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Complete Sequence of a bla_{NDM-1}-Harboring Plasmid in an *Acinetobacter bereziniae* Clinical Strain Isolated in Argentina

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The New Delhi metallo-β-lactamase (NDM-1) was initially identified in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Sweden from a patient previously hospitalized in India (1). Since then, bla_{NDM-1} has frequently been reported in *Enterobacteriaceae* and *Acinetobacter* spp., with a fast dissemination in the Indian subcontinent, the Balkan countries, China, and the Middle East (2). NDM-1 producers generally associated with *Enterobacteriaceae* species have also been reported, albeit with much lower frequency, in Latin American countries, including Guatemala, Mexico, Colombia, and Brazil (3). Moreover, production of NDM-1 in this geographic region has also been noted in *Acinetobacter baumannii* in Honduras and Brazil (3, 4) and in *Acinetobacter pittii* in Paraguay and Brazil (5, 6). Here, we report the first case of an NDM-1-producing *Acinetobacter* species in Argentina, an *Acinetobacter bereziniae* clinical isolate. We describe also the complete sequence of a bla_{NDM-1}-containing plasmid in this strain.

(Part of this work was presented at the 10th International Symposium on the Biology of Acinetobacter 2015, Athens, Greece, 3 to 6 June 2015 [7].)

A. bereziniae HPC229 was isolated on June 2014 from a blood sample of a 53-year-old female patient that underwent chemotherapy due to leukemia at a hospital located in Rosario, Argentina. The patient was treated with ciprofloxacin plus tigecycline, resulting in clinical and microbiological cure as evaluated by negative blood cultures. The patient was readmitted to the hospital 3 months later with symptoms of severe sepsis and died due to septic shock, with positive blood cultures that grew *Escherichia coli*.

HPC229, originally identified as *Acinetobacter lwoffii* by the Vitek 2 system (bioMérieux), was reclassified by DNA sequence comparison analyses of its 16S rRNA, *gyrB* (8), and *rpoB* (9) genes. The highest identity was found to the corresponding orthologs of the *A. bereziniae* ATCC 17924 type strain (99.9%, 99.7%, and 99.8%, respectively). The antibiotic susceptibility profile of HPC229 was determined by either the Vitek 2 system or the agar dilution method. The interpretation of the obtained MICs based on CLSI breakpoints (10) indicated resistance to β-lactams, including carbapenems (Table 1).

PCR amplification using specific primers for the bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{NDM}, $bla_{OXA-23-like}$, $bla_{OXA-24/40-like}$, and $bla_{OXA-58-like}$ genes (11–13) and sequencing analysis revealed the presence of bla_{NDM-1}. Evidence that bla_{NDM-1} was located in a plasmid was first obtained by plasmid curing (14) in which HPC229 was cultured in 5 ml of LB liquid medium containing 0.2 ml of 10% SDS for 48 h at 40°C. This procedure allowed the isolation of HPC229c, which showed a marked increase in susceptibility to β-lactams (Table 1). Susceptibility to aztreonam as judged by the disk diffusion assay, in contrast, showed no differences between HPC229 and HPC229c. All conjugation attempts using HPC229 as the donor and *Escherichia coli* DH5α, *A. baumannii* ATCC 17978, or *Pseudomonas aeruginosa* PAO1 as the recipient (11) were unsuccessful. The genomic relatedness between HPC229 and HPC229c was confirmed using previously described PCR-based procedures (15), and the loss of bla_{NDM-1} was confirmed by specific PCR analysis.

TABLE 1 Antimicrobial susceptibilities of *A. bereziniae* HPC229 and its plasmid-cured HPC229c derivative[c]

Antimicrobial(s)	MIC (μg/ml)	
	HPC229	HPC229c
Ampicillin-sulbactam[a]	16	≤2
Ceftazidime[b]	≥256	8
Cefotaxime[a]	≥64	8
Cefepime[b]	≥256	1
Piperacillin-tazobactam[a]	≥128	8
Imipenem[b]	128	0.125
Meropenem[a]	≥16	1
Colistin[a]	≤0.5	≤0.5
Gentamicin[a]	≤1	≤1
Amikacin[a]	≤2	≤2
Trimethoprim-sulfamethoxazole[a]	≤20	≤20
Ciprofloxacin[a]	0.5	0.5

[a] MICs were determined by the Vitek 2 system.
[b] MICs were determined by the agar dilution method.
[c] HPC229 was resistant to ampicillin-sulbactam, ceftazidime, cefotaxime, cefepime, piperacillin-tazobactam, imipenem, and meropenem, and it was susceptible to colistin, gentamicin, amikacin, trimethoprim-sulfamethoxazole, and ciprofloxacin according to CLSI breakpoints (10).

Analysis of whole-genome sequencing performed with pyrosequencing (454 Genome Sequencer FLX system; Roche Diagnostics) demonstrated that the bla_{NDM-1} metallo-β-lactamase gene was located in HPC229 in a 44,560-bp plasmid, designated pNDM229. This gene was found located inside a composite transposon bracketed by two copies of IS*Aba125* and identical to that first described in *A. baumannii* (16) and designated Tn*125* by Poirel et al. (17). The immediate genetic environment of Tn*125* in

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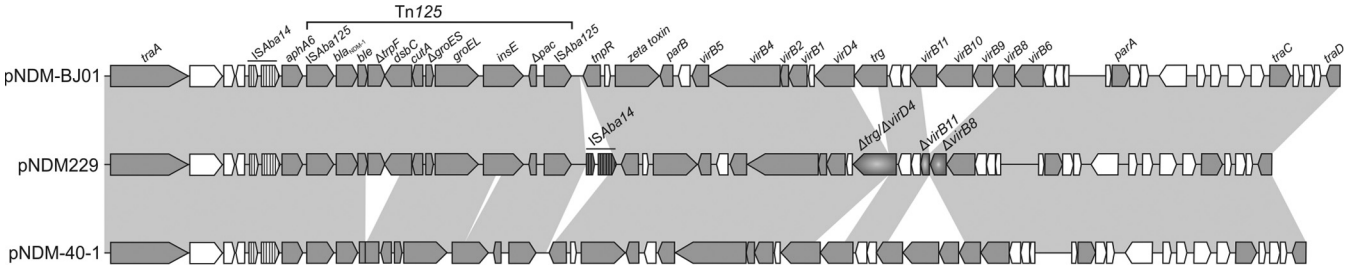

FIG 1 Comparative analysis of *Acinetobacter* bla_{NDM-1}-harboring plasmids. The linearized genetic maps of pNDM229 from *A. bereziniae* HPC229 (GenBank accession number KT072713), pNDM-BJ01 from *A. lwoffii* WJ10621 (NC_019268.1 [18]), and pNDM-40-1 from *A. bereziniae* CHI-40-1 (KF702385 [19]) are compared. Gray shading indicates more than 99% nucleotide sequence identity. In all plasmids, the IS*Aba14* element is indicated by black vertical lines. A second copy of IS*Aba14* present only in pNDM229 is shown in dark gray and exhibits 94.0% nucleotide identity with the upstream IS*Aba14* element. Partial deletions in conjugative genes in pNDM229 are indicated by radial gray gradients. Arrowheads indicate the direction of transcription, and open reading frames codifying hypothetical proteins are indicated by white arrows.

pNDM229 is most similar to that of the conjugative plasmid pNDM-BJ01 (18) present in an *A. lwoffii* clinical strain (Fig. 1). In both cases, the Tn*125* element is located immediately downstream of an *aphA6* gene, which is in turn adjacent to an upstream IS*Aba14* insertion sequence. In the case of pNDM229, however, a second IS*Aba14* copy was found immediately downstream of the Tn*125* element (Fig. 1), thus representing a novel difference from the similar arrangements previously reported (18, 19). This suggests the evolution of a novel composite transposon now bounded by two IS*Aba14* elements with the potential ability to mobilize as a whole.

In summary, we describe here the first case of an NDM-1-producing *A. bereziniae* isolate in Argentina. To our knowledge, only one other *A. bereziniae* NDM-1-producing clinical isolate has been recently reported (CHI-40-1), and the bla_{NDM-1} gene was located in this strain in a conjugative plasmid (19). However, a number of differences were noted between HPC229 and CHI-40-1 and between the plasmids carried by them (Fig. 1). First, comparison analyses of the corresponding 16S rRNA, *gyrB*, and *rpoB* genes revealed identities of 99.9%, 99.8%, and 99.6%, respectively, indicating no immediate clonal relationship between these two *A. bereziniae* strains. Second, comparison of the corresponding bla_{NDM-1}-containing platforms revealed two deletions in pNDM-40-1 which were not observed in pNDM229, one of which removed 1,298 bp from the 3′ end of *ble* to *dsbC* and the other of which removed 150 bp within *insE* (Fig. 1). Third, the above-noted second copy of IS*Aba14* downstream of Tn*125* was present only in pNDM229. Fourth, unlike with other conjugative plasmids, such as pNDM-40-1 and pNDM-BJ01, some conjugation-related genes, including *virD4*, *trg*, *virB11*, *virB8*, *virB10*, and *virB9*, were either truncated or absent in pNDM229 (Fig. 1), thus explaining conjugation failures.

The origin of *A. bereziniae* HPC229 remains obscure, since no history of overseas travel of the compromised patient or direct contact with foreigners could be identified. The above findings represent a worrisome concern, especially when considering that *Acinetobacter* species other than *A. baumannii* can act as reservoirs of bla_{NDM} genes and most probably contribute to their spreading among clinically relevant *Enterobacteriaceae* species (6, 19).

Nucleotide sequence accession numbers. The 16S rRNA, *gyrB*, and *rpoB* sequences of the HPC229 strain used for comparison with other *Acinetobacter* species have been released in GenBank under the accession numbers KP765739, KP765740, and KP765741, respectively. The 44,560 bp bla_{NDM-1}-containing pNDM229 plasmid sequence has been released under accession number KT072713.

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