First Report of Metallo-β-Lactamase NDM-5-Producing Escherichia coli in Japan

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Reports of carbapenemase-producing carbapenem-resistant Enterobacteriaceae are increasing worldwide (1). Among the newly emerged carbapenemases, New Delhi metallo-β-lactamase 1 (NDM-1) represents the latest threat to public health. Since it was first described in 2009 (2), NDM-producing organisms have become endemic on the Indian subcontinent and likely also in several Middle Eastern and Balkan countries, and numerous transfers have been recorded worldwide (3). To our knowledge, NDM is uncommon in Japan, with only six sporadic cases of NDM-1 producers reported since the first case in 2009 (1). This is the first report of the detection of an NDM-5-producing Escherichia coli clinical isolate in Japan.

Two carbapenem-resistant members of the Enterobacteriaceae (E. coli and Klebsiella pneumoniae) were recovered from fecal samples of two hospitalized patients in Teikyo University Hospital, Tokyo, Japan. They were selected by ChromID ESBL and confirmed by a double-disk synergy test (DDST) using inhibitor (4, 5). E. coli TK1044 and K. pneumoniae TK1238 were isolated from a traveler from Bangladesh in January 2013 and a traveler returning from Indonesia in February 2014, respectively. Antimicrobial susceptibilities were determined by the microdilution method according to CLSI guidelines (6). The isolates are resistant to all β-lactams, including broad-spectrum cephalosporins and carbapenems, and fluoroquinolone (Table 1). The presence of blablaNDM-1 and blablaCTX-M-15 in E. coli TK1044 and K. pneumoniae TK1238 were confirmed by a double-disk synergy test (DDST) using inhibitor (4, 5). E. coli TK1044 carries blablaNDM-5 and blablaCTX-M-15. K. pneumoniae TK1238 carries blablaNDM-1, blablaCTX-M-15, and blablaOXA-1. The genetic environment surrounding blablaNDM-5 and blablaNDM-1 were identified by DNA sequencing. Compared with blablaNDM-5-positive plasmids, a truncated insertion sequence, ISAba125, present immediately upstream of blablaNDM-5 was disrupted by the insertion of IS5, which results in a truncated transposase. The blablaMBL, blablaTPR, blablaDsbC, and blablaDsbC genes are present downstream of blablaNDM-5 (Fig. 1). The genetic environment of blablaNDM-1 has been observed to be very similar to that in previously described blablaNDM-1-positive plasmids (GenBank accession no. KF732966 and JQ314407).

Transferability of the blablaNDM gene was studied by conjugation experiments with an E. coli J53 recipient (9). Plasmid incompatibility groups were identified by the PCR-based replicon typing method (10). E. coli J53 (NDM-5) was resistant to all β-lactams except aztreonam. The blablaNDM-5 gene is on a ca. 110-kb plasmid belonging to the IncN replicon type. E. coli J53 (NDM-1) was also resistant to most β-lactams except cefmetazole and aztreonam; the blablaNDM-1-positive plasmid of ca. 100 kb was untypeable. MICs of cephalosporins and carbapenems for an NDM-5-producing isolate were reported to be higher than for an NDM-1 producer (11). NDM-5 differs from NDM-1 by two amino acid substitutions at positions 88 (Val→Leu) and 154 (Met→Leu) (11). A role for Leu154 in NDM-7 in elevating carbapenem MICs has been reported; thus, the amino acid change at position 154 in NDM-5 may be responsible for the higher carbapenemase activity (12).

Of note, K. pneumoniae TK1238 and the transconjugant E. coli J53 (NDM-1) were highly resistant to aminoglycosides (amikacin and gentamicin). Screening of 16S rRNA methylase (16S-RMTase) genes by multiplex PCR (13) identified the 16S-RMTase gene rmtC. Acquired 16S-RMTase genes among Enterobacteriaceae, including K. pneumoniae, have been reported worldwide (14, 15); however, the specific association between the blablaNDM-1 and rmtC ratio was studied by conjugation experiments with an E. coli J53 recipient (9). Plasmid incompatibility groups were identified by the PCR-based replicon typing method (10). E. coli J53 (NDM-5) was resistant to all β-lactams except aztreonam. The blablaNDM-5 gene is on a ca. 110-kb plasmid belonging to the IncN replicon type. E. coli J53 (NDM-1) was also resistant to most β-lactams except cefmetazole and aztreonam; the blablaNDM-1-positive plasmid of ca. 100 kb was untypeable. MICs of cephalosporins and carbapenems for an NDM-5-producing isolate were reported to be higher than for an NDM-1 producer (11). NDM-5 differs from NDM-1 by two amino acid substitutions at positions 88 (Val→Leu) and 154 (Met→Leu) (11). A role for Leu154 in NDM-7 in elevating carbapenem MICs has been reported; thus, the amino acid change at position 154 in NDM-5 may be responsible for the higher carbapenemase activity (12).

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Table 1: Susceptibilities of the New Delhi metallo-β-lactamase-producing clinical isolates and the E. coli J53 transconjugants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antibiotic resistance gene(s)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
<td>AMP+CLA</td>
</tr>
<tr>
<td>E. coli TK1044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDM-5, CTX-M-15</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>E. coli J53 (NDM-5)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K. pneumoniae TK1238</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>NDM-1, CTX-M-15, OXA-1, RmtC</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>E. coli J53 (NDM-1)</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>E. coli J53</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
genes has recently been identified in various countries and is the first such case in Japan (16, 17).

Multilocus sequence typing (MLST) was performed on the E. coli and K. pneumoniae isolates as previously described (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli and http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). MLST analysis revealed that K. pneumoniae TK1238 and E. coli TK104 belonged to sequence type 76 (ST76) and ST540, respectively.

To the best of our knowledge, this is the first reported clinical isolation of NDM-5-producing E. coli and NDM-1 producing K. pneumoniae cohaboring the 16S-RMTase gene rmtC in Japan. The patients had a history of international travel, and their illnesses may have been imported. Travelers contribute significantly to the global movement of microbes and resistance genes (18). Clinical laboratories must remain vigilant for these organisms to prevent the dissemination of NDM-producing organisms in Japan.

Nucleotide sequence accession number. The sequence of blaNDM-5 and its genetic environment has been deposited in NCBI GenBank under accession no. LC000627.

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REFERENCES


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Letter to the Editor

FIG 1 Schematic representation of the genetic environment of blaNDM-5-ISAba125 lies upstream of blaNDM-5 as a truncated element carrying IS5 and IS3000. The blaNDM-5, trpF, dsbC, and rmtC genes lie downstream of blaNDM-5. 

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