Characterization of Plasmids in Extensively Drug-Resistant Acinetobacter Strains Isolated in India and Pakistan

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The blaNDM-1 gene is associated with extensive drug resistance in Gram-negative bacteria. This probably spread to Enterobacteriaceae from Acinetobacter spp., and we characterized plasmids associated with blaNDM-1 in Acinetobacter spp. to gain insight into their role in this dissemination. Four clinical NDM-1-producing Acinetobacter species strains from India and Pakistan were investigated. A plasmid harboring blaNDM-1, pNDM-40-1, was characterized by whole-genome sequencing of Acinetobacter bereziniae CHI-40-1 and comparison with related plasmids. The presence of similar plasmids in strains from Pakistan was sought by PCR and sequencing of amplicons. Conjugation frequency was tested and stability of pNDM-40-1 investigated by real-time PCR of isolates passaged with and without antimicrobial selection pressure. A. bereziniae and Acinetobacter haemolyticus strains contained plasmids similar to the pNDM-BJ01-like plasmids identified in Acinetobacter spp. in China. The backbone of pNDM-40-1 was almost identical to that of pNDM-BJ01-like plasmids, but the transposon harboring blaNDM-1, Tn125, contained two short deletions. Escherichia coli and Acinetobacter pittii transconjugants were readily obtained. Transconjugants retained pNDM-40-1 after a 14-day passage experiment, although stability was greater with meropenem selection. Fragments of pNDM-BJ01-like plasmid backbones are found near blaNDM-1 in some genetic contexts from Enterobacteriaceae, suggesting that cross-genus transfer has occurred. pNDM-BJ01-like plasmids have been described in isolates originating from a wide geographical region in southern Asia. In vitro data on plasmid transfer and stability suggest that these plasmids could have contributed to the spread of blaNDM-1 into Enterobacteriaceae.

Acinetobacter baumannii is a successful nosocomial pathogen, and extensively drug-resistant strains are increasingly prevalent (1, 2). Other Acinetobacter spp. are found in the environment and can cause opportunistic infections (2). There is evidence that the gene encoding New Delhi metallo-β-lactamase-1 (NDM-1) evolved in an Acinetobacter background through fusion between the aminoglycoside resistance gene aaphA6 and a β-lactamase-encoding progenitor of blaNDM-1 (3). Subsequently, blaNDM-1 and its closely related variants have spread rapidly among many genera of Gram-negative bacteria (4, 5), and they are found on plasmids of several different incompatibility types and chromosomally (4–6). NDM enzymes hydrolyze all β-lactams except aztreonam and are commonly found with other resistance mechanisms, mediating resistance to almost all clinically available antimicrobials (5, 7).

blaNDM-1 genes are prevalent in clinical Enterobacteriaceae isolates in South Asia. Many cases of infection or colonization with NDM-producing Enterobacteriaceae around the world have been linked to travel to the Indian subcontinent (5, 8). Studies within Indian hospitals have identified NDM-1-producing Acinetobacter spp. causing infections in intensive care units (9–11). Similar cases have been reported in Europe, but most patients probably became colonized during travel to the Balkans or North Africa (12–14). In China, blaNDM-1 has been observed many times in several Acinetobacter spp., including A. baumannii, from clinical, environmental, and farm animal samples but is less commonly reported in Enterobacteriaceae (15–22).

The immediate genetic contexts of blaNDM-1 (the genes flanking blaNDM-1) in Acinetobacter spp. are well conserved. An intact ISAba125 is normally present upstream (3, 14, 16). Downstream there are usually a conserved set of genes from the bleomycin resistance gene, ble, to ISCR27. Most examples have an intact ISAba125 further downstream from ISCR27, thus capturing the entire context in a Tn125 transposon (13, 14, 16). In Chinese isolates, blaNDM-1 is usually found on plasmids, with all sequenced examples being closely related to pNDM-BJ01 from A. lwaffii WJ10621, despite being reported in many different Acinetobacter spp (15, 16, 19, 23). In most isolates identified outside Asia, blaNDM-1 is found on the chromosome (12–14).

The Acinetobacter plasmids from China share features which suggest that they could have contributed to the acquisition of blaNDM-1 by Enterobacteriaceae. We set out to see if similar plasmids were present in Acinetobacter spp. from India and Pakistan, where some studies have shown a high prevalence of Enterobacteriaceae...
producing blaNDM. We describe the whole-genome sequence (WGS) of an *A. bereziniae* isolate from Chennai, India, with such a plasmid. We further show that closely related plasmids carrying blaNDM-1 are present in *Acinetobacter* spp. isolated in Karachi, Pakistan. We also investigated the conjugation efficiencies and stabilities of these plasmids in different recipients to further explore their potential as vectors in the dissemination of blaNDM-1.

### MATERIALS AND METHODS

**Bacterial strains studied.** A full list of isolates used in this study is given in Table 1. *A. bereziniae* CHI-40-1 (11) was investigated in detail in the current study. Other *Acinetobacter* species isolates were from fecal screening samples collected at the Civil Hospital Karachi, Pakistan, in 2012. Samples from consecutive patients admitted to the hospital were collected at admission and discharge, and isolates growing on selective plates containing ertapenem were further analyzed. The isolates studied here represent all of the *blaNDM-1*-positive *Acinetobacter* spp. isolated from 717 fecal swabs processed as of January 2013. Escherichia coli UAB190 (24) and *A. pittii* AG3528 were used as recipients in mating experiments.

**Identification and antimicrobial susceptibility testing.** Initial bacterial identification was performed by matrix-assisted laser desorption ionization–time of flight mass spectroscopy (MALDI-TOF) (Bruker, Billerica, MA, USA). Confirmation of the identification was by phylogenetic analysis of 16S rRNA gene sequences for Pakistan isolates, together with ribosomal multilocus sequence typing (rMLST) for CHI-40-1 (see Fig. S1 in the supplemental material). Antimicrobial susceptibility testing was performed by Etest (bioMérieux, LaPlane, France) and MIC test strip (Liofilchem, Roseto degli Abruzzi, Italy). Interpretation was according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (version 3.1).

**WGS and analysis.** *A. bereziniae* CHI-40-1 was sequenced using an Illumina HiSeq platform at the Wellcome Trust Sanger Institute, Cambridge. A unique index-tagged insert library was prepared to allow processing of the sample data following multiplex sequencing with other libraries on eight channels of an Illumina Genome Analyzer GAII cell to give 100-bp paired-end reads, as previously described (25). Reads were assembled *de novo* using the Velvet Assembly Tool (version 1.2.10) (26). Plasmid contigs were identified using Blast searches against the sequence of plasmid pNDM-BJ01 from *Acinetobacter lwophi* strain WJ10621 (accession number JQ901791) (16). Links between contigs were confirmed by PCR and sequencing of amplicons. Detailed comparison between pNDM-40-1 and closely related plasmids was performed using nucleotide alignments created in Geneious (27). Antibiotic resistance genes were identified using RESFinder. Annotation was by transfer of annotations for genes with close nucleotide identity from reference sequences in Geneious.

**PFGE and in-gel hybridization.** Genomic DNA was prepared in 1% low-melting-point agarose plugs as described previously (28). Briefly, plugs were made using a bacterial cell suspension in Tris-EDTA (TE) buffer at a standard optical density of 1.8 to 2.0 at a wavelength of 600 nm (28). Plugs were treated with Apal (Thermo Scientific, Waltham, MA, USA) or S1 nuclease (Thermo Scientific) and pulsed-field gel electrophoresis (PFGE) performed as described previously (28, 29). Autoradiographs were prepared by in-gel hybridization of pulsed-field gels with gene probes, made using a random primer method to label *blaNDM-1* or *traA* PCR products with ³²P[CTP, as previously described (28).

**Conjugation and passage experiments.** Conjugation experiments were performed as described previously using a plate mating assay at 30°C (4), with *A. bereziniae* CHI-40-1 and *A. haemolyticus* 69122-EW as donors and *E. coli* UAB190 and *A. pittii* AG3528 (both rifampin resistant) as recipients. Selection was performed on Brilliance UTI Clarity agar (Oxoid Ltd., Basingstoke, United Kingdom) supplemented with rifampin (Sigma-Aldrich, St. Louis, MO, USA) for recipient selection, ampicillin (Sigma-Aldrich) and rifampin for UAB190 background transconjugants, or meropenem (AstraZeneca, London, United Kingdom) and rifampin for AG3528 background transconjugants. For each experiment, 5 isolated colonies were subcultured to selective media. Pure growths on subculture were tested by MALDI-TOF to confirm the species background, and the presence of *blaNDM-1* was confirmed by PCR. Mating efficiency was calculated as the number of transconjugants per recipient cell. Transconjugants obtained from mating between CHI-40-1 and each recipient background were subjected to S1 PFGE and in-gel hybridization with *blaNDM-1* and *traA*.

A passage experiment was performed on *A. bereziniae* CHI-40-1 and its transconjugants, *E. coli* UAB190NDMP2 and *A. pittii* AG3528NDMP1. Cultures from selective plates were inoculated into Luria-Bertani (LB) broth (Thermo Scientific) with and without antibiotic selection and incubated overnight at 37°C. The following day, cultures were reinoculated into a fresh broth with the same selection as the starting culture. Columbia blood agar plates (EBO Laboratories, Bonnynbridge, Scotland) were inoculated daily to check purity, and cultures were stored each day in LB broth with 10% glycerol at −80°C. This procedure was repeated for 14 consecutive days. Antibiotic selection was with meropenem at 10 μg/ml for CHI-40-1 and AG3528NDMP1 and at 1 μg/ml for UAB190NDMP2. Stored cultures were investigated by S1 PFGE and probing for *blaNDM-1* and *traA* as well as real-time quantitative PCR (qPCR) (see below).

**PCR.** A full list of PCR and sequencing primers used together with full PCR conditions is given in Table S2 in the supplemental material. The presence of plasmids similar to pNDM-BJ01 was confirmed by PCR with primers described by Hu et al. (16). Sequencing of PCR amplicons was used to resolve gaps in the pNDM-40-1 sequence and to primer walk the *blaNDM-1* context in 69122-EW. PCR amplicons for sequencing were purified using the QIAquick gel extraction kit (Qiagen, Limburg, Netherlands) as per the manufacturer’s instruction, and products were submitted to Eurofins MWG Operon (Ebersberg, Germany) for sequencing.

Real-time-quantitative PCR (qPCR) was performed to quantify changes in *blaNDM-1* and *traA* copy number present in bacterial cells over the course of the passage experiment. The single-copy chromosomal gene *rpoB* was used as the reference gene. Dual-labeled probes with fluorescent dye and quenchers were synthesized by Eurofins MWG Operon. *blaNDM-1* and *traA* fluorescence cycle threshold (*C*) values were compared to *rpoB* *C* values, and quantification was performed by the ΔΔ*C* method (30). Regression analysis was performed using Excel 2007. A validation experiment showed that ΔΔ*C* values were linear over the range of values detected in the passage experiment. All experiments were performed in triplicate.

**Nucleotide sequence accession numbers.** Accession numbers for pNDM-40-1 from *A. bereziniae* CHI-40-1 and the partial sequence for pNDM-69122 from *A. haemolyticus* 69122-EW are KP702385 and LN611576, respectively. *A. bereziniae* CHI-40-1 assembly contigs are deposited under study accession number PRJEB7120, contig accession numbers CDEL01000001 to CDEL01000324.

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**Table 1. Bacterial isolates used in this study**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Source</th>
<th>Location where isolated</th>
<th>Yr isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI-40-1</td>
<td><em>Acinetobacter</em></td>
<td>Clinical isolate (pus)</td>
<td>Tamil Nadu, India</td>
<td>2005</td>
</tr>
<tr>
<td>73261-EC</td>
<td><em>Acinetobacter</em></td>
<td>Fecal screening</td>
<td>Karachi, Pakistan</td>
<td>2012</td>
</tr>
<tr>
<td>70114-EC</td>
<td><em>Acinetobacter</em></td>
<td>Fecal screening</td>
<td>Karachi, Pakistan</td>
<td>2012</td>
</tr>
<tr>
<td>69122-EW</td>
<td><em>Acinetobacter</em></td>
<td>Fecal screening</td>
<td>Karachi, Pakistan</td>
<td>2012</td>
</tr>
<tr>
<td>74312-EC</td>
<td><em>Acinetobacter</em></td>
<td>Fecal screening</td>
<td>Karachi, Pakistan</td>
<td>2012</td>
</tr>
<tr>
<td>73668-ECT</td>
<td><em>Acinetobacter</em></td>
<td>Fecal screening</td>
<td>Karachi, Pakistan</td>
<td>2012</td>
</tr>
</tbody>
</table>

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TABLE 2 Antimicrobial MICs for *Acinetobacter* species recipients and transconjugants

<table>
<thead>
<tr>
<th>Strain</th>
<th>ATM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CAZ&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IPM</th>
<th>MEM</th>
<th>TZP</th>
<th>AMK</th>
<th>GEN</th>
<th>TOB</th>
<th>CIP</th>
<th>CST</th>
<th>FOX</th>
<th>SXT</th>
<th>TGC&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI-40-1</td>
<td>48 (R)</td>
<td></td>
<td></td>
<td></td>
<td>96  (R)</td>
<td></td>
<td>12  (R)</td>
<td></td>
<td>0.75 (S)</td>
<td></td>
<td>0.006 (S)</td>
<td></td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>69122-EW</td>
<td>3 (S)</td>
<td></td>
<td></td>
<td></td>
<td>24  (R)</td>
<td></td>
<td>8   (R)</td>
<td></td>
<td>0.75 (S)</td>
<td></td>
<td>32  (S)</td>
<td></td>
<td>4 (I)</td>
</tr>
<tr>
<td>73668-ECT</td>
<td>16 (R)</td>
<td></td>
<td></td>
<td></td>
<td>48  (R)</td>
<td></td>
<td>6   (S)</td>
<td></td>
<td>192 (R)</td>
<td></td>
<td>0.75 (S)</td>
<td></td>
<td>0.75 (S)</td>
</tr>
<tr>
<td>74312-EC</td>
<td>48 (R)</td>
<td></td>
<td></td>
<td></td>
<td>256 (R)</td>
<td></td>
<td>48  (R)</td>
<td></td>
<td>96  (R)</td>
<td></td>
<td>12  (R)</td>
<td></td>
<td>0.75 (S)</td>
</tr>
<tr>
<td>AG3528</td>
<td>16 (R)</td>
<td>3 (S)</td>
<td>0.75 (S)</td>
<td>0.75 (S)</td>
<td>2   (S)</td>
<td>2   (S)</td>
<td>0.75 (S)</td>
<td>0.75 (S)</td>
<td>4   (R)</td>
<td>1   (S)</td>
<td>16  (S)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>UAB190</td>
<td>0.125 (S)</td>
<td>0.25 (S)</td>
<td>0.38 (S)</td>
<td>0.047 (S)</td>
<td>2   (S)</td>
<td>2   (S)</td>
<td>8   (R)</td>
<td>1.5 (S)</td>
<td>0.006 (S)</td>
<td>0.5 (S)</td>
<td>3   (S)</td>
<td>0.094 (S)</td>
<td></td>
</tr>
<tr>
<td>AG3528NDMP1</td>
<td>16 (R)</td>
<td>0.75 (R)</td>
<td>0.75 (R)</td>
<td>32  (R)</td>
<td>96  (R)</td>
<td>3   (S)</td>
<td>0.75 (S)</td>
<td>0.75 (S)</td>
<td>3   (R)</td>
<td>1   (S)</td>
<td>16  (S)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>UAB190NDMP2</td>
<td>0.064 (S)</td>
<td>0.25 (R)</td>
<td>0.75 (S)</td>
<td>32  (R)</td>
<td>24  (R)</td>
<td>4   (I)</td>
<td>256  (R)</td>
<td>2   (S)</td>
<td>8   (R)</td>
<td>1.5 (S)</td>
<td>0.006 (S)</td>
<td>0.5 (S)</td>
<td>3   (S)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Antimicrobial susceptibility results based on EUCAST pharmacokinetic/pharmacodynamic (PK/PD) non-species-specific breakpoints. R, resistant; I, intermediate resistance profile; S, sensitive; ATM, aztreonam; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; TZP, piperacillin-tazobactam; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; CST, colistin sulfate; FOF, fosfomycin; RIF, rifampin; SXT, co-trimoxazole; TGC, tigecycline; ND, not determined.

<sup>b</sup> Species-specific *Acinetobacter* species breakpoints not available.

RESULTS

Species identification, antimicrobial susceptibility, and resistance genes present in *A. bereziniae* CHI-40-1. The clinical *Acinetobacter* isolate from Chennai was found to be an *A. bereziniae* strain by rMLST. Five NDM-1-producing *Acinetobacter* isolates from the fecal screening study were obtained from five different patients and included three species (Table 1; see Fig. S1 in the supplemental material). Three *Acinetobacter haemolyticus* isolates were found to be representatives of a single strain by ApaI restriction digestion and PFGE. All strains were extensively drug resistant (Table 2). In keeping with this, genes associated with resistance to β-lactams (*bla*<sub>oxa-58</sub>), aminoglycosides (*strA, strB, and aacC2*), macrolides [*msr(E) and mph(E)], trimethoprim (*dfrA1*), and sulfonamides (*sul1 and sul2*) were identified in *A. bereziniae* CHI-40-1.

Characterization of plasmid pNDM-40-1. S1 PFGE and *bla*<sub>NDM-1</sub> probing showed that *A. bereziniae* CHI-40-1 harbored multiple plasmids, with *bla*<sub>NDM-1</sub> present on plasmids corresponding to bands of ~45 kb and ~250 kb (Fig. 1a and b). However, *bla*<sub>NDM-1</sub> was present on a single ApaI restriction fragment of ~45 kb (Fig. 1c and d).

The *de novo* assembly of the *A. bereziniae* CHI-40-1 WGS produced 324 contigs, with a mean GC content of 38% and a combined size of 4.78 Mb. A 45,827-bp plasmid harboring *NDM-1* from *A. bereziniae* CHI-40-1 was closed by PCR and sequencing of amplicons. The GC content of the plasmid backbone is 36.2%, and that of the variable region (from ISAb14 to the end of Tn125, nucleotides [nt] 5427 to 16280) is 52.5%.

At the time of writing, complete sequences of nine pNDM-BJ01-like plasmids were available in GenBank. The backbone of pNDM-BJ01 is 100% identical at the nucleotide level to those of pNDM-BJ01 (bases 1 to 5684 and 17987 to 47274, accession number JQ001791), pNDM-BJ02 (JQ060896), pAbNDM-1 (JN377410), and pXM1 (AMXH01000087). pNDM-AB (KC503911), pM131_NDM-1 (JX072963), and pNDM-Iz4b (KJ547696) exhibit minor differences from the backbone of pNDM-BJ01 and from one another. pNDM-AB differs the most, because of a 3.5-kb insertion containing the genes *traD* and *msb* and a putative methyltransferase gene (Fig. 2; ever, *bla*<sub>NDM-1</sub> was present on a single ApaI restriction fragment of ~45 kb (Fig. 1c and d).

The de novo assembly of the *A. bereziniae* CHI-40-1 WGS produced 324 contigs, with a mean GC content of 38% and a combined size of 4.78 Mb. A 45,827-bp plasmid harboring *bla*<sub>NDM-1</sub> pNDM-40-1, was closed by PCR and sequencing of amplicons. The GC content of the plasmid backbone is 36.2%, and that of the variable region (from ISAb14 to the end of Tn125, nucleotides [nt] 5427 to 16280) is 52.5%.

At the time of writing, complete sequences of nine pNDM-

![FIG 1](a) Pulsed-field gel of S1 nuclease-digested genomic DNA from *A. bereziniae* CHI-40-1, recipients, and transconjugants; (b) in-gel hybridization with a *bla*<sub>NDM-1</sub> gene probe; (c) pulsed-field gel of ApaI-digested genomic DNA from CHI-40-1; (d) in-gel hybridization with a *bla*<sub>NDM-1</sub> gene probe. The molecular size marker is concatemers of λ of ~50 to 1,000 kb.
These plasmids were all identified in isolates from China and were found in five different species, including *A. baumannii*. Additionally, several *bla*NDM-1-negative *Acinetobacter* species sequences contain regions with significant identity to the backbones of pNDM-BJ01-like plasmids (see Fig. S3 in the supplemental material).

The *bla*NDM-1 gene in pNDM-40-1 is found within a Tn\_125 transposon (Fig. 2), as with other pNDM-BJ01-like plasmids (12, 13, 16). Tn\_125 in pNDM-40-1 has two deletions relative to Tn\_125 in pNDM-BJ01: a 1,298-bp deletion from the 3'=end of *ble* to *tat* and a 150-bp deletion within IS\_CR27 (see Fig. S3 in the supplemental material). pNDM-BJ02 and contig 5 from *Acinetobacter soli* TCM341 lack the 3' IS\_Aba125, while in pAB-D499 and pM131\_NDM-1, there is an IS\_Aba11 inserted at the 3' end of the element. In pNDM-AB, a large part of the context from cutA1 to the 3' IS\_Aba125 is replaced by the macrolide resistance genes *msr*(E) and *mph*(E) (23). In all pNDM-BJ01-like plasmids, the aminoglycoside resistance gene *aph*(E) and an IS\_Aba14 element are found immediately upstream of Tn\_125 (Fig. 2).

GenBank searches show that *bla*NDM-1 contexts in *Enterobacteriaceae* have high degrees of identity with the *bla*NDM-1 context from pNDM-BJ01-like plasmids. In most cases, this is restricted to genes that make up part of the full Tn25 element harboring *bla*NDM-1, with at least a fragment of the IS\_Aba125 upstream of *bla*NDM-1 and the *ble* and *trpF* genes being present in almost all cases. Four sequences from *Enterobacteriaceae* with regions of close identity to the *bla*NDM-1 context in pNDM-BJ01, which included part of the plasmid backbone, were available at the time of writing (31) (Fig. 2). These were in the plasmids pPrY2001 from *Providencia rettgeri* (KF295828), pMR0211 from *Providencia stuartii* (JN687470), pECL3-NDM-1 from *Enterobacter cloacae* (KC887917), Plasmid pPrY2001 contains the most extensive region of identity. The sequence is nearly identical to that found in pNDM-BJ01 from the
far 3' end of traA to the resolvase gene, with the main difference being the absence of the 3' ISAba125.

All pNDM-BJ01-like plasmids share a region with genes coding for a type IV secretion system (T4SS) involved in constructing the conjugation machinery and mediating conjugal transfer of plasmid DNA to recipient bacteria (16). In addition, all pNDM-BJ01-like plasmids contain genes proposed to code for a plasmid partition system (parA) and a putative zeta-toxin, which may contribute to plasmid stability through a toxin-antitoxin addiction system. It has not been possible to identify the replicase or the origin of replication of these plasmids. However, the wide range of replication strategies already described means that the lack of an identifiable replicase is not entirely surprising (6, 32).

The blaNDM-1 context in A. haemolyticus. PCR analysis revealed that the A. haemolyticus strain, but not the other two Acinetobacter species isolates from Karachi, contained several regions of a pNDM-BJ01-like plasmid backbone. The immediate context blaNDM-1 context in A. haemolyticus 69122-EW was linked to pNDM-BJ01-like backbone genes traA upstream and the resolvase gene downstream (Fig. 2). The immediate context differed from that described in pNDM-BJ01 in that most of Tn125 was missing.

A previously uncharacterized insertion sequence, ISAha3, most similar to ISAAl1 (95% amino acid [AA] identity between transposases), was inserted between 

ble

and the putative resolvase gene. No direct repeats (DRs) were observed, but this was not uncommon for other closely related ISs deposited in ISFinder. It is possible that transposition of ISAha3 resulted in deletion of the sequence often found between ble and res and also resulted in the loss of one of the DRs (33). S1 PFGE and in-gel hybridization showed that 

blaNDM-1

was present on ~45-kb plasmids in A. haemolyticus 69122-EW, pNDM-69122.

Conjugative transfer and stability of plasmids harboring 

blaNDM-1

. Transconjugants were obtained from mating experiments with A. bereziae CHI-40-1 and A. haemolyticus 69122-EW donors, in both E. coli UAB190 and A. pittii AG3528 recipients, at rates of 10⁻⁷ to 10⁻⁵ transconjugants per recipient cell. All putative transconjugants tested were found to be the recipient background species by MALDI-TOF and to be 

blaNDM-1

positive by PCR. MICs to all β-lactams except aztreonam were elevated in selected transconjugants (Table 2).

PCR analysis showed that the relaxase gene, traA, and other sections of the pNDM-BJ01-like backbone were present in all transconjugant colonies tested. In A. pittii AG3528 transconjugants, in-gel hybridization showed that both traA and 

blaNDM-1

were present on ~45-kb plasmids, as expected for pNDM-40-1. However, in E. coli UAB190 transconjugants, these genes were both present on either the chromosome or ~90-kb plasmids (Fig. 1b) (traA data not shown).

Probing of S1 PFGE gels of CHI-40-1, UAB190NDMP2, and AG3528NDMP1 over the course of a 14-day passage showed that 

blaNDM-1

-positive bands did not alter in size (see Fig. S4 in the supplemental material). The intensity of the 

blaNDM-1

bands in CHI-40-1 was similar over the course of the experiment with and without meropenem selection. The intensity of the 

blaNDM-1

bands for both transconjugant strains was stable with antibiotic selection but decreased significantly over the course of the passage without meropenem selection.

Regression analysis of 

ΔΔCt

values from qPCR experiments (see Fig. S5 in the supplemental material) showed statistically significant falls in the quantities of 

blaNDM-1

and traA template over the course of the passage experiment for transconjugant strains without meropenem selection. For the donor strain, CHI-40-1, there was little change. For all strains tested, 

blaNDM-1

and traA remained detectable throughout the 14-day passage experiment, even in the absence of antibiotic selection.

DISCUSSION

All the complete NDM-1 plasmid sequences from Acinetobacter spp. that we were able to identify in GenBank were from Chinese isolates and were similar to pNDM-BJ01. pNDM-BJ01 contains a single Apal restriction site, in keeping with the ~45-kb bands present in both the S1 and Apal gels. We conclude that CHI-40-1 harbors just one plasmid containing 

blaNDM-1

, and that the 300-kb band represented residual supercoiled plasmid DNA.

The pNDM-BJ01-like plasmids have a GC content similar to that of most Acinetobacter species WGSs deposited in GenBank (~40%). All examples of these plasmids harboring 

blaNDM-1

have very high levels of identity with one another. The small number of related sequences not associated with 

blaNDM-1

are more distantly related to pNDM-BJ01-like plasmids but are also found exclusively in Acinetobacter spp. We propose that these findings are compatible with this plasmid lineage having evolved within the Acinetobacter genus and with the acquisition of 

blaNDM-1

being a relatively recent event.

Most descriptions of conjugal transfer of 

blaNDM-1

from Acinetobacter spp. in vitro are for isolates with pNDM-BJ01-like plasmids (15, 16, 19, 23). Conjugation rates were similar for the pNDM-BJ01-like plasmids studied here into E. coli and A. pittii recipients. The stability of both 

blaNDM-1

and traA, coding for the T4SS involved in constructing the conjugation machinery and mediating conjugal transfer of plasmid DNA to recipient bacteria (16). In addition, all pNDM-BJ01-like plasmids contain genes proposed to code for a plasmid partition system (parA) and a putative zeta-toxin, which may contribute to plasmid stability through a toxin-antitoxin addiction system. It has not been possible to identify the replicase or the origin of replication of these plasmids. However, the wide range of replication strategies already described means that the lack of an identifiable replicase is not entirely surprising (6, 32).

The immediate context differed from that described in pNDM-BJ01 in that most of Tn125 was missing.

A previously uncharacterized insertion sequence, ISAha3, most similar to ISAAl1 (95% amino acid [AA] identity between transposases), was inserted between ble and the putative resolvase gene. No direct repeats (DRs) were observed, but this was not uncommon for other closely related ISs deposited in ISFinder. It is possible that transposition of ISAha3 resulted in deletion of the sequence often found between ble and res and also resulted in the loss of one of the DRs (33). S1 PFGE and in-gel hybridization showed that 

blaNDM-1

was present on ~45-kb plasmids in A. haemolyticus 69122-EW, pNDM-69122.

Conjugative transfer and stability of plasmids harboring 

blaNDM-1

. Transconjugants were obtained from mating experiments with A. bereziae CHI-40-1 and A. haemolyticus 69122-EW donors, in both E. coli UAB190 and A. pittii AG3528 recipients, at rates of 10⁻⁷ to 10⁻⁵ transconjugants per recipient cell. All putative transconjugants tested were found to be the recipient background species by MALDI-TOF and to be 

blaNDM-1

positive by PCR. MICs to all β-lactams except aztreonam were elevated in selected transconjugants (Table 2).

PCR analysis showed that the relaxase gene, traA, and other sections of the pNDM-BJ01-like backbone were present in all transconjugant colonies tested. In A. pittii AG3528 transconjugants, in-gel hybridization showed that both traA and 

blaNDM-1

were present on ~45-kb plasmids, as expected for pNDM-40-1. However, in E. coli UAB190 transconjugants, these genes were both present on either the chromosome or ~90-kb plasmids (Fig. 1b) (traA data not shown).

Probing of S1 PFGE gels of CHI-40-1, UAB190NDMP2, and AG3528NDMP1 over the course of a 14-day passage showed that 

blaNDM-1

-positive bands did not alter in size (see Fig. S4 in the supplemental material). The intensity of the 

blaNDM-1

bands in CHI-40-1 was similar over the course of the experiment with and without meropenem selection. The intensity of the 

blaNDM-1

bands for both transconjugant strains was stable with antibiotic selection but decreased significantly over the course of the passage without meropenem selection.

Regression analysis of 

ΔΔCt

values from qPCR experiments (see Fig. S5 in the supplemental material) showed statistically significant falls in the quantities of 

blaNDM-1

and traA template over
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We have no conflicts to declare.

REFERENCES


