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Plasmid Carriage of bla_{NDM-1} in Clinical *Acinetobacter baumannii* Isolates from India

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NDM-1 probably emerged in *Acinetobacter* species prior to its dissemination among *Enterobacteriaceae*, and NDM-1-like enzymes are increasingly reported in *Acinetobacter* species. Here, we report on the genetic context of bla_{NDM-1} in the earliest known NDM-1-producing organisms, clinical isolates of *Acinetobacter* from India in 2005. These strains harbor bla_{NDM-1} plasmids of different sizes. The gene is associated with the remnants of the Tn*125* transposon normally associated with bla_{NDM-1} in *Acinetobacter* spp. The transposon has been disrupted by the IS*26* insertion and subsequent movement events.

Multidrug-resistant *Enterobacteriaceae* producing the New Delhi metallo-β-lactamase-1 (NDM-1) enzyme have been isolated around the world, with many cases linked to travel, especially to South Asia (1–3). Carbapenem resistance in the opportunistic pathogen, *Acinetobacter baumannii*, is mostly associated with OXA-type β-lactamases (4), but NDM-1-like enzymes have been increasingly reported (5–10). In all bacterial species, the bla_{NDM-1} gene is associated with at least one copy of a complete or partial IS*Aba125* gene, which provides bla_{NDM-1} with a strong promoter (11–13). IS*Aba125* almost certainly originates from *Acinetobacter* spp., and there is evidence that bla_{NDM-1} was probably formed by a fusion event between the aminoglycoside resistance gene, *aphA6*, and an ancestral carbapenemase in an *Acinetobacter* background (13), thus implying that bla_{NDM-1} spread to *Enterobacteriaceae* from *Acinetobacter* spp.

A small number of studies have reported a relatively high prevalence of NDM-1-producing *A. baumannii*, causing infections in intensive care patients in Indian hospitals (10, 14). There are also several case reports of colonization and infection with these organisms from European countries, many with epidemiological links with travel to North Africa or the Balkans (5, 6, 11), and from the Middle East (9). In China, bla_{NDM-1} has been frequently identified in *Acinetobacter* spp., including *A. baumannii*, both in clinical cases and from environmental sources (7, 15–17). The genetic context of bla_{NDM-1}-like genes in *Acinetobacter* spp. shows less variation than that observed in other genera. In most strains, bla_{NDM-1} is bracketed by two copies of IS*Aba125* to form a Tn*125* transposon. The content of Tn*125* is usually conserved, with the occasional exceptions resulting from truncation by insertion of other IS elements. Tn*125* is found inserted in several different gene locations, with direct repeats at either end indicating movement by transposition. All isolates from Europe and North Africa are chromosomally located, but in China, bla_{NDM-1} is reportedly found mostly on plasmids in *Acinetobacter* spp. other than *A. baumannii* (15–18).

We received 9 *Acinetobacter* isolates that were collected in 2005 from a hospital in Tamil Nadu, India. Most isolates were from patients receiving intensive care and were isolated from blood, pus, and respiratory secretions. The initial identification and sensitivity testing were performed using a BD Phoenix system (Becton, Dickinson, Franklin Lakes, NJ, USA), with supplementary

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TABLE 1 Study isolates, specimen types, and antimicrobial MICs for *Acinetobacter* isolates

		MIC (μg/ml) for[a]:											
Isolate	Specimen type	Azt	Caz	Taz	Imp	Mem	Ami	Gent	Tob	Cip	Col	Rif	Tig
A. baumannii CHI-16	Blood	>16	≥256	>16/4	≥32	≥32	≤4	>4	2	≥32	≤1	6	2
A. baumannii CHI-18	Blood	>16	≥256	>16/4	≥32	≥32	>16	>4	>4	≥32	≤1	4	3
A. baumannii CHI-32	Blood	>16	≥256	>16/4	≥32	≥32	≤4	>4	2	≥32	≤1	6	2
A. baumannii CHI-34	Sputum	>16	≥256	>16/4	≥32	≥32	≤4	>4	>4	≥32	≤1	4	2
Acinetobacter sp CHI-40-1	Pus	>16	≥256	>16/4	≥32	≥32	>16	>4	4	≥32	≤1	≥256	0.75
A. baumannii CHI-40-2	Pus	>16	≥256	>16/4	≥32	≥32	>16	>4	>4	≥32	≤1	6	2
A. baumannii CHI-41	Sputum	>16	≥256	>16/4	≥32	≥32	>16	>4	>4	≥32	≤1	6	1.5
A. baumannii CHI-44	Endotracheal aspirate	>16	≥256	>16/4	≥32	≥32	>16	>4	>4	≥32	≤1	6	2
A. baumannii CHI-45-1	Endotracheal aspirate	>16	≥256	>16/4	≥32	≥32	>16	>4	>4	≥32	≤1	3	2

[a] MICs were determined by the BD Phoenix system except for ceftazidime, imipenem, meropenem, ciprofloxacin, rifampin, and tigecycline, which were determined by an Etest or a MIC test strip. Ami, amikacin; Azt, aztreonam; Caz, ceftazidime; Cip, ciprofloxacin; Col, colistin; Gent, gentamicin; Imp, imipenem; Mem, meropenem; Rif, rifampin; Taz, piperacillin-tazobactam; Tig, tigecycline; Tob, tobramycin.

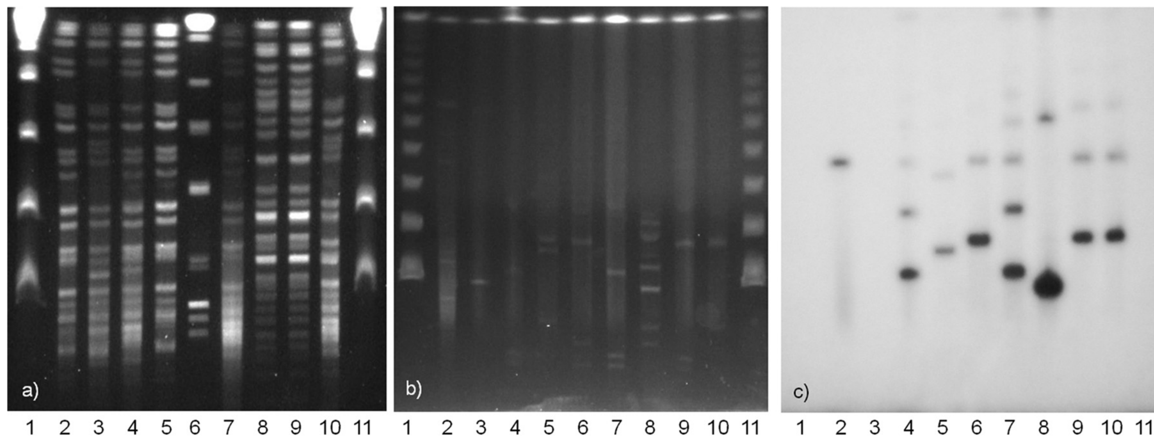

FIG 1 (a) PFGE of ApaI-digested genomic DNA. Lane 1, λ ladder (48.5-kb concatemers); lane 2, CHI-16; lane 3, CHI-18; lane 4, CHI-32; lane 5, CHI-34; lane 6, CHI-40-1; lane 7, CHI-40-2; lane 8, CHI-41; lane 9, CHI-44; lane 10, CHI-45-1; lane 11, λ ladder. (b) PFGE of S1 endonuclease-digested genomic DNA; (c) in-gel hybridization with ^{32}P-labeled bla_{NDM-1} gene probe. Lanes 1, λ ladder; lanes 2, *Klebsiella pneumoniae* KP506 (NDM positive control); lanes 3, *Escherichia coli* UAB190 (NDM negative control); lanes 4, CHI-16; lanes 5, CHI-18; lanes 6, CHI-32; lanes 7, CHI-34; lanes 8, CHI-40-1; lanes 9, CHI-40-2; lanes 10, CHI-45-1; lanes 11, λ.

sensitivity testing performed using the Etest (bioMérieux, LaPlane, France) or MIC test strip (Liofilchem, Roseto degli Abruzzi, Italy) method (Table 1). The confirmation of *A. baumannii* identification was performed by PCRs for bla_{OXA-51}-like genes. All isolates were bla_{OXA-51}-like positive by PCRs except CHI-40-1, and all were extensively drug resistant (19), with the *A. baumannii* isolates being resistant to all drugs tested except colistin and in some cases amikacin and tobramycin. Seven isolates were positive for bla_{NDM-1} by PCRs, with CHI-41 and CHI-44 being the only bla_{NDM-1}-negative isolates. All of the *A. baumannii* isolates carried bla_{OXA-23}-like genes, confirmed to be associated with IS*Aba1*, and thus having a strong upstream promoter, by PCR. All PCR amplicons were confirmed by sequencing.

A. baumannii isolates were then typed by the multilocus sequence typing (MLST) method described by Turton et al. (20) and by pulsed-field gel electrophoresis (PFGE) of ApaI-digested genomic DNA. All *A. baumannii* isolates producing NDM-1 by MLST were within group II, which corresponds with worldwide clone 1. The 2 bla_{NDM-1}-negative isolates were within group I. ApaI profiles were similar but not identical for all group II *A. baumannii* isolates and differed substantially from those for group I isolates (Fig. 1a) and CHI-40-1.

To investigate the genetic context of bla_{NDM-1}, we performed sequencing by primer walking in CHI-32, CHI-34, and CHI-45-1 using primers designed against the context in *A. baumannii* 161/07 (21). Gene probing was performed by in-gel hybridization of S1 nuclease and NotI PFGE gels. The gene probes were made using a random priming method with bla_{NDM-1}, IS*Aba125*, IS*26*, and IS*CR27* PCR products labeled with [^{32}P]CTP. As the primer walking PCR results were consistent for all three isolates studied, products were only fully sequenced for CHI-45-1. The full Tn*125* structure was present; however, IS*CR27* contains an IS*26* insertion (Fig. 2). The sequence analysis of PCR amplicons revealed that the fragment of IS*CR27* downstream of the IS*26* insertion is present both in its normal position and upstream of bla_{NDM-1}. The results of probing NotI gels with bla_{NDM-1}, IS*26*, and IS*CR27* suggest that it is the latter context which is more common. S1 gels demonstrated multiple plasmids in all *A. baumannii* isolates. The probing showed that bla_{NDM-1} was on multiple bands, ranging in size from ~45 kb to ~300 kb. We believe most of the larger bands represent

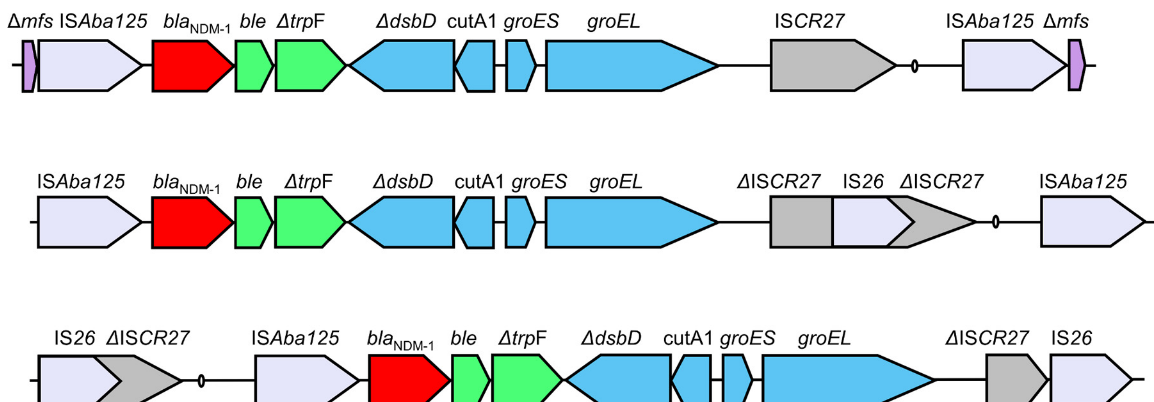

FIG 2 Gene maps of the genetic context of bla_{NDM-1} in *Acinetobacter* species. *aphA6* codes for 3′ phosphotransferase VI aminoglycoside-modifying enzyme, *ble* for bleomycin resistance protein, *trpF* for phosphoribosylanthranilate isomerase, *tat* for twin-arginine translocation pathway signal sequence domain protein, *cutA1* for periplasmic divalent cation tolerance protein, *groES* for cochaperonin, *groEL* for chaperonin, IS*CR27* for insertion sequence common repeat 27 transposase, *oriIS* for origin of insertion of IS*CR27*, and Δ*mfs* for major facilitator superfamily (MFS) metabolite/H^+ symporter. Arrows indicate the direction of the transcription of genes. The genes *tat*, *cutA1*, *groES*, and *groEL* are shaded in the same color because they are believed to be from a common source, with similar genes found in synteny in both *Xanthomonas* and *Pseudoxanthomonas* spp.

cointegrate formation rather than the presence of multiple plasmids carrying bla_{NDM-1}, since the bands increase in size by intervals of approximately the size of the smallest band for each isolate (Fig. 1b). Mating experiments were performed on plates and in broth. *A. baumannii* CHI-32, CHI-34, and CHI-45-1 were used as donors, and *Escherichia coli* UAB190 and *Acinetobacter pittii* AG3528 were used as recipients (both rifampin resistant). No transconjugants were obtained after multiple mating experiments.

The *A. baumannii* isolates analyzed in this study are among the earliest found to produce NDM-1, having been initially identified in 2005. This is the first time that the genetic context of *A. baumannii* isolates from the Indian subcontinent has been analyzed. The findings are compatible with the hypothesis that bla_{NDM-1} might have been disseminated from *Acinetobacter* to *Enterobacteriaceae* in South Asia. In these isolates, Tn*125* has been disrupted by IS*26*, and subsequent rearrangement has resulted in bla_{NDM-1} being within an IS*26* composite transposon, which might potentially mobilize bla_{NDM-1}. Otherwise, the genes usually found on Tn*125* are conserved, and so the genetic context is compatible with being the progenitor of the bla_{NDM-1} in many of the *Enterobacteriaceae* for which sequences are available. bla_{NDM-1} is located on plasmids in these isolates, which could facilitate mobilization of the gene to other bacterial species. The mating experiments suggest that the plasmids are nonconjugative, but they may be mobilizable with a helper plasmid. That these clinical isolates of *A. baumannii* producing NDM-1 were clonally related demonstrates the potential for bla_{NDM-1} establishing itself in successful strain backgrounds capable of being disseminated in the hospital environment and further compromising therapeutic options in the treatment of significant bacterial pathogens.

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