26 (50.98)	0.04–1	0.06	0.5
CTX-M-15 positive (n:8)	5 (62.5)	3 (37.5)	0.004–4	0.03	4
CTX-M-1 & CTX-M-15 positive (n:9)	4 (44.44)	5 (55.56)	0.08–4	0.015	4
CTX-M-1 & O25b-ST131 positive (n:2)	0 (0)	2 (100)	0.5–2	0.5	2
CTX-M-15 & O25b-ST131 positive (n:9)	0 (0)	9 (100)	2–16	4	16
O25b-ST131 & CTX-M-1 & CTX-M-15 positive (n:12)	0 (0)	12 (100)	4–64	16	32
O25b-ST131 & CTX-M-1 & CTX-M-15 negative (n:10)	10 (100)	0 (0)	0.004–0.03	0.015	0.03

*S: Sensitive, R: Resistant.

fosfomycin after carbapenems (96.61%) for outpatients, this option was found as nitrofurantoin (97.62%) in inpatients.

MIC values of *E. coli* strains for Cefotaxime, Cefepim demonstrating third and fourth generation cephalosporins respectively, Amikacin and Ciprofloxacin are shown in Table 4. The MIC₉₀ values of Amikacin, Cefotaxime, Cefepime and Ciprofloxacin were higher in inpatients than in outpatients, whereas Cefotaxime and Ciprofloxacin MIC₅₀ values were found to be higher in inpatients than in outpatients.

CTX-M-1 detected in 2, CTX-M-15 in 9 of the O25b-ST131 positive *E. coli* strains, also in 12 strains CTX-M-1 and CTX-M-15 were coexisted. All *E. coli* O25b-ST131 strains were resistant to ciprofloxacin (100%). Moreover, all 10 strains that are not carrying CTX-M-1 or CTX-M-15 genes and that are not ST131 clone were found to be sensitive to ciprofloxacin (Table 5).

The MIC range, MIC₅₀ and MIC₉₀ values of the Ciprofloxacin were examined according to the presence of CTX-M-1, CTX-M-15 genes and O25b-ST131 clones. In the case of CTX-M-1 and O25b-ST131 or CTX-M-15 and O25b-ST131 associations, an increase in MIC values were detected for the strains, whereas in the case of co-occurrence of O25b-ST131, CTX-M-15 and CTX-M-1 these values were found at their highest levels.

Cefotaxime MIC range, MIC₅₀ and MIC₉₀ values were analyzed according to the presence of CTX-M-1, CTX-M-15 genes and O25b-ST131. In the case of presence of one CTX-M gene and O25b-ST131

clone an increase has been noticed in MIC₉₀ values whereas the highest increase was observed in O25b-ST131, CTX-M-1 and CTX-M-15 coexistence (Table 6).

In addition, 8 out of 10 strains that were not O25b-ST131 clone and not carrying either CTX-M-1 or CTX-M-15 genes were found to be susceptible to cefotaxime. Also, all 12 O25b-ST131 clones positive strains that are carrying both CTX-M-1 and CTX-M-15 genes were found to be resistant to cefotaxime.

Discussion

E. coli is one of the most important causes of urinary tract infections in both inpatients and outpatients [17]. Particularly *E. coli* ST131 clone was considered to be an important public health problem, due to its epidemic potential, virulence and multidrug resistance ability which were dramatically higher than non ST131 clones [18]. The ST131 clone is also reported to be strongly associated with ESBLs such as the producing of CTX-M-15. This leaves limited therapeutic options for the treatment of infections caused by this clone and increases the interest in monitorization of this infectious agent [5].

When the studies conducted on the distribution of phenotypic ESBL, O25b-ST131, CTX-M-15 and CTX-M-1 cases in *E. coli* strains identified as the causative agent of UTI were examined, Namaei et al, in their study in Iran in 2017, found the frequency of O25b-

Table 6
Cefotaxime susceptibility according to CTX-M-1, CTX-M-15 and O25b-ST131 positivity.

<i>E. coli</i>	Cefotaxime				
	S	R	MIC range	MIC ₅₀	MIC ₉₀
CTX-M-1 positive (n:51)	19 (37.25)	32 (62.75)	0.015–2	0.5	1
CTX-M-15 positive (n:8)	6 (75.0)	2 (25.0)	0.003–2	0.06	2
CTX-M-1 & CTX-M-15 positive (n:9)	8 (88.89)	1 (11.11)	0.015–0.5	0.06	0.5
CTX-M-1 & O25b-ST131 positive (n:2)	0 (0)	2 (100)	0.5–2	0.5	2
CTX-M-15 & O25b-ST131 positive (n:9)	7 (77.78)	2 (22.22)	0.015–1	0.06	1
O25b-ST131 & CTX-M-1 & CTX-M-15 positive (n:12)	0 (0)	12 (100)	0.5–8	1	4
O25b-ST131 & CTX-M-1 & CTX-M-15 negative (n:10)	8 (80)	2 (20)	0.015–0.5	0.06	0.25

S: Sensitive, R: Resistant.

ST131 as 24.7%. The presence of O25b-ST131 clone in the strains isolated from outpatient and inpatients, was reported as 22.8% and 55.6%, respectively. Also, in the same study, O25b-ST131, CTX-M-1 and CTX-M-15 presence were reported in ESBL positive patients as 78.5%, 100%, 95.5%, respectively [5]. Marialouis and Santhanam, in their study conducted in India in 2016, reported ESBL presence as 47% in *E. coli* strains isolated from patients with UTI. In the same study, they reported the presence of O25b-ST131 clone in 41% of the strains [19]. Toval et al. studied on *E. coli* strains which were found as causative agents in 265 patients with UTI in Germany in 2014. O25b-ST131 clone was detected in 22 of the strains, whereas 13 were isolated from inpatients, 9 were isolated from outpatients [20]. Talan et al., studied with the strains obtained from patients with UTI in the United States in 2016, and reported ESBL positivity as 2.6% of outpatients and 12.2% of inpatients [21]. In the study conducted by Guyomard-Rabenirina et al. in France in 2016, CTX-M-15 was detected in 5, CTX-M-1 was detected in 4 out of 11 ESBL positive *E. coli* strains [22]. Can et al., reported the presence of ESBL as 24%, CTX-M-15 gene as 14%, and O25b-ST131 clone as 12% of *E. coli* strains isolated from patients with UTI in their study conducted in Istanbul at 2015 [23]. When we examine the results obtained from all these studies performed in different countries, the presence of ESBL, CTX-M-1, CTX-M-15 and O25b-ST131 clones are variable. In 2015, Goossens et al. reported that the treatment policies [24], which vary from country to country, may be effective in this difference. In addition to this, because of the lack of consensus regarding the use of molecular techniques, we believe that the different molecular techniques with different protocols used in these studies may cause differences in the results. Besides, similar to our study results, most of the studies revealed that antibiotic resistance, presence of CTX-M-1, CTX-M-15 genes, and O25b-ST131 clones were found to be higher in the strains isolated from inpatients than outpatients similar to our results. Because of the fact that inpatients strains encounter more drugs interaction than outpatients strains.

According to the antimicrobial susceptibility studies conducted on *E. coli* strains identified as causative agent of UTI; Namaei et al., reported susceptibilities to amikacin, amoxycillin-clavulanic acid, cefepim, cefotaxime, gentamycin, trimethoprim-sulfamethoxazole, ciprofloxacin, imipenem and meropenem as 97.2%, 88.4%, 79.4%, 33.4%, 67.7%, 29.9%, 23.2%, 0% and 0%, respectively in 2017 [5]. Talan et al., in their study conducted in the United States in 2016, reported antimicrobial susceptibility to ampicillin, cefazoline, ceftriaxone, ciprofloxacin, levofloxacin, gentamycin, imipenem and meropenem for outpatients and inpatients as; 45.1%, 44.9%; 92.8%, 77%; 98.4%, 85.5%; 94.7%, 80.4%; 94.9%, 83.3%; 93.7%, 87.3%; 0, 0%; and 0%, 0% respectively [21]. When all of these studies are examined, it can be seen that there is a change in antimicrobial susceptibilities by the effect of different antibiotics used from country to country, even from region to region. However, it is seen that there is a higher level of resistance profile in inpatients than outpatients, as in our study, and antibiotic options that can be used in these patients are more limited than in outpatients.

Sedighi et al., identified 97% sensitivity for amikacin in *E. coli* strains identified as the causative agent of UTI in Iran in 2014. The MIC₅₀, MIC₉₀ and MIC ranges of these strains were reported as 0.75, 1.6 and 0.125–32 µg/mL, respectively [25]. In our study, the MIC range was found as 0.5–32 µg/mL which is similar to the Sedighi et al. [25], however, our MIC₅₀ and MIC₉₀ rates were higher which are 2 µg/mL and 4 µg/mL, respectively. Cuba et al, examined the MIC values of some antibiotics commonly used in the treatment in their study conducted with *E. coli* strains isolated as UTI agent from outpatients and inpatients in Brazil in 2014. They reported 75.7% sensitivity for ceftriaxone in inpatients with a MIC range 0.016–256 µg/mL whereas they obtained %97.5 sensitivity and 0.008–256 µg/mL MIC range for outpatients. In the same study, sensitivity and MIC range for ciprofloxacin were reported as 64.3%, 0.008–32 µg/mL and 83.3%, 0.008–0.032 µg/mL in inpatients and outpatients, respectively [26]. Cerquetti et al. reported 9.8% CTX-M-15 positivity in their study conducted with strains isolated from patients with UTI in Italy in 2010, also 90% of CTX-M-15 positive strains detected as O25b-ST131 clone [27]. Suzuki et al., in 2009, reported 91.5% CTX-M-15 positivity and among these strains 21% identified as O25b-ST131 clone in Japan [28]. In 2015, Drawz et al. detected O25b-ST131 clone in 13 out of 15 CTX-M-1 positive and 6 in 10 of CTX-M-15 positive strains [29]. In our study, we detected CTX-M-15 in 9, CTX-M-1 in 2 and CTX-M-1, CTX-M-15 coexistence in 12 O25b-ST131 *E. coli* strains. The results of our study are similar to other studies indicating O25b-ST131 clone is closely related to the CTX-M-1 and CTX-M-15 genes. The fact that this clone carries these genes clearly explains the potential of this clone to develop multidrug resistance. In 2011, Ruiz et al. reported the MIC value for cefotaxime >32 µg/mL and for ciprofloxacin >8 µg/mL, when they identified the first community acquired CTX-M-15-producing O25b-ST131 *E. coli* strain in South America. They emphasized that there was an increased drug resistance especially in these strains and monitorization of the prevalence of these strains was important for the clinicians [30]. Sato et al., 2017, in their study conducted with O25b-ST131 clones, both cephalosporin sensitive and resistant strains were reported. However, when these strains examined in terms of ciprofloxacin, it was seen that all of the O25b-ST131 clones were strains were resistant. Moreover, all of these ciprofloxacin resistant strains had MIC values >128 µg/mL [31]. Röderova et al., in 2016, reported 69% O25b-ST131 positivity and high levels of ciprofloxacin resistance (> 32 µg/mL) [32]. The results of our study are similar to these studies, according to our results, in case of O25b-ST131 presence, there is a dramatic increase in MIC range, MIC₅₀ and MIC₉₀ values for ciprofloxacin.

Conclusions

As a result of our study, the presence of O25b-ST131 clone, CTX-M-1 and CTX-M-15 genes in *E. coli* strains identified as causative agents in patients with UTI in our country has been revealed. Moreover, antimicrobial susceptibility particularly for

ciprofloxacin, which is commonly used in the treatment regimens, was investigated among these strains. There is a dramatic increase in MIC range, MIC₅₀ and MIC₉₀ values for ciprofloxacin, presence of O25b-ST131 clone in strains isolated from UTIs. We believe that *E. coli* strains producing CTX-M-15 and belonging O25b-ST131 clone should be followed by reliable methods such as molecular techniques, and these molecular epidemiological data should be monitored to support clinicians.

Funding

No funding Sources.

Competing interests

None declared.

Ethical approval

The study was approved by the non-invasive clinical research ethics committee of the Medipol University School of Medicine.

References

- [1] O'Brien VP, Hannan TJ, Nielsen HV, Hultgren SJ. Drug and vaccine development for the treatment and prevention of urinary tract infections. *Microbiol Spectr* 2016;4, <http://dx.doi.org/10.1128/microbiolspec.UTI-0013-2012>.
- [2] Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014;27:543–74, <http://dx.doi.org/10.1128/CMR.00125-13>.
- [3] Szijártó V, Lukaszewicz J, Gozdiewicz TK, Magyarics Z, Nagy E, Nagy G. Diagnostic potential of monoclonal antibodies specific to the unique O-antigen of multidrug-resistant epidemic *Escherichia coli* clone ST131-O25b:H4. *Clin Vaccine Immunol* 2014;21:930–9, <http://dx.doi.org/10.1128/CVI.00685-13>. Papasian CJ, ed.
- [4] Blanco J, Mora A, Mamani R, López C, Blanco M, Dahbi G, et al. Four main serotypes among extended-spectrum-β-lactamase-producing isolates of *Escherichia coli* O25b:H4-B2-ST131: bacterial, epidemiological, and clinical characteristics. *J Clin Microbiol* 2013;51:3358–67, <http://dx.doi.org/10.1128/JCM.01555-13>.
- [5] Namaei MH, Yousefi M, Ziaee M, Salehabadi A, Ghannadkafi M, Amini E, et al. First report of prevalence of CTX-M-15-producing *Escherichia coli* O25b/ST131 from Iran. *Microb Drug Resist* 2017;23:879–84, <http://dx.doi.org/10.1089/mdr.2016.0272>.
- [6] Szijártó V, Guachalla LM, Visram ZC, Hartl K, Varga C, Mirkina I, et al. Bactericidal monoclonal antibodies specific to the lipopolysaccharide O antigen from multidrug-resistant *Escherichia coli* clone ST131-O25b:H4 elicit protection in mice. *Antimicrob Agents Chemother* 2015;59:3109–16, <http://dx.doi.org/10.1128/AAC.04494-14>.
- [7] Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA, Hultgren SJ. Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *E. coli* bladder infection. *FEMS Microbiol Rev* 2012;36:616–48, <http://dx.doi.org/10.1111/j.1574-6976.2012.00339.x>.
- [8] Johnson JR, Tchesnokova V, Johnston B, Clabots C, Roberts PL, Billig M, et al. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. *J Infect Dis* 2013;207:919–28, <http://dx.doi.org/10.1093/infdis/jis933>.
- [9] Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. *Clin Infect Dis* 2010;51:286–94, <http://dx.doi.org/10.1086/653932>.
- [10] Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. *Emerg Infect Dis* 2008;14:195–200, <http://dx.doi.org/10.3201/eid1402.070350>.
- [11] Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies. *BMC Infect Dis* 2015;15(545), <http://dx.doi.org/10.1186/s12879-015-1282-4>.
- [12] McQuiston Haslund J, Rosborg Dinesen M, Sternhagen Nielsen AB, Llor C, Bjerrem L. Different recommendations for empiric first-choice antibiotic treatment of uncomplicated urinary tract infections in Europe. *Scand J Prim Health Care* 2013;31:235–40, <http://dx.doi.org/10.3109/02813432.2013.844410>.
- [13] Miao Z, Li S, Wang L, Song W, Zhou Y. Antimicrobial resistance and molecular epidemiology of ESBL-producing *Escherichia coli* isolated from outpatients in town hospitals of Shandong Province, China. *Front Microbiol* 2017;8:63, <http://dx.doi.org/10.3389/fmicb.2017.00063>.
- [14] EUCAST, January 2016. Available at: http://www.eucast.org/clinical_breakpoints/. [Accessed January 2016] Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0; 2016.
- [15] Unlu O, Aktas Z, Tugrul HM. Analysis of virulence factors and antimicrobial resistance in *Salmonella* using molecular techniques and identification of clonal relationships among the strains. *Microb Drug Resist* 2018;6:19, <http://dx.doi.org/10.1089/mdr.2018.0042>.
- [16] Dhanji H, Doumith M, Clermont O, Denamur E, Hope R, Livermore DM, et al. Real-time PCR for detection of the O25b-ST131 clone of *Escherichia coli* and its CTX-M-15-like extended-spectrum β-lactamases. *Int J Antimicrob Agents* 2010;36:355–8, <http://dx.doi.org/10.1016/j.ijantimicag.2010.06.007>.
- [17] Damavandi M-S, Gholipour A, Latif Pour M. Prevalence of class D carbapenemases among extended-spectrum β-lactamases producing *Escherichia coli* isolates from educational hospitals in Shahrekord. *J Clin Diagn Res* 2016;10:DC01–5, <http://dx.doi.org/10.7860/JCDR/2016/17722.7739>.
- [18] Dautzenberg MJD, Haverkate MR, Bonten MJM, Bootsma MCJ. Epidemic potential of *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST258: a systematic review and meta-analysis. *BMJ Open* 2016;6:e009971, <http://dx.doi.org/10.1136/bmjopen-2015-009971>.
- [19] Marialouis XA, Santhanam A. Antibiotic resistance, RAPD-PCR typing of multiple drug resistant strains of *Escherichia coli* from urinary tract infection (UTI). *J Clin Diagn Res* 2016;10:DC05–9, <http://dx.doi.org/10.7860/JCDR/2016/16470.7389>.
- [20] Toval F, Köhler C-D, Vogel U, Wagenlehner F, Mellmann A, Fruth A, et al. Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients with urinary tract infection. *J Clin Microbiol* 2014;52:407–18, <http://dx.doi.org/10.1128/JCM.02069-13>. Forbes BA, ed.
- [21] Talan DA, Takhar SS, Krishnadasan A, Abrahamian FM, Mower WR, Moran GJ, et al. Fluoroquinolone-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* infections in patients with pyelonephritis, United States. *Emerg Infect Dis* 2016;22:1594–603, <http://dx.doi.org/10.3201/eid2209.160148>.
- [22] Guyomard-Rabenirina S, Malespine J, Ducat C, Sadikalay S, Falord M, Harrois D, et al. Temporal trends and risks factors for antimicrobial resistant Enterobacteriaceae urinary isolates from outpatients in Guadeloupe. *BMC Microbiol* 2016;16:121, <http://dx.doi.org/10.1186/s12866-016-0749-9>.
- [23] Can F, Azap OK, Seref C, İspir P, Arslan H, Ergonul O. Emerging *Escherichia coli* O25b/ST131 clone predicts treatment failure in urinary tract infections. *Clin Infect Dis* 2015;60:523–7, <http://dx.doi.org/10.1093/cid/ciu864>.
- [24] Goossens H, Ferech M, Vander Stichele R, Elseviers M, ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005;365:579–87, [http://dx.doi.org/10.1016/S0140-6736\(05\)17907-0](http://dx.doi.org/10.1016/S0140-6736(05)17907-0).
- [25] Sedighi I, Solgi A, Amanati A, Alikhani MY. Choosing the correct empirical antibiotic for urinary tract infection in pediatric: surveillance of antimicrobial susceptibility pattern of *Escherichia coli* by E-Test method. *Iran J Microbiol* 2014;6:387–91.
- [26] Cuba GT, Pignatari AC, Patekoski KS, Luchesi LJ, Kiffer CR. Pharmacodynamic profiling of commonly prescribed antimicrobial drugs against *Escherichia coli* isolates from urinary tract. *Braz J Infect Dis* 2014;18:512–7, <http://dx.doi.org/10.1016/j.bjid.2014.01.008>.
- [27] Cerquetti M, Giufrè M, García-Fernández A, Accogli M, Fortini D, Luzzi I, et al. Ciprofloxacin-resistant, CTX-M-15-producing *Escherichia coli* ST131 clone in extra-intestinal infections in Italy. *Clin Microbiol Infect* 2010;16:1555–8, <http://dx.doi.org/10.1111/j.1469-0691.2010.03162.x>.
- [28] Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y. Change in the prevalence of extended-spectrum-β-lactamase-producing *Escherichia coli* in Japan by clonal spread. *J Antimicrob Chemother* 2009;63:72–9, <http://dx.doi.org/10.1093/jac/dkn463>.
- [29] Drawz SM, Porter S, Kuskowski MA, Johnston B, Clabots C, Kline S, et al. Variation in resistance traits, phylogenetic backgrounds, and virulence genotypes among *Escherichia coli* clinical isolates from adjacent hospital campuses serving distinct patient populations. *Antimicrob Agents Chemother* 2015;59:5331–9, <http://dx.doi.org/10.1128/AAC.00048-15>.
- [30] Ruiz SJ, Montealegre MC, Ruiz-Garbajosa P, Correa A, Briceño DF, Martínez E, et al. First characterization of CTX-M-15-producing *Escherichia coli* ST131 and ST405 clones causing community-onset infections in South America. *J Clin Microbiol* 2011;49:1993–6, <http://dx.doi.org/10.1128/JCM.00045-11>.
- [31] Sato T, Suzuki Y, Shiraishi T, Honda H, Shinagawa M, Yamamoto S, et al. Tigecycline nonsusceptibility occurs exclusively in fluoroquinolone-resistant *Escherichia coli* clinical isolates, including the major multidrug-resistant lineages O25b:H4-ST131-H30R and O1-ST648. *Antimicrob Agents Chemother* 2017;61:e01654-16, <http://dx.doi.org/10.1128/AAC.01654-16>.
- [32] Röderova M, Halova D, Papousek I, Dolejska M, Masarikova M, Hanulik V, et al. Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals, and the environment in the Czech Republic. *Front Microbiol* 2016;7:2147, <http://dx.doi.org/10.3389/fmicb.2016.02147>.