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First Outbreak of KPC-2-Producing *Klebsiella pneumoniae* Sequence Type 258 in a Hospital in South Korea

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In this study, we report the first outbreak of KPC-2-producing *Klebsiella pneumoniae* isolates from three patients admitted to a neurosurgery department in a South Korean teaching hospital. Multilocus sequence typing showed that the isolates were identical to the previous KPC producers in South Korea and other countries, suggesting clonal spread.

KPC enzymes hydrolyze carbapenems and are the most clinically significant enzymes among class A carbapenemases (1). The first KPC-1-producing *Klebsiella pneumoniae* isolate was detected in 1996 in the United States (2). Thereafter, KPC-2 and its variant producers have caused severe treatment problems in hospitals around New York and have also been reported in Europe, South America, and China (1). In South Korea, only two cases of infection with KPC-2-producing *K. pneumoniae* had been reported by 2010 (3, 4). Subsequently, six bla_{KPC-2}-positive *K. pneumoniae* strains were sporadically isolated from several hospitals during nationwide surveillance in 2011 (5). To date, no outbreaks of KPC-2-producing *K. pneumoniae* have been reported in a university-affiliated hospital in South Korea.

(Some of the data in this work were presented at the 22nd European Congress of Clinical Microbiology and Infectious Diseases, London, United Kingdom, 31 March to 3 April 2012.)

In the present study, we isolated non-carbapenem-susceptible *K. pneumoniae* isolates from three patients admitted to the neurosurgery (NS) department in an 870-bed teaching hospital in South Korea over a 5-week period. All three patients were admitted for the treatment of central nervous system (CNS) lesions. One patient underwent craniotomy for the resection of a CNS tumor, and two patients received catheter insertions for intracranial hemorrhage. For postoperative care, these patients were admitted to the neurosurgical intensive care unit (ICU) from 2 to 49 days. The patients received mechanical ventilation. The periods spent by two patients in the ICU overlapped. After acute management, all three patients were transferred to the same NS general ward. On 12 July 2011, the first non-carbapenem-susceptible *K. pneumoniae* strain was isolated from one of the three patients admitted to the NS general ward, and two consecutive strains were isolated over a 5-week period. Two KPC-producing isolates were found to be the cause of pneumonia, and one isolate caused asymptomatic bacteriuria. The first patient received 4.5 g piperacillin-tazobactam three times a day for 50 days followed by 1.0 g cefepime twice daily for 28 days before the outbreak-causing strain was isolated. The second patient received 1.0 g imipenem-cilastatin twice daily for 19 days. The pneumonia cases caused by KPC-producing isolates were cured. The third patient was treated with 1.0 g meropenem twice daily for 20 days before the isolation of the KPC-producing strain (Table 1).

Bacterial identification and susceptibility tests were routinely performed using the Vitek 2 system (bioMérieux, Marcy l'Étoile, France). The antimicrobial susceptibilities were interpreted according to the Clinical and Laboratory Standards Institute 2011 guidelines (6). In previous South Korean studies, the first isolate of KPC-2-producing *K. pneumoniae* exhibited multidrug resistance to various antibiotics, including colistin (3), while the second isolate was susceptible to gentamicin, tigecycline, and colistin (4). Six surveillance isolates of KPC-2-producing *K. pneumoniae* exhibited high MICs of carbapenems (>32 mg/liter) and multidrug resistance, with the exception of susceptibility to tigecycline and colistin (5). Unlike previous studies, in our study, two isolates of KPC-producing *K. pneumoniae* exhibited intermediate susceptibility to imipenem (MIC, 2 mg/liter) and meropenem (MIC, 2 mg/liter); however, one isolate was resistant to imipenem (MIC, 4 mg/liter) and meropenem (MIC, ≥16 mg/liter) (Table 2). Carbapenemase production was confirmed by the modified Hodge test (7). In order to classify the types of carbapenem resistance mechanisms, we used a combination test of carbapenem and β-lactamase inhibitor disks (Neo-Sensitabs and Diatabs; Rosco Diagnostica, Denmark). Boronic acid exhibited inhibitory effects (≥5-mm enlargement) on all three non-carbapenem-susceptible strains, but the inhibitory zones of cloxacillin- and dipicolinic acid-containing disks were not enlarged for these strains. The bla_{KPC} gene was detected by a multiplex PCR that detects the five class A carbapenemase families (KPC, SME, IMI, NMC-A, and GES enzymes) (8). The sequence data for the bla_{KPC-2} genes were confirmed by using flanking primers (forward primer: 5′-GCT ACA CCT AGC TCC ACC TTC-3′; reverse primer: 5′-GAC AGT GGT TGG TAA TCC ATG C-3′). PCR amplification of other β-lactamase genes (i.e., bla_{TEM}, bla_{SHV}, bla_{PSE}, bla_{CTX-M}, and bla_{AmpC}) was performed using family-specific primers. All KPC-

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TABLE 1 Clinical characteristics of the patients and isolates[a]

Case	Sex and age (yr)	Underlying disease(s)	Nursery history	Hospitalization dates (day/mo/yr)	Ward	Surgery	Mechanical ventilation	Diagnosis	Specimen	Isolation date (day/mo/yr)	Previous antibiotics	Treatment	Outcome
1	M, 72	Esophageal cancer with brain metastasis	No	12/3/2011–22/7/2011	NSICU (2 days), NSGW	Craniotomy, tumor removal	Yes	Pneumonia	Sputum	18/7/2011	Piperacillin-tazobactam, cefepime	Cefepime	Cured
2	M, 64	ICH	No	29/5/2011–11/10/2011	NSICU (37 days), NSGW	ICH catheter insertion	Yes	Pneumonia	Sputum	11/8/2011	Imipenem-cilastatin	Levofloxacin	Cured
3	M, 46	ICH	Yes	9/6/2011–14/9/2011	NSICU (49 days), NSGW	ICH catheter insertion	Yes	Asymptomatic bacteriuria	Urine	19/8/2011	Meropenem	None	

[a] Abbreviations: M, male; ICH, intracranial hemorrhage; ICU, intensive care unit; NSICU, neurosurgical ICU; NSGW, neurosurgical general ward.

TABLE 2 Antimicrobial susceptibilities and MICs of KPC-2-producing *Klebsiella pneumoniae* isolates in South Korea

Antimicrobial agent	MIC (mg/liter) and susceptibility[a]						
			National surveillance		This study		
	1st isolate (Kpn-DK2)	2nd isolate (KPN 1010)	5 isolates	1 isolate	KPN MP14	KPN MP35	KPN MP52
Ampicillin	>64 (R)	>256 (R)			≥32 (R)	≥32 (R)	≥32 (R)
Amoxicillin-clavulanate		>256 (R)			≥32 (R)	≥32 (R)	≥32 (R)
Piperacillin		>256 (R)			≥128 (R)	≥128 (R)	≥128 (R)
Piperacillin-tazobactam		>256 (R)			≥128 (R)	≥128 (R)	≥128 (R)
Cephalothin		≥64 (R)			≥64 (R)	≥64 (R)	≥64 (R)
Cefoxitin		>256 (R)			≥64 (R)	≥64 (R)	≥64 (R)
Cefotetan		>256 (R)					
Cefotaxime	>128 (R)	>256 (R)	128->256 (R)	>256 (R)	8 (R)	8 (R)	8 (R)
Ceftazidime	>64 (R)	≥64 (R)	64->256 (R)	128 (R)	16 (R)	≥64	≥64 (R)
Aztreonam	>64 (R)	>256 (R)	256->256 (R)	256 (R)	≥64 (R)	≥64	≥64 (R)
Cefepime	>64 (R)	>256 (R)			4 (S)	8 (S)	8 (S)
Imipenem	32 (R)	>256 (R)	32–128 (R)	64 (R)	2 (I)	2 (I)	4 (R)
Meropenem	16 (R)	>256 (R)	64–256 (R)	32 (R)	2 (I)	2 (I)	≥16 (R)
Ertapenem		>32 (R)			>32 (R)	>32 (R)	>32 (R)
Amikacin		48 (R)	64->256 (R)	>256 (R)	≥64 (R)	≥64 (R)	≥64 (R)
Tobramycin		≥16 (R)			≥16 (R)	≥16 (R)	≥16 (R)
Gentamicin	>64 (R)	4 (S)	2–4 (S)	>256 (R)	4 (S)	4 (S)	4 (S)
Levofloxacin		≥8 (R)			≥8 (R)	≥8 (R)	≥8 (R)
Ciprofloxacin	>32 (R)	>32 (R)	256->256 (R)	256 (R)			
Trimethoprim-sulfamethoxazole		≥320 (R)			≥320 (R)	≥320 (R)	≥320 (R)
Tigecycline	4	1	0.75–1.5	1.5	2	2	2
Colistin	>64	0.25	0.25–1	1	0.25	0.25	0.25
Polymyxin B	>64	0.75					

[a] Antimicrobial susceptibility of the first isolate was tested by a broth microdilution method, that of the second isolate was tested by Vitek 2 or Etest, the six national surveillance strains were tested by Etest or agar dilution, and the isolates in this study were tested by Vitek 2 or Etest. S, susceptible; I, intermediate; R, resistant.

TABLE 3 Genetic characteristics of KPC-2-producing isolates reported in South Korea

Isolate(s)	MLST type	Genetic environment	β-Lactamase genes	Reference
Kpn-DK	ST11		bla_{TEM-1}, bla_{SHV-11}, $bla_{CTX-M-15}$	3
KPN1010	ST258	Tn*4401*, isoform *a*		4
CRKP-18, -26, -51, -53, -62, -88	ST258	Tn*4401*, isoform *a*	bla_{TEM-1}, bla_{SHV-11}, bla_{OXA-9}	5
KPN MP14, MP35, MP52	ST258	Tn*4401*, isoform *a*	$bla_{TEM-like}$, $bla_{SHV-like}$	This study

producing isolates carried three β-lactamase genes: $bla_{TEM-like}$, $bla_{SHV-like}$, and bla_{KPC-2}. However, the bla_{PSE}, bla_{CTX-M}, and bla_{AmpC} genes were not detected in the outbreak strains. The first KPC-2-producing isolate in South Korea contained a $bla_{CTX-M-15}$ gene, but the second isolate and six strains isolated from a South Korean surveillance study did not harbor this gene (3–5) (Table 3).

The genetic environments of the bla_{KPC-2} gene of the three outbreak strains and a KPN 1010 strain (4) were characterized by using specific primer pairs (9) and sequenced by PCR mapping, based on the sequence of Tn*4401*. The examined environments corresponded to that of the variant of Tn*4401*, isoform *a* (10). Plasmid analysis with S1 nuclease digestion (11) showed a ca. 180-kb band in a representative outbreak strain (data not shown). Hybridization was carried out with a digoxigenin (DIG) DNA labeling and detection kit (Roche Diagnostics GmbH, Mannheim, Germany) as described in a previous report (12), and the blotted band was hybridized with probes specific for the bla_{KPC-2} gene.

The pulsed-field gel electrophoresis patterns, obtained with XbaI digestion, of the outbreak strains were identical. Multilocus sequence typing (MLST) with seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) was performed on three KPC-producing *K. pneumoniae* isolates (13) and one contemporary extended-spectrum-β-lactamase-producing carbapenem-susceptible *K. pneumoniae* isolate as a control strain. MLST of the three bla_{KPC-2}-producing isolates resulted in one sequence type (ST), 258 (allelic profile 3-3-1-1-1-1-79), but the type of the control strain was ST11 (allelic profile 3-3-1-1-1-1-4). Although the MLST types of the first South Korean case of KPC-2-producing *K. pneumoniae* and our control strain were ST11, the remaining isolates were ST258 (3–5). The bla_{KPC-2} gene-carrying *K. pneumoniae* ST258 clone has been identified worldwide, suggesting that it may have contributed significantly to the spread of bla_{KPC-2} genes (1). Therefore, we suspect that KPC-producing *K. pneumoniae* ST258 may have spread to South Korea from other countries.

In summary, there has been no outbreak of KPC-2-producing *K. pneumoniae* in South Korea thus far. However, in our hospital, three KPC-2-producing strains were isolated during a 5-week period, and the pulsotypes and sequence types of the strains were identical. To the best of our knowledge, this is the first outbreak of KPC-2-producing *K. pneumoniae* ST258 in South Korea. Collectively, these results suggest that KPC-2-producing clones have been spreading in South Korean hospitals and their prevalence may be increasing. Therefore, more intensive efforts to control the nosocomial spread of KPC-producing isolates are warranted.

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