Transposition of Tn*125* Encoding the NDM-1 Carbapenemase in *Acinetobacter baumannii*

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The *bla*$_{NDM-1}$ gene encodes a carbapenemase that confers resistance to almost all β-lactams, including last-resort carbapenems. This is increasingly reported worldwide in nosocomial and community-acquired Gram-negative bacteria. *Acinetobacter baumannii* is an important opportunistic pathogen that is considered an intermediate reservoir for the *bla*$_{NDM-1}$ gene. In this species, the *bla*$_{NDM-1}$ gene is located within the Tn*125* composite transposon. The mechanism driving the mobility of Tn*125* has not yet been elucidated. Here we experimentally demonstrated the transposition of Tn*125* in *A. baumannii*. Systematic 3-bp duplication of the target site, being the signature of transposition, was evidenced. The target site consensus sequence for Tn*125* transposition was found to be GC enriched at the duplicated 3 bp and AT rich in the vicinity. Transposition frequency was not influenced by temperature changes or by exposure to subinhibitory concentrations of various antibiotics. This work is the first direct evidence of the functionality of a composite transposon in *A. baumannii*. It provides a mechanistic clue for the dissemination of the *bla*$_{NDM-1}$ gene in *Acinetobacter* spp. and subsequently among *Enterobacteriaceae*.

Carbapenemases are enzymes hydrolyzing most β-lactams, including penicillins, cephalosporins, and carbapenems. During the last decade, they have been increasingly reported worldwide in *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*. The spread of carbapenemases is of utmost importance for medicine, since carbapenems are last-resort antibiotics for treating the most severe and hospital-acquired infections. The class B New Delhi metallo-β-lactamase (NDM-1) is a broad-spectrum β-lactamase, hydrolyzing penicillins, cephalosporins, and carbapenems (1). NDM-1 was first identified in a *Klebsiella pneumoniae* isolate from a patient previously hospitalized in India in 2008 (2). Since then, it has been found mostly in *Enterobacteriaceae* and *A. baumannii* and to a lesser extend in *P. aeruginosa* (1, 3). The origin of the *bla*$_{NDM-1}$ resistance gene and the mechanism(s) driving its mobility remains unknown. However, a current hypothesis suggests that the *bla*$_{NDM-1}$ gene originates from an environmental bacterial progenitor species and that *A. baumannii* is an intermediate reservoir for the *bla*$_{NDM-1}$ gene (4).

A. baumannii is an opportunistic human pathogen and a common cause of sepsis, pneumonia, urinary tract infection, and primary bacteremia. Multidrug-resistant *A. baumannii* isolates are of great concern (5, 6). In *Acinetobacter* spp., the *bla*$_{NDM-1}$ gene is embedded in transposon Tn*125* (4, 7, 8). Tn*125* is a 10,099-bp composite transposon bracketed by two copies of the insertion sequence (IS) IS*Aba125* orientated in the same direction (Fig. 1A) (4, 7, 8). The 1,087-bp IS*Aba125* element belongs to the IS*30* family and encodes a 322-amino-acid-long DDE-type transposase surrounded by imperfect terminal inverted repeat sequences (IRR [inverted repeat right] and IRL [inverted repeat left], sharing 20/26 nucleotide identity) (9, 10). The *ble*$_{MBL}$ gene, which encodes a 121-amino-acid-long protein conferring resistance to bleomycin, a glycopeptide antibiotic used as an antitumor agent, is located downstream of the *bla*$_{NDM-1}$ gene (11). In addition, Tn*125* comprises six genes encoding putative proteins (*iso*, *tat*, *dct*, *groES*, *groEL*, and Δ*pac* [a truncated phospholipid acetyltransferase gene]), the IS*CR21* element, and the putative *ori*IS sequence that

defines the origin of replication of IS*CR21* (7, 8). IS*CR* elements are peculiar ISs belonging to the IS*91* family, likely mobilizing genes located at their left-hand extremity by a rolling-circle transposition process (12, 13).

Current observations suggest that the *bla*$_{NDM-1}$ gene originates from an unknown environmental bacterial progenitor species and is integrated into the chromosome of *Acinetobacter* spp. The *bla*$_{NDM-1}$-bearing Tn*125* transposon was likely subsequently built from such *Acinetobacter* spp. and then transferred onto broad-host-range plasmids, followed by horizontal transfer to *Enterobacteriaceae* and *P. aeruginosa*. This hypothesis is supported by a series of genetic features (4, 5), as follows. (i) The *bla*$_{NDM-1}$ gene displays a higher GC percentage (62%) than that of the genome of *Acinetobacter* spp. (38% to 42%), arguing in favor of a phylogenetic distance between the progenitor species and *Acinetobacter* spp. (ii) IS*CR21* may have mobilized a fragment encompassing the *bla*$_{NDM-1}$ gene that displays a similar GC percentage from an unknown bacterial progenitor. (iii) IS*Aba125* has also been identified in *Acinetobacter* species isolates without physical association with the *bla*$_{NDM-1}$ gene and shows a low GC content of 37%, consistent with a possible *Acinetobacter* species origin (4, 5).

A critical step in the dissemination process of the *bla*$_{NDM-1}$ gene is the mobility of the Tn*125* transposon in *Acinetobacter* spp. According to its genetic structure, it was presumed that Tn*125* can move through transposition. Transposition is a catalytic process, driven by an element-specific transposase. During this process,
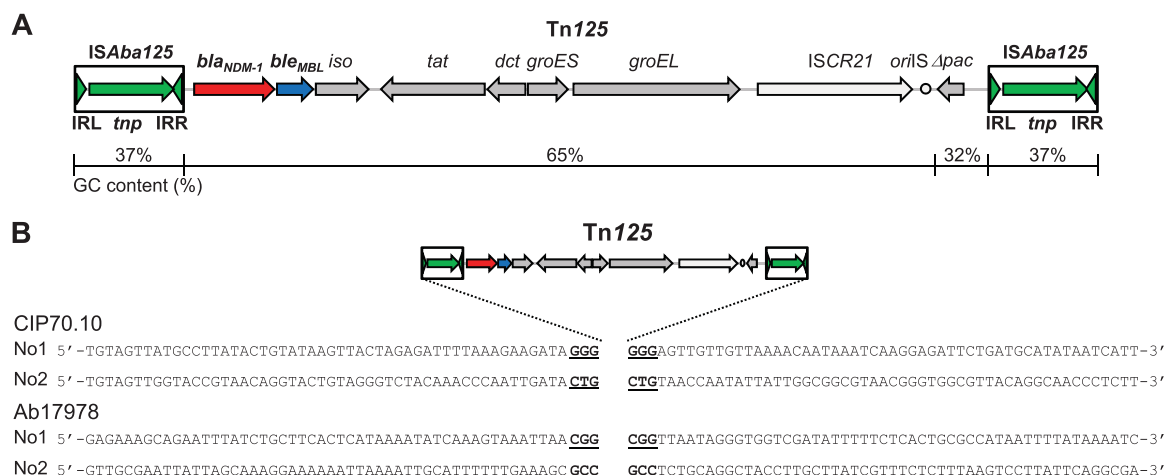
FIG 1 Transposition of Tn*125*. (A) Schematic representation of transposon Tn*125*. The total size is 10,099 bp. The ORFs are represented by arrows, and the lengths are to scale. The two IS*Aba125* elements bracketing Tn*125* are indicated in green, each with the transposase (*tnp*) gene and the IRL and IRR inverted repeats. The *bla*NDM-1 gene is in red, the *ble*MBL gene is in blue, and the 7 other ORFs are in dark gray; the IS*CR21* and the *ori*IS (circle) are in light gray. The GC content (%) for each of the following fragments is indicated: 37% for each IS*Aba125* (nucleotides 1 to 1087 and 9013 to 10099), 65% for the sequence encompassing *bla*NDM-1 to *ori*IS, and 32% for Δ*pac*. (B) Characterization of four Tn*125* transposition events in *A. baumannii* CIP70.10 and Ab17978 (*recA* mutant) strains. The duplicated 3-bp target sites are underlined. The surrounding 50 nucleotides, upstream and downstream of each transposon insertion, are shown. In *A. baumannii* CIP70.10, transposon Tn*125* transposed between ORFs designated No1 (encoding proteins deposited under accession no. CRL92855.1 and CRL92856.1) or an ORF designated No2 (encoding an outer membrane protein A precursor [accession no. CRL95910.1]); in *A. baumannii* Ab17978 (*recA* mutant), transposon Tn*125* transposed in the ORF designated No1 (encoding a hypothetical protein [accession no. AKQ26110.1]) or in the ORF designated No2 (encoding a CinA-like protein [accession no. KNZ37258.1]).

the transposase generates a short direct repeat flanking the transposon in the target DNA, corresponding to a signature of the transposition event (10). Alternative mechanisms for Tn*125* transfer might involve nonhomologous recombination or cointegration. Here, we show that the Tn*125* transposon can efficiently transpose in *A. baumannii* and that the transposition frequency is not influenced by temperature changes or antibiotic pressure.

MATERIALS AND METHODS

Strains. Transposition experiments were performed in *A. baumannii* CIP70.10 and Ab17978 (*recA*::Km) (14) reference strains. Plasmids were constructed in *Escherichia coli* TOP10 (Invitrogen).

Plasmid construction. For pTOPO-Tn*125*, the Tn*125* transposon was amplified from *A. baumannii* strain JH (8) together with 98 bp and 99 bp of the flanking genomic sequences present in upstream and downstream Tn*125*, respectively, with primers JHorfTn125-HindIII-F (5′-gatgat aagcttTCAGCAATAAATTTGTCACCAGC-3′) and JHorfTn125-XbaI-R (5′-gatgattctagaCAAGCTGCTCAAGTTAAAGATCG-3′) (the HindIII and XbaI restriction sites are underlined, and the uppercase letters correspond to the open reading frame [ORF] identified in strain JH). This amplicon was subcloned into the HindIII and XbaI restriction sites of the pCR-BluntII-TOPO plasmid (Invitrogen). The integrity of both IS*Aba125* elements and of *bla*NDM-1 was confirmed by sequencing. pTOPO-zeodel-Tn*125* was derived from pTOPO-Tn*125*, in which a frameshift in the zeocin resistance gene was generated by digestion at the unique *Fse*I site, blunting, and self-ligation. The resulting zeocin resistance protein lacks the 32 C-terminal amino acids. For the pTOPO-shuttle-Tn*125* plasmid, the *A. baumannii*-specific origin of replication was amplified from pWH1266 (a kind gift from P. Higgins) and subcloned into pTOPO-Tn*125* between the BsrGI and HindIII restriction sites. Plasmid pTOPO-shuttle-Tn*125* replicates in *E. coli*, from which it can be selected with 50 μg/ml zeocin, 100 μg/ml ampicillin, 25 μg/ml kanamycin, or 0.5 μg/ml imipenem (IPM), in *A. baumannii* CIP70.10, from which it can be selected with 25 μg/ml kanamycin, 200 μg/ml zeocin, or 1 μg/ml IPM, and finally in *A. baumannii* Ab17978 *recA*::Km, from which it

can be selected with 10 μg/ml zeocin or 1 μg/ml IPM. Full sequences of the Tn*125* plasmids are available upon request.

Transposition assays. One-hundred-nanogram amounts of pTOPO-Tn*125* or pTOPO-zeodel-Tn*125* suicide plasmids were electroporated into 25 μl of electrocompetent *A. baumannii* cells with a MicroPulser (Bio-Rad). The bacteria were resuspended in 2 ml LB and incubated for 1 h 30 min at 37°C with agitation. A total of 100 μl was plated onto LB agar plates supplemented with 1 μg/ml IPM to select the transposition events. The transformation efficiency was determined with the highly similar pTOPO-shuttle-Tn*125* plasmid (replicating in *A. baumannii*), which was electroporated and processed in parallel to the suicide plasmids. The transposition frequencies were calculated by dividing the number of transposition events by the number of transformed cells. Experiments were done in triplicate. The same procedure was used to address the effect antibiotics has on transposition, but cells were incubated for 3 h at 37°C after electroporation with the following antibiotics: the glycopeptide bleomycin (1 μg/ml and 5 μg/ml) (Molekula) or zeocin (4 μg/ml and 8 μg/ ml), the fluoroquinolone ciprofloxacin (0.05 μg/ml and 1 μg/ml) (Sigma-Aldrich), the aminoglycoside kanamycin (1.5 μg/ml and 3 μg/ml) (Roth; ThermoFisher Scientific), and the carbapenem imipenem (0.125 μg/ml and 0.25 μg/ml) (Mylan). The MIC of bleomycin for Tn*125*-containing CIP70.10 was 10 μg/ml, while it was <1 μg/ml for the parental CIP70.10 strain.

Molecular characterization of transposition events. The presence of the full-length Tn*125* was confirmed by the amplification in 5′ of a 925-bp fragment spanning from IS*Aba125* to *bla*NDM-1 with primers 125-F (5′-ACACCATTAGAGAAATTTGC-3′) and NDM-R (5′-CGGAATGGC TCATCACGATC-3′) and in 3′ of a 1,326-bp fragment spanning from Δ*pac* to IS*Aba125* with primers dpac-F (5′-CAACTGTGAGTCCTTTAC TGAC-3′) and 125-R (5′-GCAAATTTCTCTAATGGTGT-3′). The integration sites of the transposition events were characterized by shotgun cloning. Total genomic DNA was digested with EcoRV and ligated into the EcoRV-digested and dephosphorylated pBSKS-kanR vector. The libraries were electroporated into *E. coli* TOP10 and plated onto LB agar containing 1 mg/liter IPM to select for *bla*NDM-1-positive clones, which were sequenced with the primer IS125tpase_NORF-R (5′-CTCACGATA

TABLE 1 Transposition frequencies of Tn*125* in *A. baumannii* CIP70.10

		Transposition frequency $(10^{-4})^b$	
Expt[a]	Incubation drug (concn)	pTOPO-Tn*125*	pTOPO-zeodel-Tn*125*
1	None	4.5 (±0.5)	5.7 (±0.9)
2	None	11.8 (±3.0)	9.6 (±5.1)
	Bleomycin (1 mg/liter)	11.4 (±2.2)	9.0 (±3.4)
	Bleomycin (5 mg/liter)	7.6 (±3.9)	4.6 (±1.8)

[a] In experiment 1, the bacteria were incubated for 1 h 30 min at 37°C after electroporation and then plated on 1-mg/liter imipenem-containing plates. In experiment 2, the bacteria were incubated for 3 h at 37°C with the indicated concentration of bleomycin.

[b] pTOPO-Tn*125* or pTOPO-zeodel-Tn*125* was transformed as the donor of Tn*125*. The transposition frequencies are expressed as the number of transposition events relative to the number of cells transformed. Experiments were performed in triplicate. Values in parentheses are standard deviations.

GATCGTACTAGG-3′) to identify the genomic sequence upstream of Tn*125*. For each transposition event, a primer was designed to amplify a fragment spanning from the 3′ end of Tn*125* to the genomic sequence downstream of Tn*125*. These PCR amplicons were sequenced with primer IS125tpase-CORF-F (5′-CATGTCACTGAATACTCGTCC-3′).

Determination of the AT and GC contents and pictogram of the target site consensus. For the 27 transposition events characterized, the relative frequencies of each A and T, and G and C, for the region extending from 50 nucleotides upstream to 50 nucleotides downstream from the duplicated 3-bp target site were calculated and plotted onto a graph. The pictures of the relative frequencies of the bases at each position were generated with the Pictogram program (http://genes.mit.edu/pictogram .html).

Detection of IS*Aba125* in *Acinetobacter* species strains. Strains were screened by PCR with primers Tn125-F (5′-TGTATATTTCTGTGAC CCAC-3′) and Tn125-R (5′-GAAGGCGAATTCAAACATGAGGTGC-3′). A 255-bp product was amplified in the presence of IS*Aba125*.

RESULTS

Transposition of Tn*125* in *A. baumannii*. To address the transposition of Tn*125* in *A. baumannii*, the suicide plasmid pTOPO-Tn*125* was transformed as the donor for the transposon in two *A. baumannii* recipient strains, CIP70.10 (*recA* wild type) and Ab17978 (*recA* mutant) (14). The pTOPO-Tn*125* plasmid contains the entire Tn*125* transposon, together with the flanking chromosomal sequences present in the Tn*125*-positive *A. baumannii* isolate JH (98 and 99 bp upstream and downstream of the transposon, respectively) (8). Four imipenem-resistant clones, two in *A. baumannii* CIP70.10 and two in *A. baumannii* Ab17978, were stepwise characterized as follows. First, they were tested for the loss of kanamycin and zeocin resistance markers, confirming that the donor plasmid did not integrate into the genome. Second, the presence of the full-length transposon Tn*125* and the absence of the flanking sequences present in the donor plasmid were confirmed by PCR. Third, the genomic sequences flanking Tn*125* were characterized in order to map the transposition sites and analyze the target site duplication. As shown in Fig. 1B, for each of the four studied clones, the entire Tn*125* transposed into distinct loci of the *A. baumannii* chromosome. A 3-bp target site duplication was present in each case. Transposition of Tn*125* conferred resistance to cephalosporins and carbapenems, with MIC values of ceftazidime, imipenem, and meropenem being >256, >32, and >32 µg/ml, respectively.

Transposition frequencies. The transposition frequencies measured with pTOPO-Tn*125* and pTOPO-zeodel-Tn*125* as donors were 4.5×10^{-4} (±0.5×10^{-4}) and 5.7×10^{-4} (±0.9×10^{-4}) per transformed cell, respectively (Table 1, experiment 1). The frequencies with both donor plasmids were in the same range,

excluding the potential influence of the zeocin marker gene, which confers resistance to the same class of molecules (bleomycin) as the ble_{MBL} gene. Since the source of dissemination of the bla_{NDM-1} gene is likely the environment, in particularly in Asia (15, 16), where a variety of antibiotics has been widely identified (17, 18) and where temperature changes might influence the transposition frequency (19, 20), corresponding experiments were conducted. Incubation for 3 h with 1 or 5 µg/ml bleomycin, a glycopeptide antibiotic used as an antitumor agent, did not influence the transposition rate of Tn*125* (Table 1, experiment 2). Similarly, the transposition rate was not influenced by incubation for 3 h at different temperatures (25°C, 30°C, 37°C, or 44°C) or by the presence of subinhibitory concentrations of structurally nonrelated antibiotics, such as fluoroquinolone (ciprofloxacin), aminoglycoside (kanamycin), glycopeptide (zeocin), and carbapenem (imipenem) (data not shown).

Target site specificity. In order to determine a consensus target site for Tn*125* transposition, 25 additional independent transposition events, in 25 independent isolates, were characterized in *A. baumannii* CIP70.10. Among the transposition events, 19 occurred within open reading frames (ORFs), 8 in direct orientation and 11 in reverse orientation compared to the disrupted ORF, and 8 outside of ORFs. For each transposition event, a systematic 3-bp duplication of the target site was evidenced (Fig. 2A). To further characterize the features of the target site, the surrounding genomic sequences of the 27 transposition events were aligned, from 50 bp upstream up to 50 bp downstream of Tn*125*. The mean AT content for the regions distal to the duplicated target site, from −50 to −4 bp and from +4 to +50 bp relative to it, was 72% on both sides (Fig. 2B, upper graph). Around the duplicated target site, the AT content increased, with 100% at positions −3 and +3 and 83% to 90% at positions −2, −1, +1, and +2 (Fig. 2B, lower graph). By analysis of the nucleotide composition, the −3 position was found to be predominantly an A (69%), and the +3 position was found to be predominantly a T (72%) (Fig. 2C). At the duplicated target site positions, named here c1, c2, and c3, the AT content was lower (38% to 62%) (Fig. 2B, lower graph). At c1, A, T, C, and G were equally represented (24% to 28%), while G was underrepresented at c2 (10% versus 38%, 24%, and 28% for A, T, and C, respectively) and predominant at c3 (48% versus 21%, 17%, and 14% for A, T, and C, respectively) (Fig. 2C). Further analysis of sequences flanking 74 IS*Aba125* elements retrieved from GenBank confirmed the consensus derived from the analysis of Tn*125* transposition events (Fig. 2D). In conclusion, the target site specificity of Tn*125* was driven mainly both by the 3 bp surrounding the duplicated target site, being AT rich and reaching
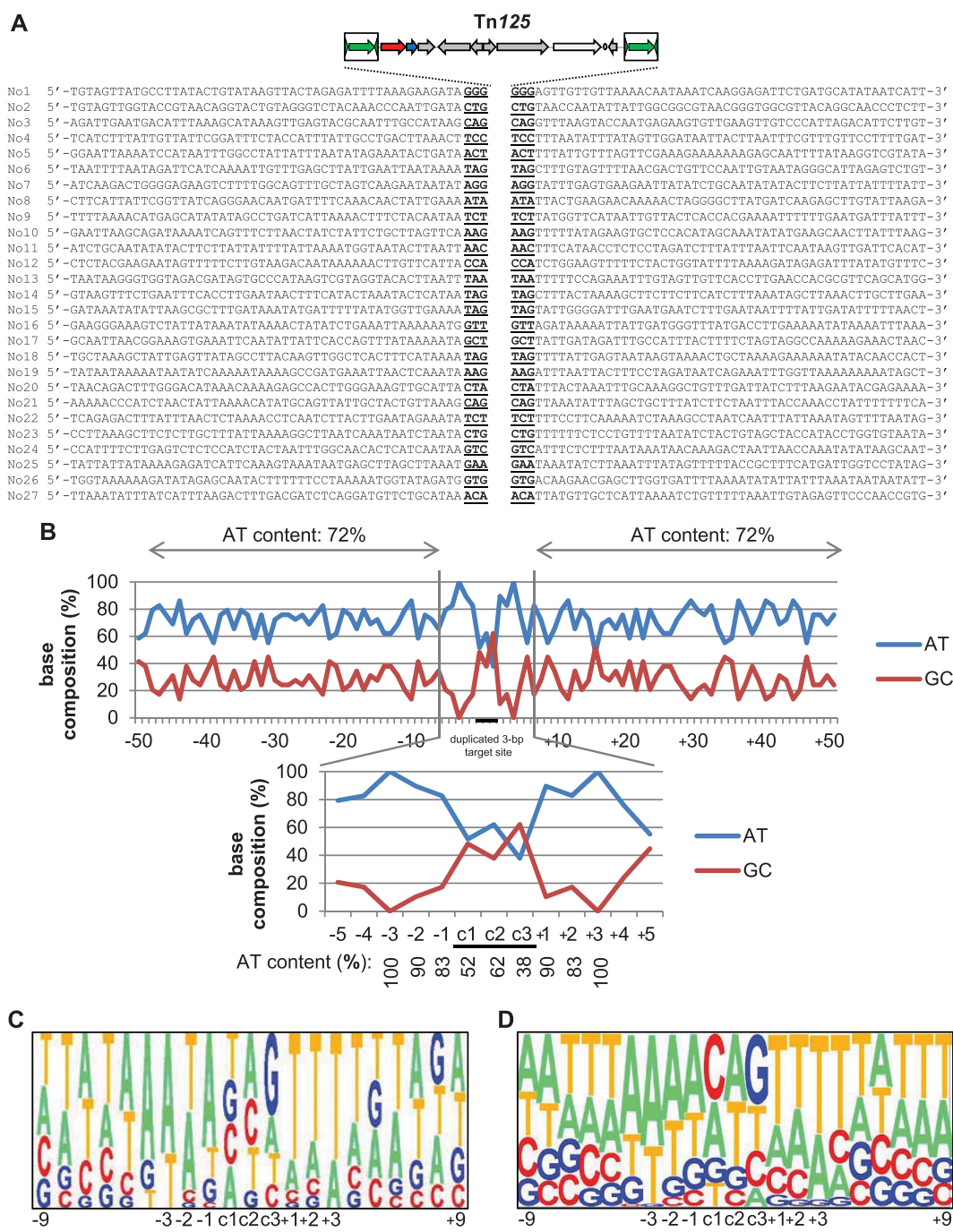
A

Tn*125*

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No1  5'-TGTAGTTATGCCTTATACTGTATAAGTTACTAGAGATTTTAAAGAAGATA GGG   GGGAGTTGTTGTTAAAACAATAAATCAAGGAGATTCTGATGCATATAATCATT-3'
No2  5'-TGTAGTTGGTACCGTAACAGGTACTGTAGGGTCTACAAACCCAATTGATA CTG   CTGTAACCAATATTATTGGCGGCGTAACGGGTGGCGTTACAGGCAACCCTCTT-3'
No3  5'-AGATTGAATGACATTTAAAGCATAAAGTTGAGTACGCAATTTGCCCATAAG CAG   CAGGTTTAAGTACCAATGAGAAGTGTTGAAGTTGTCCCATTAGACATTCTTGT-3'
No4  5'-TCATCTTTATTGTTATTCGGATTTCTACCATTTATTGCCTGACTTAAACT TCC   TCCTTTAATATTTATAGTTGGATAATTACTTAATTTCGTTTGTTCCTTTTGAT-3'
No5  5'-GGAATTAAAATCCATAATTTGGCCTATTATTTAATATAGAAATACTGATA ACT   ACTTTTATTGTTTAGTTCGAAAGAAAAAAAGAGCAATTTTATAAGGTCGTATA-3'
No6  5'-TAATTTTAATAGATTCATCAAAATTGTTTGAGCTTATTGAATTAATAAAA TAG   TAGCTTTGTAGTTTTAACGACTGTTCCAATTGTAATAGGGCATTAGAGTCTGT-3'
No7  5'-ATCAAGACTGGGGAGAAGTCTTTTGGCAGTTTGCTAGTCAAGAATAATAT AGG   AGGTATTTGAGTGAAGAATTATATCTGCAATATATACTTCTTATTATTTTATT-3'
No8  5'-CTTCATTATTCGGTTATCAGGGAACAATGATTTTCAAACAACTATTGAAA ATA   ATATTACTGAAGAACAAAAACTAGGGGCTTATGATCAAGAGCTTGTATTAAGA-3'
No9  5'-TTTTAAAACATGAGCATATATAGCCTGATCATTAAAACTTTCTACAATAA TCT   TCTTATGGTTCATAATTGTTACTCACCACGAAAATTTTTTGAATGATTTATTT-3'
No10 5'-GAATTAAGCAGATAAAATCAGTTTCTTAACTATCTATTCTGCTTAGTTCA AAG   AAGTTTTTATAGAAGTGCTCCACATAGCAAATATATGAAGCAACTTATTTAAG-3'
No11 5'-ATCTGCAATATATACTTCTTATTATTTTATTAAAATGGTAATACTTAATT AAC   AACTTTCATAACCTCTCCTAGATCTTTATTTAATTCAATAAGTTGATTCACAT-3'
No12 5'-CTCTACGAAGAATAGTTTTTCTTGTAAGACAATAAAAAACTTGTTCATTA CCA   CCATCTGGAAGTTTTTCTACTGGTATTTTAAAAGATAGAGATTTATATGTTTC-3'
No13 5'-TAATAAGGGTGGTAGACGATAGTGCCCATAAGTCGTAGGTACACTTAATT TAA   TAATTTTTCCAGAAATTTGTAGTTGTTCACCTTGAACCACGCGTTCAGCATGG-3'
No14 5'-GTAAGTTTCTGAATTTCACCTTGAATAACTTTCATACTAAATACTCATAA TAG   TAGCTTTACTAAAAGCTTCTTCTTCATCTTTAAATAGCTTAAACTTGCTTGAA-3'
No15 5'-GATAAATATATTAAGCGCTTTGATAAATATGATTTTTATATGGTTGAAAA TAG   TAGTATTGGGGATTTGAATGAATCTTTGAATAATTTTATTGATATTTTTAACT-3'
No16 5'-GAAGGGAAAGTCTATTATAAATATAAAACTATATCTGAAATTAAAAAATG GTT   GTTAGATAAAAATTATTGATGGGTTTATGACCTTGAAAAATATAAAATTTAAA-3'
No17 5'-GCAATTAACGGAAAGTGAAATTCAATATTATTCACCAGTTTATAAAAATA GCT   GCTTATTGATAGATTTGCCATTTACTTTTCTAGTAGGCCAAAAAGAAACTAAC-3'
No18 5'-TGCTAAAGCTATTGAGTTATAGCCTTACAAGTTGGCTCACTTTCATAAAA TAG   TAGTTTATTGAGTAATAAGTAAAACTGCTAAAAGAAAAAATATACAACCACT-3'
No19 5'-TATAATAAAAATAATATCAAAAATAAAAGCCGATGAAATTAACTCAAATA AAG   AAGATTTAATTACTTTCCTAGATAATCAGAAATTTGGTTAAAAAAAAAATAGCT-3'
No20 5'-TAACAGACTTTGGGACATAAACAAAAGAGCCACTTGGGAAAGTTGCATTA CTA   CTATTACTAAATTTGCAAAGGCTGTTTGATTATCTTTAAGAATACGAGAAAA-3'
No21 5'-AAAAACCCATCTAACTATTAAAACATATGCAGTTATTGCTACTGTTAAAG CAG   CAGTTAAATATTTAGCTGCTTTATCTTCTAATTTACCAAACCTATTTTTTTCA-3'
No22 5'-TCAGAGACTTTATTTAACTCTAAAACCTCAATCTTACTTGAATAGAAATA TCT   TCTTTTCCTTCAAAAATCTAAAGCCTAATCAATTTATTAAATAGTTTTAATAG-3'
No23 5'-CCTTAAAGCTTCTCTTGCTTTATTAAAAGGCTTAATCAAATAATCTAATA CTG   CTGTTTTCTCCTGTTTTAATATCTACTGTAGCTACCATACCTGGTGTAATA-3'
No24 5'-CCATTTTCTTGAGTCTCTCCATCTACTAATTTGGCAACACTCATCAATAA GTC   GTCATTTCTCTTTAATAAATAACAAAGACTAATTAACCAAATATATAAGCAAT-3'
No25 5'-TATTATTATAAAAGAGATCATTCAAAGTAAATAATGAGCTTAGCTTAAAT GAA   GAATAAATATCTTAAATTTATAGTTTTTACCGCTTTCATGATTGGTCCTATAG-3'
No26 5'-TGGTAAAAAAGATATAGAGCAATACTTTTTTCCTAAAAATGGTATAGATG GTG   GTGACAAGAACGAGCTTGGTGATTTTAAAATATATTATTTAAATAATAATATT-3'
No27 5'-TTAAATATTTATCATTTAAGACTTTGACGATCTCAGGATGTTCTGCATAA ACA   ACATTATGTTGCTCATTAAAATCTGTTTTTAAATTGTAGAGTTCCCAACCGTG-3'
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B

C   **D**

FIG 2 Target site preferences of Tn*125*. (A) Molecular characterization of 27 transposition events of Tn*125* in *A. baumannii* CIP70.10 (two of them are the same as in Fig. 1B). For each of them, the duplicated 3-bp target site is underlined. The surrounding 50 nucleotides upstream and downstream of the target sites are shown. (B) The 27 transposition sites of Fig. 2A were aligned, and the percentages of AT and GC at each position, from 50 nucleotides upstream to 50 nucleotides downstream of the target site, are shown on the graph. The 3 bp of the duplicated target site, named here c1, c2, and c3, are highlighted by a black bar. The AT percentages of regions spanning positions −50 to −4 and positions +4 to + 50 and those of the region spanning positions −3 to + 3 are indicated in the upper and lower graphs, respectively. (C) Pictogram showing the relative frequencies of each A, T, C, and G at the target site, deduced from the 27 experimental transposition events shown in panel A. (D) Pictogram showing the relative frequencies of each A, T, C, and G at the target site, deduced from 74 IS*Aba125* elements retrieved from GenBank (see Table 3 for references).

96% to 99% A or T at positions −3 and +3, and by the duplicated 3-bp target site itself, which was GC enriched, with a strong bias for nucleotide G at the position c3.

Distribution of IS*Aba125* in *Acinetobacter* spp. In order to evaluate the distribution of IS*Aba125*, its occurrence was evaluated among 17 *A. baumannii* and 16 *Acinetobacter* species clinical isolates. These strains were negative for the *bla*$_{NDM-1}$ gene, and some of them carried previously characterized resistance genes to

TABLE 2 Distribution of IS*Aba125* in *Acinetobacter* species isolates, detected by PCR

Isolate	Species[a]	Resistance gene(s) characterized[b]	IS*Aba125*	Reference or source[c]
Rem	*A. baumannii*	None	−	Collection
Son	*A. baumannii*	None	+	Collection
ROU	*A. baumannii*	None	−	Collection
4547	*A. baumannii*	None	+	Collection
MK8744	*A. baumannii*	None	−	33
AS1	*A. baumannii*	$bla_{\text{OXA-23}}$	+	34
1279 Bahe	*A. baumannii*	$bla_{\text{OXA-51}}$ oE	+	Collection
CLA-1	*A. baumannii*	$bla_{\text{OXA-40}}$	+	Collection
75510	*A. baumannii*	$bla_{\text{IMP-4}}$	+	Collection
FER	*A. baumannii*	$bla_{\text{OXA-23}}$	−	Collection
PIN	*A. baumannii*	$bla_{\text{OXA-40}}$	+	Collection
1637	*A. baumannii*	*armA*	−	Collection
AP	*A. baumannii*	$bla_{\text{OXA-91}}$, $bla_{\text{GES-14}}$	+	Collection
ELF	*A. baumannii*	$bla_{\text{OXA-40}}$, $bla_{\text{TEM-1}}$	−	Collection
614	*A. baumannii*	$bla_{\text{OXA-23}}$	+	34
133	*A. baumannii*	$bla_{\text{OXA-58}}$, $bla_{\text{OXA-64}}$	+	35
5179	*A. baumannii*	$bla_{\text{VEB-1a}}$, $bla_{\text{SCO-1}}$	+	36
CGL-3	*A. haemolyticus*	$bla_{\text{OXA-58}}$	+	Collection
7446	*A. junii*	$bla_{\text{OXA-58}}$, $bla_{\text{PER-2}}$, $bla_{\text{SCO-1}}$	+	36
7368	*A. lwoffii*	$bla_{\text{OXA-58}}$, $bla_{\text{PER-2}}$, $bla_{\text{SCO-1}}$	+	36
BER	*A. radioresistens*	$bla_{\text{OXA-105}}$	−	Collection
R864	*A. lwoffii*	$bla_{\text{OXA-134}}$	+	Collection
5400	*A. baylyi*	$bla_{\text{PER-2}}$, $bla_{\text{SCO-1}}$	+	36
0551	*A. radioresistens*	$bla_{\text{OXA-23}}$	+	Collection
CIP103788	*A. radioresistens*	$bla_{\text{OXA-23}}$, $bla_{\text{OXA-103}}$	+	Collection
CIP64.7	*A. genomospecies 6*	$bla_{\text{OXA-134}}$	+	Collection
9905	*A. johnsonii*	$bla_{\text{OXA-211}}$	+	37
CIP64.5	*A. junii*	None	−	Collection
CIP107464	*A. gerneri*	None	−	Collection
CIP107468	*A. bouvetii*	None	−	Collection
CIP107469	*A. tandoii*	None	−	Collection
CIP107470	*A. grimontii*	None	+	Collection
CIP107472	*A. towneri*	None	−	Collection

[a] *A. baumannii* is the clinically most important species.
[b] bla_{OXA} genes encode carbapenem-hydrolyzing class D oxacillinases, $bla_{\text{TEM-1}}$ and $bla_{\text{SCO-1}}$ encode penicillinases, $bla_{\text{VEB-1a}}$ and $bla_{\text{PER-2}}$ encode extended-spectrum beta-lactamases (ESBLs), $bla_{\text{IMP-4}}$ and $bla_{\text{GES-14}}$ encode carbapenemases, and *armA* encodes a 16S rRNA methylase. oE, overexpression.
[c] Collection, personal laboratory collection.

broad-spectrum β-lactams. IS*Aba125* was detected in 21 out of 33 strains (Table 2). In addition, the locations of 74 IS*Aba125* elements found in genomic sequences of 8 *A. baumannii* strains deposited in GenBank were mapped with respect to their target sites.

Half of the IS*Aba125* elements were located within ORFs. For 6 of them, the IRR of IS*Aba125*, which provides a −35 box promoter element orientated toward the flanking DNA, was found in proximity (less than 100 bp) of the ATG start codon of the adjacent

TABLE 3 Position of 74 IS*Aba125* elements with respect to genomic ORFs[a]

Strain	Accession no.	No. of IS*Aba125* copies				Reference
		Total[b]	Within ORF	Between ORFs	Promoter provider[c]	
NCGM 237	AP013357	26	14	12	2	38
LAC-4	CP007712	14	9	5	1	39
AbH12O-A2	CP009534	3	2	1	0	40
AB04-mff	CP012006	12[d]	5	6	0	41
ACICU	CP000863	7	2	5	2	29
BJAB0715	CP003847	8	3	5	0	30
TTHO-4	CP012608	4	2	2	0	Unpublished
TCDC-AB0715	CP002522	1	0	1	1	42

[a] Sequences were retrieved from 8 *A. baumannii* complete genome sequences deposited in GenBank.
[b] Number of IS*Aba125* copies found in each strain.
[c] Among IS*Aba125* intergenic copies, those copies where the distance between the end of the IRR (which provides a −35 box promoter element) and the ATG of the following ORF was less than 100 bp.
[d] Ten single IS*Aba125* elements and 1 composite transposon made of 2 IS*Aba125* copies.

ORFs (Table 3). In conclusion, IS*Aba125* was detected in more than half of the *Acinetobacter* species clinical isolates tested and potentially contributed to the genetic plasticity of those strains, either by disrupting ORFs or by potentially bringing promoter sequences to chromosomal genes.

DISCUSSION

This study actually corresponds to the first experimental demonstration of the functionality of the bla_{NDM-1}-carrying Tn*125* transposon. The ability of Tn*125* to transpose in *A. baumannii* sustains the following model: *A. baumannii* is an intermediate reservoir for the bla_{NDM-1} gene and may contribute to further dissemination of the bla_{NDM-1} gene among species. Indeed, environmental *A. baumannii* is originally likely in close contact with a still unknown progenitor of the bla_{NDM-1} gene or with disseminated bla_{NDM-1}-positive bacteria (15). It could then transfer resistance genes to *Enterobacteriaceae* in the environment or in humans.

The transposase directly influences the choice of the target site, with a preference for GC- or AT-rich DNA domains, local DNA structure, degree of supercoiling, replication or transcription level, and chromosome or plasmid location (10). The target site of Tn*125* was AT rich when the 3 bp surrounding the duplicated target site was considered, reaching 99% A and 96% T at positions −3 and +3, respectively, and was enriched in GC within the 3-bp target site, which is duplicated upon transposition, with a strong preference for G at c3 (Fig. 2). In *A. baumannii* clinical isolates, the same features are present in the chromosomal regions flanking the Tn*125* and Tn*aphA6* composite transposons, the most recently identified being composed of the *aphA6* aminoglycoside resistance gene surrounded by two IS*Aba125* elements (8, 21). The preference for AT-rich regions, previously observed for IS*Aba1* (22), is consistent with the *Acinetobacter* species origin of IS*Aba125*, whose genome is AT rich (58 to 62%). The frequencies of transposition of Tn*125* observed here were several orders of magnitude higher than, for example, those of the Tn*2006* composite transposon and the IS*Aba1* and IS*1999* elements (22, 23); unfortunately, these values are difficult to compare due to different experimental setups.

Two ISs were previously shown to be functional for transposition in *A. baumannii*, namely, IS*Aba825* (IS*982* family) (24) and IS*Aba1* (IS*4* family) (22, 25). Here, we experimentally demonstrated the functionality of IS*Aba125* (IS*30* family). Consistent with its ability to transpose, IS*Aba125* was described in association with increased resistance levels to β-lactams in *Acinetobacter* spp., for example, through duplication of the bla_{OXA-58} carbapenemase gene (26), disruption of the *carO* gene encoding an outer membrane protein acting in synergy with other resistance mechanisms (27), or insertion upstream of the bla_{ADC} cephalosporinase gene (28). In the sequenced genomes of strains listed in Table 3, IS*Aba125* was found upstream of the *ampC* gene in strain ACICU (29) or flanking the *aphA6* gene conferring resistance to amikacin in strain BJAB0715 (30).

IS*Aba125* was present in more than half of the clinical isolates tested (Table 2). In a parallel study, analysis of 131 *Acinetobacter* spp. revealed that 5 strains had 1 copy, 34 strains had few (2 to 9) copies, 12 strains exhibited numerous (≥10) copies, and 80 strains contained no copy of IS*Aba125* (31). This high frequency and high copy number of IS*Aba125* in *Acinetobacter* spp. are indicative of frequent transposition events, lateral gene transfer within *Acinetobacter* spp., a replicative mechanism of transposi-

tion (as shown for the IS*30* family), and the capacity of IS*Aba125* to expand within genomes, a general feature of ISs (9). Taken together, these characteristics are suggestive of an evolutionary role of IS*Aba125* customized for *Acinetobacter* spp., as proposed for IS*Aba1* in *A. baumannii* (32, 33).

This work demonstrates the ability of the bla_{NDM-1}-carrying Tn*125* transposon to transpose in *A. baumannii*. It underlines the importance of *A. baumannii*, which has been considered for years as playing a minor role in medical microbiology. *A. baumannii* is likely in close contact in the environment with the (still unknown) natural progenitor of the bla_{NDM-1} gene and may play a key role in the spread of the bla_{NDM-1} gene to clinically relevant bacterial species, in particular members of the *Enterobacteriaceae*.

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