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Investigation of presence of O25B-ST131 clone and *in vitro* efficacy of temocillin in *escherichia coli* isolates

Kübra HACIEMINOĞLU ULKER[1,*], Dilara SOLMAZ[2], Kenza HAMRAOUİ[2], Huda ALKARKHI[2], Geys OMAR[2], Ahmed SADEK[2], Hamza A. ABDULSATTAR ABDULSATTAR[2], Yeliz TANRIVERDİ ÇAYCI[1]

[1]Department of Medical Microbiology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[2]Medicine Faculty Student, Ondokuz Mayıs University, Samsun, Türkiye

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Abstract

Escherichia coli ST131 isolates associated with fluoroquinolone and cephalosporin resistance have increased in the last ten years. This increase has led to the emergence of multidrug-resistant *E. coli* isolates, resulting in treatment failures for urinary tract infections. The increasing antimicrobial resistance in gram-negative bacteria and the scarcity of new antimicrobials have brought old antimicrobials, such as temocillin, back into consideration. Temocillin has significant advantages and may serve as an alternative to carbapenems in treating serious Enterobacterales infections, such as systemic urinary tract infections. This study aimed to determine the presence of the O25b-ST131 clone in fluoroquinolone-resistant *E. coli* isolates and assess temocillin resistance. *E. coli* isolates obtained from urinary tract samples of patients hospitalized in the Faculty of Medicine Hospital of Ondokuz Mayıs University were included in the study. The presence of clone O25b-ST131 in these isolates was investigated using PCR. In addition, temocillin susceptibility in these isolates was determined using the Kirby-Bauer disk diffusion method. According to the PCR results, the prevalence of *E. coli* O25b-ST131 isolates was 40.8%. The findings of the antimicrobial resistance tests revealed that all isolates had low levels of carbapenem and fosfomycin resistance (2.04% and 3.06%, respectively). The temocillin resistance rate was determined to be 50%. It is known that clone O25b-ST131 is associated with fluoroquinolone and cephalosporin resistance; therefore, a high prevalence of the O25b-ST131 clone was expected in fluoroquinolone-resistant *E. coli* isolates included in the study. Temocillin is an antimicrobial agent widely used in many European countries, particularly for treating carbapenem-resistant *E. coli* infections. However, in our study, high rates of temocillin resistance (50%) were observed, despite this agent not being used in our country yet. These high resistance rates could be related to cross-antimicrobial resistance.

Keywords: *Escherchia coli*, fluoroquinolone resistance, O25b-ST131, temocillin

1. Introduction

Urinary tract infections (UTIs) are the most common type of community- and hospital-acquired infections, referring to infections in the kidney, ureter, urethra, bladder, and urinary tract. UTIs are estimated to affect approximately 150 million people annually, resulting in over 6 billion dollars in health expenditures. *Escherichia coli* is responsible for 70-90% of UTIs, and extraintestinal pathogenic *E. coli* (ExPEC) is a significant causative factor of UTIs in both developed and developing countries (1-4).

In 2008, a previously unrecognized clonal group of *E. coli*, known as 'sequence type 131 (ST131)', was identified in nine countries across three continents, including Canada, France, Portugal, Spain, Switzerland, India, South Korea, Kuwait, and Lebanon. ST131 was recognized as the dominant extraintestinal pathogenic *E. coli* (ExPEC) strain worldwide. This clone, referred to as a 'high-risk pandemic clone,' plays a significant role in the global spread of antimicrobial resistance, making it an important target for global surveillance studies. The dominant serotype of the ST131 clone is O25b:H4, which belongs to the phylogenetic group B2. This clone produces CTX-M-15 type extended-spectrum beta-lactamase (ESBL), exhibits high virulence, and is named 'O25b-ST131' (5-9).

E. coli ST131 isolates are responsible for causing community and hospital-acquired urinary tract infections and bacteremia worldwide. Moreover, the ST131 clone has been reported to be associated with other diseases, including intra-abdominal and soft tissue infections, meningitis, osteoarticular infection, myositis, and septic shock (9-13).

In the past decade, the emergence of multidrug-resistant ExPEC strains, mainly due to the increasing number of *E. coli* ST131 isolates, has posed challenges in treating UTIs, leading to antimicrobial therapy failure and increased morbidity and mortality (14-19). Carbapenems and fosfomycin are often considered appropriate treatment options for *E. coli* ST131 isolates. However, the widespread use of these antimicrobials has also led to the dissemination of carbapenem and fosfomycin resistance genes, resulting in increased resistance (1). As a result, there has been a need to explore alternative treatment options. The lack of novel antimicrobials for treating

*Correspondence: yeliztanriverdi@gmail.com

multidrug-resistant gram-negative bacterial infections has led to the resurgence of old antimicrobials, such as temocillin (20,21). Temocillin is a narrow-spectrum penicillin with limited activity against gram-positive, antipseudomonal, and anaerobic bacteria. It is recommended as an alternative antimicrobial agent for treating enteric bacterial infections. Temocillin has several advantages, including high resistance to hydrolysis by multiple ß-lactamases, including ESBL and AmpC, and minimal risk of causing *Clostridium difficile* infection. Due to these favorable properties, temocillin may be considered an alternative to carbapenems in treating severe *Enterobacterales* infections, such as systemic urinary tract infections (22-24).

This study aimed to assess the presence of the O25b-ST131 clone and temocillin resistance in fluoroquinolone-resistant *E. coli* isolates obtained from urinary tract samples.

2. Materials and Methods

2.1. Bacterial Isolates, Identification, and Susceptibility Testing

A total of 98 randomly selected fluoroquinolone-resistant *E. coli* isolates obtained from mid-stream urine samples were included in this study. These isolates were collected (one isolate per patient) at the Medical Microbiology Laboratory of Ondokuz Mayıs University between September 2020 and September 2021. Bacterial identification was performed using Vitek MS's automated system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was conducted using the Vitek 2 Compact System (bioMérieux, Marcy l'Etoile, France). Prior to the detection of the O25b-ST131 clone, the boiling method was used for DNA extraction of the isolates, and the obtained DNAs were stored at -20°C for later use as template DNA for PCR.

2.2. Detection of O25b-ST131 Clone

The presence of the O25b-ST131 clone was detected by amplifying a 347 bp fragment of the pabB gene using primers *O25pabBspe*-F and *O25pabBspe*-R in all fluoroquinolone-resistant *E. coli* isolates. The primers used in this study were selected based on a literature search (25). PCR was performed with the following conditions: initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 5 seconds, annealing at 65°C for 10 seconds, and extension at 72°C for 5 minutes. The PCR products were then subjected to agarose gel electrophoresis on a 2% agarose gel. The DNA bands of the samples were compared with a 100 bp DNA marker and analyzed using an imaging instrument.

2.3. Temocillin Susceptibility Test

The temocillin susceptibility was determined using the Kirby-Bauer disk diffusion method, following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines on Mueller-Hinton agar. Disks containing 30 µg of temocillin were used. The zone diameters were interpreted according to the EUCAST breakpoints: isolates with zone diameters of ≥50 mm were considered susceptible, while those with zone diameters of <17 mm were considered resistant to temocillin (26).

2.4. Statistical Analysis

The data obtained from the study were analyzed using IBM SPSS Statistics software, version 22.0 (SPSS Inc., IL, USA). Descriptive statistics, including percentages, were used to present patients' demographic information, clinic distribution, and antimicrobial resistance rates. The relationship between the presence of the O25b-ST131 clone and antimicrobial resistance rates was analyzed using the Pearson Chi-square test. A p-value of <0.05 was considered statistically significant for the findings.

2.5. Limitations

One limitation of our study is that the minimum inhibitory concentration (MIC) value of temocillin was not determined by the microdilution method, which is considered the gold standard method for antimicrobial susceptibility testing.

3. Results

Demographic information of the patients included in the study is presented in Table 1. Of the total patients, 63.3% were female, and 36.7% were male. The distribution of urine samples based on the clinics are shown in Table 2, with 40.8% sent from internal medicine clinics and 25.5% from surgical clinics.

Table 1. Patients' demographic information

Variables		n (%)
Gender	Women	62 (63.3)
	Men	36 (36.7)
Age Groups	24-40 age	5 (5.1)
	41-65 age	36 (36.7)
	66+ age	57 (58.2)

Table 2. The distribution of samples according to clinics

Clinics	n (%)
Emergency	5 (5.1)
Surgery Clinics	8 (8.2)
Internal Medicine	31 (31.64)
Intensive Care	13 (13.26)
Infection Diseases	5 (5.1)
Chest diseases	3 (3.06)
Gynecology and Obstetrics Surgery	6 (6.1)
Cardiology	3 (3.06)
Neurology	4 (4.08)
Orthopaedics and traumatology	1 (1.02)
Oncology	9 (9.18)
Urology	10 (10.2)
Total	98 (100)

The resistance rates of the isolates to various antibiotics are as follows: 79.6% to third-generation cephalosporins, 55.1% to gentamicin, and 29.6% to trimethoprim-sulfamethoxazole. In contrast, the resistance rates to amikacin, fosfomycin, and carbapenems were lower, with 6.1%, 3.06%, and 2.04%, respectively. The temocillin disk diffusion test results showed that 50% of the 98 fluoroquinolone-resistant *E. coli* isolates were resistant to temocillin, while the other 50% were susceptible. The antimicrobial resistance rates of all isolates are presented in Table 3.

Table 3. The antimicrobial resistance rates of isolates

Antimicrobials	All of isolates (n:98)		O25b-ST131 isolates (n:40)		Non-O25b-ST131 isolates (n:58)		p-value*
	n	%	N	%	n	%	
Amikacin	6	6.12	3	7.5	3	5.17	0.158
Amoxicillin/Clavulanic Acid	58	59.18	26	65	32	55.17	0.331
Ampicillin	91	92.85	36	90	55	94.82	0.362
Ertapenem	2	2.04	1	2.5	1	1.72	0.789
Fosfomycin	3	3.06	-	-	3	5.17	0.237
Gentamicin	29	29.59	11	27.5	18	31.03	0.460
İmipenem	1	1.02	-	-	1	1.72	0.404
Meropenem	-	-	-	-	-	-	0.404
Nitrofurantoin	5	5.1	2	5	3	5.17	0.970
Piperacillin/Tazobactam	27	27.5	10	25	17	29.31	0.616
Cefixime	76	77.5	32	80	44	75.86	0.629
Ceftazidime	73	74.48	31	77.5	42	72.41	0.644
Ceftriaxone	75	76.53	32	80	43	74.13	0.501
Cefuroxime axetil	79	80.6	33	82.5	46	79.03	0.695
Cefuroxime	80	81.63	33	82.5	47	81.03	0.706
Trimethoprim/Sulfamethoxazole	54	55.1	23	57.5	31	53.44	0.692
Temocillin	49	50	22	55	27	46.5	0.411

*: The relation between the presence of O25b-ST131 clone and antimicrobial resistance rates was analyzed using Pearson Chi-square test. Statistically significant findings were assumed with $p < 0.05$

Upon amplification, 40.8% of the isolates were identified as *E. coli* O25b-ST131. The antimicrobial resistance rates of *E. coli* O25b-ST131 and non-O25b-ST131 isolates are shown in Table 3.

However, according to the statistical analysis, there was no statistically significant difference between O25b-ST131 and non-O25b-ST131 isolates in terms of antimicrobial resistance rates (Table 3).

4. Discussion

Urinary tract infections (UTIs) are infections associated with high morbidity and mortality. The most frequently isolated bacteria in UTIs are *E. coli*, responsible for approximately 70-90% of community-acquired UTIs (27). The emergence and rapid global spread of hypervirulent *E. coli* ST131 associated with antimicrobial resistance *seriously threaten* public health. ST131 is recognized as the predominant ExPEC strain worldwide. *E. coli* ST131 isolates are often resistant to broad-spectrum cephalosporins and FQs (7,28,29). Most studies investigating the ST131 clone have focused on broad-spectrum beta-lactamase (ESBL) producing or FQ-resistant isolates. The treatment options for these problematic isolates are limited. Therefore, it is crucial to monitor the prevalence of *E. coli* ST131 and the resistance of limited agents that can be used to treat bacteria belonging to this clonal group. This study aimed to determine the prevalence of the O25b-ST131 clone and investigate temocillin resistance in FQ-resistant uropathogenic *E. coli* isolates.

In studies conducted with uropathogenic *E. coli* isolates in China, Türkiye, and Iran, the prevalence of the O25b-ST131 clone was found to be 12.5%, 22%, and 24.7%, respectively (30-32). In a study conducted with uropathogenic FQ-resistant *E. coli* isolates in Japan, the prevalence of the O25b-ST131 clone was found to be >70% (33). The results of these studies support the existence of a high prevalence of O25b-ST131 in FQ-resistant isolates. On the other hand, studies reported that O25b-ST131 clone carriage is common in long-term hospitalized patients and that the prevalence of the clone was higher in inpatients than in outpatients. High *E. coli* ST131

carriage rates of 55%, 36%, and 24%, respectively, have been found in healthcare facilities in Ireland, the UK, and the USA. (34-36). In our study, the prevalence of the O25b-ST131 clone in FQ-resistant *E. coli* isolates was 40.8%. This high rate is expected because the isolates included in the study were FQ-resistant E. coli isolates obtained from inpatient samples.

Temocillin is a narrow-spectrum penicillin primarily active against the Enterobacterales order and resistant to many beta-lactamases, including most AmpC and ESBL. In the mid-2000s, ESBL-producing *Enterobacterales* isolates became widespread, and recently, carbapenem resistance has increased considerably. New treatment options were investigated for these reasons, and interest in this old antimicrobial has increased again (20, 37). The gold standard method for determining the temocillin susceptibility is the microdilution method. However, the Kirby-Bauer disk diffusion method can also be used to determine the temocillin susceptibility. In 1985 Fuchs et al. published the first interpretative criteria for temocillin susceptibility testing. According to Fuchs et al., with the 30 µg temocillin disk, 19 mm was the susceptible zone diameter breakpoint (38). Then, breakpoints for temocillin were published for the Clinical & Laboratory Standards Institute (CLSI), British Society for Antimicrobial Chemotherapy (BSAC), Comité de l'antibiogramme de la Société Française de Microbiologie (CA-SFM), and EUCAST (26, 39-41). The susceptibility diameter breakpoints for these guidelines are 19 mm, 12 mm, 20 mm, and 50 mm with the 30 µg temocillin disk, respectively.

In studies conducted with ESBL-producing *E. coli* isolates from Türkiye and France, temocillin resistance was reported as 20.7% and 28.7%, respectively (24,42). In a study conducted with uropathogenic *E. coli* isolates in Singapore, temocillin resistance was 7% (43). In another study conducted with uropathogenic *E. coli* isolates in Korea, temocillin resistance was reported as 9.2% in ciprofloxacin-resistant isolates and 1.5% in ciprofloxacin-susceptible isolates (44). In our study, temocillin resistance was found to be 50% in FQ-resistant *E. coli* isolates and 55% in FQ-resistant O25b-ST131 *E. coli* isolates. These results are relatively high, although temocillin is not used in Turkey. The high resistance rate may be due to cross-antimicrobial resistance. In addition, temocillin resistance was found to be relatively high compared to studies conducted in our country (24,45). This result may be because studies are based on different antimicrobial susceptibility tests, guidelines, and breakpoints. Our study was performed by the Kirby-Bauer disk diffusion method based on EUCAST breakpoints.

In conclusion, the prevalence of *E. coli* O25b-ST131 was determined to be 40.8% in our study. Since all isolates are FQ-resistant, the prevalence of O25b-ST131 is expected to be high in these isolates. According to the antimicrobial susceptibility results, carbapenem and fosfomycin resistance rates were still low in all isolates (2.04% and 3.06%, respectively). Although the resistance rates of these antimicrobials, which are essential for treatment, are pleasing, continuously monitoring the resistance rates is necessary. Moreover, significant resistance differences were not observed between O25b-ST131 and non-O25b-ST131 isolates. Temocillin is one of the critical agents used in many European countries, especially in treating carbapenem-resistant *E. coli* infections. Although this agent is not yet used clinically in Türkiye, a high rate of resistance to temocillin was found in our study. This high rate may be due to cross-antimicrobial resistance.

Ethical Statement

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Ondokuz Mayis University before the start of the study (Number: B.30.2.ODM.0.20.08.667-679-707, Decision Number: 2022/449, Date: 12/10/2022).

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: K.H.Ü., Y.T.Ç., Design: K.H.Ü., Y.T.Ç., Data Collection or Processing: K.H.Ü., D.S., K.M., H.U., G.O., A.S., H.A.A.A., Analysis or Interpretation: K.H.Ü., D.S., K.M., H.U., G.O., A.S., H.A.A.A., Y.T.Ç., Literature Search: K.H.Ü., Y.T.Ç., Writing: K.H.Ü., Y.T.Ç.

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