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A *bla*$_{VIM-2}$ plasmid disseminating in extensively drug-resistant clinical *Pseudomonas aeruginosa* and *Serratia marcescens* isolates

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Infections caused by carbapenem-resistant *Enterobacteriaceae* isolates are an issue of major global concern (1). Genes coding for metallo-β-lactamases (MβLs) identified in clinical isolates are associated with mobile elements and subject to Horizontal Genetic Transfer (HGT) events (2-6). VIM-2 is present on numerous plasmids, but only pNOR-2000 from *P. aeruginosa* COL-1 from France (7-8), and pLD209 from *P. putida* LD209 from Argentina (9) have been completely sequenced. Here, we report the complete sequence and characterization of plasmid pDCPR1 harboring a bla_{VIM-2} gene cassette in a Tn*402*-type class 1 integron, which was isolated from two extensively drug-resistant strains: *P. aeruginosa* 802 (burn patient at the Hospital Municipal de Quemados, Argentina, 2005) and *S. marcescens* 68313 (Sanatorio Sagrado Corazón, Argentina, 2012).

Isolates were identified at the species level using VITEK 2 Compact (BioMérieux). Antimicrobial susceptibility was determined by the disk diffusion method performed in agar as recommended by the CLSI (10). DNA was isolated from *P. aeruginosa* 802 and *S. marcescens* 68313 using the Master Pure DNA purification kit (Epicentre, Madison, WI, USA). A library was prepared from 500 ng of total DNA. Sequencing was performed using an Illumina MiSeq and assembled using Ray (11). A single contig from the *S. marcescens* and three contigs from the *P. aeruginosa* corresponded to an identical plasmid sequence that was confirmed in the latter by three PCRs and sequencing (data not shown). The complete sequence of plasmid pDCPR1 was submitted to GenBank under accession number KJ577613.

pDCPR1 was 18,182 bp long. We observed that pDCPR1 is identical (except for 2 nt) to part of pLD209 (KF840720) (9), including the replicase (*repA*), the partitioning system (*trfB*, *parA* and *parB*), the Tn*402*-like class 1 integron harboring a bla_{VIM-2} gene cassette and several hypothetical proteins. Because only genes involved in conjugal transfer and

virulence from pLD209 (20,221 bp) are deleted in pDCPR1 (Figure 1), we discarded the possibility of a cointegration process in the formation of pLD209.The two plasmids appear to be a novel replicon type. Although not conjugative, pDCPR1 retains the putative *oriT* of pLD209 (TATCCTG'C) and should be mobilizable.

P. putida LD209 was isolated in Argentina in 2009 and *P. aeruginosa* 802 in Argentina in 2005. Therefore, the presumptive deletion of pLD209 which gave rise to pDCPR1 occurred before 2005. Since then, it is likely that both plasmids, pLD209 and pDCPR1 are circulating in Argentinean samples. Plasmid pDCPR1 was found in two different genera (*Pseudomonas* and *Serratia*) 7 years apart and no SNPs nor indels were found. It was capable of surviving in nosocomial environments while maintaining its structure. These features suggest that bacteria have found an efficient genetic platform for spreading carbapenem resistance among clinical species.

This work not only characterizes a plasmid circulating in *P. aeruginosa* and *S. marcescens*, but also it is the first report of a bla_{VIM-2} gene cassette in *S. marcescens* in Argentina. The acquisition of plasmid pDCPR1 by *S. marcescens* reinforces the global concern about the dissemination of broad-host-range plasmids involved in the evolution of pandrug resistance in almost all human pathogenic species in strongly selective environments.

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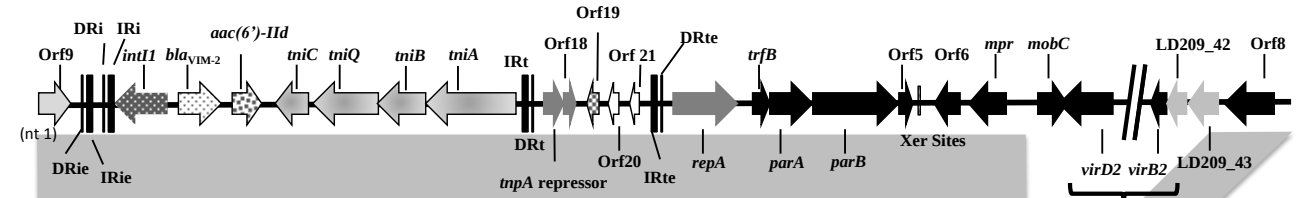
Figure 1. Structure of plasmids pLD209 and pDCPR1. The gray solid bars represent identical regions; position 1 in the figure corresponds to position 1 in the GenBank entry for pLD209 (KF840720.1, Marchiaro PM et al 2014) and position 7263 in the GenBank entry for pDCPR1 (KJ577613). Most of the additional region of pLD209 (vir Genes) has been omitted for better resolution. The 25-nt IRi and IRt represent the ends of the Tn*402*-like transposon; DRi and DRt (5'-GTTTT-3') are the initial and terminal direct repeats; the 38-nt external IRs, IRie and IRte and the external direct repeats, DRie and DRte (5'-TATTC-3') are as defined in pLD209 (KF840720.1); orf names from pDCPR1 are used in pLD209 to reflect identities.

pLD209



pDCPR1