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

Elisabet Vilacoba[a], Cecilia Quiroga[a], Mariano Pistorio[b], Angela Famiglietti[c], Hernán Rodríguez[c], Jaime Kovensky[d], Maxime Déraspe[e], Frederic Raymond[e], Paul H. Roy[e], Daniela Centrón[a#].

[a]Instituto de Microbiología y Parasitología Médica, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Tecnológicas (IMPaM, UBA-CONICET), Facultad de Medicina, Buenos Aires;

[b]Instituto de Biotecnología y Biología Molecular, CCT-CONICET-La Plata - Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata;

[c]Laboratorio de Bacteriología del Hospital de Clínicas, Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina;

[d]Hospital Municipal de Quemados, Buenos Aires, Argentina;

[e]Département de Biochimie, de Microbiologie, et de Bio-informatique, Faculté des Sciences et de Génie, Université Laval, Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec, Québec, Canada.

[#]Corresponding author. Mailing adress: Laboratorio de Investigaciones de los Mecanismos de Resistencia a Antibióticos, IMPaM, UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina. Phone: 54-115950-9500 x 2182. E-mail: dcentron@gmail.com

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.

D.C., C.Q. and M.P. are career investigators of C.O.N.I.C.E.T. E.V. is recipient of a doctoral fellowship from C.O.N.I.C.E.T. This study was supported by a grant from BID/OC ANPCyT (0034) and by UBACYT (20020100100417) Programación 2011-2014 given to D.C., Buenos Aires, Argentina.

1. **Savard P, Carroll KC, Wilson LE, Perl TM.** 2013. The challenges of carbapenemase-producing enterobacteriaceae and infection prevention: protecting patients in the chaos. Infect Control Hosp Epidemiol **34:**730-739.
2. **Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR.** 2004. Molecular characterization of a β-lactamase gene, *bla*$_{GIM-1}$, encoding a new subclass of metallo-β-lactamase. Antimicrob Agents Chemother **48:**4654-4661.
3. **Nastro M, Monge R, Zintgraff J, Vaulet LG, Boutureira M, Famiglietti A, Rodriguez CH.** 2012. First nosocomial outbreak of VIM-16-producing *Serratia marcescens* in Argentina. Clin Microbiol Infect **19:**617-619.
4. **Peleg AY, Franklin C, Bell JM, Spelman DW.** 2005. Dissemination of the metallo-β-lactamase gene *bla*$_{IMP-4}$ among gram-negative pathogens in a clinical setting in Australia. Clin Infect Dis **41:**1549-1556.
5. **Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Gottig S, Hunfeld KP, Seifert H, Witte W, Higgins PG.** 2011. Molecular characterization of *bla*$_{NDM-1}$ in an *Acinetobacter baumannii* strain isolated in Germany in 2007. J Antimicrob Chemother **66:**1998-2001.
6. **Wachino J, Yoshida H, Yamane K, Suzuki S, Matsui M, Yamagishi T, Tsutsui A, Konda T, Shibayama K, Arakawa Y.** 2011. SMB-1, a novel subclass B3 metallo-β-lactamase, associated with IS*CR1* and a class 1 integron, from a carbapenem-resistant *Serratia marcescens* clinical isolate. Antimicrob Agents Chemother **55:**5143-5149.
7. **Bonnin RA, Poirel L, Nordmann P, Eikmeyer FG, Wibberg D, Puhler A, Schluter A.** 2013. Complete sequence of broad-host-range plasmid pNOR-2000 harbouring the metallo-β-lactamase gene *bla*$_{VIM-2}$ from *Pseudomonas aeruginosa*. J Antimicrob Chemother **68:**1060-1065.

8. **Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother **44:**891-897.

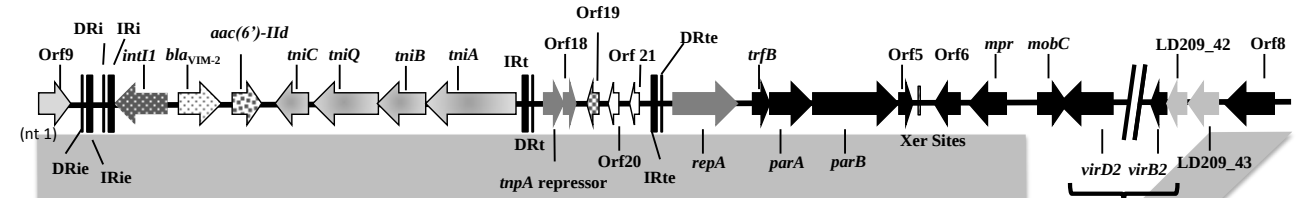
9. **Marchiaro PM, Brambilla L, Moran-Barrio J, Revale S, Pasteran F, Vila AJ, Viale AM, Limansky AS.** 2014. The complete nucleotide sequence of the carbapenem resistance-conferring conjugative plasmid pLD209 from a *Pseudomonas putida* clinical strain reveals a chimeric design formed by modules derived from both environmental and clinical bacteria. Antimicrob Agents Chemother **58:**1816-1821.

10. **CLSI.** 2014. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne (PA).

11. **Boisvert S, Laviolette F, Corbeil J.** 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. J Comput Biol **17:**1519-1533.

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