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Short communication

# Prevalence of CTX-M extended-spectrum beta-lactamases and sequence type 131 in Korean blood, urine, and rectal *Escherichia coli* isolates

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ABSTRACT

A high proportion of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* are of the ST131 lineage, but there are few estimates of ST131 prevalence among ESBL-negative *E. coli*. Without this information, it is difficult to evaluate the contribution of the ST131 lineage to the emergence and spread of ESBL *E. coli*. A total of 1658 *E. coli* isolates were collected at Gachon University Gil Medical Center in Korea from 2006 to 2008. The antibiotic resistance profile was determined for all isolates, and ESBL-positive isolates were screened for the presence of CTX-M-type ESBLs. All ESBL-positive (n = 84) and a representative sample of ESBL-negative (n = 100) isolates were screened for O25b-ST131 using a PCR-based assay. The isolates were further classified on the basis of *fumC* and *fimH* types, which allowed for a comparison of the two typing methods. 5.7% of isolates were ESBL-positive, 87% of which contained CTX-M-type ESBLs. There was no significant difference in the prevalence of ST131 between ESBL-positive and -negative groups; 14% of ESBL-positive isolates and 9% of tested ESBL-negative isolates were ST131 by CH-typing. ST131-positive isolates harbored CTX-M-1-group ESBLs (including CTX-M-15) more frequently than other CTX-M types, and exhibited greater levels of antibiotic resistance than non-ST131 isolates. Furthermore, a number of isolates identified as O25b-ST131 by PCR corresponded to non-ST131 sequence types by CH-typing, emphasizing the need to consider the testing method when comparing reported prevalences of ST131.



## 1. Introduction

*Escherichia coli* is the most common cause of urinary tract infections (UTI) and a frequent cause of bloodstream infections. UTI treatment is increasingly complicated due to the spread of antibiotic resistant organisms. Of particular concern are the extended-spectrum beta-lactamases (ESBL), which are resistant to penicillins and oxyimino-cephalosporins (Jarlier et al., 1988). The CTX-M group of ESBLs is currently the dominant type of ESBL observed in *E. coli* (Livermore et al., 2007). CTX-M-containing isolates are often multidrug-resistant, especially to the UTI treatments of choice: fluoroquinolones and trimethoprim-sulfamethoxazole (Hooton, 2012; Johnson et al., 2010; Kallen et al., 2006; Rogers et al., 2011).

The increasing prevalence of CTX-M type ESBLs among *E. coli* isolates, specifically type CTX-M-15, is attributed to the spread of sequence type 131 (ST131) (Coque et al., 2008; Kang et al., 2013; Peirano and Pitout, 2010). Due to the initial association with ESBLs, the majority of ST131 studies have described isolates that have ESBL resistance, or compare matched sets of resistant and susceptible isolates (Nicolas-Chanoine et al., 2014). In order to understand the role that ST131 has played in the spread of ESBLs, it is necessary to better estimate the prevalence of ST131 among ESBL-negative isolates.

As sequence type assignment using multi-locus sequence typing (MLST) is time-consuming and expensive, several techniques have been developed which aim to identify ST131 isolates using PCR and/or sequencing of selected genes (Nicolas-Chanoine et al., 2014). For example, Clermont et al. developed a PCR-based assay for an O25b-ST131-specific polymorphism in the *pab*B gene (Clermont et al., 2009). Weissman and coworkers proposed the use of CH-typing, determined by sequencing of internal fragments of *fimH* and *fumC*, to identify sequence types and partition them into subgroups (Weissman et al., 2012). While a comparison of the three MLST schemes and the corresponding CH-types is available, most studies make use of a single typing

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method for reasons of practicality (Clermont et al., 2015). In order to understand the effect of different typing methods on the observed prevalence of ST131 among clinical isolates, we used both a PCR-based assay for O25b-ST131 and CH-typing.

Herein, we present the prevalence of ST131 among all ESBL-positive, and a random sample of ESBL-negative blood, urine, and rectal *E. coli* isolates obtained from the Gil Medical Center in Korea between 2006 and 2008. Further, we compare the antibiotic resistance profiles and presence of CTX-M among ST131 and non-ST131 isolates.

## 2. Methods

### 2.1. Bacterial strains

The entire 2006–2008 collection from Gachon University Gil Medical Center in Korea consisted of 94 ESBL-positive isolates (76 urinary, 17 blood, and 1 rectal) and 1564 ESBL-negative isolates (707 urinary, 373 blood, and 484 rectal) as described previously (Park et al., 2014). For the current study, we included all viable ESBL-positive isolates (66 urinary, 17 blood, and 1 rectal) and a random sample of ESBL-negative isolates (using the RAND function in Excel) to represent the source distribution in the original collection; 24 blood isolates, 45 urinary isolates, and 31 rectal isolates.

The collection includes four categories of *E. coli* isolates: 1) All *E. coli* positive blood cultures from inpatients with bacteremia during January 2006 to December 2008. 2) All *E. coli* positive urinary cultures from patients with urinary tract infections (UTIs), defined as the presence of greater than $10^5$ CFUs/mL bacterial growth collected from a midstream specimen between December 2006 and December 2008. 3) *E. coli* urinary cultures from asymptomatic UTI patients, using the same definition and dates as in (2). 4) *E. coli* rectal isolates from healthy individuals who attended the Health Promotion Center of Gil Medical Center between September and December 2007. Isolates were frozen at −80 °C in Glycerol/Luria Broth (1:1) until further testing for this study.

### 2.2. Susceptibility testing

Rectal isolates were initially screened for *E. coli* with UriSelect media (Bio-Rad). Species were identified using the VITEK system (bioMérieux), and all 1658 isolates were screened for susceptibility to amikacin, ampicillin, cefotaxime, ciprofloxacin, gentamicin, imipenem, and trimethoprim-sulfamethoxazole using the disk diffusion method. ESBL producing isolates were identified using the microdilution method. Results were classified according to the CLSI criteria (CLSI, 2010).

### 2.3. PCR detection of CTX-M ESBL

Primers designed to amplify all known CTX-M variants were used (Pagani et al., 2003). PCR was carried out in 25 μL volumes using 12.5 μL GoTaq DNA polymerase (Promega), 9.5 μL water, 2.5 μL template DNA (extracted by boiling lysis), and CTX-M forward and reverse primers to a final concentration of 200 nM using the published conditions (Pagani et al., 2003). The PCR products were run on agarose gel, the resulting bands were purified (QIAquick gel extraction kit, QIAGEN), and sequenced (University of Michigan DNA Sequencing Core) using forward primers to determine the CTX-M group present. CTX-M types were determined using NCBI BLAST to compare known types with the sequencing results (Bush et al., 2015). Statistical analysis was done using SPSS (IBM, version 22) and OpenEpi. Significance was determined using the Chi-square test.

### 2.4. PCR detection of O25b-ST131

Isolates were screened for the presence of O25b-ST131 *E. coli* using primers identified by Clermont et al. (Clermont et al., 2009). PCR was carried out in 25 μL volumes, with 12.5 μL GoTaq DNA polymerase (Promega), 5 μL water, 2.5 μL template DNA (extracted using QIAcube, QIAGEN), and 2.5 μL each of 10 μM *pab*B and *trp*A. The PCR reaction was performed under the following conditions: initial denaturation at 94 °C for 4 min, 30 cycles of 5 s at 94 °C, 10 s at 65 °C, 1 min at 72 °C, followed by a final extension at 72 °C for 5 min. Results were visualized on agarose gels. Known O25b-ST131 and K-12 *E. coli* were used as positive and negative controls, respectively. A subset of the samples was previously typed by MLST and served as additional controls (Park et al., 2014).

### 2.5. fumC/fimH typing

CH-typing was performed using the published conditions (Weissman et al., 2012). PCR reactions were carried out with 12.5 μL GoTaq DNA polymerase (Promega), 2.5 μL forward and reverse primers, 2.5 μL template DNA (extracted using QIAcube, QIAGEN), and 2.5 μL water. The PCR products were run on agarose gels to confirm a band of the expected size. Subsequently, the products were purified (QIAquick PCR purification kit, QIAGEN) and sequenced with forward and reverse primers (University of Michigan DNA Sequencing Core). The resulting sequences were trimmed and aligned using CodonCode. *fumC* and *fimH* types were assigned using the available web-services (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli and https://cge.cbs.dtu.dk/services/FimTyper-1.0/) and NCBI BLAST, and compared to published CH-types (Tchesnokova et al., 2013; Weissman et al., 2012). Significance was determined using the Chi-square test or Fisher's exact test as appropriate.

## 3. Results and discussion

### 3.1. Prevalence of ESBL and ST131 among tested isolates

Overall, 5.7% of the collection's isolates had the ESBL phenotype. The ESBL phenotype was significantly more common among urinary isolates than blood isolates (9.7% versus 4.4%, $p = 0.0014$) and least common among rectal isolates (0.2%). These results are consistent with a previous report from Korea (Ko et al., 2013). Based on CH-types, there was no significant difference in the prevalence of ST131 by ESBL-phenotype or isolate source. The prevalence of ST131 was 14.3% (12/84) among ESBL-positive isolates, and 9% (9/100) among ESBL-negative isolates ($p = 0.26$). These values are near the range reported in previous Korean studies of ESBL-positive *E. coli* (19.7% to 36.2%, by MLST) (Kang et al., 2013; Kim et al., 2013; Park et al., 2012; Shin et al., 2011).

Within the ESBL-positive isolates, 15.2% (10/66) of urine isolates and 11.8% (2/17) of blood isolates were ST131 by CH-typing ($p = 1.0$). Within the ESBL-negative isolates, 15.6% (7/45) of urine isolates and 4.2% (1/24) blood isolates were ST131 ($p = 0.31$). Only 1 rectal isolate in the tested subset was ST131 (Fig. 1). With regard to CH-types, 9/12 ESBL-positive isolates had CH-type 40–30, 1 was type 40–41, 1 was type 40–29, and 1 was *fumC* type 40 and *fimH* null. To the best of our knowledge, CH-type 40–29 has not been previously described as ST131, however *fimH* type 27 has been found in ST131 isolates (Paul et al., 2013). *fimH* type 29 differs from *fimH* type 27 by only 1 base pair within the CH-typing region; therefore the isolate was assumed to be ST131. Within the ESBL-negative isolates, 5/9 were CH-type 40–30 and 4 were type 40–41.

### 3.2. Association between ST131 and CTX-M

Almost all of the ESBL-positive isolates carried CTX-M (87%); there was no significant difference in prevalence by source—although the one ESBL positive rectal isolate did not carry CTX-M. ST131 positive isolates contained CTX-M-1 group enzymes (including CTX-M-15) more frequently than other ESBL isolates (6/9 or 67% versus 32/64 or 50%).