Amended Abstract

 objetives: to assess the early occurrence of NDm-1 and other carbapenemases in a collection of Gram-negative bacilli (GNB) isolates collected in India in 2000. We previously demonstrated that NDm-1-producing isolates were present in India as early as 2006, but no data is available for prior samples.

 METHODS: Among 220 GNB isolates collected in India during 2000, 22 strains showing elevated imipenem MIC values (≥0.5 mg/L) were further evaluated for the presence of carbapenem-hydrolyzing enzymes (MHE) performance. Isolates were tested by PCR for genes encoding KPC, VIM, IMP, NMC-A, GES, OXA and IMI. Screening was performed using primers amplifying five genes located in the conserved sequences (CS).

 RESULTS: 22 GNB tested belonged to eight bacterial species, including A. baumannii, P. aeruginosa, P. stutzeri, E. cloacae, and K. pneumoniae. Among these, A. baumannii and P. stutzeri were noted for their high NDm-1 expressing rates. The P. stutzeri strain harboring blad1 was specifically and consistently detected by PCR, identified by the presence of the conserved sequence CS1170. This strain was collected in 5 cities (Mumbai, Vellore, New Delhi, Lucknow and Indore). Only one strain yielded positive PCR results for blad1 primers. No isolates were noted for the presence of other carbapenemase genes.

 CONCLUSIONS: NDm-1 producing strains were not detected in this bacterial collection from five Indian cities in 2000. However, we narrowed the interval for the emergence of NDm-1-producing strains in India. On the other hand, the detection of a D1m-1-producing strain, isolated in 2000, from a single patient in the Netherlands, suggests that this gene most likely emerged in the Indian subcontinent. Further investigations are needed to confirm these findings. The authors would like to thank L. Poirel and P. Nordmann (Bicetre Hospital, France) for kindly sharing the DIM-1-producing isolate with our group.

 Materials and Methods

 Bacterial isolates and carbapenemase screening. A total of 220 Gram-negative isolates were collected from five hospitals/cities in India during 2000. Only one isolate per patient/family was included in this study. All strains displaying an imipenem MIC value of ≤0.5 mg/L were first screened using the MUCA broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI 2012). MICs were determined with the CLSI antimicrobial standards (CLSI M100-S22). The strains were then further screened using the cephalosporinase/metallo-beta-lactamase enzyme activity assay (CLSI M100-S22). The penicillin sodium clavulanate (PSC) broth microdilution method was used to detect penicillinases. MICs were determined with the CLSI antimicrobial standards (CLSI M11-A8). The carbapenemase genes were detected using the PCR method described by Poirel et al. (2010). All strains were also screened for the presence of carbapenem-resistant Enterobacteriaceae (CRE) using the PCR method described by Poirel et al. (2010).

 Table 1. Susceptibility profiles of the Gram-negative isolates displaying imipenem MIC values ≥0.5 mg/L collected in Indian hospitals during 2000.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Organism</th>
<th>KPC</th>
<th>VIM</th>
<th>IMP</th>
<th>NMC-A</th>
<th>GES</th>
<th>OXA</th>
<th>IMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>10627</td>
<td>Pseudomonas stutzeri</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10433</td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10431</td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10430</td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10429</td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>10428</td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgment

The authors would like to thank L. Poirel and P. Nordmann (Bicetre Hospital, France) for kindly sharing the DIM-1-producing isolate with our group.

 References