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Characterisation of bla_{KPC-2}-harbouring plasmids recovered from *Pseudomonas aeruginosa* ST654 and ST235 high-risk clones

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ABSTRACT

Objective: The main objectives were to describe two bla_{KPC-2} plasmids recovered from *Pseudomonas aeruginosa* isolates belonging to the ST654 and ST235 high-risk clones, and to compare with complete sequences of bla_{KPC-2} harbouring plasmids available in public databases.
Methods: Antimicrobial susceptibility was determined according to CLSI (Clinical and Laboratory Standards Institute) guidelines. Genomes were sequenced using an Illumina MiSeq platform, and bla_{KPC-2} plasmid sequences were achieved using MinION platform. Sequences were analysed using Unicycler and RAST. In silico predictions of the isolates sequence type (ST), antimicrobial resistance genes, plasmid replicon typing and MOB relaxases were fulfilled using bioinformatics tools.
Results: PA_2047 and PA_HdC isolates corresponded to the high-risk clones ST654 and ST235, respectively. The carbapenem resistance was mediated by KPC-2. Both bla_{KPC-2} harbouring plasmids, pPA_2047 and pPA_HdC, were different among them, non-conjugative and untypable by PlasmidFinder. pPA_2047 presented high identity with a Pae-13 plasmid, and these both located bla_{KPC-2} in Tn*4401b* isoform. pPA_HdC displayed a novel architecture, and the genetic context of bla_{KPC-2} was original. Besides the bla_{KPC-2} gene, resistance genes to aminoglycosides and quinolones were detected, including the novel phosphotransferase CrpP in PA_HdC.
Conclusion: This study expands the limited knowledge about the molecular epidemiology of bla_{KPC-2} in *P. aeruginosa* from Latin America. Two novel plasmids harbouring bla_{KPC-2} were described that were untypable by their incompatibility group. The plasmid recovered from *P. aeruginosa* PA_HdC (ST235) displayed a novel architecture and an original context for bla_{KPC-2}. On the other hand, the genetic platform carrying bla_{KPC-2} in *P. aeruginosa* PA_2047 (ST654) seems to a be a classical one.

Pseudomonas aeruginosa is a leading cause of hospital-acquired infections. High morbidity-mortality rates are associated to multidrug (MDR) or extensively drug (XDR) resistant phenotypes, due in part to its remarkable capacity to develop resistance through multiple mechanisms. MDR and XDR clinical isolates of *P. aeruginosa* frequently belong to successful high-risk clones worldwide spread, including sequence type (ST) ST111, ST175, ST233, ST235, ST244, ST277, ST298 (CC445), ST308, ST357 and ST654 [1]. These lineages represent matter of major concern in several clinical settings, being frequently recognised as producer of carbapenem-hydrolysing enzymes such as class B metallo-β-lactamases (MBL) able to degrade most anti-pseudomonal β-lactams and to resist the action of currently available β-lactamase inhibitors [2]. In Argentina, MBL are the most prevalent carbapenemases in *P. aerug-*

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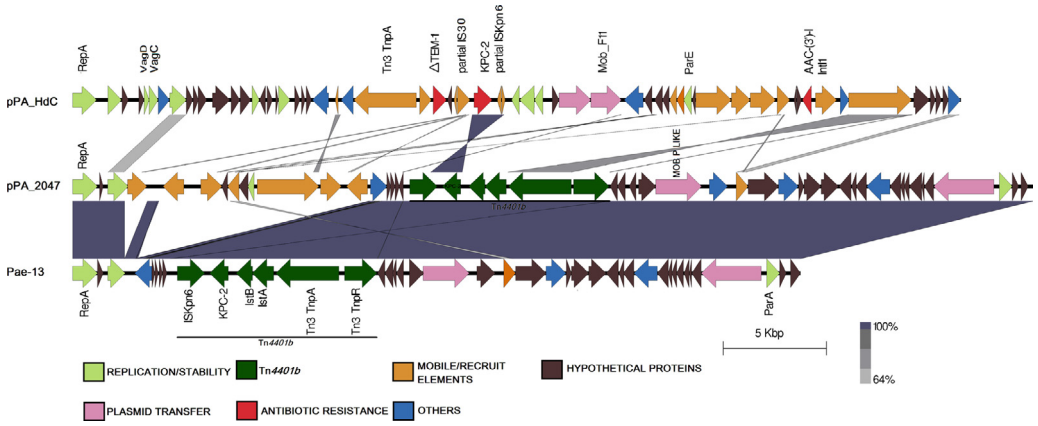

Fig. 1. Linear maps of bla_{KPC-2} harbouring plasmids. Plasmid pPA_HdC (Genbank accession no. OL780449) was recovered from *P. aeruginosa* ST635 in Argentina in 2018. pPA_2047 (GenbanK accession no. MN082782) was recovered from *P. aeruginosa* ST654 in Argentina in 2008. Plasmid Pae-13 (Genbank accession no. MT949191) was recovered from *P. aeruginosa* ST654 in Chile and reported in 2020. The figure was constructed using Easyfig tool.

inosa, although class A KPC-type carbapenemases have been sporadically detected since 2008 [3]. Both MBL and KPC are encoded by mobile genes typically located on plasmids, which can be horizontally transferred with great clinical and epidemiological impact [2]. At a global scale, bla_{KPC} from *P. aeruginosa* have been unfrequently described, and knowledge about its mobilising platforms in this species remains scarce.

The aim of this study was to describe two bla_{KPC-2} plasmids recovered from *P. aeruginosa* isolates belonging to the ST654 and ST235 high-risk clones, and to compare them with complete sequences of bla_{KPC-2} harbouring plasmids available in public databases.

PA_2047 and PA_HdC were isolated from respiratory secretions obtained from inpatients admitted at two hospitals in Buenos Aires, in 2008 and 2018, respectively [3]. Antimicrobial susceptibilities were determined by disk diffusion except for colistin where broth microdilution test was used, in accordance with CLSI guidelines (https://clsi.org/all-free-resources/). Both isolates evidenced an XDR phenotype, displaying resistance to all ß-lactams, quinolones and aminoglycosides but remaining susceptible to colistin.

Genomic DNA was extracted from overnight cultures of PA_2047 and PA_HdC isolates [4] and subjected to whole-genome sequencing with the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA), using a 2 × 250 or 2 × 300 bp paired-end approach, and with the MinION platform (Oxford Nanopore Technologies). Hybrid de novo assemblies were generated using Unicycler v.0.4.6 [5]. PubMLST analysis of the assemblies evidenced that PA_2047 belonged to ST654, while PA_HdC corresponded to ST235 (https://pubmlst.org/organisms/pseudomonas-aeruginosa). WGS resistome analysis, performed using ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder), revealed the presence of aminoglycoside modifying enzymes coding genes [PA_2047: *aph(3′)-IIb;* PA_HdC: *aadA6, aph(3′)-IIb, aac(6′)-29b*]. Resistance to quinolones was mediated by the mutation S87L in ParC in both isolates; PA_2047 carried mutations in GyrA (deletion of the amino acids 0-6 and 908), whereas PA_HdC harboured the novel phosphotransferase *crpP* gene, which mediates ciprofloxacin resistance. In both isolates a plasmid-borne bla_{KPC-2} was detected as the acquired resistance marker for carbapenems.

Annotation of plasmid sequences was carried out using RAST and manually curated. The KPC-encoding plasmid from PA_2047 (pPa_2047) was 46.22 kb in length, with 50 predicted CDS and 60% G+C content, whereas the one from PA_HDC (pPA_HDC) was 42.75 kb in length, with 52 predicted CDS and 59% G+C content. Both plasmids were untypable accordingly to PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/). However, in silico typing for MOB relaxases, using oriTfinder (https://bioinfo-mml.sjtu.edu.cn/oriTfinder/), revealed that pPA_HdC clustered in the MOB_F11 family, frequently associated to MBL harbouring plasmids from *P. aeruginosa* [4], while pPA_2047 presented a MOB_P_like relaxase. Transfer experiments failed in yielding transconjugants using *Escherichia coli* J53 as recipient, in accordance with the lack of the complete transfer operon in both PA_HdC and pPA_2047. Similarly, electrotransformation experiments using *P. aeruginosa* PAO-1 and *E. coli* DH5α as recipients were unsuccessful.

A total of 19 bla_{KPC-2} carrying-plasmid sequences were found using NCBI refseq (https://www.ncbi.nlm.nih.gov/refseq/) and Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). These plasmids were described since 2006 in *P. aeruginosa* from Brazil, Chile, China, Colombia, and the United States; four of them belonged to IncU group, three to IncP-6, one to IncQ, and the remaining 11 were untypeable (Supplementary Materials).

The sequence comparison of pPA_2047 and PA_HdC against these 19 plasmids showed that pPA_2047 displayed 100% identity and 75% coverage with Pae-13, an untypable plasmid from *P. aeruginosa* also belonging to ST654, reported in 2020 in Chile [6]. In both plasmids from ST654 isolates, bla_{KPC-2} was embedded in an intact Tn*4401b* isoform. Conversely, pPA_HdC presented a novel architecture, with no significant identity with any known bla_{KPC-2} harboring plasmids described in *P. aeruginosa* or other gram-negative bacilli so far. pPA_HdC presented a novel bla_{KPC-2} genetic context (5'-3': partial Tn*3* -Δ*bla*TEM-1 – partial IS30 family transposase - bla_{KPC-2} – partial IS*Kpn6)* (Fig. 1).

In conclusion, this study describes two bla_{KPC-2} harbouring plasmids, untypable by their incompatibility group, recovered from *P. aeruginosa* ST654 and ST235 high-risk clones in Argentina, expanding the limited knowledge about the molecular epidemiology of bla_{KPC-2} in *P. aeruginosa* from Latin America. The plasmid recovered from *P. aeruginosa* PA_HdC (ST235) displayed an original context for bla_{KPC-2}. On the other hand, the genetic platform carrying bla_{KPC-2} in *P. aeruginosa* PA_2047 (ST654), which has been circulating at least since 2008 in Argentina, resembles that recently reported in Chile.

The genome assembly of PA_2047 and the sequence of the plasmid pPA_2047 were submitted to GenBank under accession numbers JAIVGE000000000.1 and MN082782, respectively. The genome assembly of PA_HdC and the sequence of the plasmid pPA_HdC were submitted to GenBank under accession numbers JAJFEZ000000000 and OL780449, respectively.

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Competing interests

The authors declare that there are no conflicts of interest.

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's identity.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.04.017.

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