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Dissemination of Metallo-Beta-Lactamases in Europe via a Tn*5051*-like Composite Transposon: Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: *Pseudomonas aeruginosa* (PSA) isolates are routinely screened for novel metallo-beta-lactamases (MBLs) in the SENTRY Program. The genetic context of geographically distinct MBL-producing strains for 2001-2002 were investigated for the presence of mobile elements.

Methods: Isolates known to harbour MBLs were used to create genomic gene banks cloned and expressed in pk18. Gene banks were expressed in *E. coli* DH5α and recombinants isolated on media containing ceftazidime (5 mg/ml). Recombinants carrying large inserts were sequenced to identify adjacent genes and transposable elements. Sequencing was accomplished using DuPont Automated systems and sequence analysis done by DNAstar.

Results: Sequence analysis results of the genetic context of bla_{VIM-2} from Warsaw (Poland) and bla_{IMP-13} from Rome (Italy), both harboured by PSA isolates, were carried on identical mobile elements. Immediately upstream of the MBL genes was a classical Class 1 integron and downstream the genes for aacA4 and sul/qacEΔ1. Further upstream of the integron, lies a transposon displaying homology to Tn*5051* (99% identity) previously isolated from *P. putida* in New York. The class 1 integrons are inserted into an identical position approximately 200bp from the res site of the Tn*5051*-like element. This insertion site was also identical to that of a Class 1 integron containing the beta-lactamase gene cassette bla_{GES-1} isolated from a PSA isolate in France.

Conclusions: This is the first definitive report of a transposon-like element being associated with MBL genes in various geographically diverse Pseudomonads. The data indicates that the Class 1 integrons carrying different types of carbapenemases from distinctly different parts of Europe have embedded in the same transposon, a Tn*5051*-like element.

INTRODUCTION

The four groups of clinically relevant metallo-ß-lactamases are: IMP, VIM, SPM and GIM. The IMP and VIM groups have multiple members whereas at present SPM and GIM are the sole members of these two groups. The possession of metallo-ß-lactamase genes by Gram-negative pathogens is generally in combination with other antimicrobial resistance genes packaged together as gene cassettes in elements called integrons. This genetic arrangement allows the gene cassettes to move between integrons. When the integron itself is harboured by a functional transposon or composite transposon, additional levels of movement between organisms are achieved.

The combination of gene cassettes and transposons together with the broad host range plasmids, which are common in environmental and clinical bacterial isolates, enable horizontal and vertical transfer of antimicrobial resistance genes in members of the Enterobacteriaceae and Pseudomonads. Here we describe the identical genetic context of two metallo-ß-lactamase genes bla_{VIM-2} and bla_{IMP-13}. Both are harboured on an identical Tn*5051*-like composite transposon and were found in geographically distant parts of Europe.

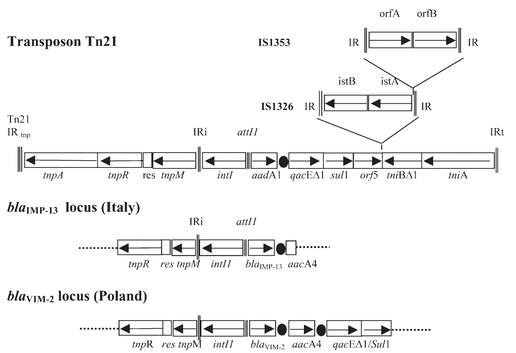
METHODS

Pseudomonas aeruginosa strains 86-14571 and 81-11963 were isolated as part of the SENTRY Antimicrobial Surveillance Program in clinical cases from Italy and Poland, respectively. Genomic DNA was isolated from both strains by the cetryl-tri-ammonium bromide (CTAB) method. Plasmids were purified by the alkaline lysis using a Qiagen miniprep kit.

For construction of the genomic library, *Sau*3A1 genomic fragments were purified after gel electrophoresis using a Qiagen gel purification kit (Qiagen). Five μg of purified size fractionated genomic fragments (>1kb) were ligated to 1 μg of pK18 that had previously been linearised and dephosphorylated using *Bam*H1 and calf intestinal alkaline phosphatase, respectively. The ligation mixture was subsequently dialysed and used to transform *E. coli* DH5α by electroporation using a Biorad Gene Pulser. Cloned metallo-ß-lactamases genes were selected on media containing 10 μg/ml ceftazidime.

Sequencing was performed on both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer and sequence analysis was performed using the Lasergene DNASTAR software package.

Figure 1. Genetic context of of *bla*VIM-2 and *bla*IMP-13 genes



Schematic representation of the bla_{IMP-13} and bla_{VIM-2} genetic loci and comparison with the 5' end of the composite mercury resistance transposon Tn*21* including the transposition (*tnp*) and integron *In2* region (GenBank accession no. AF071413). The *tnp* regions consist of genes for the transposase (*tnpA*), the resolvase (*tnpR*), the putative transposition regulator (*tnpM*) and the resolution site (*res*). The class 1 integron *In2* harboured by *Tn21* consists of a class 1 integrase gene (*intI1*) its recombination site *attI1* and the aminoglycoside resistance gene *aadA1*. The bla_{IMP-13} containing integron similarly consists of a class 1 integrase (*intI1*) and its recombination site together with at least two gene cassettes bla_{IMP-13} and an *aacA4* gene cassette as does the bla_{VIM-2} containing integron. These integrons are also harboured by Tn*21*-like transposons displaying identity to Tn*5051* and are inserted in *tnpM*, truncating this transposon gene. Arrows indicate the direction of transcription of the various genes and filled circles represent the position of 59 base elements, and dashed lines indicate vector sequence. Vertical lines represent the positions of 25-bp imperfect inverted repeats at the ends of the *In2* integron, the left hand equivalent inverted repeat in the integrons containing bla_{IMP-13} and bla_{VIM-2}, and the 38-bp inverted repeat at the "left hand" end of Tn*21*. The positions of the two insertion sequences harboured by Tn*21* are also shown. Futher PCR amplification and sequencing has confirmed that *tnp*A was upstream of *tnp*R in both 81-11963 and 86-14571 strains.

RESULTS

- Genetic analysis of the integrons containing bla_{IMP-13} and bla_{VIM-2} determined that they were both identical in respect of their genetic context (Figure 1).
- A 25bp sequence IRi was identified 174 bp upstream of the stop codon of the class I integrase gene in both isolates. This sequence represents one of the terminal inverted repeats of Tn*402* (Tn*5090*)-like elements and marks the "left hand" end of the respective integrons.
- The sequence immediately preceding the repeat sequence was used to search the GenBank data bases for similar insertion sites and a near identical insertion site was also detected for another Tn*402*-like element carrying integron *In60* which only differs by a single nucleotide deletion from the sequence harbouring bla_{IMP-13}. (Figure 2). This integron carries the extended spectrum ß-lactamase bla_{GES-1} which was identified in a *P. aeruginosa* isolate in France.
- Further upstream of the bla_{IMP-13} and bla_{VIM-2} integrons the sequence shows features characteristic of the *tnp* region of transposon Tn*21* namely, *tnp*R and *tnp*M separated by a resolution site (*res*). These genes are found in the same orientation as those found in Tn*21*.
- The *tnp*R sequence was terminated at the codon for the last amino acid by the cloning process. The presence of *tnp*A adjacent to *tnp*R was confirmed in the original isolates by PCR using specific primers. The *tnp*M-like gene sequence shares 81.4% identity with *tnp*M from Tn*21* and 98.4 % identity with *urf2* from Tn*5051*.

Figure 2 Comparison of the insertion points of integrons containing bla_{VIM-2}, bla_{IMP-13} and bla_{GES-1} in *tnp*M of Tn*5051*.

Comparison of the IRi 25bp inverted repeat sequences and flanking regions of the bla_{GES-1} containing integron In60, the integron containing bla_{IMP-13}.and the bla_{VIM-2} containing integron. The identical 25bp repeats observed at the end of the Tn*402*-like element are underlined and identical residues in the flanking sequences are indicated by vertical lines.

- The *tnp*R-like sequence codes for a putative protein displayed 98.9% identity to the TnpR protein of Tn5051 (previously characterised from *P. putida* isolated in New York), and 87% and 86% identity to the TnpR proteins of transposons Tn*21* and Tn*501*, respectively.
- This sequence information indicates that the metallo-ß-lactamase genes bla_{IMP-13} and bla_{VIM-2} were located on integrons harboured by a Tn*402*-like transposon which in turn is harboured by a Tn*21* sub-family transposon (Figure 1).

CONCLUSIONS

- Both metallo-ß-lactamase genes bla_{IMP-13} and bla_{VIM-2} in SENTRY isolates 86-14571 and 81-11963 are found in identical integrons which are harboured in Tn*5051*-like composite transposons.
- Both *P. aeruginosa* parent strains had no evidence of containing plasmids and had different ribotypes which suggests that at least two events differentiate these isolates: (A) transposition of the original Tn*5051*-type transposon, and (B) acquisition of different metallo-ß-lactamase genes.
- The insertion of the Tn402-like elements containing both bla_{IMP-13} from Italy and bla_{VIM-2} from Poland are at an identical site approximately 200 bases removed from the *res* site of a Tn*5051*-like transposon which is also shared by the element harbouring bla_{GES-1}. This suggests that the same transposon is responsible for the dissemination of both these metallo-ß-lactamase genes, as well as, bla_{GES-1} across a wide geographical area in Europe.

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