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Integration of the bla_{NDM-1} carbapenemase gene into *Proteus* genomic island 1 (PGI1-*Pm*PEL) in a *Proteus mirabilis* clinical isolate

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Objectives: To decipher the mechanisms and their associated genetic determinants responsible for β-lactam resistance in a *Proteus mirabilis* clinical isolate.

Methods: The entire genetic structure surrounding the β-lactam resistance genes was characterized by PCR, gene walking and DNA sequencing.

Results: Genes encoding the carbapenemase NDM-1 and the ESBL VEB-6 were located in a 38.5 kb MDR structure, which itself was inserted into a new variant of the *Proteus* genomic island 1 (PGI1). This new PGI1-*Pm*PEL variant of 64.4 kb was chromosomally located, as an external circular form in the *P. mirabilis* isolate, suggesting potential mobility.

Conclusions: This is the first known description of the bla_{NDM-1} gene in a genomic island structure, which might further enhance the spread of the bla_{NDM-1} carbapenemase gene among enteric pathogens.

Keywords: *P. mirabilis*, NDM-1, VEB-6, carbapenem resistance, genomic island SGI1

Introduction

While decreased susceptibility to imipenem is intrinsic in Proteeae, some *Proteus mirabilis* exhibit an increased level of resistance to imipenem (with MICs ranging from 16 to 64 mg/L) due to loss of outer membrane porin, decreased expression of PBP1a or reduced binding of imipenem by PBP2.[1] The emergence of MDR *P. mirabilis* isolates producing acquired ESBLs, AmpC and carbapenemases has also been described.[2] The carbapenemases identified previously in *P. mirabilis* are the Ambler class A β-lactamase KPC-2,[3] the class B [metallo-β-lactamases (MBLs)] VIM-1 and NDM-1,[4] and the carbapenem-hydrolysing class D β-lactamase OXA-23.[5]

The bla_{NDM-1} gene, firstly identified from a *Klebsiella pneumoniae* isolate, has been described in most enterobacterial species, but also in *Vibrio cholerae*, *Pseudomonas* spp. and *Acinetobacter* spp.[6] In Enterobacteriaceae, the bla_{NDM-1} gene is most often located on plasmids.[7] Plasmid acquisition occurs by conjugation or mobilization in Enterobacteriaceae; nevertheless, integrative mobilizable elements have been described in *Salmonella* spp., e.g. *Salmonella* genomic island 1 (SGI1).[8] The SGIs may contain MDR regions with genes encoding β-lactamases (e.g. ESBLs) and resistance to quinolones (Qnr), chloramphenicol/florfenicol, tetracycline and aminoglycosides. Variants of SGI1 have been classified from SGI1-A to SGI1-V, corresponding to variants of the MDR region.[9–13] The integration of SGI1 into the chromosome of *Salmonella* spp. occurs at the last 18 bp of the 3′-end of the *thdF* gene.[8] Since this conserved integration site has been also identified in diverse bacteria, it was hypothesized that *P. mirabilis* could be an acceptor of SGI independently of *Salmonella* spp., although SGI1 have been exceptionally detected in *P. mirabilis* isolates.[9–14] Recently a new resistance genomic island belonging to the same family as SGI1 and named PGI1 (*Proteus* genomic island 1) has been described in *P. mirabilis*.

We describe here the co-occurrence of the carbapenemase gene bla_{NDM-1} together with the ESBL gene bla_{VEB-6} in a new SGI1 variant from a clinical *P. mirabilis* isolate.

Materials and methods

Bacterial strains and antibiotic susceptibility testing

P. mirabilis PEL was isolated from a urine sample recovered from a patient hospitalized at Chambery hospital in France in 2012. *P. mirabilis* PEL was identified using the API 20E system (bioMérieux, La Balme les Grottes, France). *Escherichia coli* TOP10 (Life Technologies, Cergy-Pontoise, France), *E. coli* K-12 strain BM14[8] and *E. coli* J53 reference strain were used in cloning and conjugation experiments. MICs were determined by Etest (bioMérieux) and were interpreted according to CLSI breakpoints.[15] The production of putative carbapenemases and ESBLs was evaluated using the Carba NP test and the ESBL NDP test, respectively, as previously described.[16,17]

PCR and sequencing

A PCR approach was used to detect different types of β-lactamase genes as previously described.[4] Cloning experiments were performed using XbaI restriction, a pBKCMV plasmid, expression in *E. coli* TOP10 and selection on trypticase soy agar supplemented with ticarcillin (50 mg/L) and kanamycin (30 mg/L). Plasmid DNAs were extracted using Qiagen columns (Qiagen) and sequenced on an ABI3130 sequencer (Applied Biosystems, Les Ulis, France).[4] PCR mapping of PGI1 was performed with primers chosen in the island backbone according to sequences available in the GenBank database (AF261825), and primers PGI1circ1 and PGI1circ2 were used for the detection of a circular extrachromosomal form of SGI1.[8]

SGI mobilization assays

Conjugal transfer was attempted by liquid and solid mating-out assays using a rifampicin-resistant *E. coli* J53 strain. Before mobilization experiments, plasmid R55 was introduced into *P. mirabilis* PEL donor by liquid conjugation with *E. coli* K-12 strain BM14 carrying R55[8] and selection on agar containing gentamicin (40 mg/L) and ceftazidime (2 mg/L). Mobilization of the circular form of PGI1 was attempted by liquid and solid mating-out assays with *P. mirabilis* PEL (pR55) as a donor and *in vitro*-selected rifampicin-resistant *Salmonella enterica* serovar Typhimurium LT2 as a recipient strain. Agar plates containing ceftazidime (2 mg/L) and rifampicin (200 mg/L) were used for selection of *S. enterica* transconjugants.

Nucleotide sequence accession number

The nucleotide sequence of the PGI1 variant from *P. mirabilis* PEL was submitted to GenBank under accession number KF856624.

Results and discussion

Susceptibility testing and carbapenemase identification

P. mirabilis isolate PEL was resistant to amino- and carboxypenicillins, narrow- and broad-spectrum cephalosporins, moxalactam and aztreonam (Table S1, available as Supplementary data at *JAC* Online). It was resistant to imipenem (MIC of 32 mg/L), but susceptible to meropenem and ertapenem (MICs of 0.75 and 0.25 mg/L, respectively; Table S1). The association of reduced susceptibility to imipenem with resistance to all β-lactams including broad-spectrum cephalosporins and cefoxitin was uncommon. PCR and sequencing identified the carbapenemase and ESBL genes bla_{NDM-1} and bla_{VEB-6}. In addition, *P. mirabilis* PEL was resistant to most of the non-β-lactam antibiotics, with the exception of gentamicin, fosfomycin and rifampicin (data not shown).

Genetic structures surrounding the bla_{NDM-1} gene

Plasmid pPEL-1, recovered after XbaI cloning experiments, possessed a 28.1 kb insert and haboured the bla_{NDM-1} gene bracketed by insertion sequence IS*Aba125* and the ble_{MBL} gene encoding resistance to bleomycin, as previously identified in most bla_{NDM-1}-positive strains (Figure 1).[18] The DNA fragment encompassing IS*Aba14*, bla_{NDM-1} and IS*CR1* (*orf513*) shared 99% nucleotide identity with a plasmid-borne fragment from *Acinetobacter* spp. M131 (accession number JX072963.1) (Figure 1). IS*Aba14* had been first identified to be associated with the class A β-lactamase gene $bla_{CARB-14}$ in *Acinetobacter baumannii*[19] and also in NDM-1-producing *A. baumannii* isolates, but had not been reported from Enterobacteriaceae isolates to date. Although IS*Aba125* is systematically identified upstream of the bla_{NDM}-like genes,[7] its role in the mobilization of this gene has not yet been demonstrated. In *A. baumannii*, it is likely that composite transposon Tn*125* is at the origin of bla_{NDM} gene acquisition,[20] while the IS*CR1*-like elements might be responsible for the mobilization of bla_{NDM} in Enterobacteriaceae, through a rolling-circle transposition process or due to homologous recombination between two copies of IS*CR1*.[21] Noticeably, the bla_{NDM} gene is the product of a gene fusion event, which has implications for its expression and subsequent mobility.[22]

Genetic structure surrounding the bla_{VEB-6} gene

The close genetic environment of the bla_{VEB-6} gene shared 98% nucleotide identity with that previously reported on SGI1-V in a *P. mirabilis* isolate recovered in France in 2011.[9] However, SGI-V had a different backbone as compared with the novel PGI (named PGI-*Pm*PEL) identified here (Figure 1).

Genetic structure of the mer operon

Downstream of the bla_{NDM-1}-containing locus, a gene encoding a transposase of the Tn*3* family was identified, showing 88% nucleotide identity with that of IS*Pa40*. At the right-hand end of the complex integron, a region corresponding to the Tn*501* mercury resistance module was identified, followed by the Tn*5053* transposition module, containing four genes involved in transposition, i.e. *tniA*, *tniB*, *tniQ* and *tniR*. Sequencing of the 3′-end of plasmid pPEL showed that the antibiotic resistance gene cluster was surrounded by inverted repeats IRt and DR-R of the SGI1 type (Figure 1).[8]

Characterization of the PGI1 backbone

The 67 kb PGI1-*Pm*PEL was integrated between the *thdF* and *hipB/hipA* chromosomal genes (Figure 1). The sequences of specific recombination sites (*attP* and *attB* sites) have been identified (Figure 1).[8] The MDR region here interrupted the C1566 *orf* from *Salmonella* Heidelberg strain 7, suggesting that a large deletion had occurred from the *res* gene (C1584) to C1566 (Figure 1a). The DR-R was located 109 bp downstream of *orf* C1564. This PGI1-*Pm*PEL shared 99% identity with the two recently described PGI1-*Pm*CHA and PGI1-*Pm*CHE. Nevertheless, the same synteny was highly conserved between PGI1 and SGI1 backbones, suggesting that both islands belong to the same genomic island family.

Detection of an extrachromosomal form of PGI1

Most SGI1 have lost the ability of horizontal transfer. However, some integrative and conjugative elements (ICEs) can excise from the chromosome by a site-specific recombination, leading to the formation of circular extrachromosomal elements, which may be transferred by conjugation and integrated in a site-specific fashion into the recipient chromosome.[8] PCR experiments with PGI1circ1/2 primers confirmed the presence of a circular extrachromosomal form of PGI1-*Pm*PEL in *P. mirabilis* PEL in the presence or absence of helper plasmid pR55.[8] Mobilization *in trans* of this circular form of PGI1-*Pm*PEL was attempted after

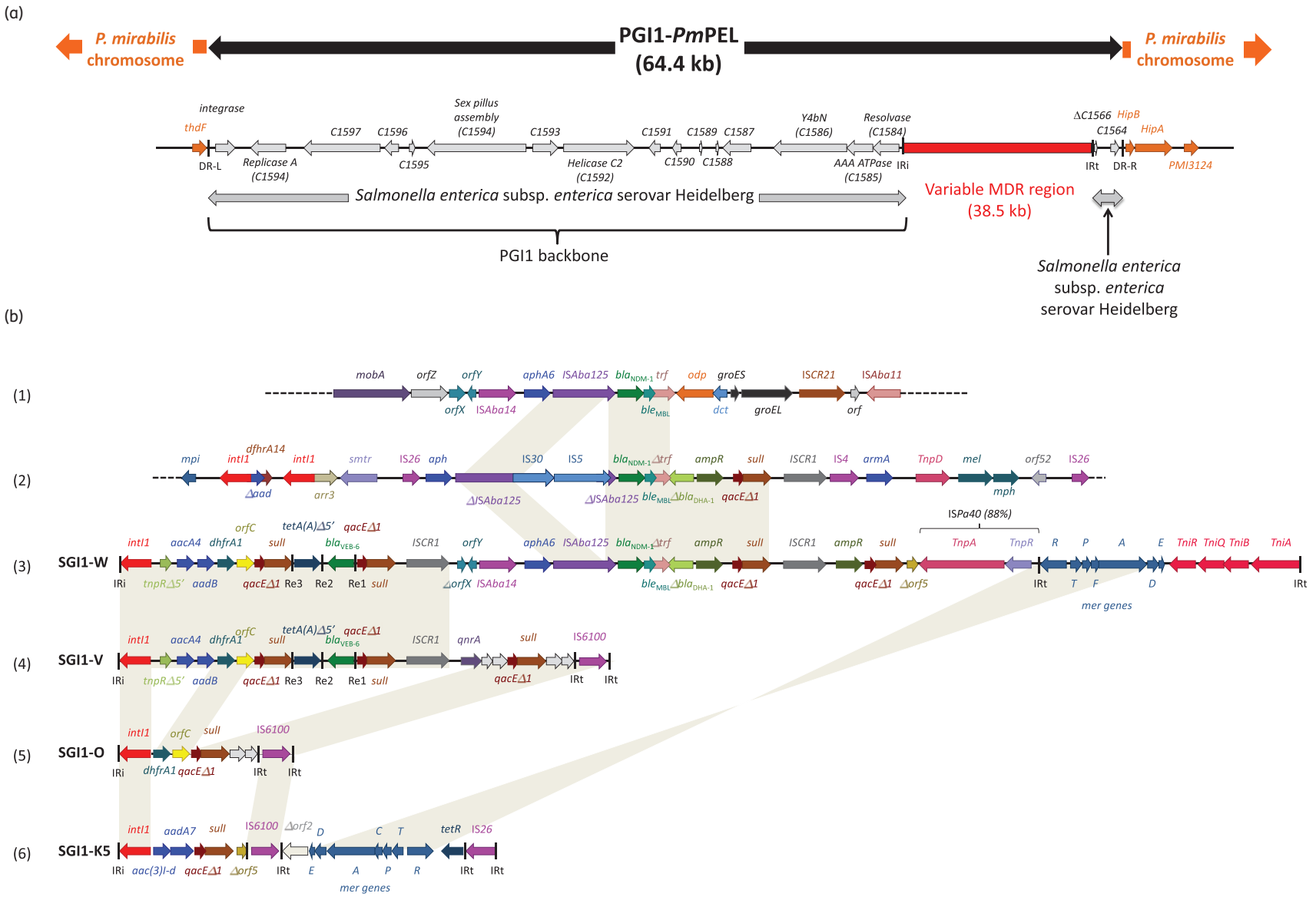

Figure 1. Schematic view of the variant SGI1-W as integrated in *P. mirabilis* strain PEL. (a) The overall structure of the SGI1 backbone is represented as reported by Doublet *et al.*[8] The 18 bp direct repeats (DR-R and DR-L) bracketing the module are shown. IRi and IRt are 25 bp imperfect inverted repeats defining the left and right ends of the MDR region encompassing the bla_{NDM-1} gene. The different parts of the MDR region of this study (b, 3) have been compared with that of the bla_{NDM-1}-harbouring plasmid pM131 from *A. baumannii* sp. (GenBank: JX072963.1) (b, 1) and pNDM-1 Saitama from *K. pneumoniae* (GenBank: AB759690) (b, 2), in SGI1-V from *P. mirabilis*[14] (b, 4), in SGI1-O from *P. mirabilis*[13] (b, 5) and in SGI1-K5 from *S. enterica*[24] (b, 6). Common features are highlighted with grey shading. Gene names are as follows: *mobA*, mobilization protein; *orf*, open reading frame; IS, insertion sequence; *aph*, aminoglycoside phosphotransferase; *bla*, β-lactamase; ble_{MBL}, bleomycin resistance protein; *trf*, phosphoribosylanthranilate isomerase; *odp*, oxidoreductase domain protein; *dct*, divalent cation tolerance protein; *groES* and *groEL*, chaperonins; *mpi*, resolvase; ISCR, insertion sequence common region; *intI1*, integrase; *aac*, aminoglycoside acetyltransferase; *dhfr*, dihydrofolate reductase; *aad*, aminoglycoside adenylyltransferase; *qacE*Δ1, ethidium bromide resistance protein; *sulI*, dihydropteroate synthase; *ampR*, *ampC* regulator gene; *tnp*, transposase; *mer*, mercury resistance genes; *tni*, transposition protein; *arr3*, rifampicin ADP-ribosylatin transferase; *smrt*, retron-type reverse transcriptase; *armA*, ArmA 16S rRNA methylase; *tnp*, transposase; *mel*, macrolide efflux protein; and *mph2*, macrolide 2′-phosphotransferase.

complementation with plasmid R55 as described by Doublet *et al.*[8] and Douard *et al.*,[23] but remained unsuccessful.

Conclusions

This study identified a new variant of PGI1 in a multiresistant *P. mirabilis* clinical isolate. This is the first known description of the bla_{NDM-1} gene, and more generally of a carbapenemase-encoding gene, in a resistance island element. Notably, the bla_{NDM-1} gene was associated with the ESBL gene bla_{VEB-6} in this same ICE, thus leading to a stable structure conferring a high level of resistance to all β-lactams, including aztreonam. The results obtained here indicate that resistance gene exchanges may occur between *Acinetobacter* spp. and *P. mirabilis*, as suggested by the occurrence of IS*Aba*14 in *P. mirabilis* PEL. The likely scenario might be that *Acinetobacter* spp. may have been the first target of bla_{NDM-1} acquisition and then the structure was captured by *Salmonella* spp. to finally target in *P. mirabilis* or directly captured by a resident PGI in *P. mirabilis*. The latter may be the most probable, as bla_{NDM-1} has not been identified on SGI1-like structures in *Salmonella* spp. to date. This is an additional clue confirming the possible role of *Acinetobacter* spp. as a source of antibiotic resistance for enterobacterial species. In addition, our study showed that such a genetic structure harbouring several clinically relevant resistance genes might be stabilized through chromosomal integration and then be vertically transmitted without risk of plasmid loss.

Note added in proof

The recent results obtained by Siebor & Neuwirth (*J Antimicrob Chemother* 2014; **69**: 3216–3220) highlighted modifications in SGI nomenclature that led us to rename the ICE in our isolate of *P. mirabilis* as PGI1.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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