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Genome Note

Genomic characterization of carbapenemase-producing *Klebsiella pneumoniae* ST307 revealed multiple introductions in Buenos Aires, Argentina

Francisco González-Espinosa[a,b], Vincenzo Di Pilato[c], Francisco Magariños[d], Jose Di Conza[a,b], Gian Maria Rossolini[e], Gabriel Gutkind[a,b], Marcela Radice[a,b], Daniela Cejas[a,b,*]

[a] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Investigaciones en Bacteriología y Virología Molecular (IBaViM), Ciudad Autónoma de Buenos Aires, Argentina
[b] CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Ciudad Autónoma de Buenos Aires, Argentina
[c] University of Genoa, Department of Surgical Sciences and Integrated Diagnostics (DISC), Genoa, Italy
[d] Hospital Donación Francisco Santojanni, Ciudad Autónoma de Buenos Aires, Argentina
[e] University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy

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ABSTRACT

Objectives: To describe at genomic level nine carbapenemase-producing *Klebsiella pneumoniae* ST307 (Kp-ST307) clinical isolates recovered in Buenos Aires during 2017 to 2021, investigating their resistome, virulome, and phylogeny.

Methods: Antimicrobial susceptibility was determined according to Clinical and Laboratory Standards Intitute (CLSI). Genomic DNA was sequenced by Illumina MiSeq and analysed using SPAdes, PROKKA, and Kleborate. Phylogeny of 355 randomly selected Kp-ST307 genomes and those from nine local isolates was inferred by a maximum-likelihood approach. The tree was visualized using Microreact.

Results: Besides resistance to ß-lactams and fluoroquinolones, six out of nine Kp-ST307 were also resistant to ceftazidime/avibactam (CZA). This difficult-to-treat resvistance phenotype was mediated by bla_{SHV-28} and GyrA-83I/ParC-80I mutations in addition to carbapenemase coding genes. Among CZA susceptible isolates, two of them harboured bla_{KPC-3} while the other harboured bla_{KPC-2}+$bla_{CTX-M-15}$. Regarding CZA-resistant isolates, three harboured bla_{KPC-3}+bla_{NDM-1}+bla_{CMY-6}, two carried bla_{KPC-2}+bla_{NDM-5}+$bla_{CTX-M-15}$, and bla_{NDM-5}+$bla_{CTX-M-15}$ were detected in the remaining isolate. Furthermore, five colistin-resistant isolates presented a nonsense mutation in *mgrB*.

Global Kp-ST307 isolates were distributed in two deep-branching lineages while local isolates were set in the main clade of the phylogenetic tree. The five isolates from the same hospital, harbouring bla_{KPC-3} or bla_{KPC-3}+bla_{NDM-1}+bla_{CMY-6}, clustered in a monophyletic subclade with Italian isolates. Also, an isolate harbouring bla_{KPC-2}+bla_{NDM-5}+$bla_{CTX-M-15}$ recovered in another hospital was closed to this group. The remaining local Kp-ST307 were grouped in other subclades containing isolates of diverse geographical origin.

Conclusion: The inferred resistome was consistent with the resistant phenotype. Phylogeny suggested multiple introduction events in our region and a single major introduction in one hospital followed by local spread.

1. Introduction

Klebsiella pneumoniae high-risk clonal lineages are already established in hospital settings and are a major cause of hospital-acquired infections; *K. pneumoniae* ST307 presents a great aptitude to adapt and persist in hospital environments. Furthermore,

* Corresponding author. Mailing address: Daniela Cejas, Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, IBaViM, Ciudad Autónoma de Buenos Aires, Argentina.
E-mail address: danielacejas@ffyb.uba.ar (D. Cejas).

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K. pneumoniae ST307 constitutes a difficult to treat resistant (DTR) pathogen and often carries transferable markers (e.g. bla_{KPC}, bla_{NDM}, bla_{OXA-48}, paired or not with $bla_{CTX-M-15}$) conferring resistance to carbapenems and novel β-lactam/β-lactam inhibitor combinations (e.g. ceftazidime/avibactam). This lineage was first recognized in the Netherlands in 2008 and Iran in 2009, followed by a period of sporadic reports from Europe, Asia, Africa, and the Americas [1]. In Argentina, it was first described in two clinical isolates recovered in two different hospitals (HA and HB) in 2017, producing KPC-2 and KPC-3, respectively [2]. Later, the clonal dissemination of *K. pneumoniae* ST307 isolates co-producing KPC-3 and NDM-1 was noticed during November 2018 to February 2019 in a single hospital [3].

The aim of this study was to describe, at genomic level, *K. pneumoniae* ST307 isolates recovered in hospitals of the Buenos Aires Metropolitan Area from 2017 to 2021, investigating their resistomes, virulomes, and phylogenies; to date, no genomic reports of this high-risk clone are available in literature from Argentina.

Nine carbapenemase-producing *K. pneumoniae* ST307 isolates recovered in four hospitals (HA, HB, HC, and HD) were included. Kp2 and Kp14 isolates were recovered in 2017 in HA and HB, respectively [2,3]. Later, M24, M25, M34, and M143 were isolated in HB from 2018 to 2020. M40 isolate was recovered in HC in 2019, and SF35 and SF39 were isolated in HD in 2021. Antimicrobial susceptibility was determined by automated systems (BD Phoenix), except for ceftazidime/avibactam and colistin, where disk diffusion and microdilution tests were used, in accordance with clinical and laboratory standards intitute (CLSI) guidelines (https://clsi.org/all- free- resources/). All isolates could be considered as DTR pathogens, being resistant to all ß-lactams and fluoroquinolones. Six of them were also resistant to ceftazidime/avibactam. Five isolates were colistin resistant but susceptible to trimethoprim/sulfamethoxazole (TMS) (Supplementary Materials).

Genomic DNA was extracted from overnight cultures using ADN PuriPrep-B Kit (InbioHigway). Whole-genome sequencing (WGS) was performed using Illumina MiSeq with 2 × 151 bp paired-end approach, assembled using SPAdes, annotated using PROKKA, and analysed using Kleborate (https://github.com/klebgenomics/Kleborate). All nine isolates carried an identical capsular locus (*KL102, wzi*173, O-locus *O1/O2v2* [O-type: *O2*]). Apart from a yersiniabactin-encoding locus (*ybt9*) detected in a single isolate (SF39), no additional acquired virulence factors could be detected using Kleborate.

The nine isolates presented the chromosomally encoded extended spectrum bla_{SHV-28} and mutations in GyrA-83I/ParC-80I associated with fluoroquinolone resistance. Three isolates (Kp2, SF35, and SF39) harboured bla_{KPC-2} + $bla_{CTX-M-15}$; among them, SF35 and SF39 also carried bla_{NDM-5}. The latter was found in M40 in addition to $bla_{CTX-M-15}$. bla_{KPC-3} was detected in five isolates (Kp14, M24, M25, M34, and M143); three of them co-harboured bla_{NDM-1}+bla_{CMY-6} (Fig. 1). The five colistin-resistant isolates (positive for bla_{KPC-3}) presented a nonsense mutation leading to a premature termination codon in *mgrB* (g59a). *drfA* was detected in those TMS-resistant isolates. Different acquired aminoglycosides resistance markers were observed among *K. pneumoniae* ST307 genomes, including the novel *rmtB* and *rmtC* (Supplementary Materials).

To investigate the phylogeny of the local isolates, 1855 *K. pneumoniae* ST307 public genomes were inspected in Pathogenwatch (https://pathogen.watch/). Of them, 355 genomes were randomly selected representing the different countries. The short reads were downloaded from the National Center for Biotechnology Information (NCBI) database, and mapped against the ST307 reference genome (ARGID_32304; Acc. No: GCA_922826555.1); recombination sites were removed using Gubbins [4]. Then, a maximum-likelihood phylogenetic tree, including all 364 genomes, was inferred using IQ-TREE following the TVM+F+ASC evolution model with 1000 random bootstrap replicates. The phylogenetic tree was visualized with metadata using Microreact (https://microreact.org/).

K. pneumoniae ST307 genomes were distributed in two deep-branching lineages in accordance with previous reports [5] (Fig. 1). One of them included 63 genomes (clade A) and the other one 301 (clade B); except for the isolates from Japan and Malta, no geographical clustering was detected. Local isolates belonged to

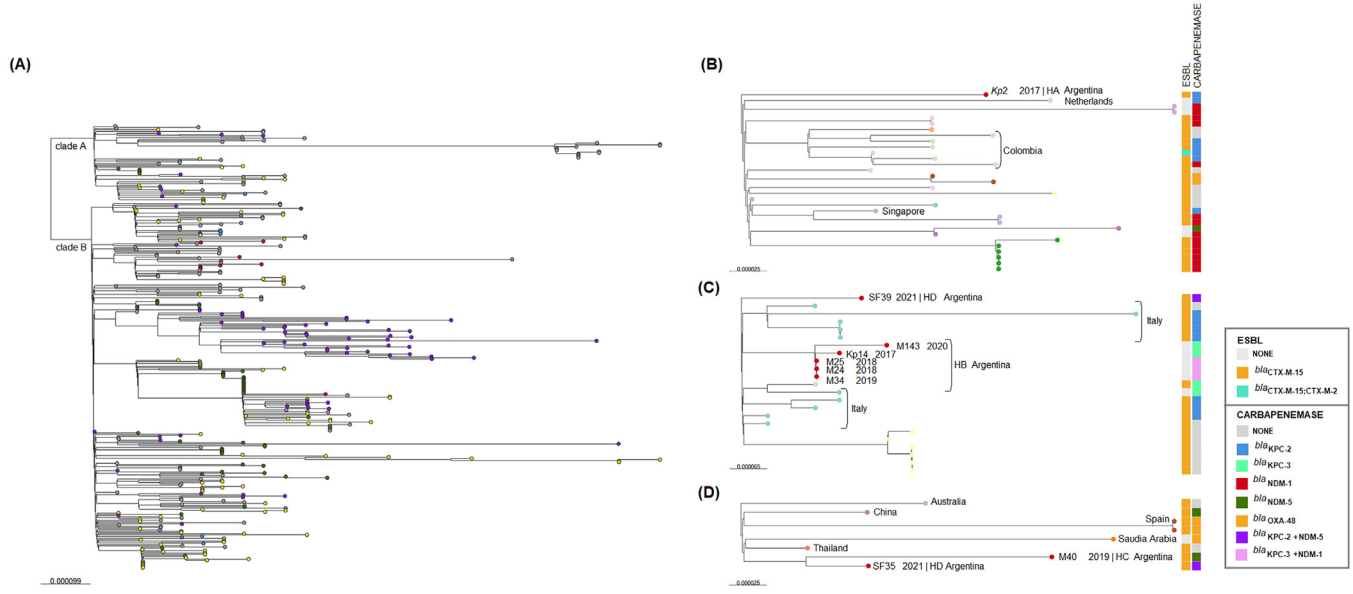

Fig. 1. Maximum likelihood phylogenetic tree of *Klebsiella pneumoniae* ST307. (A) tree containing the worldwide 364 genomes; the colors indicate the continents that different genomes belong to. Genomes from the Americas are shown in purple, but among them Argentinian genomes are marked in red. Genomes from Africa are shown in green, genomes from Asia in yellow, genomes from Europe in gray, and those from Australia, in blue. B, C, and D showed the different subclades from the main clade B that contained the Argentinian isolates. HA, HB, HC, and HD: Hospital A, B, C and D, respectively.
This tree and the metadata can be viewed in https://microreact.org/project/mcdyayxjttcgpsttahxmaf.

clade B, but they grouped in different subclades. Kp2 belonged to a subclade which contained geographically distributed isolates recovered from 2010 to 2018, mainly harbouring $bla_{CTX\text{-}M\text{-}15}$ (26/31). Besides Kp2, six isolates recovered in Colombia (n = 4), Netherlands (n = 1), and Singapore (n = 1) co-harboured $bla_{KPC\text{-}2}$. Another subclade contained the isolates recovered in HB (n = 5) harbouring $bla_{KPC\text{-}3}$ or $bla_{KPC\text{-}3}$+$bla_{NDM\text{-}1}$+$bla_{CMY\text{-}6}$. They formed a monophyletic cluster suggestive of a single major introduction in this hospital followed by local spread. This cluster was related to 10 isolates recovered in Italy from 2013 to 2019, although they carried $bla_{CTX\text{-}M\text{-}15}$ and 7/10 co-harboured $bla_{KPC\text{-}2}$, and only one co-harboured $bla_{KPC\text{-}3}$. In addition, SF39 recovered in HD, harbouring $bla_{KPC\text{-}2}$+$bla_{NDM\text{-}5}$+$bla_{CTX\text{-}M\text{-}15}$, belonged to same subclade. In turn, the $bla_{NDM\text{-}5}$+$bla_{CTX\text{-}M\text{-}15}$-harbouring M40 and $bla_{KPC\text{-}2}$+$bla_{NDM\text{-}5}$+$bla_{CTX\text{-}M\text{-}15}$-harbouring SF35 clustered together in the same subclade which contained isolates recovered from 2018 to 2021 from diverse geographical regions.

In conclusion, here we carried out the first genomic description of carbapenemase-producing *K. pneumoniae* ST307 isolates recovered in Argentina. The inferred resistome was consistent with their resistant phenotype, constituting them as DTR pathogens. Even if they grouped within the main phylogenetic lineage of *K. pneumoniae* ST307, they clustered in different branches, suggesting multiple introductions of this lineage in our region.

Sequences were deposited in Genbank under BioProject accession number PRJNA623051 and PRJNA1039806.

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Competing interests: None declared.

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 the patient's identity.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.03.017.

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