Molecular epidemiology of bloodstream-associated *Escherichia coli* ST131 H30-Rx subclone infection in a region with high quinolone resistance

Fusun Can,1 Ozlem Kurt-Azap,2 Elif Nurtop,1 Pelin Ispir,1 Ceren Seref1 and Onder Ergonul3

1Koc University, School of Medicine, Clinical Microbiology Department, Istanbul, Turkey
2Baskent University, School of Medicine, Department of Infectious Diseases, Ankara, Turkey
3Koc University, School of Medicine, Infectious Diseases Department, Istanbul, Turkey

Bloodstream infections caused by *Escherichia coli* ST131 and ST131 H30-Rx subclones have emerged worldwide. This study was carried out to evaluate the prevalence of the ST131-Rx subclone and characterize the virulence properties of the Rx isolates among the bloodstream *E. coli* isolates. A total of 297 non-duplicated *E. coli* bloodstream isolates were studied. Antibiotic susceptibilities were tested using the disc diffusion method. PCR amplification and sequencing was used to identify ST131 and H30-Rx, the virulence gene, the β-lactamase and virotype. Quinolone resistance among bacteraemic *E. coli* strains was 51%, and it was 98% among *E. coli* ST131 isolates. The ST131 isolates accounted for 16% (49) of all isolates and all ST131 isolates belonged to the extraintestinal pathogenic *E. coli*. The proportion of H30 subclone among the ST131 isolates was 98%, and 75% of H30 isolates belonged to the H30-Rx subclone. The prevalence of ST131 increased from 13 to 23% in 4 years; however, there was a decrease in the ratio of H30-Rx infections. CTX-M-15 was detected in 85% of ST131 and all of the H30-Rx isolates. The virulence genes associated with adhesion, cell protection, iron uptake and toxins (*papA*, *iha*, *kpsMTII*, *iut* and *sat*) were more common in ST131 than in non-ST131 isolates. Most of the ST131 and H30-Rx isolates were of the C virotype. All *papA*-positive isolates were in virotype C. The *E. coli* ST131 clone has increased rapidly among bloodstream isolates. However, a decrease in the proportion of the H30-Rx subclone in the quinolone-resistant population suggests the possibility of dissemination of other virulent and quinolone-resistant subclones in hospital settings.

INTRODUCTION

*Escherichia coli* is one of the major causative agents of bloodstream infections (BSI). Management of BSIs caused by *E. coli* has become complicated due to the emergence of antimicrobial resistance and highly virulent clones (Johnson et al., 2013; Peirano & Pitout, 2014; Rogers et al., 2011). Several studies in the past two decades have reported antimicrobial resistance of *E. coli* strains against quinolones, cephalosporins and carbapenems (Dhanji et al., 2010; López-Cerero et al., 2014; Olesen et al., 2013; Wu et al., 2014).

Extraintestinal pathogenic *E. coli* (ExPEC) are strains that carry special virulence factors and usually cause invasive infections. Numerous factors that influence the ExPEC pathogenesis include invasiveness, colonization, intake of nutritional elements, and stimulation of inflammation (Banerjee & Johnson, 2014). Johnson et al. (2000) and Banerjee & Johnson (2014) recently identified H30 and H30-Rx subclones of ST131. The H30-Rx subclone accounts for the majority of fluoroquinolone-resistant or extended spectrum β-lactamase (ESBL)-producing *E. coli* strains (Colpan et al., 2013). Blanco et al. (2013) described a classification system for ST131 based on four virulence factors (*afa*, *iroN*, *ibeA* and *sat*) and reported four virotypes that are associated with different epidemiological origins, based on multidrug resistance and global distribution.

A detailed subST analysis of ST131 isolates is important to understand the ongoing emergence of fluoroquinolone-resistant *E. coli*. However, there is limited information available on the global distribution, virotypes and
characteristics of ST131 sublineages of *E. coli* isolated from bloodstream infections. This study was designed to describe the characteristics of the ST131 H30-Rx sublineage isolated from bloodstream infections in a region with high rates of quinolone resistance.

**METHODS**

A total of 297 non-duplicated consequential *E. coli* strains isolated from patients with bloodstream infections at the Baskent University Hospital, Ankara, during a three-year period from 1 January 2010 to 1 July 2013 were included in this study. Susceptibility testing was performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M2-A11) (CLSI, 2010). ESBL production was determined based on the CLSI standards (CLSI, 2010). PCR amplification and sequencing for CTX-M, TEM and SHV were carried out using primers as described by Adams-Sapper *et al.* (2013). Sequences were compared with those deposited in the National Center for Biotechnology Information (NCBI) database.

For phylogenetic analysis, chuA, yjaA and TspE4.C2 fragments of DNA were tested using multiplex PCR (Johnson *et al.*, 2014). The ST131 clonal group was detected by PCR using primers for O25b rfb and allele 3 of the *pabB* gene as described by López-Cerero *et al.* (2014). The H30 subclone was detected by identifying a 354 bp PCR product of the *fimH* gene using *fimH*30F and *fimH*30R primers (Colpan *et al.*, 2013). The H30-Rx subclone was identified using a single-nucleotide polymorphism (SNP)-based PCR to detect a specific SNP (G723A) within the allantoin-encoding gene *ybbW* (Banerjee *et al.*, 2013).

PCR was used to test the presence of eight virulence genes in all isolates: *iut* (ferric aerobactin receptor), *sat* (autotransported protein, which acts as a proteolytic toxin), *papA* (major structural subunit and antigenic determinant of F fimbrae) and *kpsMTII* (Group II capsular polysaccharide synthesis) as described by Johnson & Stell (2000) and Johnson *et al.* (2000). The ExPEC was defined based on the criteria of Johnson *et al.* (2003). Multiplex PCR was used to identify virotypes by including *afa*, *ivoN*, *ibeA* and *sat* genes (Blanco *et al.*, 2013).

**Statistical analysis.** Comparisons between the categorical variables were analysed by the chi-squared test and a t-test was used to analyse continuous variables. STATA 11 was used for statistical analyses and *P*<0.05 was considered statistically significant.

**RESULTS**

The mean age of the patients was 55 years (SD 21), and 60% were female. The mean time to *E. coli* isolation from blood after admission to the hospital was 10 days (SD 13), and the case fatality rate was 16%. The ST131 clone accounted for 16% (49) of all isolates. ESBL positivity of all isolates was 45%. In addition, 31% of the ESBL-producing strains belonged to the ST131 clonal group. The proportion of H30 subclone in the ST131 clonal group was 98%, and 75% of the H30 isolates belonged to the H30-Rx subclone. The proportion of ST131 clones among the *E. coli* isolated from the bloodstream infections increased from 13% to 23% during the study period. The H30-Rx isolates accounted for 92%, 45%, 85% and 75% of the ST131 clones isolated each year (Fig. 1). The antibiotic resistance spectrum of ST131 and non-ST131 strains is presented in Table 1. The isolates in the H30-Rx subclone did not differ from the non-H30-Rx ST131 strains in their resistance to different antimicrobials. Overall quinolone resistance among bacteraemic *E. coli* strains between 2010 and 2013 was 51%, and it was 98% among *E. coli* ST131 strains (Table 1). Among the ST131 isolates, 84% were ESBL producers, and the molecular characterization of the ESBL genes showed that 85% of the isolates were positive for CTX-M-15 and 57% were positive for the TEM-1 β-lactamases. All of the ST131-H30Rx subclone isolates were CTX-M-15-positive.

The majority of the ST131 isolates carried virulence genes associated with adhesion (*papA, iha*), iron acquisition (*iut*), cell protection (*kpsMTII*) and autotransporter

**Table 1.** Resistance of ST131 and non-ST131 bloodstream infection isolates to some antimicrobials

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total isolates (n=297)</th>
<th>ST131 (N=49) n (%)</th>
<th>Non-ST131 (N=246) n (%)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/ clavulanate</td>
<td>17/19 (89)</td>
<td>37/78 (47)</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>Ampicillin/ sulbactam</td>
<td>41/44 (93)</td>
<td>99/197 (50)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>31/39 (80)</td>
<td>59/156 (38)</td>
<td>&lt;0.002*</td>
<td></td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>47/48 (98)</td>
<td>90/216 (42)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Piperacillin/ tazobactam</td>
<td>19/48 (40)</td>
<td>35/214 (16)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>41/49 (82)</td>
<td>99/233 (42)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>39/47 (83)</td>
<td>90/188 (49)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>14/15 (93)</td>
<td>26/55 (47)</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>47/48 (98)</td>
<td>90/217 (41)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant.*
toxin production (sat) (Table 2). Further, all of the ST131 isolates could be characterized as ExPEC, but only 73% of non-ST131 isolates could be characterized as ExPEC based on Johnson’s criteria (Johnson et al., 2003) (P<0.01). Among the H30-Rx subclone, 100% of the isolates were positive for the virulence gene iut while 19%, 98%, 98% and 92% were positive for papA, sat, iha and kpsMTII, respectively. The isolates belonged to three (virotype A, B and C) out of the four virotypes specified for E. coli ST131. Virotype C was the most prevalent (61%), followed by virotypes A (38%) and B (2%). None of the isolates belonged to virotype D. In the H30-Rx subclone, 53% of the isolates belonged to virotype C and 47% were virotype A. There was no significant difference between the production of CTX-M β-lactamase (89% vs 77%, P=0.089) and the antibiotic susceptibilities of the isolates that were categorized under virotype A and virotype C. All papA-positive ST131 isolates were virotype C.

DISCUSSION

Highly resistant and virulent ST131 E. coli strains have been identified in the bloodstream of patients worldwide (Can et al., 2015; Johnson et al., 2013). A high rate of quinolone resistance among bacteraemic E. coli strains (51%) and especially among E. coli ST131 strains (98%) limits the use of quinolones in clinical practice. The E. coli ST131 H30-Rx sublineage has emerged in the past 10 years and this sublineage is strongly associated with sepsis (Price et al., 2013; Santos et al., 2013). In this study, the overall prevalence of ST131 among the bloodstream isolates from the past 4 years was found to be 16% with an upward trend. Adams-Sapper et al. (2013) reported that 20% of the E. coli blood isolates from San Francisco were in the ST131 clone. A study from Canada reported that the proportion of ST131 was 46% among the fluoroquinolone-resistant bacteraemia isolates with an upward trend and rapid influx of the H30-Rx clone (Peirano & Pitout, 2014). In Taiwan, ST131 was reported to account for 29.5% of the ESBL-producing blood isolates (Chung et al., 2012) and 5.9% of the non-ESBL-producing isolates (Wu et al., 2014). A recent study in Korea reported that 30.9% of the ST131 bloodstream isolates exhibit a high resistance to ciprofloxacin (Cho et al., 2015).

H30-Rx subclone is known to exhibit high resistance to quinolones and is a CTX-M-producer (Banerjee et al., 2013; Olesen et al., 2014; Peirano & Pitout, 2014; Price et al., 2013). Peirano & Pitout (2014) have reported that a rapid influx of the H30-Rx clone is responsible for the rise in quinolone resistance. Our study was conducted in a region where quinolone resistance rates were high. Therefore, the H30-Rx subclone was highly resistant to antimicrobials and its CTX-M-15 production did not differ from the non-H30 ST131 isolates. Despite an increase in resistance to quinolones, a drop in the proportion of H30-Rx suggests that there might be other resistant subclones of ST131 responsible for high quinolone resistance. Previous studies have reported that virulence genes related with extraintestinal pathogenicity were more common in ST131 than in non-ST131 isolates (Blanco et al., 2009; Johnson et al., 2010). Moreover, isolates associated with bacteraemia are known to possess additional virulence factors to the ExPEC isolates obtained from other sites of the human body (Ewers et al., 2007). In a murine sepsis model, the presence of pap, vat, kpsMTII and iheA in any ExPEC strain was found to be strongly associated with virulence (Johnson et al., 2012). Another study published recently showed that the ST131 clone and aha/dra were associated with high virulence in bloodstream isolates (Ciesielczuk et al., 2015). In our study, both ST131 and H30-Rx subclones in the bloodstream isolates exhibited high prevalence of virulence genes and extensive ExPEC. Virotype C was the most common virotype in our study, which was also the most prevalent type in other countries in Europe and in the USA (Blanco et al., 2013; Johnson & Stell, 2000). We found that virotype C is the predominant virotype among the H30-Rx subclone isolates. ExPEC isolates are known to be able to colonize host surfaces, and invade host tissues using specific virulence factors. Virotype C was identified based on its high virulence (Blanco et al., 2013). In this study, the antibiotic resistance and CTX-M positivity of virotype C did not differ from those of the virotype A isolates. While we did not survey all known E. coli adhesins by PCR, the identification of virotype C among all papA-positive ST131 isolates is possibly an indicator of strong adhesion ability of this virotype.

| Table 2. Virulence genes of E. coli bloodstream isolates belonging to ST131 and non-ST131 clonal groups |
|--------------------------------------------------------|---------------------------------|------------------|------------------|------------------|
| Virulence factor | ST131 (N=49) n (%) | Non-ST131 (N=248) n (%) | P value |
| Adhesin | iut | 8 (16) | 97 (39) | 0.002 |
| Iron uptake | iha | 48 (98) | 68 (27) | <0.001 |
| Toxin | sat | 49 (100) | 165 (67) | <0.001 |
| Cell protection | kpsMTII | 47 (96) | 46 (19) | <0.001 |

CONCLUSIONS

E. coli ST131 clone infection is rapidly increasing among bloodstream isolates worldwide. However, the decreasing ratio of H30-Rx subclone in the quinolone-resistant population suggested that there might be other virulent and quinolone-resistant subclones disseminating this infection in hospital settings.
ACKNOWLEDGEMENTS

The authors declare no conflict of interest. No external funding was provided for this study. The study was performed at the Medical Microbiology Laboratory of Koc University, School of Medicine.

REFERENCES


