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Nosocomial Clustering of NDM-1-Producing *Klebsiella pneumoniae* Sequence Type 340 Strains in Four Patients at a South Korean Tertiary Care Hospital

Mi-Na Kim,[a] Dongeun Yong,[b] Dongheui An,[a] Hae-Sun Chung,[b] Jun Hee Woo,[c] Kyungwon Lee,[b] and Yunsop Chong[b]

Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea[a]; Department of Laboratory Medicine and Research Institute of Antimicrobial Resistance, Yonsei University College of Medicine, Seoul, South Korea[b]; and Division of Infectious Disease, Department of Internal Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea[c]

In November 2010, NDM-1-producing *Klebsiella pneumoniae* (NDMKP) was identified for the first time in South Korea from four patients with no history of traveling abroad who stayed for 21 to 205 days in a tertiary care hospital. All were sequence type (ST) 340 and had nearly identical XbaI pulsed-field gel electrophoresis (PFGE) patterns. The bla_{NDM-1}-carrying plasmids were in the IncN group, with sizes ranging from 50 to 200 kb. These findings suggest that NDMKP had already been introduced into South Korea before this clustering was found.

NDM-1 is a metallo-β-lactamase (MBL) first identified in carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* isolates from Swedish patients transferred from New Delhi, India, in 2008 (26). In a short period, NDM-1-producing *Enterobacteriaceae* have been reported in Africa, Asia, Australia, Canada, Europe, and the United States, starting in the United Kingdom, and many of those had links with India or Pakistan (15). Carbapenem-resistant *Enterobacteriaceae* (CRE) with acquired carbapenemases are rare in South Korean hospitals (8, 11).

In this study, we described four patients colonized by NDM-1-producing *K. pneumoniae* (NDMKP) who were hospitalized at a 2,700-bed tertiary care hospital in Seoul, South Korea. We characterized the genotype and phenotype of the strains. All isolates were identified as carbapenem-resistant *K. pneumoniae* by the MicroScan Neg Breakpoint Combo Panel type 44 (Siemens, West Sacramento, CA). The modified Hodge tests using ertapenem disks (4) were weakly positive, and a KPC-MBL Confirm ID kit (Rosco Diagnostica, Taastrup, Denmark) showed that only meropenem-dipicolinic acid tablets revealed an increase of the inhibition zone compared to that of meropenem tablets. Two NDMKP isolates were obtained from urine cultures, and the others were isolated from stool surveillance cultures (Table 1). Four patients were hospitalized for 21 to 205 days and received meropenem for 8 to 47 days before isolation of NDMKP. Three of them were admitted to the medical intensive care unit (MICU), but their periods in the MICU did not overlap. The remaining patient stayed at a surgical ward after a liver transplantation. There was no history of travel abroad found. None of the four patients was treated to eradicate NDMKP because all were considered to be colonizers. Stool surveillance cultures were performed on 71 patients who shared rooms or intensive care units with the patients carrying NDMKP, but only a carbapenemase-negative, carbapenem-resistant *K. pneumoniae* strain was isolated. The NDM-1-producing strains might have been introduced earlier in the hospital before this clustering was detected. Interestingly, three patients spontaneously decolonized, but one patient (case 4) carried NDMKP for more than 7 months. Prolonged colonization of NDM-1-producing *Escherichia coli* has been reported in a patient hospitalized for 13 months without exposure to carbapenems or overt infection by such bacteria (20). Prolonged carriage in the gut can be a factor facilitating the spread of NDM-1-producing *Enterobacteriaceae*.

MICs of relevant antimicrobials were confirmed by the agar dilution method (Table 2). The interpretative breakpoints for tigecycline and colistin by the European Committee on Antimicrobial Susceptibility Testing and the breakpoints for other antimicrobials by the Clinical and Laboratory Standards Institute were used (4, 6). Three strains (F181, E1454, and F528) were highly resistant to imipenem and meropenem, but one from patient 4 (E5026) was susceptible to imipenem, with a MIC of 1 μg/ml, and intermediate to meropenem. Colistin, tigecycline, and gentamicin remained active for all strains, and tobramycin, amikacin, and aztreonam were variably active. The bla_{NDM-1} gene is usually associated with high-level MICs of carbapenems (10). Emergence of strains with low-level MICs suggested that additional mechanisms, such as an efflux pump or porin loss, may be involved in carbapenem resistance in NDM-1 producers. Detection of NDM-1 CRE is impeded when strains have lower-level imipenem and meropenem MICs (3).

Extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase (PABL) production was initially screened by the combined disk tests using cefotaxime-clavulanic acid, cefotaxime disks (4), and disks with boronic acid added (23). ESBL phenotypes were positive only in strain E5026 and its transconjugant, and PABL activity was detected in all isolates (Table 2). The presence of known MBLs (bla_{VIM}, bla_{IMP}, bla_{SPM-1}, bla_{GIM-1}, bla_{AIM-1}, bla_{SIM-1}, and bla_{NDM-1}) (13, 24), ESBLs (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) (25), and PABL genes (17) were determined using PCR. We also aimed to detect IS*Aba125* and 16S rRNA methylase

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Address correspondence to Mi-Na Kim, mnkim@amc.seoul.kr, or Dongeun Yong, deyong@yuhs.ac.
M.-N.K. and D.Y. contributed equally to this work as both first authors and corresponding authors.

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TABLE 1 Clinical features of the four patients with bla_{NDM-1}-carrying *Klebsiella pneumoniae*[a]

Patient	Age (yr)/gender and underlying disease	Admission date, date discharged or expired, and clinical outcome	Ward (dates of hospital stay[b])	Period of isolation of NDMKP, specimen type, and pathogenicity	Carbapenem(s) administered before isolation of NDMKP (no. of days before isolation)
1	71/F; pyogenic spondylitis	3 June 2010; 21 January 2011; improved	GW124 (3 June–5 July), MICU1 (5 July–16 July), GW134 (16 July–30 July), MICU1 (30 July–3 August), GW134 (3 August–21 January 2011)	5 November 2010–31 December 2010; stool; colonizer	Meropenem (8)
2	52/M; dermatomyositis interstitial lung disease	7 October 2010; 16 December 2010; expired from pneumothorax	MICU1 (7 October–12 October), MICU2 (12 October–16 December)	8 November 2010–12 November 2010; urine; colonizer	Meropenem (16)
3	71/M; vertebral osteomyelitis	31 August 2010; 15 December 2010; improved	GW134 (31 August–2 September), MICU2 (2 September–17 September), GW134 (17 September–15 December)	16 November 2010/stool/colonizer	Meropenem (22), ertapenem (13)
4	59/M; liver transplantation due to HBV-associated liver cirrhosis	28 July 2010; July 2011; improved	GW91 (28 July–3 August), MICU1 (3 August–12 August), SICU (12 August–3 September), GW102 (3 September–19 September), SICU (19 September–26 September), GW102/SICU (26 September to last follow-up on 30 June 2011)	30 November 2010–6 December 2010/ urine/colonizer; 15 December 2010–22 January 2011/stool/colonizer	Meropenem (47)

[a] NDMKP, NDM-1-producing *Klebsiella pneumoniae*; GW, general ward; MICU, medical intensive care unit; SICU, surgical intensive care unit; M, male; F, female.
[b] In 2010 unless specified.

(*armA*) by using the published primers (7). All PCR products were directly sequenced. PCR assays detecting MBL genes were positive only for bla_{NDM-1}. E5026 and its transconjugant carried a $bla_{CTX-M-15}$-type gene (Table 2). All of them carried bla_{DHA-1} genes. *armA*-specific PCRs were all negative. IS*Aba125*, which is a common feature of bla_{NDM-1}-positive *Enterobacteriaceae*, was located upstream of each bla_{NDM-1} gene (19). The absence of *armA* is related to susceptibility to gentamicin, a trait which is not found in NDM-1 producers of Indian origin (26).

Plate mating was performed using *E. coli* J53 (azide resistant) as

TABLE 2 Antimicrobial susceptibility profiles of the four NDM-1-producing isolates[a]

Antimicrobial, gene, or other characteristic	MIC (μg/ml) and susceptibility, gene presence, and plasmid size(s) of each strain[b]						
	F181 from patient 1 (wild type)	E1454 from patient 2		F528 from patient 3		E5026 from patient 4	
		Wild type	Transconjugant	Wild type	Transconjugant	Wild type	Transconjugant
Antimicrobials							
Ampicillin	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R
Piperacillin	>128, R	>128, R	32, I	>128, R	64, I	>128, R	>128, R
Cephalothin	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R
Cefotaxime	>128, R	>128, R	128,R	>128, R	>128, R	>128, R	>128, R
Ceftazidime	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R
Cefoxitin	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R	>128, R
Aztreonam	0.25, S	32, R	0.25, S	64, R	0.12, S	64, R	64, R
Cefepime	128, R	128, R	8, S	>128, R	32, R	32, R	32, R
Imipenem	128, R	>128, R	8, R	16, R	8, R	1, S	2, I
Meropenem	>128, R	>128, R	2, I	32, R	1, S	2, I	2, I
Ciprofloxacin	32, R	64, R	0.06, S	128, R	0.06, S	64, R	0.06, S
Tigecycline	1, S	2, S	0.12, S	2, S	0.12, S	0.5, S	0.12, S
Colistin	0.5, S	0.5, S	0.25, S	0.5, S	0.25, S	0.5, S	0.25, S
Gentamicin	0.25, S	1, S	0.25, S	0.5, S	0.12, S	0.25, S	0.12, S
Tobramycin	8, I	16, R	0.12, S	8, I	0.12, S	0.25, S	0.12, S
Amikacin	4, S	32, I	0.25, S	8, S	0.25, S	1, S	0.25, S
Resistant genes other than bla_{NDM-1}							
$bla_{CTX-M-15}$	−	−	−	−	−	+	+[c]
bla_{TEM}/bla_{SHV}	−/+	+/+	−/−	+/+	−/−	+/+	+[d]/−
armA	−	−	−	−	−	−	−
IS*Aba125*	+	+	+	+	+	+	+
Size of plasmid (kb)	200	200, 60[e], 50[e]	50[e]	200, 70[e], 40	60[e], 40	200, 50[e]	100[e]

[a] Using the agar dilution method.
[b] I, intermediate; R, resistant; S, susceptible; −, negative; +, positive.
[c] $bla_{CTX-M-15}$ type.
[d] bla_{TEM-1} type.
[e] bla_{NDM-1}-carrying plasmid.

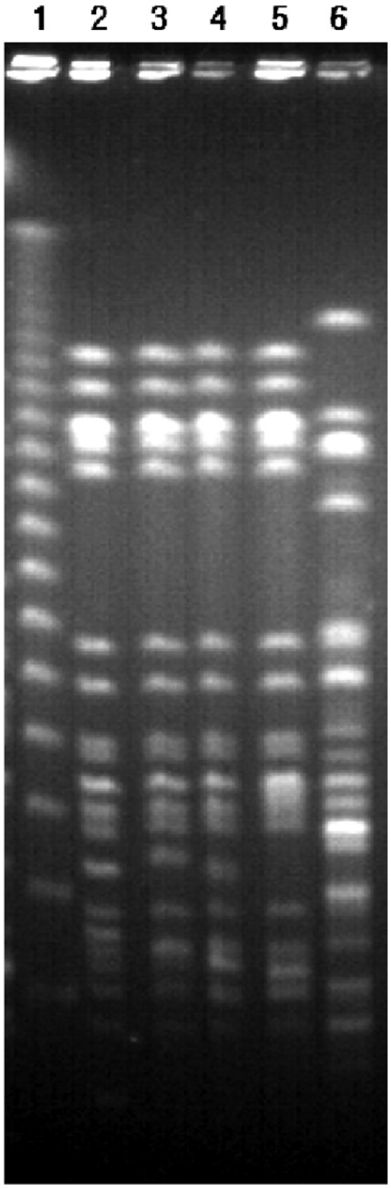

FIG 1 Comparison of PFGE patterns of XbaI-digested genomic DNA of four NDM-1-producing *K. pneumoniae* isolates. Lane 1, markers; lane 2, F181; lane 3, F528; lane 4, E1454; lane 5, E5026; lane 6, a clinical isolate of carbapenem-resistant *K. pneumoniae* without NDM-1.

a recipient, with transconjugants being selected on MacConkey agar containing 100 μg/ml of sodium azide and 8 μg/ml of ceftazidime. Plasmids in the transconjugants were typed as described previously (2). Plasmid sizes were determined by S1 nuclease restriction and pulsed-field gel electrophoresis (PFGE). The bla_{NDM-1}-carrying plasmids were successfully transferred from the three index strains (E1454, F528, and E5026) to *E. coli* J53 at frequencies ranging from ca. 10^{-5} to 10^{-7} (transconjugant/donor). Hybridization of the membrane blot with a probe recognizing bla_{NDM-1} showed that the bla_{NDM-1}-carrying plasmids varied in size, being ca. 200 kb in F181, ca. 60 and 50 kb in E1454, ca. 70 kb in F528, and ca. 50 kb in E5026 (Table 2). The bla_{NDM-1}-carrying plasmids in this study all belonged to the IncN group. These results suggested that the plasmids may be quite unstable, as reported previously (14). This type of plasmid was previously known to be involved in the transmission of VIM-1, KPC-2, and CTX-M-1 as well as NDM-1 among *K. pneumoniae* isolates and has been also prevalent in *E. coli* and *Salmonella* species (1). Various replicon types are involved in bla_{NDM-1}-carrying plasmids (19), but IncN has recently been found in only one NDM-1-positive *E. coli* strain (18). Highly efficient transmission of bla_{NDM-1}-carrying plasmids (21) may explain the diversity and worldwide spread of *bla*NDM-1-carrying *Enterobacteriaceae*.

DNA sequences of housekeeping genes were uploaded to the multilocus sequence typing (MLST) database (http://pubmlst.org) (5). All 4 strains belonged to sequence type (ST) 340. Genomic DNA was digested with the restriction enzyme XbaI (Takara, Tokyo, Japan) and separated using the CHEF-DR II apparatus (Bio-Rad, Hercules, CA). The PFGE band patterns of the four strains were different by fewer than three bands, suggesting a clonal relationship among the strains (Fig. 1). Although no clear epidemiological linkage was found among the patients, the PFGE band patterns strongly suggest clonal dissemination of a strain within the hospital. All strains yielded ST 340, which is a single-locus variant of ST 258, the dominant ST of KPC3-producing *K. pneumoniae* worldwide (9) and the ST of one of two KPC2-producing *K. pneumoniae* found in South Korea (22), and this type, like the NDMKP of this study, has been shown to be gentamicin susceptible but tobramycin and amikacin resistant (12). ST 340 has already been found in NDMKP isolates from Oman, the United Kingdom, and Canada, of which two seem to be linked to India (16, 19). The clone of the NDMKP isolate of this study may possibly have been imported from regions of NDM endemicity, even though no direct epidemiologic links were found.

In this study, the first emergence of NDMKP ST 340 strains in South Korea suggested that NDMKP strains might have been introduced earlier in the hospital and were already spread nosocomially. The one isolate revealing susceptible MICs to carbapenems implied the difficulty of detecting NDMKP, and resistance mechanism-based screening for CRE is required to prevent the spread of NDMKP.

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