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Molecular Characterization of *bla*$_{NDM-1}$ in a Sequence Type 235 *Pseudomonas aeruginosa* Isolate from France

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An NDM-1 carbapenemase-producing *Pseudomonas aeruginosa* isolate was recovered from a patient hospitalized in France after a previous hospitalization in Serbia. Genetic studies revealed that the *bla*$_{NDM-1}$ gene was surrounded by insertion sequence IS*Aba125* and a truncated bleomycin resistance gene. This *bla*$_{NDM-1}$ region was a part of the variable region of a new complex class 1 integron bearing IS common region 1 (IS*CR1*). The presence of IS*Pa7* upstream of this integron suggests insertion in a chromosomally located Tn*402*-like structure.

Since the first description in *Klebsiella pneumoniae* and *Escherichia coli*, the NDM-1 carbapenemase has been identified in many different species, including *Enterobacteriaceae* and *Acinetobacter baumannii* (1, 2). To date, the Indian subcontinent and, more recently, Balkan countries constitute a reservoir of NDM producers (2–5). The corresponding gene, which is usually plasmid borne, is spreading worldwide in *Enterobacteriaceae* (2) and also in species isolated from water supplies in India, such as *Pseudomonas putida* and *Pseudomonas pseudoalcaligenes* (6). Recent reports have described the occurrence of chromosomally located *bla*$_{NDM-1}$ in *Acinetobacter baumannii* related to transposon Tn*125* (7, 8). Before 2012, only one report of two NDM-1-producing *Pseudomonas aeruginosa* isolates was described. Both were isolated in Serbia (9). To date, the transfer mechanisms, location, and *bla*$_{NDM-1}$ genetic environment in *P. aeruginosa* remain unknown.

Here, we report the genetic environment of the *bla*$_{NDM-1}$ gene in the first NDM-1-producing *P. aeruginosa* isolate from France, HIABP11. The isolate was recovered from a urine culture of a 63-year-old female patient, who was admitted to the infectious unit of the Bégin military hospital (Saint-Mandé, France) for an acute pyelonephritis complicated with renal microabscesses (10). Three months before her admission, the patient was hospitalized in Serbia for a posttraumatic fronto-temporo-parietal subdural hematoma surgical drainage. *P. aeruginosa* HIABP11 exhibited serotype O11 (monoclonal and polyclonal antisera; Bio-Rad Laboratories, Marne-la-Coquette, France). Susceptibility testing (disk diffusion and Etest) performed and interpreted according to the EUCAST guidelines showed that *P. aeruginosa* HIABP11 was resistant to all carbapenems (imipenem, meropenem, and doripenem) and antipseudomonal cephalosporins, as well as to aminoglycosides and fluoroquinolones. The isolate remained susceptible to piperacillin-tazobactam (MIC of 12 mg/liter) and colistin (MIC of 2 mg/liter) and intermediate to aztreonam (MIC of 3 mg/liter). Metallo-β-lactamase (MBL) production in the isolate was suggested by positive EDTA (bioMérieux, Marcy l'Etoile, France) and dipicolinic acid (KPC+MBL Confirm ID Pack; Rosco Diagnostica, Taastrup, Denmark) tests with imipenem and meropenem, respectively. PCR experiments were carried out on purified DNA with primers specific for known MBL genes, as previously described (11). Sequencing of amplification products confirmed the presence of the *bla*$_{NDM-1}$ gene. Screening for additional β-lactamase genes, 16S RNA methylase genes, and *qnr* genes was negative. Interestingly, PCR experiments failed to detect the bleomycin resistance gene in *P. aeruginosa* HIABP11.

Repeated attempts to visualize a plasmid by the Kieser method (12) or to transfer by conjugation a plasmid using the recipient strain *E. coli* J53, as well as the rifampin-resistant *P. aeruginosa* strain PU21 (13), were unsuccessful, suggesting a chromosomal location for the *bla*$_{NDM-1}$ gene. To gain further insight into the genetic environment, a BamHI library of *P. aeruginosa* HIABP11 was cloned into the BamHI-restricted plasmid pK18 (Km^r). Transformants of *E. coli* DH5α were selected on Mueller-Hinton (MH) agar plates containing 30 mg/liter kanamycin and 100 mg/liter ampicillin. In comparison to recipient strain DH5α, all of the recombinant clones tested displayed high resistance to β-lactams, except aztreonam. Sequencing of the whole insert (12,732 bp) from one of these clones allowed characterization of the genetic environment surrounding the *bla*$_{NDM-1}$ gene (GenBank accession no. KC170992).

Upstream of the *bla*$_{NDM-1}$ gene, four open reading frames (ORFs) were identified (Fig. 1). The first corresponded to *orf513*, a gene encoding a transposase IS*CR*, with its origin of replication, *oriS*, region. IS*CR1* insertion sequences are often found beyond but close to the 3′-CS of class 1 integrons, belonging to the IS*91* family, and are implicated in gene resistance mobilization (14). Here, the IS*CR1* element was immediately followed by the truncated transposase gene *insB*, Δ*orfB* (IsABA14; accession no. JQ080305.1), the aminoglycoside resistance gene *aphA6*, the transposase IS*Aba125* gene, the *bla*$_{NDM-1}$ gene, the truncated bleomycin resistance gene, and the truncated *qac*/*sul* 3′ end of a class 1 integron, followed by the *orf5* gene. Detailed analysis of the 3′ flanking region of IS*Aba125* showed an identical promoter region in HIABP11, in plasmids pNDM102337 in *E. coli*, pNDM-BJ01 and pAL-1 in *A. lwoffi*, and pNDM-CIT in *C. freundii*, and in chromosomal sequences in *A. baumannii* (GenBank accession no.

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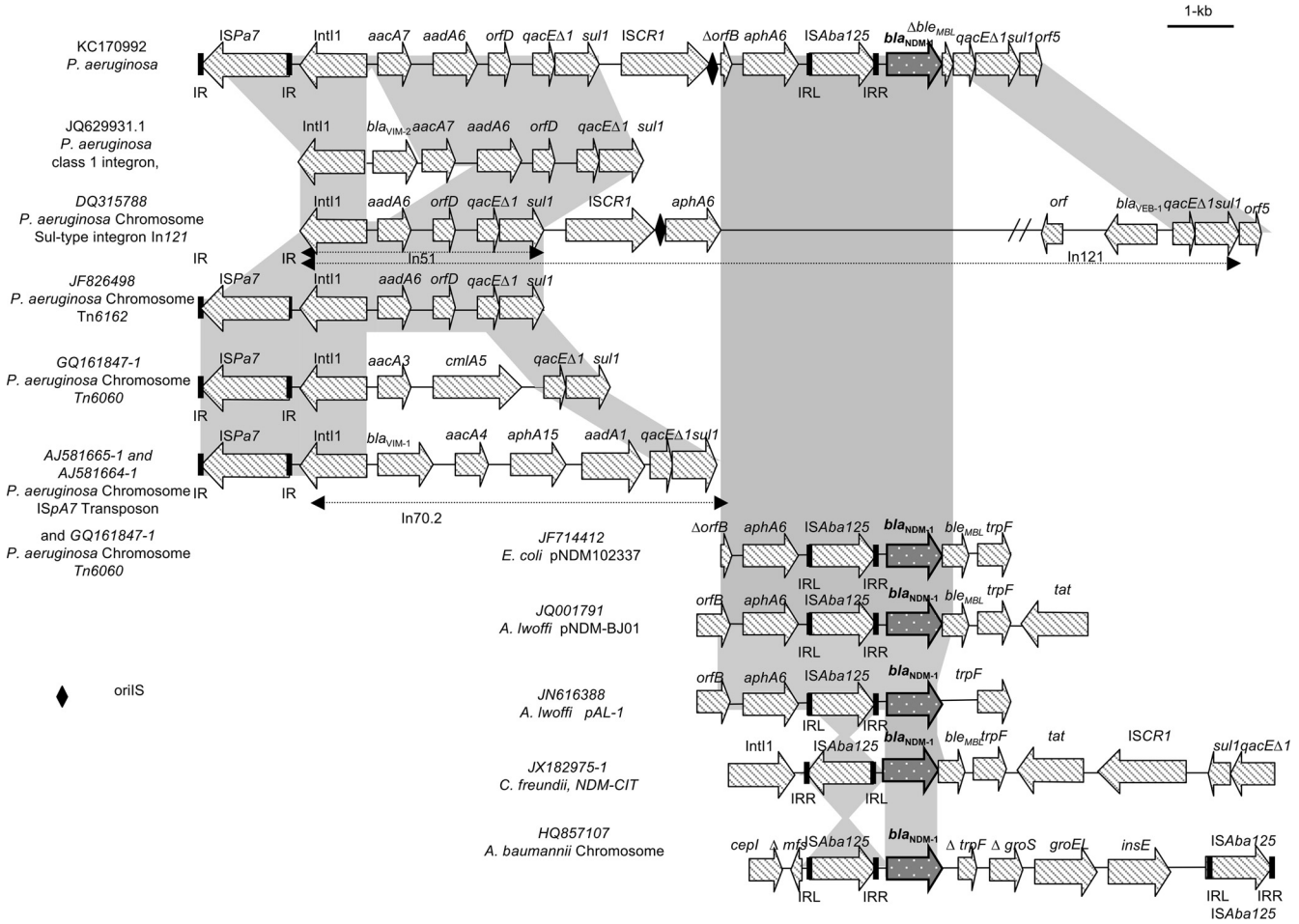

FIG 1 Schematic representation of the structure of the bla_{NDM-1}-surrounding sequences (12,735 bp) in *P. aeruginosa* HIABP11 (GenBank accession no. KC170992). Shown are alignments with (i) different chromosomally located transposon structures reported in *P. aeruginosa* isolates (some of them producing carbapenemase) and (ii) various bla_{NDM-1}-associated genetic structures identified among *Enterobacteriacea* and *Acinetobacter* spp. The truncated bleomycin resistance gene is indicated by "Δble_{MBL}." IR, inverted repeat; IRL, left inverted repeat, IRR, right inverted repeat.

JF714412, JQ001791, JN616388, JX182975, HQ857107, JN872328, and JN872329) (7, 8, 15, 16). The expression of bla_{NDM-1} was under the control of a promoter whose −35 sequence was located in the right inverted repeat of IS*Aba125*. A ribosomal binding site was found 14 bp upstream of bla_{NDM-1}. Furthermore, *in silico* analysis indicated that the 64 bp at the start of *aphA6* and bla_{NDM-1} displays 100% identity, as previously described in a chimera bla_{NDM-1} construction hypothesis (17). IS*Aba125* has often been described upstream of bla_{NDM-1}, first in *A. baumannii* and then in *A. lwoffi* and E*nterobacteriaceae*. An IS*Aba125*-related mobilization mechanism could be responsible for the *en bloc* acquisition of both bla_{NDM-1} and ble_{MBL}, mobilized from the same progenitor (8). In other bla_{NDM-1}-surrounding sequences described in *Enterobacteriaceae* and *Acinetobacter*, the bla_{NDM-1} gene was adjacent to a complete bleomycin resistance gene and a complete or truncated *trpF* gene (18). A truncated bleomycin resistance gene has not been described so far.

The region upstream of IS*CR1* corresponds to a classical class 1 integron, with the *IntI1* gene (encoding the integrase) followed by a cassette array containing 3 gene cassettes: the genes *aacA7* and *aadA6* encoding the aminoglycoside-modifying enzymes AAC-6′(I) and AADA6, respectively, and an *orfD* cassette encoding a putative polypeptide regulated by SdiA, a member of the LuxR family of transcriptional regulators. This last cassette is followed by a truncated *qac*/*sul* 3′ conserved sequence (3′-CS). The cassette array is quite related to that of JQ629931.1, which differs by the addition of a bla_{VIM-2} cassette upstream of the *aac7* cassette. The *orfD* cassette, already described in In*1* and In*51*, was generally associated with bla_{oxa-2} or *aadA6* genes (19, 20). The structure is also close to the chromosomal class 1 integron sequence in Tn*6162* with an *aacA7* cassette insertion (21) and from In*51* (19), already described in *P. aeruginosa*.

The whole structure from the *IntI1* gene to the second truncated 3′-CS (downstream of the bla_{NDM-1} and truncated bleomycin resistance genes and upstream of the *orf5* gene) corresponds to a new complex class 1 integron, with duplication of truncated 3′-CS. Duplication of 3′-CS regions, already described in many integrons, such as In*121* (22) and In*34* (23), is classically observed in IS*CR1* elements (24). The region from the integrase to the first truncated *qac*/*sul* 3′-CS is the classic integron structure, followed by a second copy of the 3′-CS. Between the 2 copies of the 3′-CS is the IS*CR*, followed by the variable region that contains resistance

genes, including bla_{NDM-1}. Thus, the mobilization of genetic sequences surrounding bla_{NDM-1} into this integron occurred probably by a rolling-circle replication event involving the IS*CR1* element and starting at the *ori*IS site (Fig. 1), which could have mobilized from a plasmid or a transposon the DNA fragment encompassing all the genes from *insB* to the truncated bleomycin resistance gene.

Upstream of the 5′ end of this complex class 1 integron, a copy of IS*Pa7* insertion sequence with inverted terminal repeats of 17 bp flanked by direct repeats of 4 bp was identified. This structure is identical to sequences found in Tn*6060* and the IS*Pa7* transposon from VIM-1 metallo-β-lactamase-producing *P. aeruginosa* isolates from Italy and Australia (24, 25). In these sequences, IS*Pa7* is inserted between two 19-bp repeats typical of the extremities of Tn*402*-like element. This transposon is known to have a unique targeting mechanism with a strong preference for insertion into or close to a *res* site (26, 27).

The average GC content of the sequence is 53.6%, with three GC islands: a first region from IS*Pa7* to *sul1* (positions 1 to 5951) with a GC content of 57.6%; a second region, including only *orf513* (positions 6999 to 7703) with a GC content of 56.5%; and a third region from bla_{NDM-1} to *orf5* with a GC content of 62%.

Analyzing the genetic environment of bla_{NDM-1} and regarding to the presence of IS*Pa7*, we suggest that bla_{NDM-1} was mobilized into a new complex class 1 integron, which is a part of a chromosomally located Tn*402*-like structure. This is the first report describing the genetic environment of bla_{NDM-1} in *P. aeruginosa*. The presence of the association of IS*Pa7* upstream of a new *CR1*-borne *sul*-type integron and a bla_{NDM-1} region, classically found in plasmids in *E. coli* or in *A. lwoffi*, highlights the high plasticity of the *P. aeruginosa* genome (21). The ability of *P. aeruginosa* to accumulate overproduction of intrinsic resistance mechanisms, as well as the combination of various genetic determinants, including nonplasmid lateral exchange of resistance regions (21), results in multidrug-resistant or extensively drug-resistant isolates. Multilocus sequence typing, conducted according to published protocols (28; http://pubmlst.org/paeruginosa/), assigned HIABP11 to sequence type 235 (ST235), which has been encountered in clinical and environmental isolates of different countries (29, 30), including Serbia (31). ST235 is the primary founder of the successful epidemic clonal complex 235 (CC235), associated with various β-lactamase genes, including PER-1 extended-spectrum β-lactamase and VIM-type metallo-β-lactamases (32, 33). Detection of NDM-1 carbapenemase in a CC235 *Pseudomonas aeruginosa* isolate underscores its potential to spread in the hospital environment.

Nucleotide sequence accession number. The nucleotide sequence of the bla_{NDM-1} region in *P. aeruginosa* HIABP11 has been registered in GenBank under accession no. KC170992.

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