MAJOR ARTICLE

Emerging *Escherichia coli* O25b/ST131 Clone Predicts Treatment Failure in Urinary Tract Infections

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Background. We described the clinical predictive role of emerging *Escherichia coli* O25b/sequence type 131 (ST131) in treatment failure of urinary tract infection.

Methods. In this prospective observational cohort study, the outpatients with acute cystitis with isolation of *E. coli* in their urine cultures were assessed. All the patients were followed up for clinical cure after 10 days of treatment. Detection of the *E. coli* O25:H4/ST131 clone was performed by multiplex polymerase chain reaction (PCR) for phylogroup typing and using PCR with primers for O25b *rfb* and allele 3 of the *pabB* gene.

Results. In a cohort of patients with diagnosis of acute urinary cystitis, 294 patients whose urine cultures were positive with a growth of $>10^4$ colony-forming units/mL of *E. coli* were included in the study. In empiric therapy, ciprofloxacin was the first choice of drug (27%), followed by phosphomycin (23%), trimethoprim-sulfamethoxazole (TMP-SMX) (9%), and cefuroxime (7%). The resistance rate was 39% against ciprofloxacin, 44% against TMP-SMX, and 25% against cefuroxime. Thirty-five of 294 (12%) isolates were typed under the O25/ST131 clone. The clinical cure rate was 85% after the treatment. In multivariate analysis, detection of the O25/ST131 clone (odds ratio [OR], 4; 95% confidence interval [CI], 1.51–10.93; $P = .005$) and diabetes mellitus (OR, 2.1; 95% CI, .99–4.79; $P = .05$) were found to be significant risk factors for the treatment failure. In another multivariate analysis performed among quinolone-resistant isolates, treatment failure was 3 times more common among the patients who were infected with ST131 *E. coli* (OR, 3; 95% CI, 1.27–7.4; $P = .012$).

Conclusions. In urinary tract infections, the *E. coli* ST131 clone seems to be a consistent predictor of treatment failure.

Keywords. *E. coli*; ST131; treatment failure; urinary.

Urinary tract infection (UTI) is one of the most common bacterial infections, with a high global burden [1]. Patients with UTI are frequently given empiric therapy, and successful treatment has become more difficult because of the rapid spread of antibiotic resistance. *Escherichia coli* is the most common causative agent of acute cystitis, and fluoroquinolones are the most commonly prescribed class for empiric treatment of UTI [1, 2]. Over the last decade, the *E. coli* sequence type 131 (ST131) clone has emerged as an important human pathogen worldwide and is recognized as a pandemic clone [3–5]. It has been shown that *E. coli* strains in the ST131 group, in addition to being resistant to most β-lactam antibiotics, are frequently resistant to aminoglycosides and fluoroquinolones [6]. The ST131 clone is strongly associated with extended-spectrum β-lactamases (ESBLs), predominantly the CTX-M-15 type [4, 7]. Emergence of the ST131 clone posed a significant threat to human health because of its combination of successful spread, capability to withstand the effect of various antimicrobial agents, and possession of high numbers of virulence factors [3].

The emergence of ST131 strains has made UTI management more problematic, leading to discordant

Received 26 May 2014; accepted 19 October 2014; electronically published 6 November 2014.

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Clinical Infectious Diseases® 2015;60(4):523–7

DOI: 10.1093/cid/ciu864

antimicrobial therapy and increased morbidity and mortality [8]. Although there are well-designed studies about emerging epidemiology of ST131, to our knowledge the most important mechanisms of transmission and risk factors are not clearly understood. Moreover, clinical outcome of uropathogenic *E. coli* ST131 infections has not yet been reported. In this study, we aimed to describe the risk factors for treatment failure in acute cystitis and to detect the clinical impact of *E. coli* ST131 and other potential parameters.

MATERIALS AND METHODS

Study Population

Urine samples were collected from consecutive outpatients with acute cystitis who had at least 1 UTI symptom, such as fever, urgency, hematuria, and dysuria, in 2011 at the outpatient clinics of Baskent University Hospital in Ankara, Turkey. Among these patients, urine analyses were done and urine samples were cultured. Those patients who had *E. coli* isolated from their urine were included in the study. Empiric antibiotic treatment was started after collection of urine samples for urine analysis and culture testing. According to the susceptibility test results, the empiric treatment regimen was switched to an appropriate choice, if resistance was detected. Empiric antibiotics, and patient's age, sex, history of hospitalization and antibiotic use, catheterization, and comorbidities were recorded. All the patients were followed up by outpatient visits, if not possible by phone calls, for clinical cure 10 days after the start of the treatment. Failure of the treatment was defined as persistence of the symptoms after 10 days of treatment or detection of resistance to empiric antibiotic and switching to the appropriate choice. This study was approved by the institutional review board of Baskent University with the project number of KA11/107 and the decision number of 11/87.

Susceptibility Testing

Susceptibility testing to 23 antibiotics (ampicillin, cefazolin, gentamicin, amikacin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefepime, cefoxitin, trimethoprim-sulfamethoxazole [TMP-SMX], ceftriaxone, ciprofloxacin, levofloxacin, imipenem, meropenem, ertapenem, aztreonam, ceftazidime, cefotaxime, norfloxacin, nitrofurantoin, and fosfomycin) was performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. *Escherichia coli* multidrug resistance was defined as resistance to 1 or more agents in ≥3 classes of antibiotics.

ESBL production was evaluated according to CLSI criteria [9]. The β-lactamase–producing isolates were searched for CTX-M positivity by polymerase chain reaction (PCR), as described previously [10]. Isolates belonging to CTX-M group 1 were further analyzed for CTX-M-15–type lactamase by sequencing. Sequences were compared with those deposited in the National Center for Biotechnology Information database.

In phylogenetic analysis, the *ChuA* and *Yja* A genes and TspE4.C2 fragments of DNA were examined by multiplex PCR [11]. Detection of O25b/ST131 clonal group was done by PCR using primers for O25b *rfb* and allele 3 of the *pabB* gene, described previously [3]. To confirm these clonal assignments, selected isolates underwent multilocus sequence typing based on a protocol published on the University College Cork website (http://mlst.ucc.ie/mlst/). The partial sequences of 7 housekeeping genes were compared: *adk*, *fumC*, *recA*, *mdh*, *purA*, *gyrB*, and *icd*. DNA sequences were assembled and multiple alignment analyses were performed by using Applied Maths version 7.0, BioNumerics. The sequencing analyses confirmed the PCR typing of ST131 isolates.

Statistical Analysis

Comparisons of categorical variables were tested by χ^2 test, and continuous variables by *t* test. Univariate and multivariate analyses for the prediction of the risk factors of treatment failure were determined by logistic regression analysis. Independent variables related to patient risk factors and virulence factors were included in the model. The independent variables were age >60 years, antibiotic use within last 3 months, hospitalization within last year, surgery within last year, chronic heart disease, diabetes mellitus, chronic renal failure, ST131 clone, quinolone resistance, ESBL production, and multidrug resistance. Statistical analyses was performed using Stata software version 11, and statistical significance was set at $P < .05$.

RESULTS

In a cohort of patients with diagnosis of acute urinary cystitis, 294 patients whose urine cultures were positive with a growth of $>10^4$ colony-forming units/mL of *E. coli* were included in the study. In empiric therapy, ciprofloxacin was the first choice of drug (27%), followed by phosphomycin (23%), TMP-SMX (9%), and cefuroxime (7%). The resistance rate was 39% against ciprofloxacin, 44% against TMP-SMX, and 25% against cefuroxime. No resistance was detected against phosphomycin and carbapenems. Resistance to ≥3 different groups of antibiotics (β-lactams, aminoglycosides, quinolones, TMP-SMX) was defined as multidrug resistance and was detected in 107 (36%) *E. coli* isolates. ESBL producers accounted for 70 of 294 (24%) isolates. CTX-M-15 β-lactamase was detected in 40 isolates (14%). Thirty-five of 294 (12%) isolates were typed under the ST131 clone. In univariate analysis, use of quinolones and antibiotics other than quinolones within the last 3 months, hospitalization within last year, surgery within last year, multidrug resistance, ESBL production, and CTX-M-15 positivity were

Table 1. Risk Factors for Presence of Sequence Type 131 Clone

Risk Factor	ST131 Isolates (n = 35), No. (%)	Non-ST131 Isolates (n = 259), No. (%)	*P* Value
Patient risk factors			
Mean age (SD)	50 (17)	53 (20)	.318
Female sex	31 (89)	221 (85)	.607
Antibiotic use other than quinolones within last 3 mo	10 (29)	55 (21)	.345
Quinolone use within last 3 mo	13 (37)	48 (19)	.011
Hospitalization within the last year	16 (46)	60 (23)	.004
Operation within the last year	11 (31)	34 (13)	.005
Bacterial factors			
Quinolone resistance	42 (100)	79 (31)	<.001
Multidrug resistance	26 (74)	81 (31)	<.001
ESBL production	21 (60)	49 (19)	<.001
CTX-M-15 positivity	13 (37)	27 (10)	<.001

Abbreviations: ESBL, extended-spectrum β-lactamase; SD, standard deviation; ST131, sequence type 131.

Table 2. Univariate Analysis for Treatment Failure

Risk Factor	Treatment Failure (n = 35), No. (%)	No Treatment Failure (n = 259), No. (%)	*P* Value
Patient risk factors			
Mean age (SD)	56 (17)	53 (20)	.277
Female sex	39 (87)	213 (86)	.843
Antibiotic use within last 3 mo	26 (58)	100 (41)	.033
Quinolone use within last 3 mo	12 (27)	49 (20)	.287
Hospitalization within last year	19 (42)	57 (23)	.006
Operation within last year	13 (29)	32 (13)	.006
Bacterial factors			
Quinolone resistance	28 (62)	86 (35)	<.001
Multidrug resistance	30 (54)	86 (28)	<.001
Belonging to ST131 clone	14 (31)	21 (8)	<.001
ESBL production	17 (38)	53 (21)	.017
CTX-M-15 positivity	10 (22)	30 (12)	.067

Abbreviations: ESBL, extended-spectrum β-lactamase; SD, standard deviation; ST131, sequence type 131.

found to be significantly associated risk factors of infection by strain of the ST131 clone (Table 1).

The clinical cure rate was 85% after treatment. Treatment failure was significantly increased with quinolone use within last 3 months, hospitalization within last year, operation within last year, quinolone resistance, multidrug resistance, CTX-M-15 positivity, belonging to the ST131 clone, and ESBL production (Table 2). Statistically significant variables in univariate analysis were included in the multivariate analysis, and having diabetes mellitus and detection of the ST131 clone were found to be significant risk factors for treatment failure (Table 3).

Another multivariate analysis was performed among quinolone-resistant isolates for prediction of treatment failure. In this analysis, none of the risk factors, except belonging to the ST131 group, were found to be significant on treatment failure. Treatment failure was 3 times more common among the patients who were infected with ST131 *E. coli* than the patients who were infected with non-ST131 group (odds ratio [OR], 3.0; 95% confidence interval [CI], 1.27–7.4; $P = .012$). Among quinolone-resistant *E. coli* isolates, CTX-M-15 positivity was more common among ST131 isolates (38%) than non-ST131 isolates (19%) ($P = .029$).

DISCUSSION

UTIs due to *E. coli* cause considerable morbidity and mortality, and management is complicated by the increasing prevalence of antimicrobial resistance [4, 12]. Empiric treatment of UTIs became a difficult clinical problem, primarily because of increasing fluoroquinolone resistance in *E. coli* [13, 14]. Recently, a highly resistant clone of *E. coli*, ST131, was reported to be a global dominating clone in extraintestinal *E. coli* infections [4, 5, 15, 16]; however, clinical implications were not clearly defined. By this prospective clinical observational study among patients with acute urinary cystitis, we investigated independent risk factors, including ST131, related to the host and the pathogen that could have an effect on treatment failure.

The clinical cure rate was 85% after the treatment. In multivariate analysis, the failure was 4 times more common among the patients who had UTI with ST131 *E. coli* than the patients infected with non-ST131 isolates (OR, 4.0; 95% CI, 1.51–10.93; $P = .005$; Table 3). According to 1 retrospective study including 300 patients, cure rates and mortality did not differ between patients with ST131 vs non-ST131 *E. coli* infections [15]. Another study performed among 100 patients with *E. coli* bacteremia did not find an effect of the ST131 clone on fatality. For detection of such an effect of ST131 on fatality, a bigger sample size is needed. We demonstrated the impact of the ST131 clone as an independent factor on treatment failure, by controlling the potential confounding parameters. As Banerjee et al indicated, retrospective studies might be biased by the selection of the infected or colonized patients [15]. One of the strong points of our study was being prospective, which yielded precise discrimination of colonized and infected patients.

This study was performed in a region where fluoroquinolone resistance is >20% [13]. To describe the role of the ST131 clone

Table 3. Univariate and Multivariate Analyses for Risk Factors of Treatment Failure

	Univariate Analysis			Multivariate Analysis		
Risk Factor	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Patient risk factors						
Age >60	1.4	.74–2.64	.299	0.9	.44–2.13	.939
Antibiotic use within last 3 mo	1.9	1.04–3.8	.035	1.1	.51–2.46	.773
Hospitalization within last year	2.4	1.27–4.76	.008	1.4	.52–3.76	.503
Operation within last year	2.8	1.3–5.79	.008	1.4	.46–4.14	.567
Chronic heart disease	2	.98–4.39	.054	1.3	.53–3.36	.536
Diabetes mellitus	2	1.05–4.13	.034	2.1	.99–4.79	.05
Chronic renal failure	2.9	.69–12.01	.144	2.6	.57–11.98	.21
Bacterial factors						
Belonging to ST131 clone	4.9	2.26–10.62	<.001	4	1.51–10.93	.005
Quinolone resistance	3.1	1.62–6.02	.001	1.1	.33–3.64	.872
ESBL production	2.2	1.14–4.4	.019	0.9	.34–2.13	.737
Multidrug resistance	2.9	1.65–5.3	<.001	1.6	.51–4.82	.421

Abbreviations: CI, confidence interval; ESBL, extended-spectrum β-lactamase; OR, odds ratio; ST131, sequence type 131.

in treatment failure of infections with fluoroquinolone-resistant isolates (n = 114), we performed another multivariate analysis, including the entire host and bacterial risk factors. Among quinolone-resistant isolates, treatment failure was 3 times more common among the patients who were infected with ST131 *E. coli* than among those in the non-ST131 group (OR, 3; 95% CI, 1.27–7.4; $P = .012$). Detection of ST131 as a significant factor in both analyses overall and in the quinolone-resistant group enhances the predictive role of ST131 on treatment failure, as an independent strong parameter. It is also possible that the higher virulence of the ST131 clone because of adherence to urinary tract epithelial cells and persistence may explain the treatment failure among the patients infected with ST131 clones [17]. In regions where fluoroquinolone resistance is >20%, they are not suggested as the first choice in empiric treatment [14]; however, in our study, we found that fluoroquinolones were the first choice in empiric treatment. In UTI, resistance rates against antibiotics is high and there are limited alternatives to fluoroquinolones in treatment. Because there are few alternatives to fluoroquinolones in UTI, and overall resistance rate against all the antibiotics was high. Therefore, detection of the ST131 clone could be important by using rapid diagnostic tools such as matrix-assisted laser desorption/ionization mass spectrometry [18, 19].

Overall, 35 of 294 (12%) isolates were typed under the ST131 clone; recently, ST131 was detected in 10%–27% of *E. coli* isolates in various studies [4, 20, 21]. The rate of ESBL, predominantly CTX-M-15 positivity, was found to be higher in the ST131 clone compared with non-ST131 isolates (60% vs 19%, $P < .001$; Table 1). Our result was in parallel with some reports [22], but in other studies, the ST131 clone was reported to be higher in non-ESBL-producing *E. coli* [3]. This could be related to the high diversity of the ST131 clone, as with other *E. coli* clones. The presence of CTX-M-15 is significantly associated with resistance to first choice of antibiotics of empiric therapy (quinolones and β-lactams), and may favor a selection pressure for the CTX-M-15–containing strains [17]. Detection of high rate of CTX-M-15 in the ST131 clone might help us to predict the increasing rate of treatment failure proportional to the increasing rate of ST131 among other *E. coli* clones. In the ST131 clone, the multidrug resistance rate was 74% ($P < .001$), which was reported as 70% in another study [23]. The rate of TMP-SMX resistance was 71% ($P < .001$) in the ST131 clone; however, it was 56% in another report [23]. In our study, all ST131 isolates were quinolone resistant. In conclusion, in UTIs, the *E. coli* ST131 clone seems to be a consistent predictor of treatment failure, which was directly related to rapidly emerging global resistance. Early and rapid detection of *E. coli* ST131 may be useful in management of UTI.

Note

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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