Dissemination of the NDM-2-producing *Acinetobacter baumannii* clone in an Israeli Rehabilitation Center

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Running head: NDM-2 producing *A. baumannii* from Israel.
Abstract

New Delhi metallo-β-lactamase (NDM-1) was initially identified in various Enterobacteriaceae and recently in Acinetobacter baumannii. This study described the clonal dissemination of a NDM-2-producing A. baumannii in an Israeli rehabilitation ward and the genetic surrounding of the gene. The bla_{NDM-2} gene was surrounded by the ble and trpF genes downstream and two copies of the IS_{Aba125} in both sides. These are the first NDM-producing A. baumannii strains in Israel from patients with no previous travel or hospitalization in the India subcontinent.
Carbapenem resistance in Gram-negative bacteria is an important worldwide problem, particularly because of the production of class A, D and B (MBLs) enzymes as a resistance mechanism, and the facility to spread by mobile genetic elements (12). The new MBL, New Delhi metallo-β-lactamase 1 (NDM-1), initially reported in Klebsiella pneumonae and Escherichia coli recovered from a Swedish patient who was previously hospitalized in India (23), has disseminated to several countries and other Enterobacteriaceae (4, 9, 13, 15-18, 22). Recently, cases of NDM-producing Acinetobacter baumannii have been described in India, Egypt and China (2, 6, 8).

Five carbapenem-resistant A. baumannii isolates were recovered from female patients at the TA-Sourasky-MA Rehabilitation hospital in Israel Tel-Aviv (Table 1). The 5 elderly patients (mean age 81) were hospitalized in the same geriatric rehabilitation ward. The cultures were taken as a point prevalence study from 70 patients hospitalized in two wards in the rehabilitation center. Surveillance skin cultures were taken from 6 body sites (armpit, thigh, and groins, bilaterally). Four of the all 5 patients were admitted to rehabilitation after orthopedic surgery in 2 different orthopedic wards located in the same hospital, adjacent to the Rehabilitation center. Three of the patients, shared a room with each other at a point during their hospital stay, and others shared with them common facilities. None of the patients had any clinical culture that grew Acinetobacter spp., and no signs of infection due to Acinetobacter were evident. There was no specific history taken regarding travel. (Table 1). Isolates were initially identified using the VITEK-2 automatic system (bioMerieux, Marcy, France) and confirmed by amplified ribosomal DNA restriction analysis (ARDRA) (20). The epidemiological relationship was corroborated by pulsed-field gel electrophoresis (PFGE) under conditions described elsewhere (10). PFGE results showed an identical pattern for all the strains. Multiplex PCR to identify clonal lineages
(19) showed that the strains did not belong to pan-European clones I, II or III. Multilocus sequence typing (MLST) indicated that strain corresponded to sequence type (ST) 103 according to Pasteur system (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html), which is in agreement with the ST found in the NDM-2 producing A. baumannii isolate reported from Egypt (6).

Antibiotic susceptibility was performed by MicroScan (Siemens, CA, USA) and the results were interpreted according to CLSI guidelines (1). The strains were resistant to aztreonam, cefepime, ceftazidime and amikacin with a MIC of $\geq 64\ \text{mg/L}$, ampicillin-sulbactam ($\geq 16/8\ \text{mg/L}$), ciprofloxacin ($\geq 4\ \text{mg/L}$), gentamicin ($\geq 16\ \text{mg/L}$), imipenem and meropenem ($\geq 16\ \text{mg/L}$), piperacillin ($\geq 128\ \text{mg/L}$), piperacillin-tazobactam ($\geq 128\ \text{mg/L}$), ticarcillin ($\geq 128\ \text{mg/L}$). MICs of tigecycline and colistin were 2 mg/L and $\leq 0.5\ \text{mg/L}$, respectively. MBL production was confirmed by Etest strips (AB Biodisk, Sweden). The MICs of imipenem were $\geq 256\ \text{mg/L}$ and imipenem/EDTA $\leq 1\ \text{mg/L}$.

Multiplex PCR for class D $\beta$-lactamases ($bla_{OXA-51}$, $bla_{OXA-23}$, $bla_{OXA-24}$ and $bla_{OXA-58}$) (21) was only positive for $bla_{OXA-51}$ in all the strains. PCR for class B $\beta$-lactamases $bla_{IMP}$, $bla_{VIM}$, $bla_{SIM}$, $bla_{SPM}$, $bla_{GIM}$ (11) and $bla_{NDM}$ (NDM-1F, 5’ CCAATATTATGCACCCGGTCG and NDM-1R, 5’ ATGCGGGCCGTATGAGTGATTG) was performed with specific primers. All strains were positive for $bla_{NDM}$. Sequence analysis of the PCR products showed 99 % identity with the $bla_{NDM-1}$ previously reported (23). The sequence of the $bla_{NDM}$ gene detected in our study showed a double nucleotide substitution from C to G at position 82 and A to G at position 468 from the start codon. Only the first change resulted in an amino acid substitution from P (proline) to A (alanine) at position 28 as was already described and
named bla\textsubscript{NDM-2} (6), and the other was a silent mutation. Although the armA gene has been associated with bla\textsubscript{NDM-1} in A. baumannii (8), PCR to detect the 16S rRNA methylase-encoding genes \textit{rmtA}, \textit{rmtB}, \textit{rmtC}, \textit{rmtD}, \textit{armA} (3) and \textit{npmA} (npmA-F, 5’ CTCAAGGAACAAAGACCGTTG 3’ and npmA-R, 5’ GTTTCTGGCCATGTCTCAAAC 3’) were negative in our strains in agreement to the report by Kaase et al. (6).

Plasmid identification by the Kado and Liu method (7) and conjugation experiments using an \textit{A. baumannii} ciprofloxacin R/imipenem S as a recipient, were unsuccessful. Southern blot analysis was performed by digestion with the S1 nuclease. Digested genomic DNA was first separated by PFGE and then hybridized with the \textit{bla\textsubscript{NDM-1}} probe marked with the PCR DIG probe synthesis kit (Roche, Barcelona, Spain). Detection was performed with anti-digoxigenin antibody conjugated to alkaline phosphatase and CDP-Star-chemiluminescence substrate (Roche). The data showed two plasmids of approximately 70 and 200 Kb with no signal hybridization with the probe, but it was clearly demonstrated that \textit{bla\textsubscript{NDM-2}} is located in the chromosome (Fig. 1).

In order to determine the genetic structure surrounding of the \textit{bla\textsubscript{NDM-2}} gene, DNA from strain AB-I1 was digested with \textit{RsaI} (Promega). The fragments obtained were autoligated at 16 °C with T4 DNA ligase (Promega). The fragment of DNA containing the \textit{bla\textsubscript{NDM-2}} gene was used as a template for an inverse PCR with primers designed from the \textit{bla\textsubscript{NDM-1}} gene sequence (NDM-inv-F, 5’-TGCGAGACTGAGCACTA-3’ and NDM-inv-R, 5’-GGTCGCCAGTTCATTGC-3’). Analysis of the genetic surrounding showed that the \textit{bla\textsubscript{NDM-2}} gene was similar to that described for plasmid pNDM-HK (5) with the \textit{ble} (bleomycin resistance) and \textit{trpF} (N-(5’-phosphoribosyl) anthranilate isomerase) genes downstream, however two copies of the insertion sequence IS\textit{Aba125}, one upstream
close to the promoter region and the second at the 3′ end of the truncated \textit{trpF} gene, with the respective left (IRL) and right inverted repeats (IRR) was observed in our strain (Fig. 2). A promoter made of -35 (TTGAAT) and -10 (TACAGT) sequences separated by a distance of 17 bp were found at 104 bp from the \textit{bla\textsubscript{NDM-2}} start codon. Similar position of the promoter has been reported (5) suggesting NDM enzymes are under the control of the promoter upstream of the gene.

Sequence alignment of our strain AB-I1 with the previously reported arrays of \textit{E. coli} 271, pNDM-HK and pkpANDM-1 (5, 14) showed high homology in the region corresponding to the IRR of the IS\textsubscript{Aba125} gene, suggesting the presence of truncated structures by the participation of mobile genetic elements that can move from one site to another by transposition. With this, \textit{bla\textsubscript{NDM-1}} or \textit{bla\textsubscript{NDM-2}} flanked by IS can also be shuttled between plasmids (2) and the chromosome. Therefore, we can hypothesized that the IS\textsubscript{Aba125} element specific from \textit{A. baumannii} could be the origin of the dissemination among \textit{Enterobacteriaceae} plasmids.

Despite the epidemiological evidence that travel to the Indian subcontinent is related to infection caused by \textit{bla\textsubscript{NDM-1}} (9), the occurrence of sporadic colonizers and their clonal dissemination in the same unit, as observed in the present study, may be possible without any association with previous travels or hospitalization in the Indian subcontinent.

In conclusion, we report for the first time, a clonal dissemination of a NDM-2-producing \textit{A. baumannii} in an Israeli rehabilitation ward and the genetic surrounding of the gene. Epidemiological control and adequate identification of NDM-producing \textit{A. baumannii} will prevent an increase in resistance and better applications of therapeutic measures.
Nucleotide sequence accession number. The GenBank accession number for the strain AB-I1 is JF821215.

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REFERENCES


### Table 1. Epidemiological information of *Acinetobacter baumannii* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date of hospitalization</th>
<th>SEX</th>
<th>AGE</th>
<th>Source of isolate</th>
<th>Days-Patient hospitalization</th>
<th>Co-morbidities</th>
<th>Surgery</th>
<th>Invasive device</th>
<th>Treatment</th>
<th>Screening CRA</th>
<th>Date of screening CRA</th>
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<tr>
<td>I-15</td>
<td>14/06/2009-01/07/2009</td>
<td>Female</td>
<td>79</td>
<td>skin</td>
<td>18</td>
<td>CVD</td>
<td>Limbs</td>
<td>UC, ID</td>
<td>cephalosporins</td>
<td>positive</td>
<td>09/07/2009</td>
</tr>
<tr>
<td>I-1</td>
<td>02/07/2009-20/07/2009</td>
<td>Female</td>
<td>84</td>
<td>skin</td>
<td>24</td>
<td>CVD</td>
<td>Limbs, joints</td>
<td>UC, ID</td>
<td>cephalosporins</td>
<td>positive</td>
<td>09/07/2009</td>
</tr>
<tr>
<td>I-16</td>
<td>08/07/2009-20/08/2009</td>
<td>Female</td>
<td>85</td>
<td>skin</td>
<td>43</td>
<td>CVD, CLD</td>
<td>Limbs, joints</td>
<td>UC, ID</td>
<td>cephalosporins</td>
<td>positive</td>
<td>12/07/2009</td>
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<tr>
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<td>09/07/2009-07/08/2009</td>
<td>Female</td>
<td>81</td>
<td>skin</td>
<td>29</td>
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<td>UC, ID, Tr.</td>
<td>no</td>
<td>positive</td>
<td>09/07/2009</td>
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<td>Female</td>
<td>75</td>
<td>skin</td>
<td>84</td>
<td>CVD, DM</td>
<td>Limbs, joints</td>
<td>UC, ID</td>
<td>cephalosporins</td>
<td>positive</td>
<td>12/07/2009</td>
</tr>
</tbody>
</table>

**Abbreviations:** CVD: Cardiovascular disease, CLD: chronic lung disease, DM: diabetes mellitus, UC: Urinary catheter, ID: intravascular device, Tr: tracheostomy, CRA: carbapenem-resistant *Acinetobacter baumannii*
FIG. 1. PFGE analysis of *Acinetobacter baumannii* strains (a) Plasmid identification by digestion with S1 nuclease (b) Hybridization with *bla*<sub>NDM-1</sub> probe (c). Lanes 1, *A. baumannii* AB-I1, lane 2, AB-I2, lane 3, AB-I3, lane 4, AB-I4 and lane 5, AB-I5. Lane 6 to 8, *A. baumannii* European clones EC-I (Strain RUH-875), EC-II (Strain RUH-134) and EC-III (Strain RUH-5875), respectively. Bands with white arrows indicate the presence of plasmids without signal hybridization with the *bla*<sub>NDM-1</sub> probe and black arrow indicate the chromosomal position with positive hybridization with the *bla*<sub>NDM-1</sub> probe.
FIG. 2. Genetic surrounding of the bla<sub>NDM-2</sub> in an <i>A. baumannii</i> strain AB-II. Left inverted repeats (IRL), right inverted repeated (IRR). P: promoter, <i>ble</i>: bleomycin resistance gene, <i>trpF</i>: N-(5'-phosphoribosyl) anthranilate isomerase.