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Extensively Drug-Resistant *Escherichia coli* Sequence Type 1642 Carrying an IncX3 Plasmid Containing the *bla*$_{KPC-2}$ Gene Associated with Transposon Tn*4401a*

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Background: Extensively drug-resistant (XDR) *Enterobacteriaceae* carrying the *bla*$_{KPC}$ gene have emerged as a major global therapeutic concern. The purpose of this study was to analyze the complete sequences of plasmids from KPC-2 carbapenemase-producing XDR *Escherichia coli* sequence type (ST) 1642 isolates.

Methods: We performed antimicrobial susceptibility testing, PCR, multilocus sequence typing (MLST), and whole-genome sequencing to characterize the plasmid-mediated KPC-2-producing *E. coli* clinical isolates.

Results: The isolates were resistant to most available antibiotics, including meropenem, ampicillin, ceftriaxone, gentamicin, and ciprofloxacin, but susceptible to tigecycline and colistin. The isolates were identified as the rare ST1642 by MLST. The isolates carried four plasmids: the first 69-kb conjugative IncX3 plasmid harbors *bla*$_{KPC-2}$ within a truncated Tn*4401a* transposon and *bla*$_{SHV-11}$ with duplicated conjugative elements. The second 142-kb plasmid with a multireplicon consisting of IncQ, IncFIA, and IncIB carries *bla*$_{TEM-1b}$ and two class 1 integrons. This plasmid also harbors a wide variety of additional antimicrobial resistance genes including *aadA5, dfrA17, mph(A), sul1, tet(B), aac(3')-IId, strA, strB,* and *sul2*.

Conclusions: The complete sequence analysis of plasmids from an XDR *E. coli* strain related to persistent infection showed the coexistence of a *bla*$_{KPC-2}$–carrying IncX3-type plasmid and a class 1 integron-harboring multireplicon, suggesting its potential to cause outbreaks. Of additional clinical significance, the rare ST1642, identified in a cat, could constitute the source of human infection.

Key Words: *Escherichia coli*, ST1642, *bla*$_{KPC}$, Tn*4401a*, IncX3

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INTRODUCTION

Klebsiella pneumoniae carbapenemase (KPC) is the most common class A carbapenemase in the world; the extensive spread of KPC-producing *Enterobacteriaceae* has become a major therapeutic concern in clinical settings [1]. To date, a total of 21 KPC variants (KPC-2 to KPC-22) have been identified; of these, KPC-2 and KPC-3 are the most prevalent [1]. KPCs are found predominately in *K. pneumoniae* and less frequently in *Escherichia coli* [1, 2]. Similar to other acquired antimicrobial resistance determinants, the *bla*$_{KPC}$ gene is disseminated by two means: 1) clonal spread of bacteria carrying the gene, and 2) horizontal transfer of the gene carried on mobile elements such as plasmids and transposons [1, 2].

Molecular epidemiological studies on KPC-producing *E. coli* by multilocus sequence typing (MLST) have revealed that sequence type (ST) 131 strains are the most pervasive, followed by ST410, and less frequently ST69, ST93, ST167, ST354, and ST3948 strains [3-6]. In *E. coli* isolates, both bla_{KPC-2} and bla_{KPC-3} genes are prevalent, while bla_{KPC-8} is rarely identified [4]. The bla_{KPC-2} gene is carried by IncFIA-, IncFIIk-, IncN-, and IncA/C-type plasmids in the USA; IncN-, IncA/C- and IncF-type plasmids in China; an IncFIIA-type plasmid in France; an IncFIIk-type plasmid in Greece; and IncFIIs-, IncN-, and IncHI2- plasmids in Israel; while the bla_{KPC-3} gene is associated with IncFIA-, IncFIIk-, IncN-, and IncA/C-type plasmids in the USA; an IncFIIk-type plasmid in Italy; IncFII-, IncA/C-, and ColE-type plasmids in Spain; and IncFIIs-, IncN-, and IncHI2-plasmids in Israel [4, 6]. Although an IncX3-type plasmid is prevalent in *K. pneumoniae* carrying the bla_{KPC} gene [7], it has rarely been reported in *E. coli*.

The highly mobile Tn3-based transposon Tn*4401* facilitates dissemination of the bla_{KPC} gene [8]. Tn*4401* is comprised of *tnpA* (transposase), *tnpR* (resolvase), and two insertion sequences (ISs), IS*Kpn7* and IS*Kpn6*, as well as the bla_{KPC} gene. The 10-kb transposon has 39-bp imperfect left- and right-inverted repeats and is flanked by 5-bp direct repeats [9]. Seven Tn*4401* isoforms, Tn*4401a* to Tn*4401g*, have been identified; these are differentiated based on the size (68- to 255-bp) of the deletion between IS*Kpn7* and bla_{KPC} compared with the prototype Tn*4401b* [10].

Here, we investigated the plasmids present in KPC-producing *E. coli* clinical isolates to elucidate the mechanisms underlying the acquisition of multi-drug resistance, including resistance to carbapenems.

METHODS

1. Patient description

An 85-year-old woman with a history of hypertension was transferred from Hyemin general hospital (Seoul, Korea) to Gangnam Severance hospital (Seoul, Korea) in January 2014 for the management of her cerebral hemorrhage. Brain computed tomography and magnetic resonance imaging revealed a subacute intracerebral hemorrhage in the right thalamus. The patient complained of urinary frequency and retention, and a fever of over 38.6°C developed on the 8th day post admission. An *E. coli* isolate (EcU443) was recovered from a urinary specimen on the 8th day, and subsequent *E. coli* isolates (EcU213 on the 18th day and EcU120 on the 42nd day) were serially recovered from urinary specimens.

2. Bacterial isolates and antimicrobial susceptibility testing

This study involved two *E. coli* clinical isolates, EcU443 and EcU120, serially recovered from urinary specimens of a patient with a 34-day interval. The isolates were identified as *E. coli* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics GmbH, Leipzig, Germany). Antimicrobial susceptibilities were determined using VITEK 2 AST N211 cards (bioMérieux Vitek Inc., Hazelwood, MO, USA) and disk diffusion tests on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) according to the CLSI guidelines [11]. Carbapenemase production was confirmed using the KPC+MBL Confirm ID Kit (Rosco Diagnostica, Taastrup, Denmark) with tablets containing meropenem (10 μg) alone or supplemented with dipicolinic acid (1,000 μg), phenylboronic acid (400 μg), and cloxacillin (750 μg) [12].

3. Genotyping of β-lactamases

The genomic DNA of each isolate was extracted by the boiling lysis method [13]. PCR was performed to detect genes encoding extended-spectrum β-lactamases (CTX-M-1-, CTX-M-9-, TEM-, and SHV-type) and carbapenemases (IMP-1-type, VIM-2-type, NDM, KPC, GES, and OXA-48-like), as previously described [14]. Both strands of the amplicons were sequenced using an automatic sequencer (model 3730xl; Applied Biosystems, Weiterstadt, Germany), and the nucleotide sequences were compared using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast).

4. Multilocus sequence typing

MLST was carried out using partial sequences of seven *E. coli* housekeeping genes, including *adk, fumC, gyrB, icd, mdh, purA,* and *recA*. Nucleotide sequences were compared with those in the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) to identify allelic types and STs.

5. Bacterial conjugation

E. coli strain EcU443 was used as the donor and sodium azide-resistant *E. coli* J53 was used as the recipient for the standard agar mating method [15]. Following overnight mating at 37°C on brain-heart infusion (BHI; MB cell, Los Angeles, CA, USA) agar, transconjugants were selected on BHI supplemented with 100 μg/mL sodium azide and 0.5 μg/mL imipenem.

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6. Whole genome sequencing

The complete DNA sequences of the plasmids present in EcU443 were obtained via Single-Molecule Real-Time sequencing on a PacBio RSII instrument (Pacific Biosciences, Menlo Park, CA, USA), according to the manufacturer's instructions. The sequences were annotated using Prokka 1.11 (http://www.vicbioinformatics.com/software.prokka.shtml). Resistance genes, IS elements, replication origins, virulent elements, and toxins and antitoxin systems were identified using ResFinder (https://cge.cbs.dtu.dk//services/ResFinder/), IS-finder (https://www-is.biotoul.fr/), plasmid finder (https://cge.cbs.dtu.dk//services/Pla-smidFinder/), the Virulence Factor Database (http://www.mgc.ac.cn/VFs/), and TA Finder 1.0 (http://202.120.12.133/TAfinder/index.php), respectively.

7. GenBank accession numbers

Nucleotide sequence data for plasmids pECSEV_01 and pECSEV_02 are available under GenBank accession numbers KX683283 and KX683284, respectively.

RESULTS

1. Antimicrobial susceptibilities and molecular typing

The antimicrobial susceptibility profiles of the clinical *E. coli* strains are presented in Table 1. The isolates exhibited resistance to most antibiotics tested, including meropenem, ampicillin, ceftriaxone, gentamicin, and ciprofloxacin, but were susceptible to tigecycline and colistin. Phenotypic carbapenemase differentiation tests showed positive results for KPC production in both isolates (EcU443 and EcU120; Table 2). The MLST assay

Table 1. Antimicrobial susceptibilities of clinical *Escherichia coli* isolates*

Antibiotics	MIC (mg/L)			Antibiotics	Zone diameter (mm)		Interpretation‡
	EcU443†	EcU213†	EcU120†		EcU443	EcU120	
AMP	**≥32**	**≥32**	**≥32**	AMP	**6**	**10**	R
SAM	**≥32**	**≥32**	**≥32**	SAM	**6**	**6**	R
TZP	**≥128**	**≥128**	**≥128**	PIP	**10**	**10**	R
ATM	**≥64**	**≥64**	**≥64**	TIC	**10**	**10**	R
CAZ	4	4	**16**	CRO	**15**	**16**	R
GEN	**≥16**	**≥16**	**≥16**	GEN	**7**	**7**	R
LVX	**≥8**	**≥8**	**≥8**	CIP	**6**	**6**	R
CFZ	**≥64**	**≥64**	**≥64**	IMP	**20**	**19**	I
MEM	**≥16**	**≥16**	**≥16**	MEM	**20**	**20**	I
ERM	**4**	**4**	**≥8**	ERM	**17**	**17**	R
CTM	**≥320**	**≥320**	**≥320**	CST	14	15	-
TGC	≤0.5	≤0.5	≤0.5	TGC	24	24	-

*The breakpoints were applied according to the Clinical and Laboratory Standards Institute (CLSI) guidelines; the resistance values are in bold; †EcU443, EcU213, and EcU120 were isolated on the 8th, 18th, and 42nd day post admission, respectively; ‡The EcU443 disk diffusion tests results were interpreted according to the CLSI guidelines; the results for colistin and tigecycline are not shown because of the lack of suggested breakpoints.
Abbreviations: AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CFZ, cefazolin; CIP, ciprofloxacin; CRO, ceftriaxone; CST, colistin; ERM, ertapenem; GEN, gentamicin; I, intermediate; IMP, imipenem; LVX, levofloxacin; MEM, meropenem; MIC, minimum inhibitory concentration; PIP, piperacillin; R, resistant; SAM, ampicillin-sulbactam; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; TIC, ticarcillin; TZP, piperacillin-tazobactam.

Table 2. Results of carbapenemase differentiation tests and sequence types of *Escherichia coli* isolates

E. coli isolates*	Carbapenemase differentiation test				MLST							
	MEM	MEM+DPA	**MEM+PBA†**	MEM+CLX	**ST**	*adk*	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
EcU443	19	19	**24**	20	**1642**	6	4	5	18	11	8	6
EcU120	20	20	**25**	21	**1642**	6	4	5	18	11	8	6

*EcU443 and EcU120 were isolated on the 8th and 42nd day post admission, respectively; †A difference between MEM+PBA and MEM >5 mm indicated KPC production.
Abbreviations: CLX, cloxacillin; DPA, dipicolinic acid; MEM, meropenem; MLST, Multilocus sequence typing; PBA, phenylboronic acid.

assigned both isolates as an identical ST1642 (6-4-5-18-11-8-6). PCR and sequencing for β-lactamase genes demonstrated the presence of bla_{KPC-2}, bla_{SHV-11}, and bla_{TEM-1} in both isolates.

2. Plasmids pECSEV_01 and pECSEV_02

E. coli strain EcU443 had a 4,769,071-bp chromosome and four plasmids. The chromosome did not contain any acquired antimicrobial resistance determinants. The 69,409-bp IncX3 plasmid (pECSEV_01) carried bla_{KPC-2} and bla_{SHV-11} for β-lactam resistance; the 142,708-bp multireplicon (IncFIA, IncFIB, and IncQ) plasmid (pECSEV_02) included *strA, strB, aadA5*, and *aac(3)-IId* for aminoglycoside resistance, bla_{TEM-1b} for β-lactam resistance, *mph(A)* for macrolide resistance, *sul1* and *sul2* for sulfonamide resistance, *tet(B)* for tetracycline resistance, and *dfrA17* for trimethoprim resistance; and the 118,130-bp plasmid (pECSEV_03) harbored bla_{TEM-1b} for β-lactam resistance and *qnrS1* for quinolone resistance. Moreover, a cryptic 110,571-bp plasmid was also identified.

Plasmid pECSEV_01 carrying the bla_{KPC-2} gene belonged to the IncX3 group (Fig. 1). This plasmid could be transferred to recipient *E. coli* J53 by surface mating. The bla_{KPC-2} gene was located within a truncated Tn*4401* transposon; Δ*ISKpn7-bla*$_{KPC-2}$-*ISKpn6* had a 99-bp deletion between *ISKpn7* and bla_{KPC} indicating it was a Tn*4401a* isoform. The plasmid also harbored the bla_{SHV-11} gene encoding a broad-spectrum β-lactamase. The replication origin *repB* gene belonged to the IncX3 incompatibility type. The remaining sections of pECSEV_01 consisted of duplicated type IV secretion systems and conjugative elements.

Plasmid pECSEV_02 possessed three replication origins for the IncFIA, IncFIB, and IncQ groups (Fig. 2). The plasmid carried two identical copies of a class 1 integron, In54, containing the *dhfrA17-aadA5-emrE* gene cassettes for trimethoprim-, aminoglycosides-, and multidrug-resistance, as well as a truncated Tn*3* transposon harboring bla_{TEM-1b}. The *macB*, *mph(A), aac(3)-IId, strA, strB*, and *tet(B)* drug resistance genes were also identified. In addition to drug-resistance determinants, pECSEV_02 also included a virulence gene cluster containing the *iucA, iucB, iucC, iucD,* and *iutA* genes involved in hydroxamate siderophore aerobactin synthesis and two toxin/antitoxin systems, *vapBC* and *ccdAB*.

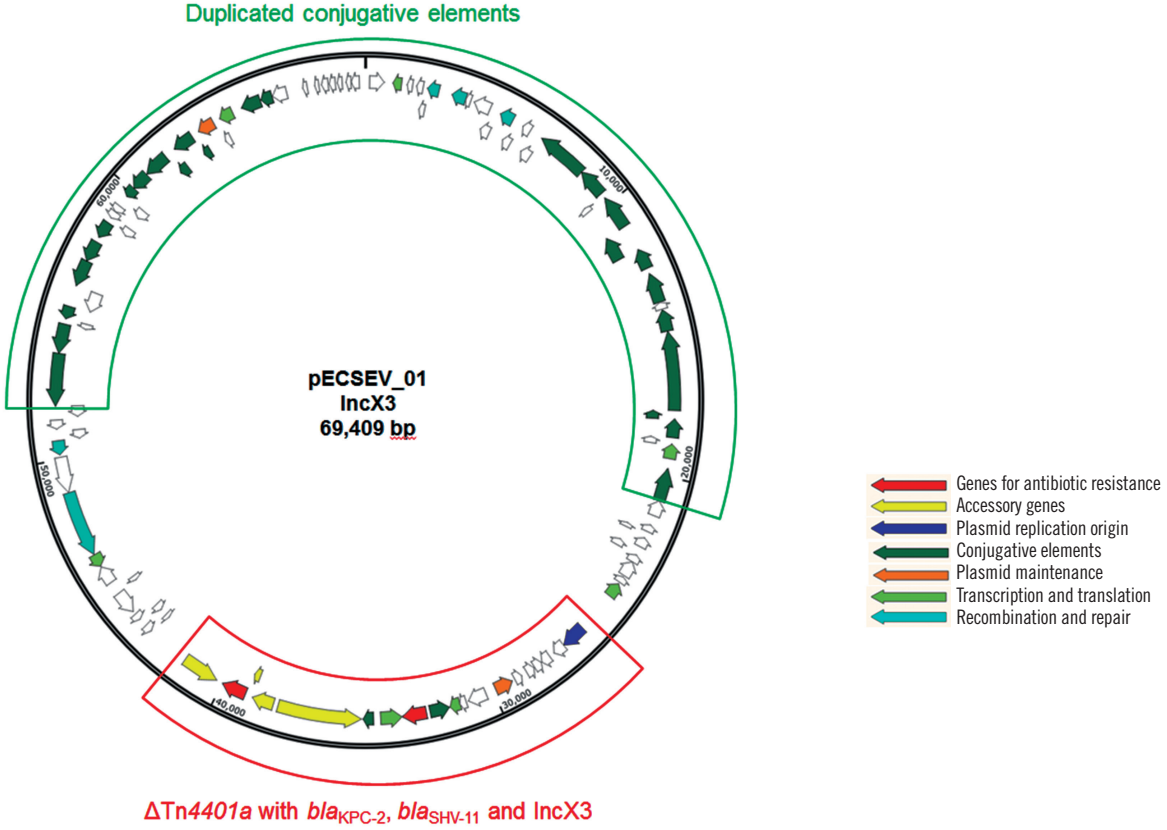

Fig. 1. Circular map of pECSEV_01 containing bla_{KPC-2}, bla_{SHV-11}, and duplicated conjugative elements.

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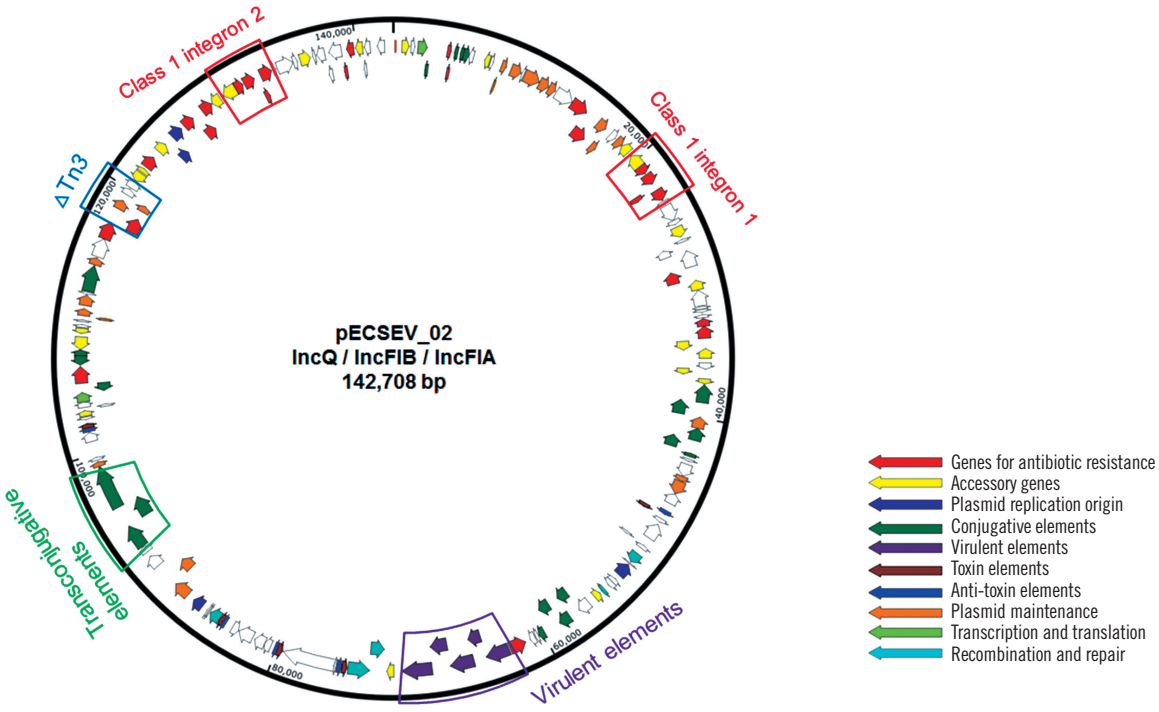

Fig. 2. Circular map of pECSEV_02 containing *bla*$_{TEM-1}$, two class 1 integrons, virulence elements, and transconjugative elements.

DISCUSSION

E. coli clinical isolates EcU443 and EcU120 were serially recovered from urinary specimens of a patient with a 34-day interval. Both strains belong to ST1642, exhibit resistance or intermediate resistance to all classes of antimicrobial agents tested, except for tigecycline and colistin, and possess the *bla*$_{KPC-2}$, *bla*$_{SHV-11}$, and *bla*$_{TEM-1}$ β-lactamase genes, indicating that this clone was responsible for the persistent infection in the patient over the 34 days. To the best of our knowledge, *E. coli* ST1642, first isolated from a pet cat as an extraintestinal pathogenic strain [16], has not been previously reported as a KPC-producer. Although ST131 strains are the most frequently identified in KPC-producer *E. coli* infections [17], three cases of KPC-2-producer infections due to ST69, ST393, and a new *E. coli* ST were recently identified in Busan [18]. The three *E. coli* strains carried an IncX3 plasmid similar to that in our strains, although the *E. coli* strains were epidemiologically unrelated.

E. coli strain EcU443 possesses four plasmids. The *bla*$_{KPC-2}$ gene is on IncX3 plasmid pECSEV_01 containing the *bla*$_{SHV-11}$ gene. The plasmid showed 99% identity with IncX3 plasmid pKpS90 from a *K. pneumoniae* ST258 strain (GenBank accession number JX461340) isolated from a blood culture during a hospital outbreak in France [19]. In pECSEV_01, the truncated Tn*4401a* carrying the *bla*$_{KPC-2}$ gene is integrated in the opposite direction, but at the same location as in pKpS90. The truncated Tn*4401a* has also been found in IncX3 plasmid pKPC_Kp01 from a clinical *K. pneumoniae* isolate responsible for a hospital outbreak in Busan [18].

Plasmid pECSEV_02, containing multiple replicons of IncFIA, IncFIB, and IncQ, carries various genes conferring resistance to diverse classes of antimicrobial agents. Interestingly, pECSEV_02 harbors two identical copies of class 1 integron In54. An *Enterobacter cloacae* strain carrying multiple non-identical class 1 integrons in a plasmid has been identified [20]. Plasmid pECSEV_02 also carries genes for virulence factors and for toxin/antitoxin systems that can enhance bacterial fitness in a human host, thus explaining its long persistence in the patient. The third plasmid, pECSEV_03, also contributed to the extensively drug-resistant (XDR) *E. coli* via *bla*$_{TEM-1b}$ and *qnrS1*.

This study reports a persistent infection case caused by an XDR *E. coli* ST1642 strain carrying an IncX3 plasmid containing *bla*$_{KPC-2}$ associated with a truncated Tn*4401a* transposon and a plasmid with multireplicons of IncQ, IncFIA, and IncFIB, which contains genes conferring resistance to multiple classes of antimicrobial agents. Sporadic or epidemic infection cases caused by KPC-producers have been increasingly reported in Korea; the *bla*$_{KPC}$ genes are spreading to *K. pneumoniae* as well as other

species in the family *Enterobacteriaceae*, including *E. coli*, via R plasmids. Further studies are needed to investigate the current status of KPC-producers in Korea.

Authors' Disclosure of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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REFERENCES

1. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic Context, treatment options, and detection methods. Front Microbiol 2016;7:895.
2. Chen YT, Lin JC, Fung CP, Lu PL, Chuang YC, Wu TL, et al. KPC-2-encoding plasmids from *Escherichia coli* and *Klebsiella pneumoniae* in Taiwan. J Antimicrob Chemother 2014;69:628-31.
3. Adler A, Miller-Roll T, Assous MV, Geffen Y, Paikin S, Schwartz D, et al. A multicenter study of the clonal structure and resistance mechanism of KPC-producing *Escherichia coli* isolates in Israel. Clin Microbiol Infect 2015;21:230-5.
4. Piazza A, Caltagirone M, Bitar I, Nucleo E, Spalla M, Fogato E, et al. Emergence of *Escherichia coli* Sequence Type 131 (ST131) and ST3948 with KPC-2, KPC-3 and KPC-8 carbapenemases from a Long-Term Care and Rehabilitation Facility (LTCRF) in Northern Italy. Adv Exp Med Biol 2016;901:77-89.
5. Chavda KD, Chen L, Jacobs MR, Bonomo RA, Kreiswirth BN. Molecular diversity and plasmid analysis of KPC-producing *Escherichia coli*. Antimicrob Agents Chemother 2016;60:4073-81.
6. Xu G, Jiang Y, An W, Wang H, Zhang X. Emergence of KPC-2-producing *Escherichia coli* isolates in an urban river in Harbin, China. World J Microbiol Biotechnol 2015;31:1443-50.
7. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, et al. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. Plasmid 2012;68:43-50.
8. He S, Chandler M, Varani AM, Hickman AB, Dekker JP, Dyda F. Mechanisms of evolution in high-consequence drug resistance plasmids. MBio 2016;7:e01987-16.
9. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the β-lactamase *bla*$_{KPC}$ gene. Antimicrob Agents Chemother 2008;52:1257-63.
10. Naas T, Cuzon G, Truong HV, Nordmann P. Role of IS*Kpn7* and deletions in *bla*$_{KPC}$ gene expression. Antimicrob Agents Chemother 2012;56:4753-9.
11. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Twenty-sixth informational supplement, M100-S26. Wayne, PA: National Committee for Clinical Laboratory Standards, 2016.
12. Kim MN, Yong D, An D, Chung HS, Woo JH, Lee K, et al. Nosocomial clustering of NDM-1-producing *Klebsiella pneumoniae* sequence type 340 strains in four patients at a South Korean tertiary care hospital. J Clin Microbiol 2012;50:1433-6.
13. Chen L, Mediavilla JR, Endimiani A, Rosenthal ME, Zhao Y, Bonomo RA, et al. Multiplex real-time PCR assay for detection and classification of *Klebsiella pneumoniae* carbapenemase gene (*bla*$_{KPC}$) variants. J Clin Microbiol 2011;49:579-85.
14. Jeong S, Kim JO, Jeong SH, Bae IK, Song W. Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in gram-negative clinical isolates. J Microbiol Methods 2015;113:4-9.
15. Jeong SH, Lee KM, Lee J, Bae IK, Kim JS, Kim HS, et al. Clonal and horizontal spread of the *bla*$_{OXA-232}$ gene among *Enterobacteriaceae* in a Korean hospital. Diagn Microbiol Infect Dis 2015;82:70-2.
16. Harada K, Nakai Y, Kataoka Y. Mechanisms of resistance to cephalosporin and emergence of O25b-ST131 clone harboring CTX-M-27 β-lactamase in extraintestinal pathogenic *Escherichia coli* from dogs and cats in Japan. Microbiol Immunol 2012;56:480-5.
17. O'Hara JA, Hu F, Ahn C, Nelson J, Rivera JI, Pasculle AW, et al. Molecular epidemiology of KPC-producing *Escherichia coli*: occurrence of ST131-fimH30 subclone harboring pKpQIL-like IncFIIk plasmid. Antimicrob Agents Chemother 2014;58:4234-7.
18. Kim JO, Song SA, Yoon EJ, Shin JH, Lee H, Jeong SH, et al. Outbreak of KPC-2-producing *Enterobacteriaceae* caused by clonal dissemination of *Klebsiella pneumoniae* ST307 carrying an IncX3-type plasmid harboring a truncated Tn*4401a*. Diagn Microbiol Infect Dis 2017;87:343-8.
19. Kassis-Chikhani N, Frangeul L, Drieux L, Sengelin C, Jarlier V, Brisse S, et al. Complete nucleotide sequence of the first KPC-2- and SHV-12-encoding IncX plasmid, pKpS90, from *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2013;57:618-20.
20. Plante I, Centrón D, Roy PH. Direct sequencing and PCR mapping of integrons reveals multiple class 1 integrons in the multiresistant strain *Enterobacter cloacae* SCH88040794. FEMS Microbiol Lett 2003;221:59-62.

https://doi.org/10.3343/alm.2018.38.1.17