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Short Communication

The clinical impact of ST131 H30-Rx subclone in urinary tract infections due to multidrug-resistant *Escherichia coli*

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ABSTRACT

In this study, risk factors for ST131 H30 and H30-Rx subclones among urinary tract infections (UTIs) caused by multidrug-resistant (MDR) *Escherichia coli* were described. Urine samples were collected from consecutive outpatients registered to the outpatient clinics of Başkent University Hospital (Ankara, Turkey) with complaints of acute cystitis in 2011. A total of 107 MDR *E. coli* isolates were included in the study. Of the 107 isolates studied, 26 (24.3%) were typed as ST131 clone. Extended-spectrum β-lactamase (ESBL)-producers accounted for 59 (55.1%) of the 107 isolates. Among the 59 ESBL-positive isolates, 18 (31%) were found to belong to the ST131 clone. Of the 18 ESBL-positive ST131 isolates, 17 (94%) were defined as H30 subclone, among which 16 (94%) represented the H30-Rx subclone. Among the 48 ESBL-negative isolates, 8 (17%) ST131 isolates were detected, 7 (88%) of which belonged to H30 subclone; 5 (71%) of the H30 subclone isolates were classified under H30-Rx subclone. In multivariate analysis, hospitalisation within last year was the only host risk factor associated with MDR *E. coli* ST131 H30-Rx subclone UTI (OR = 3.5, 95% CI 1.04–12.17; $P = 0.042$). CTX-M-15 production was found to be highly associated with the presence of ST131 H30-Rx subclone (OR = 4.8, 95% CI 1.54–15.32; $P = 0.007$). In conclusion, urinary MDR *E. coli* ST131 H30-Rx subclone was found to be important in the dissemination of MDR UTIs in the community. Approximately 20% of the MDR isolates were H30-Rx subclone. Infection with this subclone was found to be healthcare-associated.

1. Introduction

Multidrug-resistant (MDR) *Escherichia coli* strains are the leading cause of serious urinary tract infections (UTIs) in community and healthcare settings [1]. *Escherichia coli* clone ST131 has been detected at an alarming frequency worldwide [2,3] and is associated with high extraintestinal virulence and multidrug resistance [3,4]. It is also strongly associated with extended-spectrum β-lactamases (ESBLs), predominantly CTX-M-15 type [5]. The combination of antimicrobial resistance and virulence factors is an advantage for ST131 in being dominant among other *E. coli* clones that are less virulent and/or more susceptible [2]. Recently, Johnson et al. have shown that a subclone within ST131, called H30, has been spreading significantly among fluoroquinolone-resistant *E. coli* after the year 2000 [6]. Some isolates in the H30 subclone are defined as H30-Rx; they contain a variant of the type 1 fimbrial adhesin gene *fimH* and comprise almost one-half of all recent fluoroquinolone-resistant *E. coli* isolates [7,8].

There are only a few studies regarding the prevalence and resistance phenotypes of the H30 and H30-Rx ST131 subclones [9] and, to our knowledge, there is no study on the clinical impact of these subclones among patients with acute urinary cystitis infected with MDR *E. coli* isolates. In this study, clinical risk factors for ST131 clone as well as H30 and H30-Rx subclones among UTIs caused by MDR *E. coli* are described.

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2. Methods

2.1. Isolates

A total of 107 MDR *E. coli* isolates from consecutive patients with acute cystitis who presented to the outpatient clinics of Başkent University Hospital (Ankara, Turkey) in 2011 were included in the study. The MDR isolates were resistant to at least one agent in three or more different classes of antibiotics [β-lactams, fluoroquinolones, trimethoprim/sulfamethoxazole (SXT) and aminoglycosides].

2.2. Microbiological laboratory procedures

Susceptibility testing was performed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. ESBL production was determined according to CLSI standards [10]. For the detection of ESBLs and penicillinases, PCR amplification and sequencing were performed using primers for $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} [11]. Sequences were compared with those deposited in the National Center for Biotechnology Information (NCBI) database.

Identification of ST131 clonal group was done by PCR using primers for O25b *rfb* and allele 3 of the *pabB* gene as previously described [12]. The H30 subclone was identified by detection of amplified 354-bp PCR products of the *fimH* gene using fimH30F and fimH30R primers. The H30-Rx subclone was determined by PCR for identification of a specific single nucleotide polymorphism (SNP) (G723A) within the allantoin-encoding gene *ybbW* [7].

Strain relatedness of the ST131 isolates was assessed by repetitive sequence-based PCR (rep-PCR) (DiversiLab®; bioMérieux, Marcy-l'Étoile, France). Strains with similarity <95% were evaluated as unrelated.

This study was approved by the Institutional Review Board of Başkent University.

2.3. Statistical analysis

Categorical variables were compared by χ^2 test and continuous variables by *t*-test. Univariate and multivariate analyses for the prediction of risk factors for infection with ST131 H30-Rx clone among MDR *E. coli* isolates were determined by logistic regression. The independent variables were age >50 years, female sex, UTI within the last year, quinolone use within the last 3 months, hospitalisation within the last year, immunosuppression, chronic heart disease, diabetes mellitus and CTX-M-15 production. Statistical analyses were performed using STATA 11 (StataCorp LP, College Station, TX) and statistical significance was set as $P < 0.05$.

3. Results

In total, 107 consecutive MDR *E. coli* isolates were included in the study. Of the 107 isolates, 26 (24.3%) were typed as ST131 clone; 24 (92%) of the 26 ST131 isolates were H30 subclone, among which 21 (88%) were H30-Rx. ESBL-producers accounted for 59 (55.1%) of the 107 isolates. Among the 59 ESBL-positive isolates, 18 (31%) were found to be belong to the ST131 clone. Of the 18 ESBL-positive ST131 isolates, 17 (94%) were defined as H30 subclone, among which 16 (94%) were represented by H30-Rx subclone. Among the 48 ESBL-negative isolates, 8 (17%) ST131 isolates were detected, among which 7 (88%) belonged to H30 subclone; 5 (71%) of the H30 isolates were classified under H30-Rx subclone. The prevalence of ST131 isolates was not found to be significantly different between ESBL-positive and ESBL-negative isolates ($P = 0.096$). Similarly, the prevalence of H30 subclones among ESBL-positive and ESBL-negative isolates was not found to be different ($P = 0.079$).

The resistance rates for all of the isolates were as follows: ampicillin and nitrofurantoin, 100%; SXT, 91%; ciprofloxacin, 89%; norfloxacin, 86%; ampicillin/sulbactam (SAM), 66%; cefazolin, 60%; cefuroxime, 59%; ceftazidime, 51%; ceftriaxone, 51%; and gentamicin, 49%. No resistance was detected against fosfomycin and carbapenems. Both in ESBL-positive and ESBL-negative groups, no significant difference in antimicrobial resistance was detected between ST131 and non-ST131 isolates. All of the ST131 H30 isolates were found to be resistant to ciprofloxacin. ESBL-positive ST131 H30 isolates were significantly more resistant to ceftriaxone ($P = 0.002$), cefuroxime ($P = 0.023$) and SAM ($P < 0.001$) than ESBL-negative ST131 H30 isolates. CTX-M-15 β-lactamase was detected in 14 (58%) of 24 ST131 H30 isolates and the remaining isolates were found to be positive for CTX-M-3 ($n = 5$) or CTX-M-1 ($n = 3$) β-lactamases. CTX-M-15 β-lactamase positivity in ST131 H30 isolates was found to be higher than in non-ST131 isolates (58% vs 30%; $P < 0.012$). The positivity of TEM-1 among ST131 H30 isolates was 35%, and no SHV was detected in any of the strains.

The majority of the ST131 isolates were found to be different from each other by rep-PCR fingerprinting profiles. However, eight strains were clustered together with ≥95% similarity index. One ST131 strain could not be recovered by subculturing and was not included in the rep-PCR analysis. All of the strains in the cluster were identified as H30-Rx; CTX-M-15 production was detected in four of the eight isolates (Fig. 1).

Hospitalisation within the last year was found to be the only significant host risk factor associated with MDR *E. coli* ST131 H30-Rx subclone UTI in univariate (Table 1) and multivariate analysis [odds ratio (OR) = 3.5, 95% confidence interval (CI) 1.04–12.17; $P = 0.042$] (Table 2). CTX-M-15 production was found to be a consistent predictor of ST131 H30-Rx infection in univariate and multivariate analyses (OR = 4.8, CI 1.54–15.32; $P = 0.007$) (Table 2).

4. Discussion

The emergence of MDR *E. coli* strains complicates the management of UTIs due to its spread through populations and the lack of effective therapy [5]. The powerful infectious capacity of ST131 H30 subclone generally associated with its high transmissibility, its ability to colonise the urinary epithelium and, moreover, its ESBL-producing ability enhances the development of recurrent UTIs and sepsis [6,8]. We described the clinical impact of urinary MDR *E. coli* ST131 H30-Rx subclone among patients with acute cystitis. This study was unique because it was performed among a MDR *E. coli* population and integrated detailed clinical and molecular data.

The prevalence of ST131 clone differs according to region (4% in France [13], 27% in the USA [6] and 12% in Turkey [5]). In a study performed among MDR *E. coli* from various sources, the prevalence of ST131 reached 67–69% of fluoroquinolone- and extended-spectrum cephalosporin-resistant isolates [6]. However, H30 subclone was reported as a predominant subclone of ST131, and H30-Rx was the predominant sublineage of H30 that contains extensively drug-resistant isolates in all of the studies [4]. In the current study, the prevalence of ST131 clone was 24% of urinary MDR *E. coli* isolates, and H30 and H30-Rx subclones were dominant. It was reported that H30-Rx subclone was associated with extensively antimicrobial-resistant infections and that isolates were found as overwhelmingly quinolone resistant [4,7]. In the current study, H30 and H30-Rx subclones were found to be 100% resistant to quinolones, and the CTX-M-15-producing isolates were highly prevalent in these clones.