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Genome Note

Emergence of colistin resistance in *Klebsiella pneumoniae* ST15 disseminating bla_{KPC-2} in a novel genetic platform

Verónica E. Álvarez[a], Mariana G. Massó[a], Gabriela D'amico González[a], Anahí S. Gambino[a], Camila A. Knecht[a], Bárbara Prack Mc Cormick[a,b], Carolina Leguina[a], María Piekar[a], Tomás Poklepovich[c], Josefina Campos[c], Sonia Arduino[d], Daniela Centrón[a], María P. Quiroga[a,*]

[a] *Laboratorio de Investigaciones en Mecanismos de Resistencia a Antimicrobianos, Instituto de Investigaciones en Microbiología y Parasitología Médica, Facultad de Medicina, Universidad de Buenos Aires–Consejo Nacional de Investigaciones Científicas y Técnicas (IMPaM, UBA-CONICET), Ciudad Autónoma de Buenos Aires, Argentina*

[b] *Universidad Nacional de Lomas de Zamora, Facultad de Ciencias Agrarias, RP N°4 km 2 (1836), Llavallol, Buenos Aires, Argentina*

[c] *Plataforma de genómica y bioinformática, INEI-ANLIS 'Dr Carlos G. Malbrán', Ciudad Autónoma de Buenos Aires, Argentina*

[d] *CentraLab, Ciudad Autónoma de Buenos Aires, Argentina*

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ABSTRACT

Objectives: Isolation of colistin- and carbapenem-resistant *Klebsiella pneumoniae* (CCR-Kp) is increasing in hospital settings worldwide, which is related to increased morbidity, mortality and healthcare costs. The aim of this work was to perform whole-genome sequencing (WGS), genomic and phylogenetic analysis, and conjugation assays of an extensively drug-resistant (XDR) CCR-Kp isolate from Argentina.

Methods: WGS of strain KpS26 isolated from a bloodstream infection was performed using Illumina MiSeq-I, and de novo assembly was achieved using SPAdes v.3.11. A maximum likelihood tree was created using MEGA7 based on core genome single nucleotide polymorphisms from whole-genome alignment of *K. pneumoniae* isolates identified in silico as sequence type 15 (ST15). The resistome, plasmids and integrons were analysed using ResFinder, AMRFinderPlus, ISfinder, plasmidSPAdes, PlasmidFinder and IntegronFinder. Standard conjugation was performed.

Results: KpS26 belonged to ST15, which is less common than ST258, ST25 and ST11 that are globally reported as responsible for CCR-Kp outbreaks. Fourteen transferable antimicrobial resistance genes (ARGs), including bla_{KPC-2} in a novel genetic platform transferable by conjugation, were detected contributing to the XDR phenotype. The amino acid substitution T157P in the protein encoded by the *pmrB* gene of KpS26, previously reported as being responsible for resistance to colistin in *K. pneumoniae* lineages globally disseminated, was also identified in this strain.

Conclusion: The XDR CCR-Kp isolate analysed here shows that ST15 is also disseminating bla_{KPC-2} in Argentina alongside other ARGs, evidencing that KPC epidemiology continues to be shaped by intricate and assorted ways of lateral gene transfer.

The worldwide epidemiology of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* (KPC-Kp) shows that different lineages are circulating in different geographical regions, with sequence type 258 (ST258) being predominant in Europe and the USA and ST11 being the most frequent in East Asia [1]. In Argentina, a change in epidemiology has been observed in the last 5 years, since more virulent lineages were detected in a multicentre study displacing KPC-Kp ST258 isolates [2]. ST15 was recently identified as the cause of several nosocomial infections, including hospital outbreaks of carbapenem-resistant *K. pneumoniae* (CR-Kp) in Asia, Europe, Africa and, recently, Brazil [3]. The aim of this work was to carry out whole-genome sequencing of an extensively drug-resistant (XDR) CR-Kp from Argentina to

* Corresponding author. Dept. Microbiología, IMPaM (UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, P-12, Ciudad Autónoma de Buenos Aires, Argentina. Tel.: +54 11 5950 9500 ext. 2171.
E-mail address: quirogamp@gmail.com (M.P. Quiroga).

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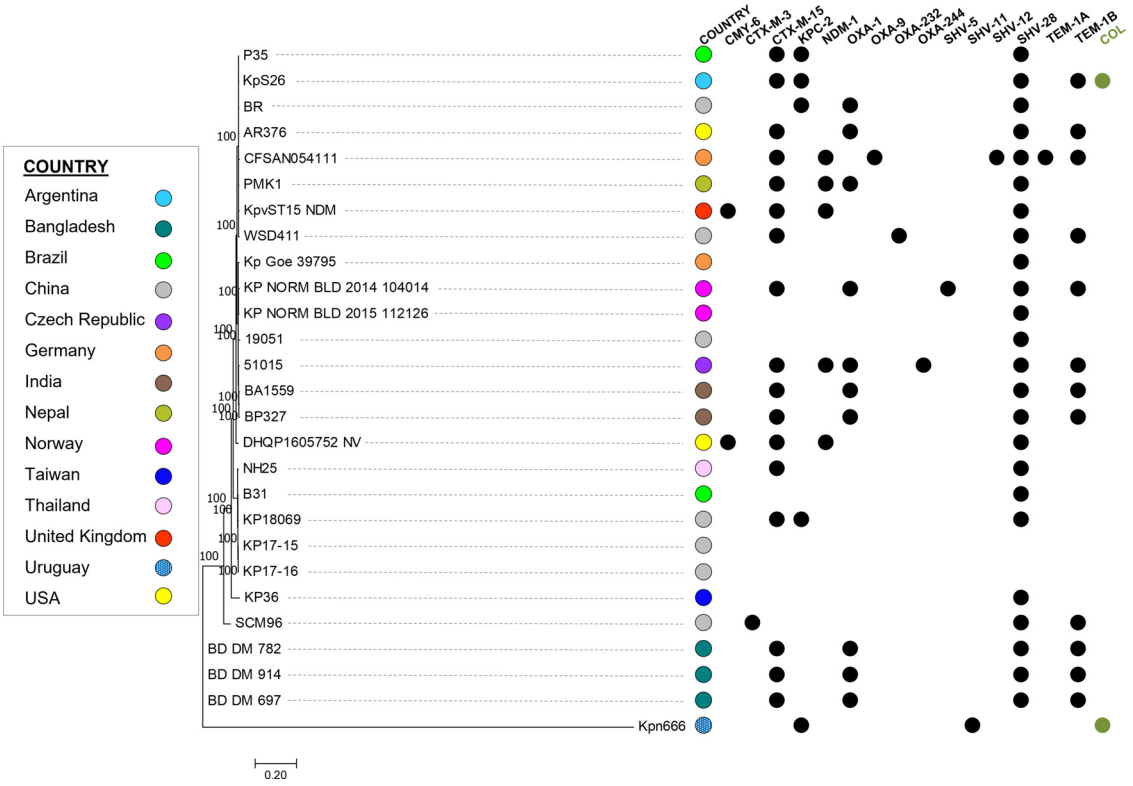

Fig. 1. Maximum likelihood tree based on the single nucleotide polymorphisms (SNPs) of the core genome of *Klebsiella pneumoniae* KpS26 and the 25 complete genomes of *K. pneumoniae* ST15 from GenBank and *K. pneumoniae* Kpn666 (ST258) as outgroup. The complete genomes of *K. pneumoniae* were downloaded from GenBank (13 October 20). Multilocus sequence typing (MLST) was performed in silico using the MLST database and schema for *K. pneumoniae* at the Institut Pasteur MLST website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The phylogenetic tree was created with the GTR model and 100 bootstraps using MEGA7. The strains used to build the tree were 19051 (**CP022023.1**), 51015 (**CP050376.1**), AR376 (**CP029137.1**), B31 (**CP035929.1**), BA1559 (**CP036187.1**), BD_DM_697 (**CP046939.1**), BD_DM_782 (**CP046381.1**), BD_DM_914 (**CP046949.1**), BP327 (**CP036335.1**), BR (**CP015990.1**), CFSAN054111 (**CP028176.1**), DHQP1605752_NV (**CP022127.1**), Kp_Goe_39795 (**CP018458.1**), KP_NORM_BLD_2014_104014 (**CP034045.1**), KP_NORM_BLD_2015_112126 (**CP034053.1**), KP17-15 (**CP034076.1**), KP17-16 (**CP034077.1**), KP18069 (**CP059889.1**), KP36 (**CP017385.1**), Kpn666 (**PVNV00000000.1**), KpS26 (**JAHQJD000000000.1**), KpvST15_NDM (**CP040593.1**), NH25 (**CP024874.1**), P35 (**CP053035.1**), PMK1 (**CP008929.1**), SCM96 (**CP028716.1**) and WSD411 (**CP045674.1**). Resistome analysis was done using information from the entire genomes, not only the core genome. Country of isolation and presence of antimicrobial resistance genes encoding CMY-6, CTX-M-3, CTX-M-15, KPC-2, NDM-1, OXA-1, OXA-9, OXA-232, OXA-244, SHV-5, SHV-11, SHV-12, SHV-28, TEM-1A, TEM-1B and colistin resistance (COL) are detailed for each strain.

identify acquired antimicrobial resistance genes (ARGs) as well as epidemiological and phylogenetic traits. Since the isolation of colistin-resistant CR-Kp (CCR-Kp) is growing in hospital settings in our country, the XDR CCR-Kp isolate KpS26 was selected for further studies. KpS26 was isolated from a bloodstream infection of a 28-year-old male inpatient in a hospital setting in Ciudad Autónoma de Buenos Aires in February 2020. KpS26 showed resistance to imipenem, meropenem, aztreonam, ertapenem, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, sulfamethoxazole, trimethoprim, chloramphenicol, piperacillin/tazobactam, tetracycline, ciprofloxacin, streptomycin and colistin according to Clinical and Laboratory Standards Institute (CLSI) 2020 breakpoints. KpS26 showed susceptibility to gentamicin, tigecycline, minocycline and fosfomycin. Genomic DNA sequencing, de novo assembly, gene prediction and annotation, contig orientation, multilocus sequence typing (MLST), maximum likelihood phylogenetic tree construction, genomic, plasmid, insertion sequence (IS) and integron content analysis, and conjugation were performed as described previously [3,4]. The resistomes of all of the strains analysed here were assessed using ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/) and AMRFinderPlus (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/). Contigs were re-ordered using the ST15 genome *K. pneumoniae* PMK1 (**CP008929.1**). The draft genome sequence of KpS26 consisted of 151 contigs, a total sequence of 5 693 576 bp with an N_{50} contig size of 188 484 bp. The GC% average was 57.04%. A total of 5342 coding sequences (CDS) and 121 RNAs were identified. MLST classified KpS26 as ST15. A total of 14 transferable ARGs were identified in the genome of KpS26 [*aadA2, aph(3′′)-Ib, aph(6)-Id,* $bla_{\text{CTX-M-15}}$, $bla_{\text{KPC-2}}$, $bla_{\text{TEM-1B}}$, *fosA, catA1, oqxA, oqxB, sul1, sul2, dfrA12* and *dfrA14* with 100% identity in 100% query cover to previous reports]. Transfer of the $bla_{\text{KPC-2}}$ gene was carried out by biparental conjugation as previously described [3], with sodium azide-resistant *Escherichia coli* J53 as the recipient strain. Transconjugants were selected on Luria–Bertani agar supplemented with sodium azide (100 μg/mL) plus meropenem (1 μg/mL); transconjugants showed resistance to aztreonam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem and trimethoprim/sulfamethoxazole. The $bla_{\text{KPC-2}}$ gene was located on a plasmid transferable by conjugation; BLASTn analysis identified that $bla_{\text{KPC-2}}$ was found in node 49 of 9097 bp in length, in a ΔΔTn*1721*-like–$bla_{\text{KPC-2}}$-related structure rendering a novel genetic platform as IS*4321*–Tn*21tnpA*–Tn*21tnpR*–Tn*1721* IR_{L1}–*ISKpn6*-like–$bla_{\text{KPC-2}}$–Tn*3*. The 3786 bp of this arrangement, IS*4321*–IS*4321* IRL–Tn*21tnpA*–Tn*21tnpR* (positions 1–3786 from node 49), showed 100% of query cover and 99.97% identity with plasmid pNDM-MAR from ST15 NDM *K. pneumoniae* strain isolated in Morocco (**JN420336.1**, positions 128 556–132 341). The remaining 4051 bp downstream of $bla_{\text{KPC-2}}$, Tn*1721* IR_{L1}–*ISKpn6*-like (positions 3787–7837 from node 49), showed 100% of query cover and 100% identity with pHS092839 from KPC-Kp HS092839 reported in China (**KF724506.1**, positions 3957–8007). Upstream

of bla_{KPC-2}, a total of 378 bp were found in node 49. The flanking 106 bp were identical to pHS092839 (**KF724506.1**, positions 8890-8995) and the remaining 272 bp corresponded to Tn*3*; insertion of IS*Kpn8* was not detected (**KF724506.1**). Three gene cassettes were also identified: *dfrA14* within a class 1 integron and *dfrA12–aadA2* in a cluster of *attC* sites lacking integron-integrases (CALIN). The protein encoded by the *pmrB* gene had the amino acid substitution T157P previously reported as being responsible for overexpression of *pmrCAB* and *pmrHFIJKLM* operons leading to resistance to colistin [5]. Also, KpS26 harboured the multidrug efflux pump gene *kdeA*, which is similar to the deduced amino acid of MdfA of *E. coli* that confers resistance to macrolides. Although the fosfomycin resistance gene *fosA* (**NG_047881.1**) from the FosA5 family fosfomycin resistance glutathione transferase was found, no reduced susceptibility to this antibiotic was identified in this strain. The *gyrA* mutations leading to D87A and S83F amino acid substitutions linked to ciprofloxacin resistance were also identified. As expected, a variant of SHV (bla_{SHV-28}) was detected. It is likely that the high-level of resistance to β-lactams shown by this strain is mediated by the contribution of several types of β-lactamases, namely KPC-2 carbapenemase, TEM-1B, and the CTX-M-15 and SHV-28 extended-spectrum β-lactamases. Analysis of KpS26 contigs with plasmidSPAdes and PlasmidFinder with a threshold of ID = 95% revealed the presence of FIA(HI1), FIB(K), FII(K), M1 and Col(pHAD28) plasmid type replicons with 92.37–99.59% identity with reference sequences (**AF250878.1, JN233704.1, CP000648.1, U27345.1** and **KU674895.1**, respectively). We found a total of eight Argentinian *K. pneumoniae* genomes in the GenBank database at contig assembly level ($n = 7$) and chromosome assembly level ($n = 1$). Only one genome was identified as ST15 (**PDFE00000000.1**) corresponding to strain KN-ST15 isolated in 2013. Also, a total of 25 of 556 complete genomes of *K. pneumoniae* from GenBank were identified as ST15 (October 2020). The phylogenetic tree based on core genome single nucleotide polymorphisms showed several clusters among the ST15 lineage, with KpS26 located in a cluster shared with strain P35 isolated from Brazil (Fig. 1). Several β-lactamases were identified in ST15 strains, with $bla_{CTX-M-15}$ being found in 17 of 26 strains distributed among all clusters (Fig. 1). The *pmrB* allele identified in KpS26 was the only colistin resistance determinant found in the ST15 genomes analysed in this study (Fig. 1). Resistance to colistin was uncommon in ST15, and the KpS26 strain represents the emergence of CCR-Kp ST15 in Argentina (Fig. 1) aztrenama.

Taken together, these findings evidenced the ability of *K. pneumoniae* ST15 to acquire ARGs usually associated with lateral gene transfer, including bla_{KPC-2} in a novel genetic platform. Furthermore, our results show the potential of ST15 to evolve to a colistin-resistant phenotype, as also did other genotypes previously reported in globally disseminated KPC-Kp isolates [5]. In conclusion, molecular surveillance of KPC-producing CCR-Kp should be performed in our region to further investigate spread of this lineage.

Competing interests

None declared.

Nucleotide sequence accession nos

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. **JAHQJD000000000**. The version described in this paper is version **JAHQJD010000000**.

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Ethical approval

Not required.

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