Short Communication

First case of NDM-1 producing *Klebsiella pneumoniae* in Caribbean islands

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**Summary**

**Objectives:** Characterize a NDM-1 producing *K. pneumoniae* isolate recovered from a patient hospitalized in Guadeloupe, French West Indies, after its transfer from Cuba

**Methods:** Antibiotic susceptibilities were determined by the disk diffusion method, and E-test. Carbapenemase production was assessed using the Carba NP test. Antibiotic resistance determinants and their surrounding structures were characterized by PCR mapping and DNA sequencing. Transfer of the β-lactam resistance marker was attempted by liquid mating-out assays

**Results:** Here we reported the first NDM-1 producing enterobacterial isolate recovered from Caribbean islands. This *K. pneumoniae* isolate belongs to a new sequence type (ST1649). The *blaNDM-1* gene together with the *aacA4* gene were carried on a self conjugative IncR plasmid of c.a. 80 kb.

**Conclusion:** This study describes the first identification of a NDM-1 producer in Caribbean islands. The uncommon incompatibility group of the *blaNDM-1* carrying plasmid and the uncommon ST type of the *K. pneumoniae* strain suggest a possible local emergence of NDM producers.

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The metallo-β-lactamase (MBL) group of enzymes are carbapenemases that inactivate most of the β-lactam molecules except aztreonam, and are frequently associated with genes conferring resistance to several other classes of antibiotics. Among the MBLs, the New Delhi metallo-β-lactamase-1 (NDM-1) was first described in a *Klebsiella pneumoniae* isolate. Initially, the spread of NDM-1 was mostly identified from the Indian sub-continent. Since then, NDM-1 producers have been described worldwide. Here, we report the emergence of a NDM-1 producing *K. pneumoniae* recovered from a patient hospitalized in Guadeloupe, French West Indies after its transfer from Cuba.

In June 2014, a 67-year-old German traveler was hospitalized in La Havana, Cuba, for an ischemic cerebrovascular failure. On day 8, the patient developed a pneumonia that was empirically treated with cefazidime, ciprofloxacin, and metronidazole. The clinical status of the patient worsened necessitating intubation and mechanical ventilation on day 11, and his transfer on day 15 to the intensive care unit of the University hospital of Pointe-à-Pitre, Guadeloupe, France. As recommended for the detection of carbapenemase producers, a rectal swab was collected at admission, leading to the isolation of the multidrug resistant *K. pneumoniae* KHU strain.

Antimicrobial drug susceptibilities were determined by the disk diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines as updated in 2014. The minimal inhibitory concentrations (MICs) were determined by using E-test (bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar at 37 °C. The *K. pneumoniae* KHU

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Table 1

<table>
<thead>
<tr>
<th>β-Lactam (s)</th>
<th>K. pneumoniae KHU</th>
<th>Tc(^a) E. coli J53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Amoxicillin + CLA</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Ticarcillin + CLA</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt; 256</td>
<td>128</td>
</tr>
<tr>
<td>Piperacillin + TZB</td>
<td>&gt; 256</td>
<td>128</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt; 256</td>
<td>64</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt; 512</td>
<td>&gt; 512</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Meropenem</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>Doripenem</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^a\) CLA, clavulanic acid; TZB, tazobactam, at 4 µg/mL.

was resistant to all β-lactams except aztreonam, including imipenem (MIC = 16 µg/mL), meropenem (MIC = 32 µg/mL), ertapenem (MIC = 32 µg/mL), and doripenem (MIC = 12 µg/mL) (Table 1). It was also resistant to all aminoglycosides except to amikacin, to fluoroquinolones, to trimethoprim-sulfamethoxazole, to doxycycline, and to tetracycline but remained susceptible to colistin, fosfomycin, and tigecycline. In addition, three other multidrug resistant strains were isolated as a carrier stage, an extended-spectrum β-lactamase (CTX-M-15) producing Escherichia coli, a methicillin-resistant Staphylococcus aureus and an OXA-23 producing Acinetobacter baumannii. To the contrary of the NDM-1 producing K. pneumoniae, the latter two bacteria also were isolated in the respiratory secretions.

Using the Carba NP test, a carbapenemase activity was rapidly detected. Then PCR amplification followed by sequencing on whole-cell DNA as described identified the bla\(_{NDM-1}\) gene together with the aacA4 gene encoding the AAC(6’)-Ib acetyltransferase that confers high-level resistance to aminoglycosides, except to amikacin. PCR for the bla\(_{CTX-M}\), bla\(_{TEM}\), bla\(_{OXA-1}\), bla\(_{OXA-9}\) and bla\(_{OXA-10}\) genes were negative. Multi-locus sequence typing (MLST) analysis showed that the K. pneumoniae KHU isolate belonged to a new sequence type (ST) variant, ST1649, that is single locus variant of ST129 that has never been associated with NDM producers.

Plasmid DNA of K. pneumoniae KHU was extracted and analyzed using the Kieser method, as described. Two plasmids were identified, being ca. 80-kb and 160-kb in size. Transfer of the β-lactam resistance marker into E. coli J53 was attempted by liquid mating-out assays at 37 °C. E. coli transconjugants were obtained being resistant to all β-lactams except aztreonam, including to carbapenems (MICs of imipenem, meropenem, ertapenem, and doripenem were 6, 6, 4, and 6 µg/mL, respectively) (Table 1). They harbored a ca. 80-kb plasmid carrying the bla\(_{NDM-1}\) and aacA4 genes. PCR-based repilon typing method was performed as described, and showed that this bla\(_{NDM-1}\)-positive plasmid belonged to the IncR incompatibility group. Although bla\(_{NDM-1}\) carrying is usually located on IncA/C-, IncF-I, IncI1/M-, IncN2-, IncH1B- or untypable-type plasmids, an IncR bla\(_{NDM-1}\) carrying plasmid has been recently reported in Czech Republic. Genetic structures surrounding the bla\(_{NDM-1}\) gene, performed by PCR mapping as previously described, identified a truncated insertion sequence ISAba125 and the bleomycin resistance gene ble\(_{MIR}\) upstream and downstream of the bla\(_{NDM-1}\) gene, respectively. This genetic environment is known for most NDM-1-producers in Enterobacteriaceae.

This study describes the identification of a NDM-1 producer in Caribbean islands. As the patient was a traveler from Germany, an intestinal colonization before travelling to Cuba cannot be excluded. However, the patient had never been hospitalized before, which is the most important risk factor for multidrug resistant bacteria acquisition. No apparent link of this patient with the Indian subcontinent was established. Combined with the uncommon incompatibility group of the bla\(_{NDM-1}\) carrying plasmid and the uncommon ST of the K. pneumoniae strain, all these elements suggest a local emergence of NDM-1 producers. This is of major concern as Cuba is considered a major hub for all forms of human migrations, and to the United States, Europe and other Caribbean islands. Furthermore, few hospitals in Cuba can implement all the measures necessary to prevent nosocomial infections but also act as a barrier between the community and the hospital. Further work shall determine the prevalence rate of NDM and carbapenemase producers in Cuba to search for a possible endemia of this multidrug resistance trait.

Acknowledgments

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References