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Dissemination of New Delhi metallo-\(\beta\)-lactamase-1-producing Acinetobacter baumannii in Europe

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Abstract

Multidrug-resistant and New Delhi metallo-\(\beta\)-lactamase 1 (NDM-1) -producing Acinetobacter baumannii are increasingly reported. A collection of five NDM-1-positive A. baumannii isolates recovered in four European countries were analysed. Genotyping was performed by pulsed-field gel electrophoresis, multiplex PCR sequence typing, Diversilab and multilocus sequence typing. Three distinct sequence types were identified. All isolates harboured a chromosomally located sequence typing. Three distinct sequence types were identified. Genotyping was performed by pulsed-field gel electrophoresis, multiplex PCR sequence typing, Diversilab and multilocus sequence typing. Three distinct sequence types were identified. All isolates harboured a chromosomally located bla\(_{NDM-1}\) gene within a Tn125-like transposon. One isolate co-expressed another unrelated carbapenemase OXA-23. This report constitutes the first epidemiological study of NDM-1-producing A. baumannii from four countries.

Keywords: Carbapenemase, Gram-negative rods, New Delhi metallo-\(\beta\)-lactamase, Tn125

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Acinetobacter baumannii is an opportunistic pathogen that is an important source of nosocomial infections, mostly pneumonia [1]. The treatment of infections due to this microorganism is becoming a serious clinical concern because A. baumannii is frequently resistant to multiple antibiotics [2]. Resistance to carbapenems in A. baumannii is mostly related to the production of carbapenem-hydrolysing class D \(\beta\)-lactamases and to a lesser extent to metallo-\(\beta\)-lactamases [2]. Recent reports showed that the bla\(_{NDM-1}\) gene encoding New Delhi metallo-\(\beta\)-lactamase 1 (NDM-1) is spreading worldwide among gram-negative bacteria [3]. The bla\(_{NDM-1}\) gene was initially identified in Klebsiella pneumoniae and Escherichia coli isolates but has been additionally reported in many other Gram-negative rods [3,4]. In particular, the bla\(_{NDM-1}\) and bla\(_{NDM-2}\) genes have been recently identified in A. baumannii [5–9] and in other Acinetobacter species [10,11]. Notably, both genes have been identified as located on the same composite transposon named Tn125, being 10 099-bp long and comprising two copies of an identical insertion sequence IS\(\text{Aba125}\) bracketing the bla\(_{NDM-1/2}\) genes [12,13].

Our study aimed to study the clonal relationship of NDM-1-producing A. baumannii and the genetic context of bla\(_{NDM-1}\) responsible for dissemination of this resistance trait, by analysing a collection of bla\(_{NDM-1}\)-positive A. baumannii isolates recovered from different European countries. Isolate 161/07 was recovered in Germany in 2007 with a Balkan origin [5], isolate Slo was from a respiratory sample taken in Slovenia in 2008, isolate JH was collected in Switzerland in 2010 with a Balkan origin [12], and the two remaining isolates were isolated in France in 2011, one of those two being imported in France from Algeria (Table 1) [14].

The isolates were identified by 16S rRNA gene sequencing [15]. Susceptibility testing was performed by disc-diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) and interpreted according to updated CLSI guidelines [16]. The MICs of \(\beta\)-lactams including imipenem, meropenem and doripenem were determined by Etest (AB bioMérieux; Solna, Sweden) as described previously [17]. All isolates were resistant to \(\beta\)-lactams including carbapenems (Table 1). The production of metallo-\(\beta\)-lactamases was assessed using a combined disc-test, based on the inhibition of the metallo-\(\beta\)-lactamase activity by EDTA as described elsewhere [18]. All isolates were positive for the production of metallo-\(\beta\)-lactamases. The PCR experiments performed to detect carbapenemase genes as described previously [19], followed by sequence analysis, led to the identification of the bla\(_{OXA-S1}\) gene in the five isolates in addition to naturally occurring bla\(_{OXA-51}\)-like genes (respectively encoding OXA-64, OXA-69 or OXA-94) (Table 1). In addition, the bla\(_{OXA-23}\) gene, coding for the
Gene names are abbreviated according to their corresponding proteins: **extracted from** *A. baumannii* and by electroporation of a plasmid DNA suspension arrows. The lengths of the target genes and the exact location of the target site are not to scale. The *ori* of ISCR1 is indicated by a circle. Gene names are abbreviated according to their corresponding proteins: **iso** for phosphoribosylanthranilate isomerase; **tat** for twin-arginine translocation pathway signal sequence protein; **dor** for divalent cation tolerance protein; **Δpac** for truncated phospholipid acetyltransferase. IRL and **IRR** are for inverted repeat left and right, respectively. The Tn125 complete was found in isolates JH, Slo and 161/07 and the truncated isoform of Tn125 (**Δ**Tn125) was found in isolates Ora-1 and StN.

**TABLE 1.** Features of NDM-1 producing *Acinetobacter baumannii*

<table>
<thead>
<tr>
<th><em>A. baumannii</em> isolates</th>
<th>Country of isolation</th>
<th>Year of isolation</th>
<th>Sample</th>
<th>Carbenapenemase genotyping</th>
<th>MICs</th>
<th>Pulsotype</th>
<th>Support</th>
<th>Genetic vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>JH</td>
<td>Switzerland</td>
<td>2010</td>
<td>Rectal swab</td>
<td>NDM-1, OXA-23, OXA-69</td>
<td>&gt;256  &gt;256  &gt;32  &gt;32  &gt;32  l/l   A</td>
<td>Chromosomal</td>
<td>Tn125</td>
<td></td>
</tr>
<tr>
<td>Slo</td>
<td>Slovenia</td>
<td>2008</td>
<td>Respiratory sample</td>
<td>NDM-1, OXA-64</td>
<td>&gt;256  &gt;256  &gt;32  &gt;32  &gt;32  nd/25  B</td>
<td>Chromosomal</td>
<td>Tn125</td>
<td></td>
</tr>
<tr>
<td>161/07</td>
<td>Germany</td>
<td>2007</td>
<td>Skin, respiratory sample</td>
<td>NDM-1, OXA-64</td>
<td>&gt;256  &gt;256  &gt;32  &gt;32  &gt;32  nd/25  B</td>
<td>Chromosomal</td>
<td>Tn125</td>
<td></td>
</tr>
<tr>
<td>Ora-1</td>
<td>France</td>
<td>2011</td>
<td>Rectal swab</td>
<td>NDM-1, OXA-94</td>
<td>&gt;256  &gt;256  &gt;32  &gt;32  &gt;32  nd/85  C</td>
<td>Chromosomal</td>
<td>ΔTn125</td>
<td></td>
</tr>
<tr>
<td>StN</td>
<td>France</td>
<td>2011</td>
<td>Rectal swab</td>
<td>NDM-1, OXA-94</td>
<td>&gt;256  &gt;256  &gt;32  &gt;32  &gt;32  nd/85  C</td>
<td>Chromosomal</td>
<td>ΔTn125</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 1.** Schematic representation of transposon Tn125 carrying the *bla*NDM-1 gene. Genes and their transcription orientations are indicated by arrows. The lengths of the target genes and the exact location of the target site are not to scale. The **ori** of ISCR1 is indicated by a circle. Gene names are abbreviated according to their corresponding proteins: **iso** for phosphoribosylanthranilate isomerase; **tat** for twin-arginine translocation pathway signal sequence protein; **dor** for divalent cation tolerance protein; **Δpac** for truncated phospholipid acetyltransferase. IRL and **IRR** are for inverted repeat left and right, respectively. The Tn125 complete was found in isolates JH, Slo and 161/07 and the truncated isoform of Tn125 (**Δ**Tn125) was found in isolates Ora-1 and StN.
the manufacturer’s instructions (bioMérieux, La Balme-les-Grottes, France), and by pulsed-field gel electrophoresis as described [23]. The pulsed-field gel electrophoresis analysis showed that the five isolates were grouped into three distinct clones named A to C (Table 1), with strains Ora-1 and StN (from France) sharing identical patterns, strains Slo (from Slovenia) and 161/07 (from Germany) being clonally related according to the Tenover’s criteria (two bands of difference) [24], and strain JH (from Switzerland) belonging to a different clone. Analysis by the Diversilab technique resulted in the same interpretation (Fig. 2). Further analysis with sequence-type multiplex PCR showed that isolate JH belonged to European clone I although other isolates did not correspond to any defined European clone (Table 1). Multilocus sequence typing analysis showed that isolate JH belonged to ST1, isolates Slo and 161/07 belonged to ST25, and the two remaining isolates belonged to ST85 (Table 1). ST1-type isolates are widely distributed throughout the world whereas ST25 and ST85 strains have been rarely reported (http://www.pasteur.fr/cgi-bin/genopole/PF8/mlstdb-net.pl?file=acin_isolates.xml).

In conclusion, this report highlights scattered diffusion of NDM-1 producing A. baumannii in Europe from the west to the east. This dissemination was neither due to a single clone nor to any plasmid diffusion but rather to different clones carrying the transposon Tn125 or Tn125-derivatives which are truncated. While this study was in progress, two NDM-1-producing A. baumannii were isolated in Belgium and Czech Republic, [25,26]. The clinical isolate from the Czech Republic belonged to ST1, similar to isolate JH from Switzerland; whereas the clinical isolate from Belgium belonged to European clone II (which corresponds to ST2 in the multilocus sequence typing Pasteur Institute scheme) [25,26]. These two reports reinforce the fact that the spread of the blaNDM-1 gene in A. baumannii is not linked to a clonal spread but to the spread of a genetic structure. The spread of transposon Tn125 in Acinetobacter species harbouring blaNDM genes mirrors what has been observed with the blaKPC carbapenemase gene, which is associated with transposon Tn4401 [27].

Interestingly, we report here the first occurrence of an NDM-1 producer in Slovenia and therefore further confirm that Balkan countries constitute a significant reservoir for NDM-1-producing bacteria.

**Nucleotide Sequence Accession Number**

The nucleotide sequence data of the ΔTn125 reported in this work has been deposited in the GenBank nucleotide database under accession no. JX000237.

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**Transparency Declaration**

Nothing to declare.

**References**