Detection of blaNDM-4 in Escherichia coli from hospital sewage

New Delhi metallo-β-lactamase (NDM-1) has been identified as a novel class of carbapenemase found in Enterobacteriaceae, first isolated from a Swedish patient of Indian origin (Kumarasamy et al., 2010; Yong et al., 2009). Later, many reports on the emerging trends of NDM-1 from different parts of the world have been published (Khan & Nordmann, 2012a, b). Moreover, new variants of NDM-1 have also been identified from different countries (Cuzon et al., 2013; Göttig et al., 2013). Here we report the finding of an NDM-4-producing Escherichia coli in the sewage of an Indian hospital. NDM-4 differs by a single amino acid substitution (Met154Leu) from NDM-1. Kinetic data showed that NDM-4 hydrolysed imipenem more than did NDM-1 [kcat/Km (μM⁻¹·s⁻¹) ratio for NDM-4/NDM-1 for imipenem was 2.20]. Further, the MICs of imipenem and ertapenem were also found to be higher for Escherichia coli expressing NDM-4 than that expressing NDM-1, suggesting that the Leu154 residue is involved in the higher carbapenemase activity (Nordmann et al., 2012).

Water samples were collected from three different outlets (three sites) of sewage water within 10 m of the washroom of private and general wards, before they merged into the main drainage system, at Aligarh Hospital situated in the northern part of India. Three samples, each of 5 ml, were collected in sterilized tubes from the three different sites and were taken to the laboratory for further processing. Samples were centrifuged at 3000 g for 30 s to precipitate the debris. Samples (100 μl) of 10–100-fold diluted samples were grown at 37 °C overnight on Mueller–Hinton agar containing 0.5 mg meropenem l⁻¹, under aseptic conditions. A total of 52 colonies were randomly picked from different plates as carbapenem resistant strains.

Species identification was performed using different biochemical tests (Geldreich et al., 1964). One of the strains (AK1) was found to be Escherichia coli, which was further confirmed using 16S rRNA gene sequence analysis (KJ184354). The primers used to amplify the 16S rRNA gene were 5’-CTTACGGGAGGCAGCAGTAG-3’ and 5’-CAACAGAGCTTTACGATCCG-3’ (Shemesh et al., 2007).

An antibiogram analysis of the AK1 strain was performed using different biochemical tests (Geldreich et al., 1964). One of the strains (AK1) was found to be Escherichia coli, which was further confirmed using 16S rRNA gene sequence analysis (KJ184354). The primers used to amplify the 16S rRNA gene were 5’-CTTACGGGAGGCAGCAGTAG-3’ and 5’-CAACAGAGCTTTACGATCCG-3’ (Shemesh et al., 2007).

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Table 1. MICs and genetic profile of blaNDM-4

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>MIC (μg ml⁻¹)</th>
<th>Plasmid type</th>
<th>Genetic environment of blaNDM-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>AK-1</td>
<td>IPM 512, MEM 256, ATM &gt;1024, CAZ &gt;1024,CTX &gt;1024, FOX 1024, TCC 128, IPC &gt;1024</td>
<td>IncK</td>
<td>ISAb125 complete, blaSMB present</td>
</tr>
</tbody>
</table>

Detection of metallo-β-lactamase activity was performed by using two imipenem discs (10 mg), one containing 10 μl of 0.1 M anhydrous EDTA. The discs were placed 25 mm apart on Mueller–Hinton plates (Franklin et al., 2006). PCR amplification and sequence analysis of DNA from the AK1 strain using primers as described previously (Khan & Nordmann, 2012a) revealed the presence of the blaNDM-4 gene (KJ184353).

Conjugal transfer was performed using AK1 as the donor and a sodium azide-resistant Escherichia coli J53 as the recipient strain, with selection based on growth on agar in the presence of cefoxitin (10 μg ml⁻¹) and sodium azide (100 μg ml⁻¹) (Poirel et al., 2011a). Transconjugants (T-AK-1) were obtained with the frequency of about 10⁻² and were tested for susceptibility against clinically used antibiotics (Table 2). A plasmid profile performed using the Kieser technique (Kieser, 1984) revealed that the AK1 strain harboured a single plasmid of a size of about 154 kb. A PCR-based replicon typing method (Carattoli et al., 2005) revealed the incompatibility group Inc K for the blaNDM-4-carrying plasmid in the AK1 strain (Table 1). This is the first report of this to the best of our knowledge, although blaCTX-M and blaIPC have been shown to be present on Inc K in earlier reports (Cottell et al., 2011; da Costa et al., 2014).
Enterobacter cloacae genetic analysis revealed that in truncated with bleomycin reverse: 5'-TTT GC-3' IS Aba125. In another study it was found truncated with bleomycin gene in a strain of Escherichia coli (Poirel et al., 2010).

To the best of our knowledge, this is the first report on NDM-4-producing Escherichia coli isolates from hospital sewage from one of the North Indian hospitals. Although other reports identified NDM-4-producing isolates in Denmark (Dortet et al., 2012), Cameroon (Nordmann et al., 2012), France (Jakobsen et al., 2014) and the Czech Republic (Papagianisis et al., 2013), in this study it was identified on a different plasmid type associated with complete ISAba125, which has not been shown before (Dortet et al., 2012; Nordmann et al., 2012; Jakobsen et al., 2014). The spread of the blaNDM-1 gene and other variants in Enterobacteriaceae in India and its dissemination to other countries have already been considered as a prime issue for the Indian Health Ministry so that policies can be implemented in order to control the threat of the NDM-4 producers as a new variant. Moreover, the emergence of blaNDM-4 and other variants in India should definitely be taken prudently; further surveillance is needed to evaluate the prevalence of multidrug-resistant bacteria and to develop strategies to prevent their spread.

**Acknowledgements**

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Asad U. Khan and Shadab Parvez

Medical Microbiology and Molecular Biology Laboratory, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202002, India

Correspondence: Asad U. Khan (asad.k@rediffmail.com)


**Table 2. Antimicrobial susceptibility profile**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>IPM</th>
<th>MEM</th>
<th>FOX</th>
<th>CTX</th>
<th>CAZ</th>
<th>TCC</th>
<th>ATM</th>
<th>GM</th>
<th>AN</th>
<th>CIP</th>
<th>CS</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK-1 (donor)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>E-J53, azide resistant (recipient)</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Transconjugant</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
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