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A novel class 1 integron array carrying *bla*$_{VIM-2}$ genes and a new insertion sequence in a *Pseudomonas aeruginosa* strain isolated from a Spanish hospital

Pseudomonas aeruginosa is a pathogen commonly implicated in nosocomial infections. Carbapenems are an effective treatment option, but *P. aeruginosa* resistant to these agents are increasingly reported. The zinc-dependent metallo-β-lactamases (MBLs) are one of the most clinically important enzymes that hydrolyse carbapenems, penicillins and extended-spectrum cephalosporins, but not aztreonam (Walsh *et al.*, 2005). Their activity is inhibited by the effect of metal chelators such as EDTA. The MBLs most frequently detected worldwide are the IMP and VIM types, which have been reported within genetic elements such as integrons (Walsh *et al.*, 2005; Partridge *et al.*, 2009). Integrons have the ability to capture, integrate and express gene cassettes involved in the resistance to different antimicrobial families (e.g. β-lactams, carbapenems and aminoglycosides) (Partridge *et al.*, 2009). This facilitates the dissemination and co-selection of multiresistant *P. aeruginosa* strains and limits the therapeutic options (Bonomo & Szabo, 2006).

P. aeruginosa strain W37 was recovered from a urinary sample in a Spanish hospital in 2007. Susceptibility testing was performed by the disc-diffusion method (CLSI, 2010). This strain showed resistance to imipenem, meropenem, ticarcillin, ceftriaxone, cefotaxime, gentamicin, tobramycin and sulfonamides; intermediate resistance to cefepime, aztreonam and ciprofloxacin; and susceptibility to ceftazidime, amikacin and colistin. The MICs of imipenem (IPM) and meropenem (MEM), determined by the agar dilution method (CLSI, 2010), were >256 and 64 mg l^{-1}, respectively. The MBL Etest (AB bioMérieux) and the double-disc (IPM/0.5 M EDTA/MEM) (Lee *et al.*, 2001) methods proved an MBL-positive phenotype in this strain.

Multilocus sequence typing (MLST) showed in *P. aeruginosa* W37 a new allelic combination of the seven sequenced housekeeping genes (*acsA6*, *aroE5*, *guaA6*, *mutL5*, *nuoD4*, *ppsA94*, *trpE7*) that was registered and named ST973 in the MLST database (http://pubmlst.org/paeruginosa/) (Curran *et al.*, 2004). This sequence type is a single-locus variant of ST641 and no clonal complex is yet described (Nemec *et al.*, 2010).

MBL genes were detected and characterized by multiplex-PCR and subsequent sequencing (Ellington *et al.*, 2007). The presence of class 1 and 2 integrons was determined by PCR, using primers targeting genes in the integron conserved segments (*intI1*, *intI2* and *qacE*Δ1 + *sul1*). Integron variable regions were analysed by PCR mapping and sequencing (Sáenz *et al.*, 2004).

P. aeruginosa W37 harboured the *bla*$_{VIM-2}$ gene and two class 1 integrons. One of these integrons contained only a *bla*$_{VIM-2}$ gene cassette in its variable region, but the other one had a variable region of 5429 bp that included *bla*$_{VIM-2}$ + *aac(6′)-Ib′* + *aadA1* gene cassettes followed by a new open reading frame (ORF) and another *bla*$_{VIM-2}$ gene cassette (Fig. 1). The presence of two copies of the *bla*$_{VIM-2}$ gene is remarkable in this last integron. The *aac(6′)-Ib′* gene encodes the AAC(6′)-Ib′ aminoglycoside acetyltransferase, which has an amino acid substitution (Leu119→Ser) with respect to AAC(6′)-Ib, and confers resistance to gentamicin, tobramycin, kanamycin and netilmicin, a typical phenotype of an AAC(6′)-II enzyme (Lambert *et al.*, 1994).

The new ORF (1059 bp) encoded a putative transposase of 352 amino acids which is 62 % similar to the IS*1618* transposase. This ORF was part of a new IS of 1216 bp, designated IS*Pa34* by IS Finder (http://www-is.biotoul.fr/). IS*Pa34* belongs to the IS*110* family and IS*1111* group, and it was inserted into the *attC* of the *aadA1* gene cassette, whose mobilization could be affected. According to the IS*110* family characteristics, no direct target repeats are created by IS*Pa34* (Mahillon & Chandler, 1998).

The nucleotide sequence of the novel class 1 integron array determined in this study was deposited in the GenBank database with the accession number GU354325.

Several studies have described class 1 integrons containing the *bla*$_{VIM-2}$ gene cassette (Walsh *et al.*, 2005; Santos *et al.*, 2010; Samuelsen *et al.*, 2010; Hammami *et al.*, 2010), but to our knowledge, this is the first report of the double detection of *bla*$_{VIM-2}$ gene cassettes in the variable region of the same integron in *P. aeruginosa*. In addition, the presence of a new insertion sequence in the surrounding environment could be implicated in the mobilization of these gene cassettes.

The polymorphisms of the promoter (Pc) responsible for the expression of inserted gene cassettes were characterized by PCR and sequencing in the two class 1 integrons of strain W37. The Pc hybrid 1 (PcH1) was detected in both integrons, characterized by the TGGACA and TAAACT sequences at the -35 and -10 hexamer positions, respectively. An *in silico* study has previously described (Jové *et al.*, 2010) that the PcH1 variant is associated with weak expression of gene cassettes, and the resulting integrase with high excision activity, which favours the capacity for rearrangement of gene cassettes, in order to place the required gene cassette closer to PcH1.

The location of the class 1 integrons was studied by independent genomic DNA digestions with the nuclease S1 (8 U per plug) and the endonucleases I-*Ceu*I and

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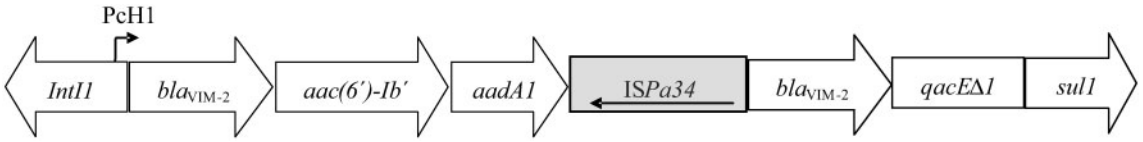

Fig. 1. Structure of the new class 1 integron of *P. aeruginosa* W37 (GenBank accession number GU354325).

*Spe*I (10 U per plug) (New England Biolabs), and subsequent PFGE separations. Bacterial DNA embedded in agarose plugs was prepared as described by Kaufmann (1998). The PFGE conditions used were as follows: pulse time ranging from 5 s to 45 s for 16 h after nuclease S1 digestion, from 50 s to 90 s for 22 h after I-*Ceu*I digestion, and two ramps were used after *Spe*I digestion, from 5 s to 15 s for 10 h and from 15 s to 45 s for another 10 h. All gels were run at 6 V cm^{-2} and at 14 °C. Digested PFGE gels were analysed by Southern blotting and hybridization using bla_{VIM-2}, *intI1*, IS*Pa34* and 16S rRNA gene probes. The bla_{VIM-2} and *intI1* genes hybridized in two bands with sizes between 97 and 145.4 kb in the *Spe*I-PFGE gel. DNA digestion with I-*Ceu*I showed that the bla_{VIM-2}, *intI1*, IS*Pa34* and 16S rRNA probes hybridized only with chromosomal DNA. A DNA plasmid extraction and subsequent hybridization was performed and this also confirmed that the integron is chromosomally located.

In summary, a novel class 1 integron that contains two copies of the bla_{VIM-2} gene and a new insertion sequence IS*Pa34* is reported. The accumulation of genes encoding MBL in *P. aeruginosa* strains is worrisome, especially when these structures could be mobilized by plasmids or transposons and transferred to other bacteria.

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