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Genome Note

Genomic characterization of *bla*$_{VIM-11}$-harbouring plasmids recovered from *Pseudomonas aeruginosa*

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ABSTRACT

Objectives: To the best of our knowledge, no genomic descriptions of *bla*$_{VIM-11}$-harbouring plasmids are available in literature so far. The aim of this study was to describe the genomic features of three *bla*$_{VIM-11}$-harbouring plasmids recovered from *Pseudomonas aeruginosa* isolated in Argentina in different periods.
Methods: *bla*$_{VIM-11}$-harbouring plasmids from three clinical *P. aeruginosa* isolates were transferred by transformation into *P. aeruginosa* PAO-1. Then, genomic DNA of these transformants was extracted and sequenced using NovaSeq 6000 System-Illumina. *De novo* assemblies were generated using Unicycler program and reads were mapped against a reference genome of *P. aeruginosa* PAO-1. Plasmids sequences were predicted identifying the reads that did not map the reference sequence of PAO-1. These reads were recovered and assembled *de novo*. *In silico* predictions were carried out using bioinformatics tools.
Results: One Plasmid (pP6VIM-11) was distributed in 2 contigs, a second plasmid (pPOta2VIM-11) was found in a single contig, and the last one (pP936401VIM-11) was fragmented into 4 contigs. pP6VIM-11 and pPOta2VIM-11 belonged to the IncP-1β group, displaying 64% of coverage and 83.9% of identity among them. pP936401VIM-1 plasmid corresponded to the IncN group. The bioinformatic analysis revealed that *bla*$_{VIM-11}$ was located in a class 1 integron, flanked by insertion sequences, exhibiting potential for its dissemination. However, none of the plasmids were conjugative.
Conclusion: This study corresponded to the first description and deposit of *bla*$_{VIM-11}$-harbouring plasmids in *P. aeruginosa*, which expands the limited knowledge about their molecular epidemiology.

1. Introduction

Pseudomonas aeruginosa is a leading cause of hospital-acquired infections. High morbidity-mortality rates are associated to resistant phenotypes. Carbapenem-hydrolyzing enzymes, such as class B metallo-β-lactamases (MBL), are able to inactivate most anti-pseudomonal β-lactams and are resistant to currently available β-lactamase inhibitors [1]. Different MBL have been described in *P. aeruginosa*, including VIM, IMP, AIM, SPM, GIM, SIM, DIM, and NDM. Among them, VIM (Verona integron-mediated) MBL are the most frequent enzymes in carbapenem-resistant *P. aeruginosa* isolates. To date, 86 VIM variants have been reported, belonging to five clusters (VIM-1-group, VIM-2-group, VIM-5-group, VIM-7-group, and VIM-13-group) (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#) [2]. Previous studies in Argentina showed that MBL production is responsible for ca. 10% of carbapenem resistance in *P. aeruginosa*, with VIM-2 and VIM-11 being the most prevalent MBL. Aside from this country, VIM-11 was reported in India, Malaysia, Pakistan, Saudi Arabia, and Mexico. VIM-11 differs from VIM-2 in a unique nonsynonymous mutation (N165S) and belongs to VIM-2-like cluster. VIM-11 displays greater hydrolytic efficiency for imipenem compared with meropenem; and a slightly better catalytic efficiency for ceftazidime, cefepime, and cefpirome respecting VIM-2 [3]. *bla*$_{VIM-11}$ was described as a gene cassette in the variable region of class 1 integrons, located on plasmids

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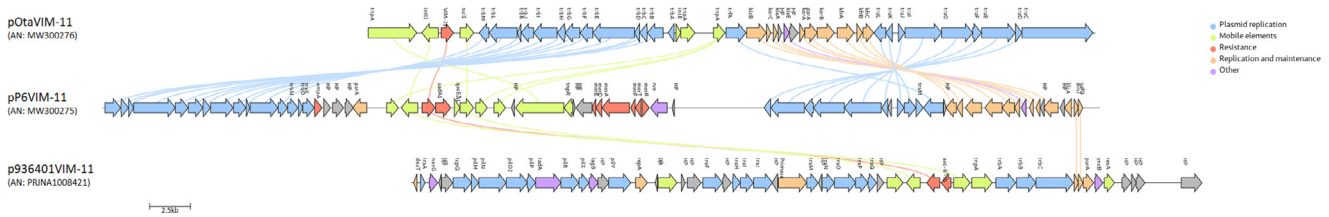

Fig. 1. Linear maps of bla_{VIM-11}-harbouring plasmids. Plasmid pP6VIM-11 (Genbank Accession no. MW300275) was recovered from *Pseudomonas aeruginosa* C6 ST635 in Argentina in 2012. Plasmid pP936401VIM-11 (Genbank Accession no. PRJNA1008421) was recovered from *P. aeruginosa* 936401 ST2735 in Argentina in 2016. Plasmid pPOta2VIM-11 (Genbank Accession no. MW300276) was recovered from *P. aeruginosa* Ota2 ST94 in Argentina in 2018. The figure was constructed using clinker and clustermap.js.

and chromosome, not only in *P. aeruginosa* but also in *Enterobacterales* [4]. To the best of our knowledge no genomic descriptions of bla_{VIM-11}-harbouring plasmids are available in literature nor National Center for Biotechnology Information (NCBI) Reference Sequences (refseq) (https://www.ncbi.nlm.nih.gov/refseq/). The aim of this study was to describe the genomic features bla_{VIM-11}-harbouring plasmids recovered from *P. aeruginosa* isolates.

Ninety-two carbapenem-resistant *P. aeruginosa* isolates recovered from inpatients in Buenos Aires from 2012 to 2018 were delivered to IBaViM Institute as part of prospective studies or to confirm the suspicion of MBL production. Twenty-three of 92 were MBL producers (VIM-2 n: 11, VIM-11 n: 11, and IMP-13 n: 1). bla_{VIM-11} harbouring plasmids were extracted using a phenol-chloroform method and transformed by electroporation into *P. aeruginosa* PAO-1 receptor strain. Plasmids from the electroporants were used as template for incompatibility groups identification using PCR-based replicon typing method. Only one plasmid from 936401 isolate was typeable and corresponded to the IncN group. When addressing the determination of toxin/antitoxin systems using the technique proposed by Mnif *et al.*, none of the plasmids could be typed. These results suggested a compelling difference between the replicons and plasmid addiction systems of *P. aeruginosa* and *Enterobacteriaceae*, for which these techniques were originally proposed. Consequently, bla_{VIM-11}-carrying plasmids from three *P. aeruginosa* isolates recovered in different periods were selected. *P. aeruginosa* C6 belonging to ST699 was recovered in 2012, *P. aeruginosa* 936401 belonging to ST2735 in 2016, and *P. aeruginosa* Ota2 belonging to ST94 in 2018. Genomic DNA from 6-PAO-1, 936401-PAO-1, and Ota2-PAO-1 electroporants were extracted using QIAamp DNA Mini Kit (Qiagen, Germany). Whole-genome sequencing was carried out using a 2 × 250 bp pair-end reads approach (NovaSeq 6000 system, Illumina, San Diego, California). *De novo* assemblies were generated using Unicycler program version 0.4.8.0 with the default parameters to consider contigs >500 bp. Reads were mapped against a reference genome of *P. aeruginosa* PAO-1 (Accession number: GCF_000006765.1) using bbmap v38.96 (https://sourceforge.net/projects/bbmap/). Reads that did not map the reference sequence were recovered and assembled *de novo* as mentioned. Plasmid pP6VIM-11 was distributed in 2 contigs, while plasmid pPOta2VIM-11 was found in a single contig, and the plasmid pP936401VIM-11 was fragmented into 4 contigs. Plasmid ORFs were annotated using RAST (http://rast.nmpdr.org) and manually curated. Plasmids are shown in Figure 1. pP6VIM-11 presented 61kb, 70 CDS, and corresponded to the IncP-1 group. The *dinJ/yafQ* toxin-antitoxin system (T-AT) was detected. A class 1 integron harbouring bla_{VIM-11} and *aadA1,* flanked by two IS*26*, was recognized. This novel structure was named In*2031* by INTEGRALL (http://integrall.bio.ua.pt/). pPOta2VIM-11 presented 44.6kb, 43 CDS, an IncP-1 replicon, no T-AT could be found. bla_{VIM-11} was the only cassette gene of a class 1 integron flanked upstream by an IS*Pa38* and downstream by a complete *trb* operon.

Manual analysis of the 4 contigs of plasmidic nature of p936401VIM-11 using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) suggested a structure of 48.5 kb in size with 53 CDS (Supp. 1). p936401VIM-11 corresponded to the IncN group and harboured the *relE/yafQ* T-AT. *aac-(6')-Ib* and *blaVIM-11* were detected as first and second cassette genes in a class 1 integron, flanked by an IS*2000* and IS*10A*.

All plasmids lacked a complete *tra* operon; however pP6VIM-11 and pPOta2VIM-11 presented an *oriT*, making these plasmids mobilizable.

IncP-1 plasmids have been identified worldwide in clinical and environmental distant phylogenetically bacterial species. pP6VIM-11 and pPOta2VIM-11 displayed 64% of coverage and 83.9% of identity among them but presented a different organization (Fig. 1). IncP-1 plasmids can be divided into 5 subgroups: α, β, γ, δ, and ε, according to *trfA* sequence [5]. In order to classify the pP6VIM-11 and pPOta2VIM-11 within IncP-1 subgroups, a phylogenetic analysis was carried out including 43 *trfA* sequences downloaded from GenBank and applying a maximum likelihood approach under the model of evolution model HKY+ γ and 1000 random bootstrap replicates (Supp. 2). Both pP6VIM-11 and pPOta2VIM-11 belonged to the main subgroup, Inc-P1β, which is represented by resistance plasmids mainly recovered from wastewater treatment plants and environmental bacteria.

This study constitutes the first full description of bla_{VIM-11}-harbouring plasmids. bla_{VIM-11} was located in class 1 integrons flanked by different IS showing the key role of IS in the integration of resistance genes into plasmids.

Considering the environmental context of these plasmids, their presence in *P. aeruginosa* is not striking given the versatility of this species, which is recovered in both clinical and environmental settings.

These plasmids were deposited in Genbank under the accession numbers MW300275 (pPC6VIM-11), PRJNA1008421 (pP936401VIM-11), and MW300276 (pPOta2VIM-11).

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Competing interests: None declared

Ethical approval: The ethics committee of FFyB-UBA approved this study (Res CD 894-2019). The isolates were delivered anonymized from Hospitals to IBaViM-FFyB-UBA in order to preserve patient identity.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.12.014.

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