Integration of the bla_{NDM-1} carbapenemase gene into Proteus genomic island 1 (PGI1-PmPEL) 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-PmPEL 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 Proteaeae, 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. The emergence of MDR P. mirabilis isolates producing acquired ESBLs, AmpC and carbapenemases has also been described. The carbapenemases identified previously in P. mirabilis are the Ambler class A β-lactamase KPC-2, the class B [metallo-β-lactamases (MBLs)] VIM-1 and NDM-1, and the carbapenem-hydrolysing class D β-lactamase OXA-23.

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. In Enterobacteriaceae, the bla_{NDM-1} gene is most often located on plasmids. 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). The SGI1s 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. The integration of SGI1 into the chromosome of Salmonella spp. occurs at the last 18 bp of the 3′-end of the thdF gene. 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. 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 BM146 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.

The production of putative carbapenemases and ESBLs was evaluated using the Carba NP test and the ESBL NDP test, respectively, as previously described.
PCR and sequencing

A PCR approach was used to detect different types of β-lactamase genes as previously described. Cloning experiments were performed using XbaI restriction, a pBKCMV plasmid, expression in E. coli TOP10 and selection on tetracycline 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). 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.

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 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 carbapenem, 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 EBL genes blaNDM-1 and blaVEB-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 blaNDM-1 gene

Plasmid pPEL–1, recovered after XbaI cloning experiments, possessed a 28.1 kb insert and harbour the blaNDM-1 gene bracketed by insertion sequence ISAba125 and the blaVEB-6 gene encoding resistance to bleomycin, as previously identified in most blaNDM-1-positive strains (Figure 1). The DNA fragment encompassing ISAba14, blaNDM-1 and ISCR1 (orfS13) shared 99% nucleotide identity with a plasmid-borne fragment from Acinetobacter spp. M131 (accession number JX072963.1) (Figure 1). ISAba14 had been first identified to be associated with the class A β-lactamase gene blaCARB-14 in Acinetobacter baumannii and also in NDM-1-producing A. baumannii isolates, but had not been reported from Enterobacteriaceae isolates to date. Although ISAba125 is systematically identified upstream of the blaNDM-1-like genes, its role in the mobilization of this gene has not yet been demonstrated. In A. baumannii, it is likely that composite transposon Tn125 is at the origin of blaNDM gene acquisition, while the ISCR1-like elements might be responsible for the mobilization of blaNDM in Enterobacteriaceae, through a rolling-circle transposition process or due to homologous recombination between two copies of ISCR1. Noticeably, the blaNDM gene is the product of a gene fusion event, which has implications for its expression and subsequent mobility.

Genetic structure surrounding the blaVEB-6 gene

The close genetic environment of the blaVEB-6 gene shared 98% nucleotide identity with that previously reported on SGI-I in a P. mirabilis isolate recovered in France in 2011. However, SGI-V had a different backbone as compared with the novel PGI (named PGI–PmPEL) identified here (Figure 1).

Genetic structure of the mer operon

Downstream of the blaNDM-1-containing locus, a gene encoding a transposase of the Tn3 family was identified, showing 88% nucleotide identity with that of ISPa40. At the right-hand end of the complex integron, a region corresponding to the Tn501 mercury resistance module was identified, followed by the Tn505 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).

Characterization of the PGI1 backbone

The 67 kb PGI1–PmPEL was integrated between the thrF and hipB/hipA chromosomal genes (Figure 1). The sequences of specific recombination sites (attP and attB sites) have been identified (Figure 1). 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–PmPEL shared 99% identity with the two recently described PGI1–PmCHA and PGI1–PmCHE. 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. PCR experiments with PGI1circ1/2 primers confirmed the presence of a circular extrachromosomal form of PGI1–PmPEL in P. mirabilis PEL in the presence or absence of helper plasmid pR55. Mobilization in trans of this circular form of PGI1–PmPEL 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. The 18 bp direct repeats (DR-R and DR-L) bracketing the module are shown. IRr and IRt are 25 bp imperfect inverted repeats defining the left and right ends of the MDR region encompassing the blaNDM-1 gene. The different parts of the MDR region of this study (b, 3) have been compared with that of the blaNDM-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 (b, 4), in SGI1-O from P. mirabilis (b, 5) and in SGI1-K5 from S. enterica (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, b-lactamase; bleMBL, bleomycin resistance protein; trf, phosphoribosylanthranilate isomerase; oep, oxidoeductase domain protein; dct, divalent cation tolerance protein; groES and groEL, chaperonins; mpi, resolvase; ISCR, insertion sequence common region; intII, integrase; aac, aminoglycoside acetyltransferase; dhfr, dihydrofolate reductase; aad, aminoglycoside adenylyltransferase; qacE, ethidium bromide resistance protein; sulI, dihydroteoroate synthase; ampR, ampC regulator gene; tnp, transposase; mer, mercury resistance genes; tri, transposition protein; arr, rifampicin ADP-ribosylatil transferase; smr, retron-type reverse transcriptase; armA, ArmA 16S rRNA methylase; trp, transposase; mel, macrolide efflux protein; and mph2, macrolide 2′-phosphotransferase.
complementation with plasmid R55 as described by Doubelt et al. and Douard et al., 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 blaNDM-1 gene, and more generally of a carbapenemase-encoding gene, in a resistance island element. Notably, the blaNDM-1 gene was associated with the ESBL gene blaPER-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 ISAba14 in P. mirabilis PEL. The likely scenario might be that Acinetobacter spp. may have been the first target of blaNDM-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 blaNDM-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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Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

