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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

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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 blaNDM-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 Confirmed 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), and 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 blaNDM-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, ceftoxime 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 (blaSIM-1, blaNDM-1, blaAIM-1, blaIMP, blaSHV, blaTEM, and blaCTX-M) (13, 24), ESBLs (blaTEM, blaSHV, and blaCTX-M) (25), and PABL genes (17) were determined using PCR. We also aimed to detect IS*Abba*125 and 165 rRNA methylase.
TABLE 1 Clinical features of the four patients with blaNDM-1-carrying Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/gender and underlying disease</th>
<th>Admission date, date discharged or expired, and clinical outcome</th>
<th>Ward (dates of hospital stay)</th>
<th>Period of isolation of NDMKP, specimen type, and pathogenicity</th>
<th>Carbapenem(s) administered before isolation of NDMKP (no. of days before isolation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>52/M; dermatomyositis interstitial lung disease</td>
<td>7 October 2010; 16 December 2010, expired from pneumothorax</td>
<td>MICU1 (7 October–12 October), MICU2 (12 October–16 December)</td>
<td>8 November 2010–12 November 2010; urine; colonizer</td>
<td>Meropenem (16)</td>
</tr>
<tr>
<td>3</td>
<td>71/M; vertebral osteomyelitis</td>
<td>31 August 2010; 15 December 2010, improved</td>
<td>GW134 (31 August–2 September), MICU2 (2 September–17 September), GW134 (17 September–15 December)</td>
<td>16 November 2010/stool/colonizer</td>
<td>Meropenem (22), ertapenem (13)</td>
</tr>
<tr>
<td>4</td>
<td>59/M; liver transplantation due to HBV-associated liver cirrhosis</td>
<td>28 July 2010; July 2011, improved</td>
<td>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)</td>
<td>30 November 2010–6 December 2010/urine/colonizer; 15 December 2010–22 January 2011/stool/colonizer</td>
<td>Meropenem (47)</td>
</tr>
</tbody>
</table>

**TABLE 2 Antimicrobial susceptibility profiles of the four NDM-1-producing isolates**

<table>
<thead>
<tr>
<th>Antimicrobial, gene, or other characteristic</th>
<th>MIC (µg/ml) and susceptibility, gene presence, and plasmid size(s) of each strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F181 from patient 1 (wild type) Wild type Transconjugant F1454 from patient 2 Wild type Transconjugant F528 from patient 3 Wild type Transconjugant E5026 from patient 4 Wild type Transconjugant</td>
</tr>
<tr>
<td>Antimicrobials</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.25, S 0.25, S 0.25, S 0.25, S 0.25, S 0.25, S 0.25, S 0.25, S 0.25, S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>128, R 128, R 8, R 8, S 8, S 8, S 8, S 8, S 8, S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>32, R 64, R 0.06, S 128, R 0.06, S 64, R 0.06, S 64, R 0.06, S</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1, S 2, S 0.12, S 2, S 0.12, S 0.12, S 0.12, S 0.12, S</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5, S 0.5, S 0.25, S 0.5, S 0.25, S 0.5, S 0.25, S 0.5, S 0.25, S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.25, S 1, S 0.25, S 0.5, S 0.25, S 0.5, S 0.25, S 0.5, S 0.25, S</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>8, I 16, R 0.12, S 8, I 0.12, S 0.12, S 0.12, S 0.12, S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>4, S 32, I 0.25, S 8, S 0.25, S 8, S 0.25, S 8, S 0.25, S</td>
</tr>
</tbody>
</table>

**Resistant genes other than blaNDM-1**

- **blaCTX-M-15:**
- **blaTE3a:**
- **blaTEM-1:**
- **armA:**
- **ISAba125:**
- **Size of plasmid (kb):**

**a** Using the agar dilution method.

**b** I, intermediate; R, resistant; S, susceptible; –, negative; +, positive.

**c** **blaCTX-M-15** type.

**d** **blaTEM-1** type.

**e** **blaNDM-1**-carrying plasmid.
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.

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.

REFERENCES


