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<td><strong>Author(s)</strong></td>
<td>Ho, PL; Chu, YPS; Lo, WU; Chow, KH; Law, PYT; Tse, CWS; Ng, TK; Cheng, VCC; Que, TL</td>
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High prevalence of *Escherichia coli* sequence type 131 among antimicrobial-resistant *E. coli* isolates from geriatric patients

Pak-Leung Ho1*, Yuki Pui-Shan Chu2, Wai-U Lo1, Kin-Hung Chow1, Pierra Y. Law1, Cindy Wing-Sze Tse3, Tak-Keung Ng4, Vincent Chi-Chung Cheng1, Tak-Lun Que2

1Department of Microbiology and Carol Yu Centre for Infection, University of Hong Kong, Hong Kong SAR, PR China
2Department of Clinical Pathology, Tuen Mun Hospital, Hong Kong SAR, PR China
3Department of Clinical Pathology, Kwong Wah Hospital, Hong Kong SAR, PR China
4Department of Clinical Pathology, Princess Margaret Hospital, Hong Kong SAR, PR China

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*Correspondence: Pak-Leung Ho, Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Pokfulam, Hong Kong, China.

Tel: +852 22554892; Fax: +852 28551241.

E-mail address: plho@hkucc.hku.hk (P.L. Ho)
ABSTRACT

Previous work on the subclones within *Escherichia coli* ST131 predominantly involved isolates from Western countries. This study assessed the prevalence and antimicrobial resistance attributed to this clonal group. A total of 340 consecutive, nonduplicated urinary *E. coli* isolates originating from four clinical laboratories in Hong Kong in 2013 were tested. ST131 prevalence among the total isolates was 18.5% (63/340) and was higher among inpatients isolates (23.0%) than outpatient isolates (11.8%, *P*<0.001); and higher among isolates from patients aged ≥65 years than from patients aged 18-50 years and 51-64 years (25.4% vs. 2.4% and 4.0%, respectively, *P*<0.001). Of the 63 ST131 isolates, 43 (68.3%) isolates belonged to the H30 subclone, whereas the remaining isolate belonged to H41 (n=17), H54 (n=2) and H22 (n=1). All H30 isolates were ciprofloxacin-resistant of which 18.6% (8/43) belonged to the H30-Rx subclone. Twenty-six (41.3%) ST131 isolates were ESBL-producers of which 19 had *bla*\(_{CTX-M-14}\) (12 non-H30-Rx, two H30-Rx and five H41), six had *bla*\(_{CTX-M-15}\) (five non-H30-Rx and one H30-Rx) and one was *bla*\(_{CTX-M}\) negative (H30). In conclusion, ST131 accounts for a large share of the antimicrobial-resistant *E. coli* isolates from geriatric patients. Unlike previous reports, ESBL-producing ST131 strains mainly belonged to non-H30-Rx rather than the H30-Rx subclone with *bla*\(_{CTX-M-14}\) as the dominant enzyme type.
INTRODUCTION

The incidence of infections due to antimicrobial-resistant Escherichia coli is increasing worldwide (Barber et al., 2013). Resistance rates for cotrimoxazole, fluoroquinolones and third generation cephalosporins, which are often used for empirical therapy, are now above the 15-20% threshold recommended for choosing first-line antimicrobial agents for empirical treatment in Hong Kong (Ho et al., 2007b; Ho et al., 2010). Discordant therapy may cause treatment failure, persistence and recurrence of infection leading to more patient morbidity and mortality (Barber et al., 2013; Shin et al., 2012). Emerging resistance in E. coli involves acquisition of resistance determinant by susceptible strain and the expansion of preexisting resistant clones (Naseer & Sundsfjord, 2011). ST131 is a highly successful E. coli clone which has received considerable attentions due to its wide geographic distribution, ability to cause a wide range of extra-intestinal infections and association with CTX-M β-lactamases and multidrug resistance (Nicolas-Chanoine et al., 2014).

ST131 can be divided into different subclones by other typing methods, of which sequencing the type 1 fimbrial adhesion gene fimH is one widely used approach (Weissman et al., 2012). H30, designated according to the fimH30 variant is currently the most prevalent subclone of ST131 (Nicolas-Chanoine et al., 2014). Two studies involving whole genome sequencing of ST131 isolates collected from multiple countries came to the same conclusion that
fluroquinolone resistance within ST131 was confined almost entirely to the H30 subclone and 
that CTX-M-15 producers clustered within a nested subclone, designated as H30-Rx (Petty et 
al., 2014; Price et al., 2013). Reported prevalence of H30 and H30-Rx among ST131 isolates 
ranged 66.7%-95.8% and 16.9%-66.2%, respectively, depending on the isolate sources and 
selection criteria (Banerjee et al., 2013b; Peirano et al., 2014; Peirano & Pitout, 2014; Price 
et al., 2013). Majority of the ST131 isolates that have been tested for the H30 and H30-Rx 
subclones were collected from North America and Europe; relatively few isolates were from 
Asia (Banerjee et al., 2013a; Banerjee et al., 2013b; Colpan et al., 2013; Johnson et al., 2013; 
Johnson et al., 2014; Peirano et al., 2014; Tchesnokova et al., 2013). Additionally, few 
studies have assessed the association of host factors with the two ST131 subclones (Banerjee 
& Johnson, 2014). Here, we used an unselected collection of urinary E. coli isolates from 
four laboratories in Hong Kong to evaluate the relationship between patient demographics, 
antimicrobial-resistant phenotypes, ST131 and its major subclones.

METHODS

Study design. A total of 340 non-duplicated, urinary E. coli isolates were studied. The 
isolates were consecutive single-patient E. coli isolates from four clinical microbiology 
laboratories in Hong Kong over a two week period, from May to June 2013. The laboratories 
together served about a quarter of the Hong Kong populations in different geographic
districts. The inclusion criteria were: (1) patient age 18 years or above, (2) mid-stream urine specimen, and (3) significant growth at $\geq 10^5$ CFU/ml. Patient identities was kept anonymous.

The following information was provided by the submitting laboratories: sex, age, date of collection and patient location (outpatient or inpatient). One isolate per patient was included.

**Microbiological methods.** The VITEK GNI system (bioMerieux Vitek Inc., Hazelwood, MO) was used for bacterial identification. Antibiotic susceptibilities were tested by the disc diffusion method using Mueller-Hinton agar (Oxoid, Basingstoke, UK) and interpreted according to the Clinical and Laboratory Standard Institute. All antibiotic discs were obtained commercially (BBL, Becton Dickinson, Cockeysville, MD, USA). The double disk synergy test was used for detection of extended-spectrum $\beta$-lactamases (ESBL) (Ho *et al.*, 2010). The susceptibility testing of all isolates were performed in a central laboratory at the University of Hong Kong. On each day of testing, standard strains (ATCC 25922 and 35218) were included as quality controls. For each isolate, the resistance score was the number of antimicrobials (including ampicillin, amoxicillin-clavulanate, cefuroxime, ceftriaxone, ertapenem, nalidixic acid, ciprofloxacin, co-trimoxazole, gentamicin, nitrofurantoin and fosfomycin which were chosen to represent 11 classes) for which it exhibited resistance (including both intermediate and resistant categories).
**Molecular studies.** PCR assays were used to assign the *E. coli* isolates to phylogroups A, B1, B2, C, D, E and F (Clermont *et al.*, 2013). Phylogroup B isolates were investigated for ST131 status by PCR assays targeting SNPs in *mdh* and *gyrB* (Johnson *et al.*, 2009), and the O25b variant and SNPs in *pabB* (Clermont *et al.*, 2009). A subset of the isolates were further tested by multilocus sequence typing (MLST) for confirmation (Wirth *et al.*, 2006).

ST131-associated O serotype, *fimH* subtype and the *H*30-Rx subsubclone were determined by established methods (Banerjee *et al.*, 2013b; Clermont *et al.*, 2007; Weissman *et al.*, 2012). The *bla*<sub>CTX-M</sub> genes were detected by PCR and sequencing using primers with specificity for the CTX-M subgroups (*bla*<sub>CTX-M1G</sub>, *bla*<sub>CTX-M2G</sub>, *bla*<sub>CTX-M8G</sub>, *bla*<sub>CTX-M9G</sub>, and *bla*<sub>CTX-M25G</sub>) (Ho *et al.*, 2007a; Ho *et al.*, 2012). Alleles were assigned by sequencing the full length of *bla*<sub>CTX-M</sub> as previously described (Ho *et al.*, 2012).

**Statistical analysis.** The Chi-square, Fisher’s exact test or Student’s *t*-test were used for statistical analysis. Univariate and multivariate analyses were used to assess risk factors associated with ST131 subclones. The following parameters were included in the multivariate analysis: age, sex, laboratory source, and patient care location. The values of parameters are given as mean (± standard deviation) where appropriate. A two-tailed *P* value of <0.05 was considered significant. All analyses were performed using statistical software (SPSS, version 14.0; SPSS Inc; Chicago, IL).
RESULTS

Patient demographics

A total of 340 urinary isolates were included in the study; 204 (60.0%) from inpatients, 136 (40.0%) from outpatients; 259 (76.2%) from females and 81 (23.8%) from males. Each laboratory contributed 82 to 89 isolates. Overall, 50 (14.7%) were obtained from patients aged 18-50 years, 58 (17.1%) from patients aged 51-64 years and 232 (68.2%) from patients aged ≥65 years. The patients had mean age of 69.7 ± 17.3 years.

Distribution of phylogroups and ST131 by patient sources

Phylogroup B2 predominated among the isolates with similar frequencies among isolates from different age groups (62.1%-66.0%) and among inpatients (63.7%) and outpatients (63.2%) isolates (Table 1). Allele-specific PCR assays targeting mdh and gyrB identified 63 isolates as ST131 of which 45 isolates were also positive for O25b and pabB. One isolate was pabB positive and mdh-negative, gyrB-positive. MLST confirmed the isolate as ST131. Another 22 isolates were randomly chosen for MLST and all were confirmed to be ST131. The prevalence of ST131 among all urinary isolates was 18.5% (63/340) overall, but this varied according to isolate sources (Table 1). The prevalence of ST131 was higher among isolates from patients aged ≥65 years (25.4%) than the other age groups (3.4%-4.0%), and higher among inpatients (23.0%) than in outpatients (11.8%). Of the 63 ST131 isolates, 45
(71.4%) were serogroup O25b, 17 (27.0%) were serogroup O16 and one (1.6%) was O-non-typeable. Forty-three (68.3%) ST131 isolates belonged to the H30 subclone, whereas the remaining 20 isolates belonged to H41 (n=17), H54 (n=2) and H22 (n=1). All H30 isolates were ciprofloxacin-resistant and 18.6% (8/43) of H30 isolates belonged to the H30-Rx subclone. In general, serogroup O25b isolates were of H30 (93.3%, 42/45) subclone and serogroup O16 were of H41 subclone (100%, 17/17). The frequency of H30 subclone was higher among patients aged ≥65 years and inpatients while those for H41 and other fimH subtypes were similar among the patient subsets. In multivariate analysis, aged ≥65y was the only factor significantly associated with ST131 (OR 8.9, 95% CI 3.1-25.1, P<0.001), H30 (OR 7.3, 95% CI 2.2-24.1, P=0.001) and H41 (OR 7.9, 95% CI 1.04-60.6, P=0.046).

**Distribution of antibiotic resistance by ST131 status**

Among ST131 isolates, resistance rates for 8 of the 11 antimicrobials were high, ranging from 33.3% to 87.3% while those for nitrofurantoin (1.6%) and fosfomycin (1.6%) were rare. All isolates including all ST131 subclones were susceptible to ertapenem. ST131 isolates were significantly more likely than non-ST131 isolates to be resistant to ampicillin (87.3% vs. 67.9%, respectively), amoxicillin-clavulanate (33.3% vs. 18.8%), cefuroxime (41.3% vs. 20.9%), nalidixic acid (100% vs. 69.0%), ciprofloxacin (71.4% vs. 32.5%) and gentamicin (38.1% vs. 25.6%), and to be ESBL-producers (41.3% vs. 18.8%). Resistance rates among
$H_{30}$ and $H_{41}$ isolates were similar except for resistance to ciprofloxacin which is
substantially higher among $H_{30}$ isolates (100% for $H_{30}$ vs. 11.8% for $H_{41}$, $P<0.001$). The
resistance score was highest for $H_{30}$ isolates (5.1±2.0), followed by $H_{41}$ isolates (3.5±1.4)
and non-ST131 isolates (3.0±2.4). Within $H_{30}$ isolates, resistance score for $H_{30}$-Rx (4.9 ±
2.7) and non-$H_{30}$-Rx (5.1 ± 1.8) isolates were similar ($P = 0.737$).

Rates of ESBL production, and ciprofloxacin, cotrimoxazole and gentamicin resistance were
similar among isolates from different age groups. ST131 accounted for 33.3%, 33.3%, 25.3%
of all ESBL-producing, ciprofloxacin-resistant, and gentamicin-resistant $E. coli$ populations,
respectively. In contrast, prevalence of ST131 among the antimicrobial-sensitive counterparts
were significantly lower ($P<0.05$ for all comparisons), being 14.1% for ESBL-negative
isolates, 8.8% for ciprofloxacin-sensitive isolates and 15.9% for gentamicin-sensitive isolates.
The prevalence of ST131 among cotrimoxazole-resistant (17.4%) and -sensitive (19.2%)
isolates was similar. Stratification by age groups revealed that there were variations in the
resistant populations attributed to ST131 (Fig. 1). Among the ST131 isolates, 26 (41.3%)
were ESBL-producers. PCR and sequencing showed that 19 had $bla_{CTX-M-14}$ (12 non-$H_{30}$-Rx,
two $H_{30}$-Rx and five $H_{41}$), six had $bla_{CTX-M-15}$ (five non-$H_{30}$-Rx and one $H_{30}$-Rx) and one
was $bla_{CTX-M}$ negative ($H_{30}$).
DISCUSSION

We evaluated 340 *E. coli* urine isolates, collected from four laboratories in 2013 for the ST131 clonal group and its subclones. The prevalence of ST131 among total *E. coli* isolates (18.5%) is concordant with other studies in the United State (17%-27%) and Europe (12%-22%) (Nicolas-Chanoine *et al.*, 2014). Our findings showed that the prevalence of ST131 and its *H*30 subclone were higher among older age, inpatients and antimicrobial-resistant isolates. These findings indicated that expansion of ST131 is an important mechanism of increased antimicrobial resistance in the geriatric population. The reason for the higher prevalence of ST131 among geriatric patients is not clear but could possibly be related to selection from over-prescription of broad-spectrum antimicrobials (third generation cephalosporins, fluoroquinolones), institutional acquisition from exposure in old age homes and hospitals, and underlying comorbidities (Ho *et al.*, 2014). In previous studies, approximately 25% of hospitalized patients and elderly residents of long-term care facilities were found to carry ST131 in their feces (Banerjee & Johnson, 2014), comparing with <5% among healthy young adults (Kudinha *et al.*, 2013; Leflon-Guibout *et al.*, 2008); suggesting institutions may pose risk for ST131 transmission. However, a recent study found that only 2.1% of 240 residents from 11 nursing homes in Germany had fecal carriage of ST131 (Arvand *et al.*, 2013).
We found that H30 comprised 68.3% of all ST131 isolates which is lower than the proportions reported for unselected clinical isolates from the United States (87.3%) and France (86.5%) (Lafolie et al., 2014). The prevalence of H30-Rx subclone among our H30 isolates was 18.6%, which is substantially lower than the >70% among H30 ST131 isolates preselected by specific resistance phenotypes (Banerjee et al., 2013b; Peirano et al., 2014).

H30-Rx described previously among isolates from Europe and North America was almost always ESBL-positive and had CTX-M-15 (Peirano et al., 2014; Petty et al., 2014; Price et al., 2013). Here, only three of the eight H30-Rx isolates were ESBL-producers. Unlike previous reports (Peirano et al., 2014; Petty et al., 2014; Price et al., 2013), ESBL-producing ST131 strains in the present study mainly belonged to non-H30-Rx rather than the H30-Rx subclone. Among all ST131 subclones, CTX-M-14 was the predominant ESBL found. Plasmid IncF family played a major role in the dissemination of CTX-M-15 in Europe and the United States (Nicolas-Chanoine et al., 2014). In Asia, IncF plasmids were found to more often carry CTX-M-14 instead of CTX-M-15 (Ho et al., 2007a; Nicolas-Chanoine et al., 2014). Among ST131 isolates, IncF plasmids carrying CTX-M-14 have been reported from Hong Kong, mainland China and South Korea (Ho et al., 2012; Nicolas-Chanoine et al., 2014). In Japan, CTX-M-14 was detected in 44% and 73% of ESBL-producing ST131-O25b and ST131-O16 isolates, respectively, comparing with 18% and 8% for CTX-M-15, respectively. (Matsumura et al., 2012).
In summary, this study found that antimicrobial-resistant *E. coli* from geriatric patients are substantially more likely to be caused by ST131 than those from younger patients, and that the *H*30 and *H*41 subclones possess certain resistance traits different from those reported in other locales.
Conflict of interest statement

All authors have no competing interests

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Escherichia coli phylo-typing method revisited: improvement of specificity and detection of

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### Table 1. Distribution of phylogroups and ST131 among 340 urinary *E. coli* isolates

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<td>ST131</td>
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<td></td>
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<tr>
<td>H30 subclone</td>
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<tr>
<td>H41 subclone</td>
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<td>1 (2.0)</td>
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<td>Others*</td>
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<td>H30-Rx</td>
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* Including H54 (two isolates) and H22 (one isolate).
Fig 1. Antimicrobial-resistant *E. coli* populations attributed to ST131 according to age groups.

The *P* values are indicated for between age groups comparison.