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Tn*125*-Related Acquisition of bla_{NDM}-Like Genes in *Acinetobacter baumannii*

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A multidrug-resistant *Acinetobacter baumannii* isolate recovered from a patient hospitalized in Switzerland after a transfer from Serbia produced the NDM-1 carbapenemase. The bla_{NDM-1} gene was part of a chromosomally located Tn*125* composite transposon bracketed by two copies of the same insertion sequence, IS*Aba125*. This transposon was also associated with the acquisition and expression of the bla_{NDM-2} gene in an *A. baumannii* isolate in Germany. Tn*125* appears to be the main vehicle for dissemination of bla_{NDM} genes in that species.

The carbapenemase NDM-1, initially identified in *Escherichia coli* and *Klebsiella pneumoniae*, has been found mostly in enterobacterial species (12, 16, 17). However, recent reports have described the occurrence of bla_{NDM} genes in *Acinetobacter baumannii*. Several NDM-1-positive *A. baumannii* isolates have been identified in India (10), and two NDM-positive *A. baumannii* isolates have been recovered in Germany, one being from a patient transferred from a Serbian hospital and producing NDM-1 (6), whereas the other produced NDM-2 (one amino acid substitution with respect to NDM-1) and had been recovered from a patient transferred from an Egyptian hospital (isolate ML) (9). Although the Indian subcontinent is considered a reservoir of NDM-1 producers (17), recent reports indicate that at least the Balkan states and the Middle East regions could also be potential reservoirs (13, 21).

In a recent work, Pfeifer et al. (18) reported that the bla_{NDM-1} gene identified in *A. baumannii* isolate 161/07 from Germany (from a patient transferred from Serbia) (6) was located inside a composite transposon bracketed by two copies of insertion sequence IS*Aba125*.

A retrospective survey focusing on multidrug-resistant Gram-negative isolates identified several non-clonally related NDM-1-producing isolates from three patients who had been hospitalized in Geneva University Hospitals, Switzerland, from March 2009 to October 2010. One *E. coli* and one *K. pneumoniae* isolate were recovered from the same patient, who had been transferred from Serbia (22). In both isolates, the bla_{NDM-1} gene was identified on the same 150-kb IncA/C-type plasmid (20). Further investigations showed that this patient also carried a multidrug-resistant *A. baumannii* isolate that had been recovered from rectal swabs.

A. baumannii isolate JH was resistant to all β-lactams, including carbapenems (MICs of imipenem, ertapenem, doripenem, and meropenem measured by Etest [AB bioMérieux; Solna, Sweden] were all >32 μg/ml) according to the CLSI guidelines (3). It was also resistant to gentamicin, amikacin, chloramphenicol, tetracycline, and fluoroquinolones and remained susceptible to tobramycin and netilmicin, with MICs of colistin, rifampin, and tigecycline being at 0.5, 1, and 1 μg/ml, respectively.

PCR and sequencing revealed that *A. baumannii* JH harbored the bla_{NDM-1} gene. Screening for additional β-lactamase genes and for 16S RNA methylase genes as reported previously (1, 23) showed that *A. baumannii* JH was coharboring another carbapenemase gene, namely, bla_{OXA-23}, whereas no 16S RNA methylase gene was identified, in accordance with the susceptibility observed for some aminoglycosides. Multilocus sequence typing was performed, following the Institut Pasteur scheme, as described previously (4), and showed that isolate JH belonged to the ST1 type, whereas an NDM-2-positive *A. baumannii* ML isolate recently identified from Egypt belonged to the ST103 type (9), both types differing significantly. Interestingly, ST1-type *A. baumannii* isolates have recently been identified in Greece (carbapenem resistant by production of OXA-58) (14) and Italy (carbapenem susceptible or resistant by production of OXA-58) (5).

In order to investigate the genetic structures at the origin of the bla_{NDM-1} gene in *A. baumannii* JH and of the bla_{NDM-2} gene in *A. baumannii* ML, cloning experiments were performed as described previously (24). Expression in *E. coli* TOP10 gave *E. coli* TOP10(pAbNDM1) and *E. coli* TOP10(pAbNDM-2), expressing NDM-1 and NDM-2, respectively. They exhibited the exact same resistance pattern, with identical MICs of all β-lactams, in accordance with our previous observations (9).

Sequencing of the whole insert of both recombinant plasmids (an XbaI 15-kb fragment for pAbNDM1 and an HindIII 17-kb fragment for pAbNDM2) allowed characterization of the structures surrounding the bla_{NDM-1} and bla_{NDM-2} genes. Both genes were located inside the exact same composite transposon, named Tn*125*, made of two copies of insertion sequence IS*Aba125* bracketing a 7,925-bp fragment, thus forming a 10,099-bp transposon. IS*Aba125* is a 1,087-bp element belonging to the IS*30* family, containing a single open reading frame corresponding to the 322-amino-acid-long transposase. The two copies of IS*Aba125* were located in the same direct orientation, and they differed by 6 bp, leading only to three amino acid changes. However, their respective inverted repeats were identical.

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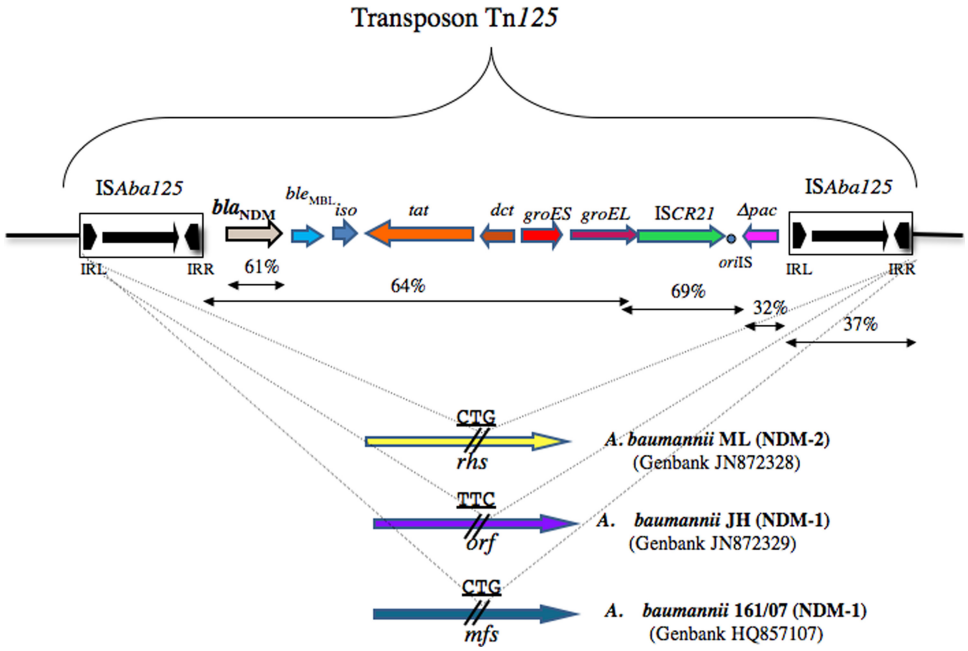

FIG 1 Features of transposon Tn*125*, carrying the bla_{NDM-1} and bla_{NDM-2} genes. Genes and their transcription orientations are indicated by arrows. The lengths of the target genes and the exact location of the target site are not to scale. *ori*IS of IS*CR21* is indicated by a circle. The 3-bp Tn*125* target sites identified in each isolate are underlined and uppercase. GC content is indicated in percentages. Gene names are abbreviated according to their corresponding proteins: *iso* for phosphoribosylanthranilate isomerase; *tat* for the twin-arginine translocation pathway signal sequence protein; *dvt* for the divalent cation tolerance protein; Δ*pac* for truncated phospholipid acetyltransferase; *rhs* for the Rhs protein; *orf* for an unknown open reading frame; and *mfs* for major facilitator superfamily metabolite/H^+ symporter. IRL and IRR indicate inverted repeat left and right, respectively.

In both isolates, transposon Tn*125* was bracketed by a 3-bp target site duplication (CTG in isolate ML and TTC in isolate JH) corresponding to the signature of the transposition process. Further sequence analysis showed that transposon Tn*125* was located on the chromosome in both isolates. In *A. baumannii* JH, it was inserted into a gene similar to that identified in the chromosome of several *A. baumannii* strains (*in silico* analysis) and encoding a putative protein of unknown function. In *A. baumannii* ML, it was inserted into the *rhs* gene, encoding an Rhs protein of unknown function, though Rhs elements are known to be accessory elements often present as multiple copies in genomes (27). These data suggest that the same transposition targeted independently the chromosomes of two distinct *A. baumannii* strains.

Detailed analysis of the Tn*125* content revealed that the distance between IS*Aba125* and the bla_{NDM-1} or bla_{NDM-2} gene was 93 bp, as previously observed in bla_{NDM-1}-positive enterobacterial isolates in which either a truncated or complete copy of IS*Aba125* had been identified (Fig. 1) (20). The expression of the bla_{NDM-1}/bla_{NDM-2} genes was therefore under the control of a hybrid promoter whose −35 sequence was located in the left inverted repeat of IS*Aba125*, as demonstrated in *E. coli* (19). Downstream of bla_{NDM-1}/bla_{NDM-2}, eight open reading frames (ORFs) were identified (Fig. 1). The first corresponded to the *bleMBL* gene, encoding a 121-amino-acid-long protein conferring resistance to bleomycin, as previously found in enterobacterial isolates (19, 20). Then, a gene encoding a 212-amino-acid-long putative phosphoribosylanthranilate isomerase was identified, whose product shares 98% amino acid identity with the protein encoded by a gene identified downstream of bla_{NDM-1} on plasmid pNDM-HK from *E. coli* (Fig. 1). It was followed by a gene encoding a 343-amino-acid-long putative twin-arginine translocation pathway signal sequence domain protein sharing similarities with that of *Brevundimonas diminuta* (GenBank accession no. EEGF96543.1) and then by a gene encoding a 135-amino-acid long periplasmidic divalent cation tolerance protein 64% identical with that of *Xanthomonas albilineans* (GenBank accession no. CBA14859.1). Downstream, genes encoding the GroES and GroEL chaperonin proteins, respectively, were identified. The GroES protein (97 amino acids long) shared 85% identity with those encoded by genes located on IncA/C-type and bla_{CMY-2}-positive plasmids in *E. coli* (2) and *Salmonella enterica* serovar Typhimurium (28). The GroEL protein (547 amino acids long) shared 92% identity with that encoded by an IncHI2 plasmid from *E. coli* APEC01 (8). Finally, the mobilized fragment bracketed by the two IS*Aba125* elements ended with a gene encoding the putative transposase of an IS*CR*-like element, termed IS*CR21*, sharing 96% identity with that identified in *E. coli* APEC01 and 93% with that of IS*CR19* involved in the acquisition of the bla_{OXA-18} gene in *Pseudomonas aeruginosa* (15). IS*CR* elements are peculiar insertion sequences belonging to the IS*91* family, likely mobilizing genes located at their left-hand extremity by rolling-circle transposition (25). According to the sequence of its transposase, IS*CR21* belongs to the IS*CR3*/IS*CR5* group (26). The putative *ori*IS sequence defining the origin of replication of IS*CR21* was identified, sharing a high degree of identity (16 out of 19 bp) with those of IS*CR3*. At the right-hand extremity of IS*CR21* and before the IS*Aba125* right copy of Tn*125*, a truncated gene encoding a putative phospholipid acetyltransferase was identified, with the corresponding protein sequence sharing 91% amino acid identity with that of *Acinetobacter junii* (GenBank accession no. EEY94339.1) (Fig. 1).

Analyzing both the location of the diverse mobile elements and

the GC content of the genes identified in transposon Tn*125* (Fig. 1), our hypothesis here is that the original mobilization of the bla_{NDM-1} gene occurred through a rolling-circle transposition process involving IS*CR21*. This IS could have mobilized a DNA fragment encompassing all the genes from *groEL* and *groES* to bla_{NDM-1} (actually exhibiting similar GC percentages) from its still-unknown bacterial progenitor. Then, this transposed fragment may have targeted the chromosome of an *A. junii*-related strain (corresponding to sequences with a much lower GC content) (Fig. 1). Then, two copies of IS*Aba125* (known to be present in different *Acinetobacter* species, since it is has been found in *A. baumannii* and *Acinetobacter* genomospecies 3/*Acinetobacter pittii* according to the GenBank databases) could have targeted the bla_{NDM-1}-surrounding sequences, thus forming Tn*125*, which could transpose to other *Acinetobacter* sp. isolates.

It is noteworthy that the NDM-1-producing *A. baumannii* isolate 161/07 from Germany belonged to ST25, distinct from our two isolates; however, the bla_{NDM-1} gene was also identified as part of a composite transposon (18). *In silico* analysis revealed that this isolate harbored an identical transposon Tn*125*. Its target site was also chromosomal but corresponded to another gene, namely, *mfs*, encoding a putative major facilitator superfamily metabolite/H^+ symporter (18). A detailed analysis using sequences available in the GenBank databases (accession no. HQ857107) showed that the IS*Aba125* extremities were wrongly annotated in that study, the target site duplication being consequently 3 and not 8 bp long.

Transposon Tn*125* was bracketed by two copies of insertion sequence IS*Aba125* that are identical to those reported to be multiple copies in the chromosome of *A. baumannii* ACICU (7) (Fig. 1). It is noteworthy that the target site duplication bracketing Tn*125* in isolate 161/07 corresponded to CTG, exactly identical to that identified in isolate ML, despite the fact that the two target genes have no genetic link. This would suggest that this CTG sequence may represent a hot spot of transposition for IS*Aba125* and consequently for transposition of Tn*125*.

The current dissemination of bla_{NDM-1} and bla_{NDM-2} in *A. baumannii* is likely linked to a Tn*125* and not plasmid related, in contrast to what is observed in the *Enterobacteriaceae*, in which it is supported mainly by plasmids in which bla_{NDM-1}-surrounding sequences contain a single copy of IS*Aba125* that is most often truncated.

Nucleotide sequence accession number. The nucleotide and protein sequences of the Tn*125* transposons and their close genetic environments have been registered in GenBank under accession no. JN872328 (isolate ML) and JN872329 (isolate JH).

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