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Dissemination of an NDM-2-Producing *Acinetobacter baumannii* Clone in an Israeli Rehabilitation Center▽

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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 an NDM-2-producing *A. baumannii* isolate in an Israeli rehabilitation ward and the genetic surroundings of the gene. The bla_{NDM-2} gene was surrounded by the *ble* and *trpF* genes downstream and two copies of the IS*Aba125* on both sides. These are the first NDM-producing *A. baumannii* strains in Israel from patients with no previous travel or hospitalization on the Indian subcontinent.

Carbapenem resistance in Gram-negative bacteria is an important worldwide problem, particularly because of the production of class A, D, and B metallo-β-lactamase enzymes (MBLs) 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 pneumoniae* 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 (1, 6, 8).

Five carbapenem-resistant *A. baumannii* isolates were recovered from female patients at the TA-Sourasky-MA Rehabilitation hospital in Tel Aviv, Israel (Table 1). The five 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 six body sites (armpit, thigh, and groin, bilaterally). Four of the five patients were admitted to rehabilitation after orthopedic surgery in two 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 (bioMérieux, Marcy, France) and confirmed by amplified rRNA gene 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 clone I, II, or III. Multilocus sequence typing (MLST) indicated that the strain corresponded to sequence type (ST) 103 according to the 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), and the results were interpreted according to CLSI guidelines (2). The strains were resistant to aztreonam, cefepime, ceftazidime, and amikacin (MIC, ≥64 mg/liter), ampicillin-sulbactam (>16/8 mg/liter), ciprofloxacin (≥4 mg/liter), gentamicin (≥16 mg/liter), imipenem and meropenem (≥16 mg/liter), piperacillin (32 to ≥128 mg/liter), piperacillin-tazobactam (32 to ≥128 mg/liter), and ticarcillin (≥128 mg/liter). MICs of tigecycline and colistin were 2 mg/liter and ≤0.5 mg/liter, respectively. MBL production was confirmed by Etest strips (AB Biodisk, Sweden). The MIC of imipenem was ≥256 mg/liter, and that of imipenem/EDTA was ≤1 mg/liter.

Multiplex PCR for class D β-lactamases (bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58}) (21) was positive only for bla_{OXA-51} in all the strains. PCRs for class B β-lactamases $_{bla}$IMP, bla_{VIM}, bla_{SIM}, bla_{SPM}, bla_{GIM} (11), and bla_{NDM} (NDM-1F, 5′-CCAA TATTATGCACCCGGTCG; NDM-1R, 5′-ATGCGGGCCG TATGAGTGATTG) were 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_{NDM-2} (6), and the other was a silent mutation. Although the *armA* gene has been associated with bla_{NDM-1} in *A. baumannii* (8), PCRs to detect the 16S rRNA methylase-encoding genes *rmtA*, *rmtB*, *rmtC*, *rmtD*,

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TABLE 1. Epidemiological information on *Acinetobacter baumannii* strains[a]

Strain	Dates of hospitalization	Sex	Age (yr)	Source of isolate	Days of hospitalization	Comorbidity[b]	Surgery site(s)	Invasive devices[c]	Treatment	Screening CRA[d]	Date of screening CRA
I-15	14/06/2009–01/07/2009	Female	79	Skin	18	CVD	Limbs	UC, ID	Cephalosporins	Positive	09/07/2009
I-1	02/07/2009–26/07/2009	Female	84	Skin	24	CVD	Limbs, joints	UC, ID	Cephalosporins	Positive	09/07/2009
I-16	08/07/2009–20/08/2009	Female	85	Skin	43	CVD, CLD	Limbs, joints	UC, ID	Cephalosporins	Positive	12/07/2009
I-2	09/07/2009–07/08/2009	Female	81	Skin	29	CVD, DM	Head-neck	UC, ID, Tr	No	Positive	09/07/2009
I-17	09/07/2009–01/10/2009	Female	75	Skin	84	CVD, DM	Limbs, joints	UC, ID	Cephalosporins	Positive	12/07/2009

[a] Dates are given as day/month/year.
[b] CVD, cardiovascular disease; CLD, chronic lung disease; DM, diabetes mellitus.
[c] UC, urinary catheter; ID, intravascular device; Tr, tracheostomy.
[d] CRA, carbapenem-resistant *Acinetobacter baumannii*.

armA (3), and *npmA* (npmA-F, 5′-CTCAAAGGAACAAAG ACGGTTG-3′; npmA-R, 5′-GTTTCTGGCCATGTTCAAA AC-3′) were negative in our strains, in agreement with the report by Kaase et al. (6).

Plasmid identification by the Kado and Liu method (7) and conjugation experiments using a ciprofloxacin-resistant, imipenem-susceptible *A. baumannii* isolate 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 $bla_{\text{NDM-1}}$ probe marked with the PCR DIG probe synthesis kit (Roche, Barcelona, Spain). Detection was performed with antidigoxigenin 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 $bla_{\text{NDM-2}}$ is located in the chromosome (Fig. 1).

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

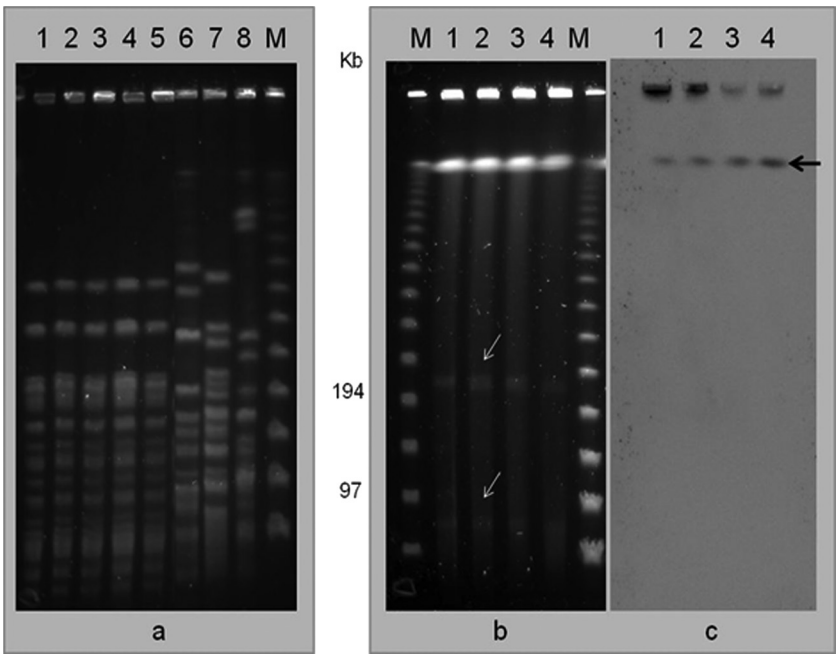

FIG. 1. (a) PFGE analysis of *Acinetobacter baumannii* strains. (b) Plasmid identification by digestion with S1 nuclease. (c) Hybridization with $bla_{\text{NDM-1}}$ probe. Lanes: 1, *A. baumannii* AB-I1; 2, AB-I2; 3, AB-I3; 4, AB-I4; 5, AB-I5. Lanes 6 to 8, *A. baumannii* European clones EC-I (strain 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 $bla_{\text{NDM-1}}$ probe; black arrow indicates the chromosomal position with positive hybridization with the $bla_{\text{NDM-1}}$ probe.

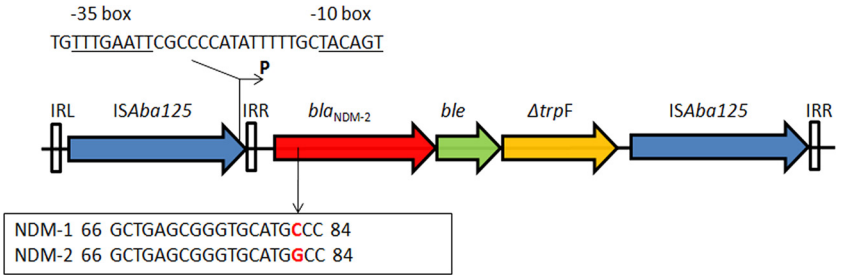

FIG. 2. Genetic surroundings of the bla_{NDM-2} in *A. baumannii* strain AB-I1. IRL, left inverted repeat; IRR, right inverted repeat. P, promoter; *ble*, bleomycin resistance gene; *trpF*, *N*-(5′-phosphoribosyl) anthranilate isomerase.

moter has been reported (5), suggesting that 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 *E. coli* 271, pNDM-HK, and pkpANDM-1 (5, 14) showed a high degree of homology in the region corresponding to the IRR of the IS*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, bla_{NDM-1} or bla_{NDM-2} flanked by IS can also be shuttled between plasmids (1) and the chromosome. Therefore, we can hypothesize that the IS*Aba125* specific element from *A. baumannii* could be the origin of the dissemination among plasmids from the *Enterobacteriaceae*.

Despite the epidemiological evidence that travel to the Indian subcontinent is related to infection caused by bla_{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 on the Indian subcontinent.

In conclusion, we report for the first time a clonal dissemination of an NDM-2-producing *A. baumannii* isolate in an Israeli rehabilitation ward and the genetic surroundings of the gene. Epidemiological control and adequate identification of NDM-producing *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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