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Emergence of *Escherichia coli* Sequence Type ST131 Carrying both the bla_{GES-5} and $bla_{CTX-M-15}$ Genes[▽]

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Escherichia coli **clinical isolate BD07372 of sequence type ST131 recovered from a bed sore specimen exhibited high-level resistance to ceftazidime and cefotaxime but exhibited susceptibility to imipenem and meropenem. The isolate harbored two β-lactamase genes, the $bla_{CTX-M-15}$ gene carried by an ~250-kbp plasmid carrying the FIA and FIC replicons and the bla_{GES-5} gene carried by a class 1 integron in the chromosome.**

The class A carbapenemases, which have been identified in Gram-negative rods, involve chromosome-borne NMC-A and SME enzymes and plasmid-borne KPC, GES, and IMI enzymes (15). To date, 17 variants of GES-type extended-spectrum β-lactamases (ESBLs) have been identified (http://www.lahey.org/Studies/), but the carbapenem hydrolytic activity has been demonstrated only in some variants, including GES-2, GES-4, GES-5, GES-6, and GES-14, with a substitution at the Gly170 residue (4, 15). The bla_{GES-5} gene was first detected when carried by a plasmid of *Escherichia coli* 365-02 from Greece in 2004 (14). Then, detection of the bla_{GES-5} gene carried by *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* has been reported in many parts of the world (15).

E. coli clinical isolate BD07372 was recovered from a bed sore sampled on 17 May 2007 from a 79-year-old female who has home nursing care. She had been hospitalized for 35 days at a secondary-care hospital in Bundang, Republic of Korea, for the treatment of chronic renal failure, type II diabetes mellitus, and hypertension. Multilocus sequence typing was performed on the isolate using the seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) following use of the protocols available at the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli), and the isolate was identified as sequence type ST131 (allelic profile, 53-40-47-13-36-28-29).

A disk diffusion assay was performed (5), and the isolate exhibited resistance to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefoxitin, aztreonam, ceftazidime, cefotaxime, cefepime, gentamicin, tobramycin, amikacin, and ciprofloxacin and was susceptible to imipenem, meropenem, and trimethoprim-sulfamethoxazole. Synergy was observed between the amoxicillin-clavulanic acid (20/10-μg) disk and the ceftazidime (30-μg), cefotaxime (30-μg), cefepime (30-μg), and aztreonam (30-μg) disks (Becton Dickinson, Spark, MD) in double-disk synergy tests, indicating the production of ESBL (12). Agar dilution MIC testing on Muller-Hinton agar (Difco Laboratories, Detroit, MI) with an inoculum of 10^4 CFU per spot confirmed high-level MICs of ceftazidime (>256 μg/ml) and cefotaxime (>256 μg/ml) and low-level MICs of imipenem (0.5 μg/ml) and meropenem (0.25 μg/ml) for the isolate BD07372 (5). Clavulanic acid (Sigma, St. Louis, MO) at a fixed concentration of 4 μg/ml lowered the MICs of ceftazidime and cefotaxime to 1 μg/ml and 8 μg/ml, respectively (Table 1).

Isolate BD07372 showed a positive result in the Hodge test, which was performed using a MacConkey agar plate with an ertapenem disk, indicating the production of a carbapenemase (7). However, the isolate showed a negative result in the EDTA-based double disk synergy test, which was performed using a Mueller-Hinton agar plate with an imipenem disk and a disk containing EDTA (750g) plus sodium mercaptoacetic acid (2 mg), indicating that the isolate does not produce metallo-β-lactamases (8). In the isoelectric focusing analysis, three β-lactamase activities with isoelectric points of 5.8, 6.1, and 8.6, corresponding to GES-5, OXA-17, and CTX-M-15, respectively, were detected.

PCR and sequencing experiments were performed to detect genes encoding CTX-M-, GES-, PER-, SHV-, TEM-, and VEB-type ESBLs as previously described (11). Isolate BD07372 carried two β-lactamase genes, the $bla_{CTX-M-15}$ and the bla_{GES-5} genes. The isolate transferred cefotaxime resistance to the *E. coli* J53 azide-resistant recipient in mating experiments (3), in which transconjugants were selected on MacConkey agar (Difco Laboratories, Detroit, MI) plates supplemented with cefotaxime (2 μg/ml) and sodium azide (100 μg/ml). The location of the β-lactamase genes was identified by the hybridization of I-CeuI-digested genomic DNA or S1 nuclease-treated linearized plasmids with probes specific for the β-lactamase genes, various replicons of plasmids, and 16S rRNA genes as described previously (2, 9). The probe specific for the $bla_{CTX-M-15}$ gene hybridized with an ~250-kbp plasmid in both isolate BD07372 and its transconjugant, but that specific for the bla_{GES-5} gene did not. The ~250-kbp plasmid carried two types of replicon, FIA and FIC. Replicon sequence

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TABLE 1. MICs of *E. coli* wild-type strain BD07372, its transconjugant, and the recipient J53

Antimicrobial agent[a]	*E. coli* MIC (μg/ml)		
	Wild-type strain BD07372[b]	Transconjugant trcBD07372[c]	Recipient J53
Amoxicillin-clavulanic acid	256	256	6
Piperacillin-tazobactam	>256	>256	1.5
Cefoxitin	128	96	12
Ceftazidime	>256	16	0.25
Ceftazidime-clavulanic acid	8	0.5	0.12
Cefotaxime	>256	32	0.06
Cefotaxime-clavulanic acid	1	0.06	0.06
Cefepime	>256	16	0.06
Aztreonam	64	64	0.25
Imipenem	0.5	0.25	0.19
Imipenem-clavulanic acid	0.25	0.125	0.125
Meropenem	0.25	0.06	0.06
Meropenem-clavulanic acid	0.06	0.06	0.06
Ertapenem	1.5	1	0.023
Ertapenem-clavulanic acid	0.25	0.25	0.023

[a] Clavulanic acid was added at a fixed concentration of 4 μg/ml.
[b] Carries bla_{GES-5} and $bla_{CTX-M-15}$.
[c] Carries $bla_{CTX-M-15}$.

typing was performed on the plasmid as previously described (13), and the sequence types of the replicons were identified to be FIA (1) and FIC (1). This result is in agreement with the previous study, which has confirmed that all the variants of ST131 producing CTX-M-15 ESBL harbored the corresponding gene on the IncF-type plasmid (6). The probe specific for the bla_{GES-5} gene hybridized with an I-CeuI macrorestriction fragment of ~270 kbp carried by isolate BD07372. The probe specific for 16S rRNA genes also hybridized with the I-CeuI macrorestriction fragment, indicating the chromosomal location of the bla_{GES-5} gene in the isolate.

To investigate genetic environments surrounding the bla_{GES-5} gene, several overlapping PCR fragments obtained from whole DNA of the *E. coli* isolate were sequenced with primers corresponding to the internal region of the class 1 integron as previously described (1). Identical to the bla_{GES-5} gene carried by a plasmid of *K. pneumoniae* CHAK36, the bla_{GES-5} gene was carried by a class 1 integron as a gene cassette downstream of the *attI1* recombination site. The integron had three unique gene cassettes [bla_{GES-5}–aac(6′)-IIa–bla_{OXA-17}/*orf4*] between the two conserved elements, the 5′-conserved segment (5′-CS) and the 3′-CS, but the 59-base element of the third gene cassette was interrupted by a putative transposase gene, *orf4*.

In summary, *E. coli* isolate BD07372 of ST131 harbored two β-lactamase genes, the $bla_{CTX-M-15}$ gene carried by an ~250-kbp plasmid carrying the FIA and FIC replicons and the bla_{GES-5} gene carried by a class 1 integron in a chromosome. Recently, the intercontinental dissemination of an *E. coli* clone of ST131 producing CTX-M-15, which was carried mostly by IncF plasmids, has been reported (10). Emergence of an *E. coli* clone of ST131 simultaneously producing CTX-M-15 and GES-5 could be a serious threat to global health.

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