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Genome Note

Integral genomic description of *bla*$_{NDM-5}$-harbouring plasmids recovered from *Enterobacterales* in Argentina.

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ABSTRACT

Objective: To characterise four *bla*$_{NDM-5}$-harbouring plasmids recovered in *Enterobacterales* isolated in Argentina.
Methods: DNA was sequenced by Illumina and Oxford Nanopore Technologies, assembled using Unicycler, analysed using PlasmidFinder, MOB-Typer, IslandViewer4, and Resfinder, and visualised by Proksee and Clinker. *bla*$_{NDM-5}$-harbouring plasmids were compared with similar deposited plasmids using PLSDB.
Results: Two plasmids belonged to incompatibility group IncFII where *bla*$_{NDM-5}$ was located in a previously described genetic context, immersed in an antimicrobial resistance island (ARI). Local IncFII plasmids displayed high similarity (≥90% shared hashes (sh)), with four deposited in PLSDB. The other two local plasmids belonged to the multi-replicon group IncFIB-HI1B, harbouring *bla*$_{NDM-5}$ in a novel variant of the genetic context. Both multi-replicon plasmids presented two ARIs, one containing *bla*$_{NDM-5}$ in addition to another antimicrobial resistance marker (ARM), and the second ARI carrying *bla*$_{CTX-M-15}$ and a class 1 integron. Plasmids deposited in PLSDB showed low similarity to local multi-replicon plasmids. The most similar plasmids (n = 5) displayed less than 60% shared hashes and showed the same Inc groups but lacked ARM.
Conclusions: This study broadens the limited understanding of *bla*$_{NDM-5}$-harbouring plasmids in Latin America. Furthermore, it represents the first description of *bla*$_{NDM-5}$ in a novel variant of common genetic platform and its location in multi-replicon IncFIB-IncHI1B plasmids, which were not previously associated with any ARM.

Carbapenemase-producing *Enterobacterales* are a rising and worrisome threat to public health (https://www.who.int). New Delhi metallo-β-lactamases (NDMs) have replaced *Klebsiella pneumoniae* carbapenemase (KPC) as the most prevalent enzymes in our region [1]. NDMs confer resistance to penicillins, cephalosporins, and carbapenems, but not to monobactams, and are not inhibited by classic (such as clavulanic acid) or new β-lactamase inhibitors (such as avibactam, vaborbactam, and relebactam). *bla*$_{NDM}$ is commonly located on plasmids mostly associated with other antimicrobial resistance markers (ARMs). *bla*$_{NDM-5}$ was first reported in 2011 in the UK and, together with *bla*$_{NDM-1}$, constitutes the most prevalent variant worldwide. *bla*$_{NDM-5}$ has mainly been described in IncX3 plasmids as well as other Inc plasmids such as IncFII and IncI1, and in multi-replicon plasmids [2,3].

In Argentina, *bla*$_{NDM-5}$ was first reported in 2021 in a clinical *Escherichia coli* isolate (Ec265) [4]. Genomic descriptions of plasmids containing *bla*$_{NDM-5}$ from our region are scarce in the literature and there are no deposits available in PLSDB (https://ccb-microbe.cs.uni-saarland.de/plsdb).

The aim of this study was to fully characterise *bla*$_{NDM-5}$-harbouring plasmids recovered from *Enterobacterales* isolates in Argentina.

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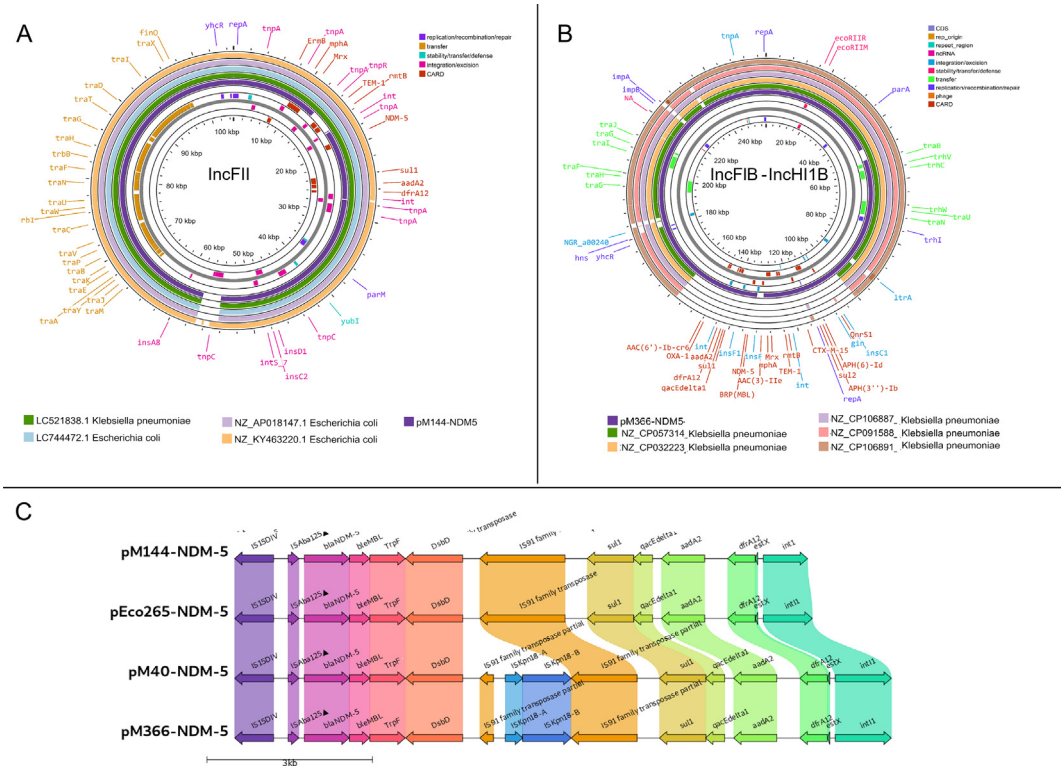

Fig. 1. (A) Comparison among local and most similar IncFII plasmids from PLSDB. pEco265-NDM-5 was used as a template. (B) Comparison among local and most similar multi-replicon IncFIB-HI1B from PLSDB. pM40-NDM-5 was used as a template. Graphics were generated using Proksee, with relevant features added using CARD and mobileOG-DB. (C) bla_{NDM-5} genetic context. Graphics were generated using Clinker.

Three *Klebsiella pneumoniae* (M40, M144, and M366) and one *E. coli* (Ec265) isolates were recovered from in-patients between 2018 and 2022 at three hospitals in Buenos Aires Metropolitan Area (Suppl. data #1). These isolates were resistant to aminopenicillins, cephalosporins, carbapenems, fluoroquinolones, and trimethoprim-sulfamethoxazol. Only Ec265 was susceptible to aztreonam.

All isolates were subjected to plasmid conjugation assays using *E. coli* J53Az^R as the recipient strain. Transconjugants were selected on Müeller-Hinton agar plates containing sodium azide (150 µg/mL) and ceftriaxone (10 µg/mL). bla_{NDM-5}-harbouring plasmids were successfully transferred, except from *K. pneumoniae* M366.

Genomic DNA was extracted from transconjugants and M366 using commercial kits and sequenced by Illumina (Illumina Inc., San Diego, CA, USA) and Oxford Nanopore Technologies (Oxford, UK). Unicycler, PlasmidFinder, MOB-Typer, Island Viewer4, and Resfinder were used for sequence assembly and analysis. Both BAKTA and manual curation were used for genome annotation. bla_{NDM-5}-harbouring plasmids were compared with those already deposited using PLSDB, and the circular plasmids with higher shared hashes (sh) were further analysed. The genetic context of bla_{NDM-5} was studied using the blastn tool against the National Center for Biotechnology Information database (https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi). Proksee and Clinker were used for visualisation.

All plasmids presented complete *tra* genes except for pM366-NDM-5, in which *traK* was interrupted and *traE* was absent, consistent with conjugation results. Two plasmid types were detected regarding their maintenance and structural features. pEco256-NDM-5 and pM144-NDM-5 belonged to IncFII, coded for a MOB-F relaxase and a MazE addiction system, and presented a size of 105 and 98 kb, respectively (Suppl. data #1). The IncFII group was previously reported in bla_{NDM-5}- and bla_{NDM-1}-harbouring plasmids in 2017 and 2012, respectively [5] (https://ccb-microbe.cs.uni-saarland.de/plsdb). On the other hand, pM40-NDM-5 and pM366-NDM-5 were associated with multi-replicon IncFIB-IncHI1B plasmids, coding for MOB-H relaxase and a ParB-like addiction system of 252 and 258 kb in size, respectively (Suppl. data #1). It is worth noting that multi-replicon IncFIB-HI1B plasmids were not previously associated with ARM in the analysed database.

All plasmids presented the previously described bla_{NDM-5} genetic context (5′–3′: IS*15DIV*, an isoform of IS*26*, -IS*Aba125*Δ-bla_{NDM-5}-*bleMBL-trpF-dsbD*-IS*91* family transposase), followed by a class 1 integron containing *dfrA12* and *aad2* in its variable region [3,4]. However, the IS*91* transposase coding gene was interrupted by IS*Kp18* in the IncFIB-HI1B plasmids, resulting in a novel variant of this genetic context (Fig. 1). These plasmids, with the exception of pM144-NDM-5, harboured *rmt*B, which codes for a 16S ribosomal RNA methyltransferase capable of conferring high-level resistance to aminoglycosides.

Multi-replicon plasmids (pM40-NDM-5 and pM366-NDM-5) also carried $bla_{CTX-M-15}$, which codes for an extended spectrum β-lactamase able to inactivate penicillins, cephalosporins, and monobactams. Furthermore, these plasmids harboured *aac(6′)-Ib-cr*, which are involved in aminoglycoside and fluoroquinolone resistance, and a *ter* operon responsible for tellurite resistance. In both multi-replicon plasmids, ARMs were distributed in two antimicrobial resistance islands (ARIs): 23 kb ARI-1 contained bla_{NDM-5}, in addition to another seven ARMs; and ARI-2 presented as 8 kb in size and harboured $bla_{CTX-M-15}$ and a class 1 integron containing *aph(6)-Id, aph(3′′)-Ib*, and *sul2* (Suppl. data #3). Plasmids deposited in PLSDB showed low similarity to local multi-replicon IncFIB-HI1B plasmids. The most similar plasmids ($n = 5$) displayed 74–76% of coverage and 58–60% sh against those from local isolates, sharing the same Inc groups but lacking ARM (Fig. 1) (Suppl. data #2).

Additionally, local IncFII plasmids only harboured ARI-1. These plasmids displayed high similarity (≥90% sh) with four plasmids deposited in PLSDB, sharing the same Inc group as well as ARI-1 (Fig. 1) (Suppl. data #2 and #3).

In conclusion, this study provides an integral characterisation of bla_{NDM-5}-harbouring plasmids in Argentina, including the first isolate carrying this marker, which additionally broadens the limited understanding of their molecular epidemiology in Latin America. Furthermore, we have described bla_{NDM-5} in a novel variant of the common genetic platform and its location in multireplicon IncFIB-IncHI1B plasmids, reporting its association with ARM.

Plasmid sequences were deposited in GenBank under the following accession numbers: PQ247031 (pM40-NDM-5), PQ247032 (pM366-NDM-5), PQ241462 (pEco265-NDM-5), and PQ241463 (pM144-NDM-5).

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Declaration of competing interests: None declared.

Ethical approval: The ethics committee of *Facultad de Farmacia y Bioquímica - Universidad de Buenos Aires* (FFyB-UBA) approved this study (res. CD 894–2019). The isolates were delivered in an anonymised manner from hospitals to IBaViM Institute of 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.2024.10.258.

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