AAC Journals.ASM.org

Association of Fluoroquinolone Resistance, Virulence Genes, and IncF Plasmids with Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Sequence Type 131 (ST131) and ST405 Clonal Groups

Yasufumi Matsumura,[a] Masaki Yamamoto,[a] Miki Nagao,[a] Yutaka Ito,[b] Shunji Takakura,[a] Satoshi Ichiyama,[a] for the Kyoto-Shiga Clinical Microbiology Study Group

Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto, Japan[a]; Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto, Japan[b]

The global increase of extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* is associated with the specific clonal group sequence type 131 (ST131). In order to understand the successful spread of ESBL-producing *E. coli* clonal groups, we characterized fluoroquinolone resistance determinants, virulence genotypes, and plasmid replicons of ST131 and another global clonal group, ST405. We investigated 41 ST131-O25b, 26 ST131-O16, 41 ST405, and 41 other ST (OST) ESBL-producing isolates, which were collected at seven acute care hospitals in Japan. The detection of ESBL types, fluoroquinolone resistance-associated mutations (including quinolone resistance-determining regions [QRDRs]), virulence genotypes, plasmid replicon types, and IncF replicon sequence types was performed using PCR and sequencing. bla_{CTX-M}, specifically $bla_{CTX-M-14}$, was the most common ESBL gene type among the four groups. Ciprofloxacin resistance was found in 90% of ST131-O25b, 19% of ST131-O16, 100% of ST405, and 54% of OST isolates. Multidrug resistance was more common in the ST405 group than in the ST131-O25 group (56% versus 32%; $P = 0.045$). All ST131-O25b isolates except one had four characteristic mutations in QRDRs, but most of the isolates from the other three groups had three mutations in common. The ST131-O25b and ST405 groups had larger numbers of virulence genes than the OST group. All of the ST131-O25b and ST405 isolates and most of the ST131-O16 and OST isolates carried IncF replicons. The most prevalent IncF replicon sequence types differed between the four clonal groups. Both the ST131-O25b and ST405 clonal groups had a fluoroquinolone resistance mechanism in QRDRs, multidrug resistance, high virulence, and IncF plasmids, suggesting the potential for further global expansion and a need for measures against these clonal groups.

The global increase in extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* is closely related to a pandemic clonal group: CTX-M-type ESBL-producing *E. coli* with sequence type 131 belonging to the O25b serogroup and the B2 phylogenetic group (ST131-O25b) (1, 2). In addition to the ST131 clonal group, a CTX-M-producing ST405 clonal group belonging to phylogenetic group D (ST405) has also been detected worldwide (3–5). Our regional surveillance program in Japan previously demonstrated that not only the ST131-O25b group, but also the B2-ST131-O16 (ST131-O16) and ST405 clonal groups, contributed to the recent increase in prevalence of ESBL-producing *E. coli* (6).

The success of the ST131-O25b clonal group has been explained by its acquisition of fluoroquinolone resistance and additional virulence factors (2). However, data for the ST131-O16 and ST405 clonal groups are lacking. Plasmids could be an important component of these clonal groups, because ESBL genes are generally located on plasmids (1). Clonal groups that were determined by multilocus sequence typing (MLST) have chromosomal genetic similarity. To characterize plasmids, replicon typing to assess the incompatibility group and sequence typing to identify a specific replicon have been developed (7). Addiction systems encoded by plasmids contributed to the promotion of plasmid spread and adaptation to the host (8). However, there have been few reports on the characterization of plasmids in these clonal groups, which is important for explaining the success of these clonal groups.

The aim of this study was to investigate the fluoroquinolone resistance mechanisms, virulence genotypes, and plasmid replicons of the ST131-O25b, ST131-O16, and ST405 clonal groups compared to isolates that are not from an ST131 or ST405 clonal group. We performed this study to enhance our understanding of the global spread of these specific clonal groups.

MATERIALS AND METHODS

Bacterial isolates. Between April 2001 and December 2010, a total of 581 clinical *E. coli* isolates were collected at seven acute care hospitals in the Kyoto and Shiga regions of Japan. The following clonal groups were previously investigated and characterized: ST131-O25b ($n = 185$), ST131-O16 ($n = 26$), ST405 ($n = 41$), D-ST69 ($n = 7$), and D-ST393 ($n = 2$) (6). To further characterize the ST131 and ST405 clonal groups, this study included 41 ST131-O25b, 26 ST131-O16, 41 ST405, and 41 other ST (OST; isolates from groups other than the previously listed three clonal groups) isolates. The 41 ST131-O25b and 41 OST isolates were randomly selected to match the number of ST405 isolates. Bacterial DNAs were isolated using a QIAamp DNA minikit (Qiagen, Hilden, Germany) and were used in the subsequent analyses.

Received 4 April 2013 Returned for modification 2 July 2013
Accepted 9 July 2013
Published ahead of print 15 July 2013
Address correspondence to Yasufumi Matsumura, yazblood@kuhp.kyoto-u.ac.jp.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.00641-13.

doi:10.1128/AAC.00641-13

β-Lactamase identification. The presence of ESBL or plasmid-mediated AmpC β-lactamase (pAmpC) genes was detected by PCR amplification and sequencing of the bla_{CTX-M}, bla_{TEM}, bla_{SHV}, and bla_{OXA-1} genes and the six main groups of pAmpC-type genes, as described previously (6).

Detection of clonal groups. Phylogenetic grouping, PCR O typing, B2-ST131-O25b *pabB* allele-specific PCR, ST405 *adk* allele-specific PCR, and MLST were used to determine clonal groups (6). MLST was performed according to the Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli), using seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) (9). If single-locus variants of the founding ST of an ST complex (STC), which are defined in the MLST database, were identified, they were considered to belong to that STC.

Susceptibility testing. Antibiotic susceptibility was evaluated by microdilution using Eiken dry plates (Eiken, Tokyo, Japan) and included testing with piperacillin-tazobactam, gentamicin, tobramycin, amikacin, imipenem, minocycline, and trimethoprim-sulfamethoxazole. Susceptibilities to ciprofloxacin and nalidixic acid were evaluated using Etest (Sysmex bioMérieux, Tokyo, Japan). The results were interpreted using the 2012 CLSI breakpoints (10). Intermediate susceptibility to each antibiotic was considered to be the same as resistance to that antibiotic. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial categories (11).

Fluoroquinolone resistance. The quinolone resistance-determining regions (QRDRs) of the *gyrA* (12) and *parC* (13) genes and the AcrAB efflux pump regulatory genes, *marOR* and *acrR*, were sequenced (13), and the correlating amino acids were compared with the corresponding regions of *E. coli* K-12 (GenBank accession no. NC000913). The *marOR* and *acrR* mutations affecting the AcrAB efflux pump and fluoroquinolone resistance were interpreted as described by Lindgren et al. (13). Plasmid-mediated quinolone resistance (PMQR) determinants [*qnrA*, *qnrB*, *qnrC*, *qnrS*, *qepA*, *aac(6′)-Ib-cr*, and *oqxAB*] were detected by PCR (14, 15).

Virulence genotypes. The presence of 29 extraintestinal pathogenic *E. coli* (ExPEC)-associated virulence genes was searched for by multiplex PCR (16). Isolates were defined as ExPEC if they were positive for two of the following genes or gene sets: *papA* and/or *papC*, *sfa-focDE*, *afa-draBC*, *kpsM* II, and *iutA* (17). The virulence score represents the number of virulence genes detected and was adjusted for multiple detection of the *pap*, *sfa*, *foc*, and *kpsM* II operons.

Plasmid addiction systems. Five plasmid protein antitoxin-regulated systems (*pemK-pemI*, *ccdA-ccdB*, *relB-relE*, *parD-parE*, and *vagC-vagD*) and three plasmid antisense RNA-regulated systems (*hok-sok*, *pndA-pndC*, and *srnB-srnC*) were searched for by PCR (8).

Plasmid replicon typing. Plasmid replicons were determined using the PCR-based replicon typing scheme (18). IncF replicon sequence typing was also performed, and the FAB (FII:FIA:FIB) formula represents the allele type and number identified for each replicon (7).

Statistical analysis. The ST131-O25b, ST131-O16, and ST405 groups were compared with the OST group. The categorical variables were compared using Fisher's exact test. The continuous variables were compared using the Mann-Whitney U test and the Kruskal-Wallis test. *P* values of <0.05 were considered statistically significant. We conducted our statistical analysis using Stata, version 11.2 (StataCorp, College Station, TX).

RESULTS

OST reference group. All 41 OST isolates underwent MLST (see Table S1 in the supplemental material). Twenty-eight different STs and 6 STCs that had more than one isolate were identified. STC38 (20%) and phylogenetic group D (56%) were the most prevalent groups.

Antibiotic resistance and β-lactamase genes. Table 1 shows that most of the ST131-O25b isolates (90%) and all of the ST405 isolates were resistant to ciprofloxacin, whereas only 19% of the ST131-O16 isolates had statistically significant resistance compared to the OST isolates (54%). For nalidixic acid, the resistance rates of the ST131-O16 (69%) and OST groups (78%) were similar.

The ST131-O25b group had ciprofloxacin and gentamicin resistance and minocycline susceptibility, the ST131-O16 group had ciprofloxacin susceptibility, and the ST405 group had ciprofloxacin, gentamicin, and tobramycin resistance. MDR was more common in the ST405 group (56%) than in the ST131-O25 group (32%) and was less common in the ST131-O16 (8%) group than in the OST group (39%).

The bla_{CTX-M} group, especially $bla_{CTX-M-14}$, was the dominant ESBL gene type in all four clonal groups. The ST131-O16 and ST405 groups had $bla_{CTX-M-14}$ (73% [each]) more frequently than the OST group did (46%). $bla_{CTX-M-15}$ was the second most prevalent bla_{CTX-M} gene in the ST131-O16, ST405, and OST groups, whereas $bla_{CTX-M-27}$ was the second most prevalent bla_{CTX-M} gene in the ST131-O25b group. $bla_{CTX-M-27}$ was not found in the ST131-O16 and ST405 groups. The majority of the MDR isolates had $bla_{CTX-M-14}$ (10 ST131-O25b, 2 ST131-O16, 16 ST405, and 6 OST isolates). The ST131-O25b group had bla_{TEM-1} more frequently than the OST group did.

Fluoroquinolone resistance mechanisms. The details of the fluoroquinolone resistance-associated genes are shown in Table 2, and those of the QRDRs are shown in Table 3. Nearly all of the ciprofloxacin-resistant isolates had more than two QRDR mutations; 97% of the ST131-O25b isolates had four mutations (S83L and D87N in GyrA and S80I and E84V in ParC; "LNIV" genotype). The most prevalent mutations in the other three groups were three mutations that led to the "LNIE" genotype (60 to 73% of isolates). One ST405 isolate had only two QRDR mutations, but it had an *acrAB*-upregulating mutation (deletion and frameshift in *acrR*). Ciprofloxacin-resistant and nalidixic acid-susceptible isolates had one or two QRDR mutations. The *acrAB*-upregulating mutation was found less frequently in the ST131-O25b group than in the OST group ($P = 0.027$). PMQR determinants were not common in any of the groups.

Virulence genotypes. Table 4 shows that the ST131-O25b and ST405 groups had higher virulence scores than the OST group. In addition, the ST131-O25b group had ExPEC status more often than the OST group. Conversely, the ST131-O16 group had ExPEC status less often than the OST group (23% versus 46%; $P = 0.072$).

Plasmid addiction systems. The number of plasmid addiction systems did not differ between the four groups. However, *relBE*, *ccdAB*, and *pemKI* were found more frequently in the ST131-O25b group, *relBE* in the ST131-O16 group, and *pemKI* and *hok-sok* in the ST405 group. The ST405 group had *vagCD* and *pndAC* less frequently than the case for the other groups.

Plasmid replicon types. IncF replicons were highly prevalent in the ST131-O16 (92%) and OST (85%) groups and were also present in the ST131-O25b and ST405 groups; all of the isolates from these groups possessed IncF replicons. ColE was the second most prevalent replicon in the ST131-O25b and OST groups. IncU and IncB/O were the second most prevalent replicons in the ST131-O16 and ST405 groups, respectively. IncF replicon sequence typing found 15, 12, 25, and 28 different FAB types in the ST131-O25b, ST131-O16, ST405, and OST groups, respectively (see Table S2 in the supplemental material). F1:A2:B20 was the most prevalent replicon (41%) in the ST131-O25b group and was found significantly more frequently than in the other three groups, even though some of the other isolates had it. Seventeen

TABLE 1 Antibiotic resistance and β-lactamase genes in *E. coli* B2-ST131-O25b, B2-ST131-O16, D-ST405, and OST clonal groups[g]

Characteristic	No. (%) of isolates				*P* value[a]			
						OST vs:		
	ST131-O25b ($n = 41$)	ST131-O16 ($n = 26$)	ST405 ($n = 41$)	OST ($n = 41$)	Overall	ST131-O25b	ST131-O16	ST405
Antimicrobial resistance								
Ciprofloxacin	37 (90)	5 (19)	41 (100)	22 (54)	<0.001	<0.001	0.006	<0.001
Nalidixic acid	37 (90)	18 (69)	41 (100)	32 (78)	<0.001	<0.001	0.565	<0.001
Amikacin	0 (0)	0 (0)	2 (5)	1 (2)	0.630	1.000	1.000	1.000
Gentamicin	18 (44)	3 (12)	17 (41)	6 (15)	0.001	0.007	1.000	0.013
Tobramycin	15 (37)	3 (12)	21 (51)	7 (17)	0.001	0.080	0.729	0.002
Minocycline	3 (7)	15 (58)	9 (22)	18 (44)	<0.001	<0.001	0.322	0.059
Trimethoprim-sulfamethoxazole	18 (44)	13 (50)	28 (68)	26 (63)	0.452	0.121	0.317	0.816
Piperacillin-tazobactam	2 (5)	3 (12)	7 (17)	4 (10)	0.038	0.675	1.000	0.519
Multidrug resistance	13[a] (32)	2 (8)	23[a] (56)	16 (39)	<0.001	0.645	0.005	0.184
ESBL genes								
Any $bla_{\text{CTX-M}}$ gene	39 (95)	23 (88)	41 (100)	39 (95)	0.168	1.000	0.369	0.494
$bla_{\text{CTX-M-14}}$	23 (56)	19 (73)	30[b] (73)	19[b] (46)	0.041	0.508	0.044	0.024
$bla_{\text{CTX-M-15}}$	5 (12)	2 (8)	12[b] (29)	7[b,c] (17)	0.111	0.756	0.465	0.295
$bla_{\text{CTX-M-27}}$	8[a] (20)	0 (0)	0[a] (0)	2 (5)	0.001	0.088	0.518	0.494
$bla_{\text{CTX-M-2}}$	2 (5)	1 (4)	0 (0)	5 (12)	0.098	0.432	0.392	0.027
$bla_{\text{CTX-M-3}}$	1 (2)	0 (0)	0 (0)	4 (10)	0.094	0.359	0.641	0.058
Other $bla_{\text{CTX-M}}$ genes[d]	0 (0)	1 (2)	1 (2)	3 (7)	0.442	0.116	1.000	0.616
bla_{TEM}[e]	1 (2)	1 (4)	0 (0)	2[c] (5)	0.699	1.000	1.000	0.494
bla_{SHV}[f]	1 (2)	2 (8)	0 (0)	1 (2)	0.331	1.000	0.555	0.500
Other β-lactamase genes								
$bla_{\text{TEM-1}}$	28 (68)	9 (35)	16 (39)	15 (37)	0.012	0.008	1.000	1.000
$bla_{\text{OXA-1}}$	2 (5)	0 (0)	3 (7)	2 (5)	0.654	1.000	1.000	1.000

[a] Comparison between the ST131-O25b and ST405 groups revealed that significant differences were found only in multidrug resistance ($P = 0.045$) and carriage of $bla_{\text{CTX-M-27}}$ ($P = 0.005$).
[b] Two ST405 isolates and one OST isolate were positive for both $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$.
[c] One OST isolate was positive for both $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-20}}$.
[d] $bla_{\text{CTX-M-55}}$ was found in one ST131-O16 isolate, and $bla_{\text{CTX-M-24}}$ was found in one ST405 isolate. $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-24}}$, and $bla_{\text{CTX-M-55}}$ were each found in three OST isolates.
[e] $bla_{\text{TEM-20}}$ was found in two OST isolates, and $bla_{\text{TEM-12}}$ was found in the other two isolates.
[f] $bla_{\text{SHV-12}}$ was found in one ST131-O16 isolate, and $bla_{\text{SHV-2}}$ was found in the other three isolates.
[g] All isolates in this study were susceptible to imipenem. Multidrug resistance was defined by resistance to at least one agent in three or more antimicrobial categories.

ST131-O25b isolates with F1:A2:B20 were associated with *ccdAB* ($n = 17$), *srnBC* ($n = 17$), *pemKI* ($n = 16$), $bla_{\text{CTX-M-14}}$ ($n = 9$), $bla_{\text{CTX-M-27}}$ ($n = 8$), and $bla_{\text{CTX-M-3}}$ ($n = 1$). F2:A1:B− was the second most prevalent replicon in the ST131-O25b group. All six isolates had *ccdAB*, *pemKI*, *vagCD*, and *hok-sok*, four isolates had $bla_{\text{CTX-M-15}}$, and two isolates had $bla_{\text{CTX-M-14}}$. F29:A−:B10 was the most prevalent replicon (19%) in the ST131-O16 group and was not found in the other three groups, except for one ST405 isolate ($P = 0.03$). Five ST131-O16 isolates with F29:A−:B10 were associated with $bla_{\text{CTX-M-14}}$ ($n = 2$), $bla_{\text{CTX-M-15}}$ ($n = 1$), $bla_{\text{CTX-M-2}}$ ($n = 1$), and $bla_{\text{SHV-2}}$ ($n = 1$). F1:A6:B20 was the most prevalent replicon in the ST405 group (22%) and was not found in the ST131-O16 and OST groups ($P = 0.001$). Nine ST405 isolates with F1:A6:B20 were associated with *ccdAB* ($n = 9$), *srnBC* ($n = 9$), *pemKI* ($n = 8$), *hok-sok* ($n = 8$), and $bla_{\text{CTX-M-14}}$ ($n = 9$). F18:A−:B1 (10%), F−:A1:B1 (7%), and F1:A7:B23 (7%) were the most common replicons in the OST group.

DISCUSSION

This study characterized ST131 and ST405, the major ESBL-producing *E. coli* clonal groups found in a recent multicenter surveillance in Japan. We found that the ST405 isolates shared characteristics such as fluoroquinolone resistance, MDR, and high virulence with the ST131-O25b isolates, and we elucidated the differences between the ST131-O25b and ST131-O16 groups.

Non-ST131 and non-ST405 isolates were selected as the reference OST group. This group was composed of diverse STs, but D-ST38 was the most frequent one (20%). In a previous Japanese nationwide study conducted between 2002 and 2003, ST38 (18%) was the second most common ST, followed by ST131-O25b (21%) (19). Studies from Canada, the Netherlands, Spain, and Korea have indicated that ST38, ST648, and STC10 also have a global prevalence (4, 5, 20, 21).

$bla_{\text{CTX-M-15}}$ is most closely associated with the ST131 clonal group and thus is the most widely distributed $bla_{\text{CTX-M}}$ type (2). $bla_{\text{CTX-M-14}}$ was reported to be the second most common type. In a previous Japanese study (19), $bla_{\text{CTX-M-14}}$ was the dominant type among ST131 and ST38 isolates, and our study also showed $bla_{\text{CTX-M-14}}$ predominance in all four groups. The second most prevalent $bla_{\text{CTX-M}}$ type in the ST131-O25b group, $bla_{\text{CTX-M-27}}$, has rarely been reported outside Japan (5, 22). $bla_{\text{CTX-M-15}}$ was the second most prevalent type in the other three groups and was found most frequently in the ST405 group (29%). Most ST405 isolates outside Japan have been reported to carry $bla_{\text{CTX-M-15}}$

TABLE 2 Fluoroquinolone resistance mechanisms[a]

Resistance mechanism	No. (%) of isolates					
	ST131-O25b	ST131-O16		ST405	OST	
	CIP^r NAL^r ($n = 37$)	CIP^r NAL^r ($n = 5$)	CIP^s NAL^r ($n = 13$)	CIP^r NAL^r ($n = 41$)	CIP^r NAL^r ($n = 22$)	CIP^s NAL^r ($n = 10$)
QRDRs with >2 mutations	37 (100)	5 (100)	0 (0)	40 (98)	22 (100)	0 (0)
acrAB-associated mutations in *marOR* or *acrR*	4 (11)	0 (0)	2 (15)	15 (37)	11 (50)	2 (20)
Mutation in MarA activator binding site of *marO*	0 (0)	0 (0)	0 (0)	5[b] (12)	1 (5)	0 (0)
Loss of RNA binding site in *marO*	0 (0)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
Deletion and frameshift in *marR*	0 (0)	0 (0)	1 (8)		0 (0)	0 (0)
IS*1* insertion in *acrR*	3 (8)	0 (0)	1 (8)	1 (2)	0 (0)	0 (0)
Insertion and frameshift in *acrR*	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Deletion and frameshift in *acrR*	0 (0)	0 (0)	0 (0)	8[b] (20)	5 (23)	0 (0)
Point mutation (stop codon) in *acrR*	1 (3)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Point mutation (amino acid change) in *acrR*	0 (0)	0 (0)	1 (13)	2 (5)	5 (23)	2 (20)
PMQR determinants						
aac(6′)-Ib-cr	3 (8)	0 (0)	0 (0)	4 (10)	0 (0)	3 (30)
oqxAB	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)

[a] OST, other sequence types; CIP, ciprofloxacin; NAL, nalidixic acid; r, resistant; s, sensitive; QRDRs, quinolone resistance-determining regions, including GyrA and ParC; PMQR, plasmid-mediated quinolone resistance. One nalidixic acid-susceptible B2-ST131-O16 isolate had a point mutation (amino acid change) in *acrR*. The *qepA* gene was not found in this study. Only one nalidixic acid-susceptible OST isolate was positive for *qnrB4*. No other fluoroquinolone resistance mechanism was detected among nalidixic acid-susceptible isolates.

[b] Five isolates with mutation in the MarA activator binding site also had a deletion and frameshift in *acrR*.

(5, 22). The results described here may support the hypothesis that $bla_{CTX\text{-}M\text{-}15}$ was imported to Japan with the ST405 clonal group, not with ST131-O25b.

ESBL-producing *E. coli* strains often have resistance to non-β-lactam antibiotics. A Canadian study reported that 30% of ESBL-producing *E. coli* strains currently have resistance to three or more non-β-lactam antibiotics; the most common resistances in MDR were to ciprofloxacin (89%), tobramycin (67%), and

TABLE 3 Genotypes of quinolone resistance-determining regions[a]

QRDR genotype	No. (%) of isolates					
	ST131-O25b	ST131-O16		ST405	OST	
	CIP^r NAL^r ($n = 37$)	CIP^r NAL^r ($n = 5$)	CIP^s NAL^r ($n = 13$)	CIP^r NAL^r ($n = 41$)	CIP^r NAL^r ($n = 22$)	CIP^s NAL^r ($n = 10$)
Four mutations						
LNIV	36 (97)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LNIG	0 (0)	0 (0)	0 (0)	4 (10)	3 (14)	0 (0)
LNIK	0 (0)	0 (0)	0 (0)	3 (7)	0 (0)	0 (0)
Three mutations						
LNIE	0 (0)	3 (60)	0 (0)	26 (63)	16 (73)	0 (0)
LYIE	0 (0)	0 (0)	0 (0)	7 (17)	0 (0)	0 (0)
LNRE	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)
LNSK	1 (3)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
LGRE	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
LVRE	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
Two mutations						
LDIE	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (10)
LESE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
One mutation						
LDSE	0 (0)	0 (0)	10 (77)	0 (0)	0 (0)	6 (60)
SGSE	0 (0)	0 (0)	3 (23)	0 (0)	0 (0)	0 (0)
ADSE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
SNSE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
Wild type (SDSE)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a] OST, other sequence types; CIP, ciprofloxacin; NAL, nalidixic acid; r, resistant; s, sensitive; QRDR, quinolone resistance-determining region. QRDR genotypes are shown sequentially by the deduced amino acids of GyrA codons 83 and 87 and ParC codons 80 and 84. All nalidixic acid-susceptible isolates in any clonal group had wild-type QRDRs.

TABLE 4 Virulence genotypes, plasmid addiction systems, and plasmid replicon types[b]

Characteristic	No. (%) of isolates (unless indicated otherwise)				*P* value[a]			
	ST131-O25b ($n = 41$)	ST131-O16 ($n = 26$)	ST405 ($n = 41$)	OST ($n = 41$)	Overall	OST vs: ST131-O25b	ST131-O16	ST405
Virulence genotype								
ExPEC status	31 (76)	6 (23)	22 (54)	19 (46)	<0.001	0.012	0.072	0.659
Virulence score	6 (5–6)	5 (5–6)	6 (6–8)	4 (3–6)	<0.001	0.002	0.193	<0.001
No. of plasmid addiction systems	4 (3–4)	3 (2–4)	3 (2–4)	3 (2–4)	0.480	0.305	0.777	0.806
vagCD	8 (20)	5 (19)	2 (5)	15 (37)	0.004	0.139	0.174	0.001
relBE	10 (24)	7 (27)	0 (0)	1 (2)	<0.001	0.007	0.004	1.000
ccdAB	41 (100)	21 (81)	26 (63)	26 (63)	<0.001	<0.001	0.174	1.000
srnBC	28 (68)	16 (62)	31 (76)	27 (66)	0.624	1.000	0.796	0.467
pemKI	35 (85)	16 (62)	34 (83)	21 (51)	0.001	0.002	0.458	0.004
hok-sok	12 (29)	8 (31)	29 (71)	18 (44)	0.001	0.252	0.315	0.025
pndAC	7 (17)	7 (27)	6 (15)	15 (37)	0.088	0.080	0.439	0.041
parDE	0 (0)	0 (0)	0 (0)	0 (0)	1.000	1.000	1.000	1.000
Replicon type								
IncF	41 (100)	24 (92)	41 (100)	35 (85)	0.003	0.026	0.469	0.026
IncFII	40 (98)	23 (88)	39 (95)	32 (78)	0.019	0.014	0.343	0.048
IncFIA	38 (93)	16 (62)	21 (51)	17 (41)	<0.001	<0.001	0.136	0.507
IncFIB	30 (73)	23 (88)	32 (78)	35 (85)	0.400	0.276	1.000	0.569
ColE	18 (44)	2 (8)	5 (12)	18 (44)	<0.001	1.000	0.002	0.003
IncI1	6 (15)	2 (8)	7 (17)	11 (27)	0.247	0.276	0.064	0.424
IncB/O	5 (12)	1 (4)	9 (22)	7 (17)	0.205	0.756	0.138	0.781
IncU	9 (22)	4 (15)	1 (2)	7 (17)	0.040	0.781	1.000	0.057
IncN	3 (7)	1 (4)	0 (0)	1 (2)	0.422	0.616	1.000	1.000
IncP	0 (0)	0 (0)	0 (0)	3 (7)	0.064	0.241	0.277	0.241
IncA/C	1 (2)	0 (0)	0 (0)	0 (0)	1.000	1.000	1.000	1.000
IncL/M	0 (0)	0 (0)	0 (0)	1 (2)	1.000	1.000	1.000	1.000
Nontypeable	0 (0)	2 (8)	0 (0)	3 (7)	0.050	0.241	1.000	0.241

[a] Comparison between the ST131-O25b and ST405 groups revealed that statistical differences were found only in carriage of *relBE* ($P = 0.013$), FIA ($P < 0.001$), ColE ($P = 0.003$), and IncU ($P = 0.014$).
[b] OST, other sequence types; ExPEC, extraintestinal pathogenic *E. coli*. Continuous variables are presented as medians (interquartile ranges).

trimethoprim-sulfamethoxazole (65%) (23). Similarly, 32 to 56% of isolates in our study exhibited MDR, except for those in the ST131-O16 group. The acquisition and maintenance of MDR are possible with ESBL plasmids, which may simultaneously have multiple resistance determinants. For example, $bla_{CTX\text{-}M\text{-}15}$ plasmids isolated in the United Kingdom often confer resistance to aminoglycosides, trimethoprim-sulfamethoxazole, and tetracycline (24). The MDR ST131-O25b and ST405 isolates in our study had different resistance patterns and frequently carried $bla_{CTX\text{-}M\text{-}14}$.

Fluoroquinolone resistance in ESBL-producing *E. coli* strains is common worldwide. This higher resistance rate has been established for ST131-O25b, with four specific QRDR changes (the "LNIV" genotype) (25), and often with *aac*(6′)-*Ib-cr* (2). The "LNIV" genotype was also observed in our isolates, but *aac*(6′)-*Ib-cr* was not frequently found. In accordance with our ST405 isolates, all of the 22 ST405 isolates found in Canada or the Netherlands were shown to be ciprofloxacin resistant (5), but their resistance mechanism has not been reported. We found that three mutations giving the "LNIE" genotype in QRDRs were prevalent in the ST405 group and that one-third of the ST405 isolates had *acrAB*-upregulating genotypes. This QRDR genotype was also common in our ST131-O16 and OST groups; the same genotype is also common in Asia and worldwide (12), but in previous studies, the STs were not reported. As far as we know, the relationships between specific clonal groups and the fluoroquinolone-related efflux pump have not been investigated. We found that *acrAB*-upregulating genotypes were common in the ST405 and OST groups but not in the ST131-O25b group. Fluoroquinolone-resistant *E. coli* usually has three or more mutations in four QRDRs and an *acrAB*-upregulating mutation (13). PMQR determinants have little effect on increasing the MIC to its breakpoint. Our data could explain the presence of ciprofloxacin resistance in all of the isolates. Among ciprofloxacin-susceptible isolates in the ST131-O16 and OST groups, isolates with nalidixic acid resistance were commonly found to have one or two mutations in QRDRs. With persistent use and pressure of quinolones, these fluoroquinolone-susceptible isolates may be at risk for the acquisition of resistance.

Both ST131-O25b and ST405 clonal groups are known to be highly virulent, as judged by virulence genes and animal models (22, 26, 27). The ExPEC statuses and virulence scores obtained for the ST131-O25b and ST405 groups confirmed their high virulence. However, the ST131-O16 group appeared to have low virulence and to differ from the ST131-O25b group. Plasmid replicon types were successfully determined for almost all of the isolates, and as expected, IncF was the predominant replicon in all four groups. The F1:A2:B20 replicon had the strongest association with the ST131-O25b group. This replicon sequence type was reported to be present in a CTX-M-27-producing ST131-O25b iso-

late in the United Kingdom and in CTX-M-15-producing ST131-O25b and ST405 isolates in South Korea, although the number of isolates was small (22, 24). In the United Kingdom, CTX-M-15-producing ST131 isolates typically had a plasmid that carried *ccdAB*, *pemKI*, *vagCD*, *hok-sok*, and the F2:A1:B− replicon (24). These characteristics were in accordance with the plasmid pEK499, which was originally found in United Kingdom epidemic clonal group A. Four of our ST131-O25b isolates had the identical pattern. A Spanish study identified $bla_{\text{CTX-M-14}}$ plasmids that were associated with an IncK replicon (28). However, reports from France (29) and Hong Kong (30) found that IncF replicons had high prevalences. The $bla_{\text{CTX-M-14}}$ plasmid in Hong Kong was associated with F2:A−:B:− and F35:A−:B− but was rarely carried by the ST131-O25b or ST405 clonal group. F2:A−:B− was found in four of our ST405 isolates with $bla_{\text{CTX-M-14}}$, but F1:A6:B20 and $bla_{\text{CTX-M-14}}$ were associated with nine ST405 isolates.

This study has several limitations. Not all of the ST131-O25b and OST isolates found in our previous study were investigated here. The isolates were collected regionally, not nationwide. However, the previously reported findings regarding the ST131 and ST405 clonal groups were similar to our findings, which may support both our new findings and the hypothesis that our isolates belong to the same globally spread clonal group. In addition to ST405 and ST131, ST38 is another clonal group that is contributing to the spread of ESBL-producing *E. coli*. A detailed characterization of ST38 was not performed.

In summary, we found similar characteristics in the ST405 and ST131-O25b groups, including ciprofloxacin resistance, MDR, and a high virulence score, although the prevalences of the IncF replicon sequence types differed. These results suggest that the ST405 group has the potential to spread as a pandemic clonal group following ST131-O25b. In Japan, the ST131-O25b group could have plasmids different from those of the global ST131-O25b group. This hypothesis is supported by the observation that our isolates had F1:A2:B20 replicon sequence types in association with $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-27}}$ rather than $bla_{\text{CTX-M-15}}$. However, high ciprofloxacin resistance with the LNIV genotype, MDR, and high virulence were common. Taking into account many of the different characteristics of the ST131-O16 group compared to the ST131-O25b group (less MDR, less ciprofloxacin resistance, the "LNIE" genotype, low virulence, and IncF replicon sequence types) and the abrupt worldwide spread of the ST131-O25b group with the "LNIV" genotype (25), the ST131-O16 group may be a clonal group distinct from ST131-O25b and may have limited importance. This study indicates that monitoring for and analysis of ESBL-producing *E. coli* ST131-O25b and ST405 clonal groups should continue, because these groups may emerge as a public health concern. The IncF plasmids, which are present in both the ST131-O25b and ST405 groups, should also be studied further, as they may play an important role in the success of clonal spread.

ACKNOWLEDGMENTS

The members of the Kyoto-Shiga Clinical Microbiology Study Group include Naohisa Fujita, Toshiaki Komori, Yukiji Yamada, Tsunehiro Shimizu, Akihiko Hayashi, Tamotsu Ono, Naoko Fujihara, Takeshi Higuchi, Kunihiko Moro, Masayo Shigeta, Kaneyuki Kida, Fusayuki Tsuboi, and Yoshihisa Sugimoto.

We thank Michio Tanaka and Sayo Shitashiro for technical assistance.

This study was supported by the Charitable Trust Laboratory Medicine Research Foundation of Japan.

All authors declare no conflicts of interest.

REFERENCES

1. **Rossolini GM, D'Andrea MM, Mugnaioli C.** 2008. The spread of CTX-M-type extended-spectrum beta-lactamases. Clin. Microbiol. Infect. **14**(Suppl 1):33–41.
2. **Rogers BA, Sidjabat HE, Paterson DL.** 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J. Antimicrob. Chemother. **66:**1–14.
3. **Kim J, Bae IK, Jeong SH, Chang CL, Lee CH, Lee K.** 2011. Characterization of IncF plasmids carrying the $bla_{\text{CTX-M-14}}$ gene in clinical isolates of *Escherichia coli* from Korea. J. Antimicrob. Chemother. **66:**1263–1268.
4. **Peirano G, van der Bij AK, Gregson DB, Pitout JD.** 2012. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J. Clin. Microbiol. **50:**294–299.
5. **Van der Bij AK, Peirano G, Pitondo-Silva A, Pitout JD.** 2012. The presence of genes encoding for different virulence factors in clonally related *Escherichia coli* that produce CTX-Ms. Diagn. Microbiol. Infect. Dis. **72:**297–302.
6. **Matsumura Y, Yamamoto M, Nagao M, Hotta G, Matsushima A, Ito Y, Takakura S, Ichiyama S, Kyoto-Shiga Clinical Microbiology Study Group.** 2012. Emergence and spread of B2-ST131-O25b, B2-ST131-O16 and D-ST405 clonal groups among extended-spectrum-β-lactamase-producing *Escherichia coli* in Japan. J. Antimicrob. Chemother. **67:**2612–2620.
7. **Villa L, García-Fernández A, Fortini D, Carattoli A.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. **65:**2518–2529.
8. **Mnif B, Vimont S, Boyd A, Bourit E, Picard B, Branger C, Denamur E, Arlet G.** 2010. Molecular characterization of addiction systems of plasmids encoding extended-spectrum beta-lactamases in *Escherichia coli*. J. Antimicrob. Chemother. **65:**1599–1603.
9. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151.
10. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
11. **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. **18:**268–281.
12. **Uchida Y, Mochimaru T, Morokuma Y, Kiyosuke M, Fujise M, Eto F, Harada Y, Kadowaki M, Shimono N, Kang D.** 2010. Geographic distribution of fluoroquinolone-resistant *Escherichia coli* strains in Asia. Int. J. Antimicrob. Agents **35:**387–391.
13. **Lindgren PK, Karlsson A, Hughes D.** 2003. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. Antimicrob. Agents Chemother. **47:**3222–3232.
14. **Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC.** 2009. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob. Agents Chemother. **53:**639–645.
15. **Liu BT, Wang XM, Liao XP, Sun J, Zhu HQ, Chen XY, Liu YH.** 2011. Plasmid-mediated quinolone resistance determinants *oqxAB* and *aac(6′)-Ib-cr* and extended-spectrum β-lactamase gene $bla_{\text{CTX-M-24}}$ co-located on the same plasmid in one *Escherichia coli* strain from China. J. Antimicrob. Chemother. **66:**1638–1639.
16. **Johnson JR, Stell AL.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
17. **Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG.** 2009. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob. Agents Chemother. **53:**2733–2739.
18. **Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, Nolan LK.** 2007. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Appl. Environ. Microbiol. **73:**1976–1983.

19. **Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y.** 2009. Change in the prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* in Japan by clonal spread. J. Antimicrob. Chemother. **63:**72–79.
20. **Park SH, Byun JH, Choi SM, Lee DG, Kim SH, Kwon JC, Park C, Choi JH, Yoo JH.** 2012. Molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* in the community and hospital in Korea: emergence of ST131 producing CTX-M-15. BMC Infect. Dis. **12:**149. doi:10.1186/1471-2334-12-149.
21. **Oteo J, Diestra K, Juan C, Bautista V, Novais A, Pérez-Vázquez M, Moyá B, Miró E, Coque TM, Oliver A, Cantón R, Navarro F, Campos J, Spanish Network in Infectious Pathology Project (REIPI).** 2009. Extended-spectrum beta-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Int. J. Antimicrob. Agents **34:**173–176.
22. **Shin J, Kim DH, Ko KS.** 2011. Comparison of CTX-M-14- and CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from patients with bacteremia. J. Infect. **63:**39–47.
23. **Lowe CF, McGeer A, Muller MP, Katz K, Toronto ESBL Working Group.** 2012. Decreased susceptibility to noncarbapenem antimicrobials in extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in Toronto, Canada. Antimicrob. Agents Chemother. **56:**3977–3980.
24. **Doumith M, Dhanji H, Ellington MJ, Hawkey P, Woodford N.** 2012. Characterization of plasmids encoding extended-spectrum β-lactamases and their addiction systems circulating among *Escherichia coli* clinical isolates in the UK. J. Antimicrob. Chemother. **67:**878–885.
25. **Johnson JR, Tchesnokova V, Johnston B, Clabots C, Roberts PL, Billig M, Riddell K, Rogers P, Qin X, Butler-Wu S, Price LB, Aziz M, Nicolas-Chanoine MH, Debroy C, Robicsek A, Hansen G, Urban C, Platell J, Trott DJ, Zhanel G, Weissman SJ, Cookson BT, Fang FC, Limaye AP, Scholes D, Chattopadhyay S, Hooper DC, Sokurenko EV.** 2013. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. J. Infect. Dis. **207:**919–928.
26. **Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G.** 2008. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J. Antimicrob. Chemother. **61:**1024–1028.
27. **Mihaila L, Wyplosz B, Clermont O, Garry L, Hipeaux MC, Vittecoq D, Dussaix E, Denamur E, Branger C.** 2010. Probable intrafamily transmission of a highly virulent CTX-M-3-producing *Escherichia coli* belonging to the emerging phylogenetic subgroup D2 O102-ST405 clone. J. Antimicrob. Chemother. **65:**1537–1539.
28. **Diestra K, Juan C, Curiao T, Moyá B, Miró E, Oteo J, Coque TM, Pérez-Vázquez M, Campos J, Cantón R, Oliver A, Navarro F, Red Española de Investigación en Patología Infecciosa (REIPI), Spain**2009. Characterization of plasmids encoding bla_{ESBL} and surrounding genes in Spanish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. J. Antimicrob. Chemother. **63:**60–66.
29. **Marcadé G, Deschamps C, Boyd A, Gautier V, Picard B, Branger C, Denamur E, Arlet G.** 2009. Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum beta-lactamases. J. Antimicrob. Chemother. **63:**67–71.
30. **Ho PL, Yeung MK, Lo WU, Tse H, Li Z, Lai EL, Chow KH, To KK, Yam WC.** 2012. Predominance of pHK01-like incompatibility group FII plasmids encoding CTX-M-14 among extended-spectrum beta-lactamase-producing *Escherichia coli* in Hong Kong, 1996–2008. Diagn. Microbiol. Infect. Dis. **73:**182–186.