






Molecular Characterization of the Convergent Carbapenem-Resistant and Hypervirulent *Klebsiella pneumoniae* Strain K1-ST23, Collected in Chile during the COVID-19 Pandemic

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ABSTRACT The aim of this study was to investigate the genomic features of a carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-hvKp) isolate (K-2157) collected in Chile. Antibiotic susceptibility was determined using the disk diffusion and broth microdilution methods. Whole-genome sequencing (WGS) and hybrid assembly were performed, using data generated on the Illumina and Nanopore platforms. The mucoid phenotype was analyzed using both the string test and sedimentation profile. The genomic features of K-2157 (e.g., sequence type, K locus, and mobile genetic elements) were retrieved using different bioinformatic tools. Strain K-2157 exhibited resistance to carbapenems and was identified as a high-risk virulent clone belonging to capsular serotype K1 and sequence type 23 (ST23). Strikingly, K-2157 displayed a resistome composed of β -lactam resistance genes (*bla*_{SHV-190r}, *bla*_{TEM-1r}, *bla*_{OXA-9r}, and *bla*_{KPC-2}), the fosfomycin resistance gene *fosA*, and the fluoroquinolones resistance genes *oqxA* and *oqxB*. Moreover, several genes involved in siderophore biosynthesis (*ybt*, *iro*, and *iuc*), bacteriocins (*clb*), and capsule hyperproduction (plasmid-borne *rmpA* [*rmpA*] and *rmpA2*) were found, which is congruent with the positive string test displayed by K-2157. In addition, K-2157 harbored two plasmids: one of 113,644 bp (KPC+) and another of 230,602 bp, containing virulence genes, in addition to an integrative and conjugative element (ICE) embedded on its chromosome, revealing that the presence of these mobile genetic elements mediates the convergence between virulence and antibiotic resistance. Our report is the first genomic characterization of a hypervirulent and highly resistant *K. pneumoniae* isolate in Chile, which was collected during the coronavirus disease 2019 (COVID-19) pandemic. Due to their global dissemination and public health impact, genomic surveillance of the spread of convergent high-risk K1-ST23 *K. pneumoniae* clones should be highly prioritized.

IMPORTANCE *Klebsiella pneumoniae* is a resistant pathogen involved primarily in hospital-acquired infections. This pathogen is characterized by its notorious resistance to last-line antibiotics, such as carbapenems. Moreover, hypervirulent *K. pneumoniae* (hvKp) isolates, first identified in Southeast Asia, have emerged globally and are able to cause infections in healthy people. Alarming, isolates displaying a convergence phenotype of carbapenem resistance and hypervirulence have been detected in several countries, representing a serious threat to public health. In this work, we analyzed the genomic characteristics of a carbapenem-resistant hvKp isolate recovered in 2022 from a patient with COVID-19 in Chile, representing the first analysis of this type in the country. Our results

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will provide a baseline for the study of these isolates in Chile, which will support the adoption of local measures aimed at controlling their dissemination.

KEYWORDS carbapenem-resistant *Klebsiella pneumoniae*, WHO priority pathogens, hypervirulence, genomic surveillance, carbapenem resistance

Klebsiella pneumoniae has emerged as a relevant opportunistic pathogen causing a broad spectrum of infections, including urinary tract infections (UTIs), bloodstream infections, and lung and abdominal cavity infections in hospitalized patients (1). This species is considered among the most common nosocomial pathogens worldwide (2) and belongs to the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) group of bacterial organisms, so called due to their ability to evade commonly used antibiotics (3). Accordingly, *K. pneumoniae* isolates resistant to carbapenems and producing extended-spectrum β -lactamases (ESBLs) have been classified as top-priority pathogens by the World Health Organization (WHO) (4). Globally, infections caused by carbapenem-resistant *K. pneumoniae* (CR-Kp) isolates have elevated mortality rates (5), highlighting their relevance to human health.

On the other hand, hypervirulent *K. pneumoniae* (hvKp) isolates have been emerging worldwide recently (6). In this sense, the first hvKp isolate was detected in the mid-1980s in Taiwan and differed from classical *K. pneumoniae* (cKp) in its ability to generate infections in healthy patients (7). As such, hvKp isolates have been associated with pyogenic liver abscesses, lung abscesses, and metastatic infections, affecting the brain and eyes in healthy adult patients (7). In contrast, cKp isolates are associated with hospital-acquired infections, affecting debilitated patients; hence, hvKp isolates are defined as more virulent than cKp strains (8).

Frequently, cKp isolates possess elevated rates of antibiotic resistance, including to last-line drugs such as third-generation cephalosporins and carbapenems, whereas hvKp isolates are normally susceptible to these drugs (2, 9). Commonly, resistant cKp and hvKp strains belong to different clonal groups (CGs), such as CG15 and CG23, respectively (9). However, there is increasing evidence of the emergence of convergent *K. pneumoniae* isolates, which are both highly pathogenic and resistant to practically all antibiotics commonly utilized for their treatment (2). This convergence is the result of the mobilization of virulence and resistance genes that are carried on mobile genetic elements, such as plasmids (2). Due to the relevance of the convergence of virulence and resistance in *K. pneumoniae*, we here present the genomic features of a carbapenem-resistant (CR) hvKp strain (K-2157) recovered in a Chilean hospital.

K. pneumoniae K-2157 was recovered in April 2022 from tracheal aspirate fluid from an intubated patient with coronavirus disease 2019 (COVID-19) in Santiago, Chile. K-2157 was cultured in tryptic soy agar medium and incubated at 37°C for 18 h under aerobic conditions. Antimicrobial susceptibility testing was carried out according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (10). The string test was performed on blood agar to identify the hypermucoviscous phenotype (11), while capsular polysaccharide (CPS) quantification was carried out according to Park et al. (12). In addition, we performed the BlueCarba test (13) and conjugation experiments. Virulence biomarkers (*ybtS*, *clbA*, *iucA*, plasmid-borne *rmpA* [*p*_{rmpA}], *p*_{rmpA2}, K1, and K2) and carbapenemase genes (*bla*_{KPC}-like, *bla*_{NDM}-like, *bla*_{VIM}-like, *bla*_{IMP}-like, and *bla*_{OXA-48}-like) were screened for using PCR (14, 15).

K-2157 was subjected to whole-genome sequencing (WGS) utilizing the Illumina NextSeq 2000 and Oxford Nanopore GridION sequencing platforms. A Nanopore library was constructed following the SQK-RAD004 protocol (Oxford Nanopore Technologies, Oxford, UK). R9.4.1 flow cells were loaded with 30 μ L of DNA from the Nanopore library, and the GridION Mk1 instrument was run for approximately 24 h, until no further sequencing reads could be collected. Fast5 files were base called using Guppy v5.1.12, and output DNA sequence reads with quality values of >8 were saved as Fastq files. A hybrid assembly

approach was conducted using Unicycler assembler v0.5.0 (16). Species identification was confirmed using the Tetra Correlation Search (TCS) (<https://jspecies.ribohost.com/jspeciesws/#home>) with the assembled genome. The genome was analyzed using the Bandage tool (17) to identify the chromosome and plasmid(s). Antibiotic resistance genes (ARGs), sequence type (ST), and virulence factors were screened for using Kleborate v2.2.0 (18). Integrating conjugative elements (ICE) and integrative and mobilizable elements (IME) were detected using the ICE Finder server (<https://bioinfo-mml.sjtu.edu.cn/ICEfinder/ICEfinder.html>). Those contigs previously identified as plasmids were corroborated using MOB-suite (19), the PlasmidFinder database with ABRicate v1.01.1, and the plasmid database (PLSDB) (20). Seventeen deposited genomes of hvKp isolates collected between 2000 and 2022 were retrieved from GenBank. The inclusion criteria used were to add (i) hvKp from the continent and (ii) genomes of closely related isolates. The genomes were annotated using Prokka v1.14, and the core genome was determined using Roary v3.13.0. Finally, a maximum likelihood (ML) single nucleotide polymorphism (SNP) tree was built using FastTree v2.1.1.

K-2157 was confirmed as *K. pneumoniae* by the TCS analysis. It displayed resistance to imipenem (IPM), meropenem (MEM), ertapenem (ETP), cefotaxime (CTX), ceftriaxone (CRO), piperacillin-tazobactam (TZP), aztreonam (ATM), and amoxicillin-clavulanic acid (AMC), whereas it was intermediate to ceftazidime (CAZ) and ciprofloxacin (CIP) and susceptible to amikacin (AMK), gentamicin (GEN), colistin (CST), levofloxacin (LEV), and sulfamethoxazole-trimethoprim (SXT). K-2157 was positive for the BlueCarba test, which is congruent with the detection of *bla*_{KPC-2}. K-2157 tested positive using the string test in blood agar and showed high CPS production (12). In addition, PCR screening evidenced the presence of virulent biomarkers, revealing that K-2157 exhibits the convergence of carbapenem resistance and a hypervirulent phenotype.

The resistome of K-2157 included the *bla*_{TEM-1}, *bla*_{SHV-190}, *bla*_{OXA-9}, *bla*_{KPC-2}, *fosA*, *oqxA*, and *oqxB* genes. Furthermore, K-2157 belongs to the ST23 clone associated with the K1 capsular type. Our results are congruent with previous works, since K1-ST23 isolates have been described as an hvKp with moderate virulence in the *Galleria mellonella* model (21). Classically, the K1-ST23 clone is defined as susceptible to antibiotics, including cephalosporins and carbapenems (18). In this regard, we previously characterized a K1-ST23 hvKp isolate recovered in Chile which was susceptible to carbapenems (22). Since its first description in 2012 in Russia and Germany, this lineage has been identified in several European countries (21, 23). In Europe, the emergence of the convergence phenomenon of K1-ST23 hvKp isolates with carbapenem resistance is mainly mediated by the acquisition of an IncL plasmid harboring the *bla*_{OXA-48} gene (21). However, hvKp K-2157 presented an IncFIB plasmid of 113,644 bp (p113_K2157) harboring the *bla*_{KPC-2}, *bla*_{OXA-9}, and *bla*_{TEM-1} genes (Fig. 1A). In this sense, p113_K2157 is similar to a plasmid previously detected in *K. pneumoniae* recovered in the United States in 2013 (Fig. 1A). Moreover, the *bla*_{KPC-2} gene was carried on a Tn4401 transposon. In addition to the KPC-containing plasmid, K-2157 has another plasmid of 230,602 bp (pVir230_K2157) (Fig. 1B). This plasmid harbored the siderophores genes *iro*, *iut*, and *iuc*, in addition to the *pmpA*, *pmpA2*, and *peg-344* genes, which are all considered biomarkers for hvKp (24). pVir230_K2157 belongs to the IncHI1B type and is similar to a plasmid identified in a *K. pneumoniae* isolate recovered in China in 2017 (Fig. 1B). Importantly, conjugation experiments using *Escherichia coli* J53 as the recipient at 25°C and 37°C were negative. Previously, it was asserted that the convergence of virulence and resistance in this clone is mediated by the presence of a fused single plasmid (25), differing from our results. Additionally, K-2157 presented an ICE of 104,622 bp on its chromosome, which is related to ICEKp10, carrying the yersiniabactins *ybtS*, *ybtX*, *ybtQ*, *ybtP*, *ybtA*, *ybtU*, *ybtT*, *ybtE*, *irp1*, *irp2*, and *fyuA* and the colibactin operon *CibABCDEFGHIJKLMNPOQR*. These results show that the high risk K1-ST23 clone integrates virulence-related ICEKp on its chromosome and can capture additional virulence and resistance epidemic plasmids, producing the convergent phenotype. It is important to highlight the context of the isolation of K-2157, since it was recovered from a COVID-19 patient. Although the evidence is scarce, there is a report from Italy in which hvKp isolates were collected from 36 COVID-19 patients (26).

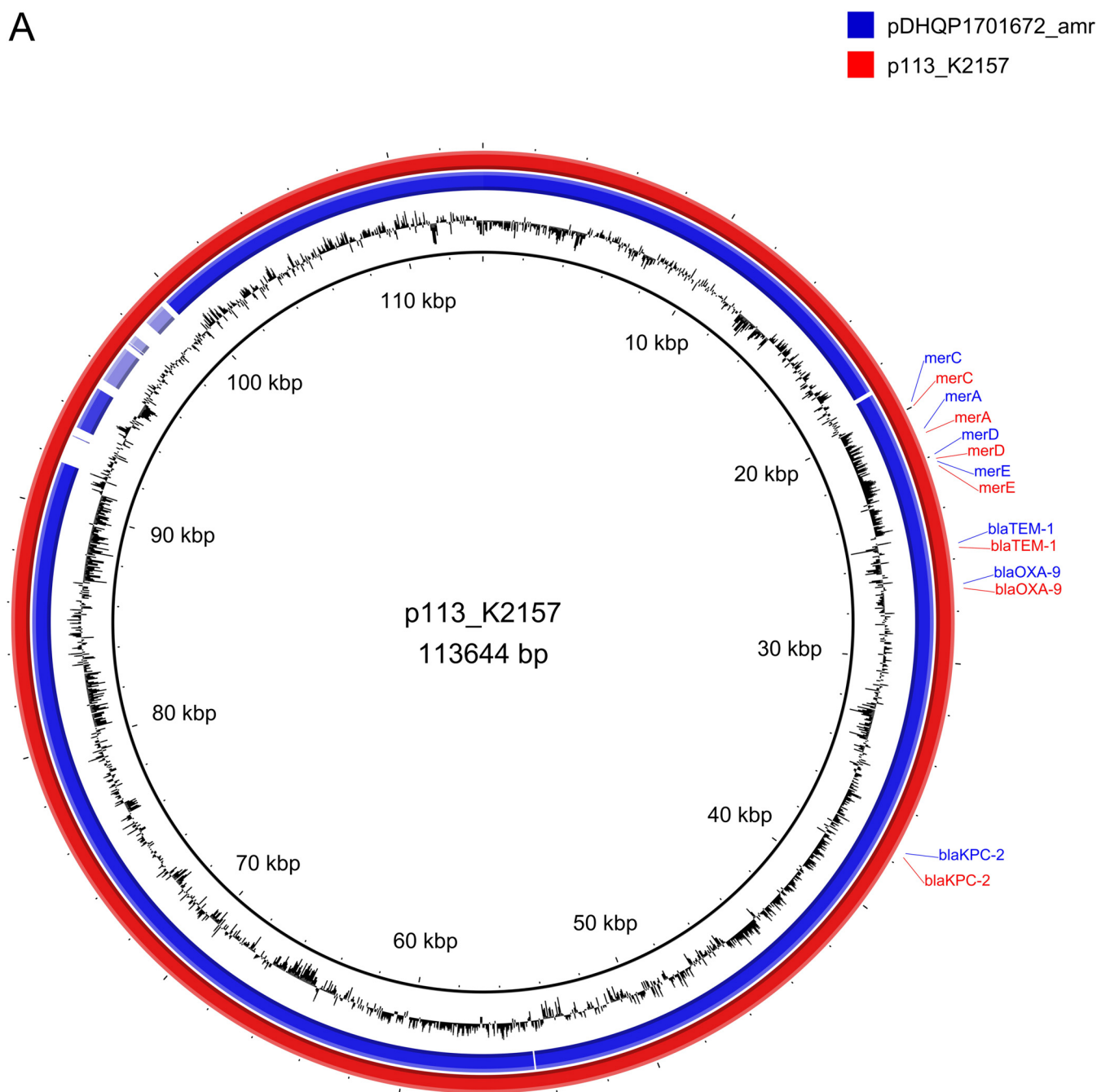






FIG 1 Comparative analysis of resistance plasmid p113_K2157 and virulence plasmid pVir230_K2157 found in strain K-2157 with plasmids deposited in public databases. (A) Resistance plasmids. Plasmid pDHQP1701672_amr (GenBank accession number [NZ_CP037744.1](#)) corresponds to the first hypervirulent KPC-2-producing *K. pneumoniae* isolate collected in the Americas. (B) Virulence plasmids. Plasmid pVir_3214 ([NZ_CP028852.1](#)) matches 100% with pVir230_K2157. Plasmid pVIR_CR_hvKp4 ([NZ_MF437313.1](#)) was identified in *K. pneumoniae* ST11. Plasmid pDHQP ([NZ_CP037743.1](#)) was identified in the first hypervirulent KPC-2-producing *K. pneumoniae* isolate collected in the Americas, and plasmid pLVPK ([NC_005249.1](#)) corresponds to the first hypervirulent plasmid described in *K. pneumoniae*.

In this case, the infections produced in these susceptible patients were associated with high mortality rates (26), highlighting the relevance of this pathotype during the pandemic.

Moreover, we determined that isolate K-2157 was closely related to hvKp isolates from Chile, China, and the United States (Fig. 2). Interestingly, K-2157 was the only hvKp K1-ST23 isolate containing a carbapenemase gene (*bla_{KPC-2}*) (Fig. 2) that determines the emergence of the convergent pathotype. These results suggest that these isolates are circulating in distant geographical areas; therefore, genomic surveillance is paramount to prevent its

 pLVPK
 pDHQP1701672_hv
 pVIR_CR_hvKp4
 pVIR_3214
 pVir230_K2157

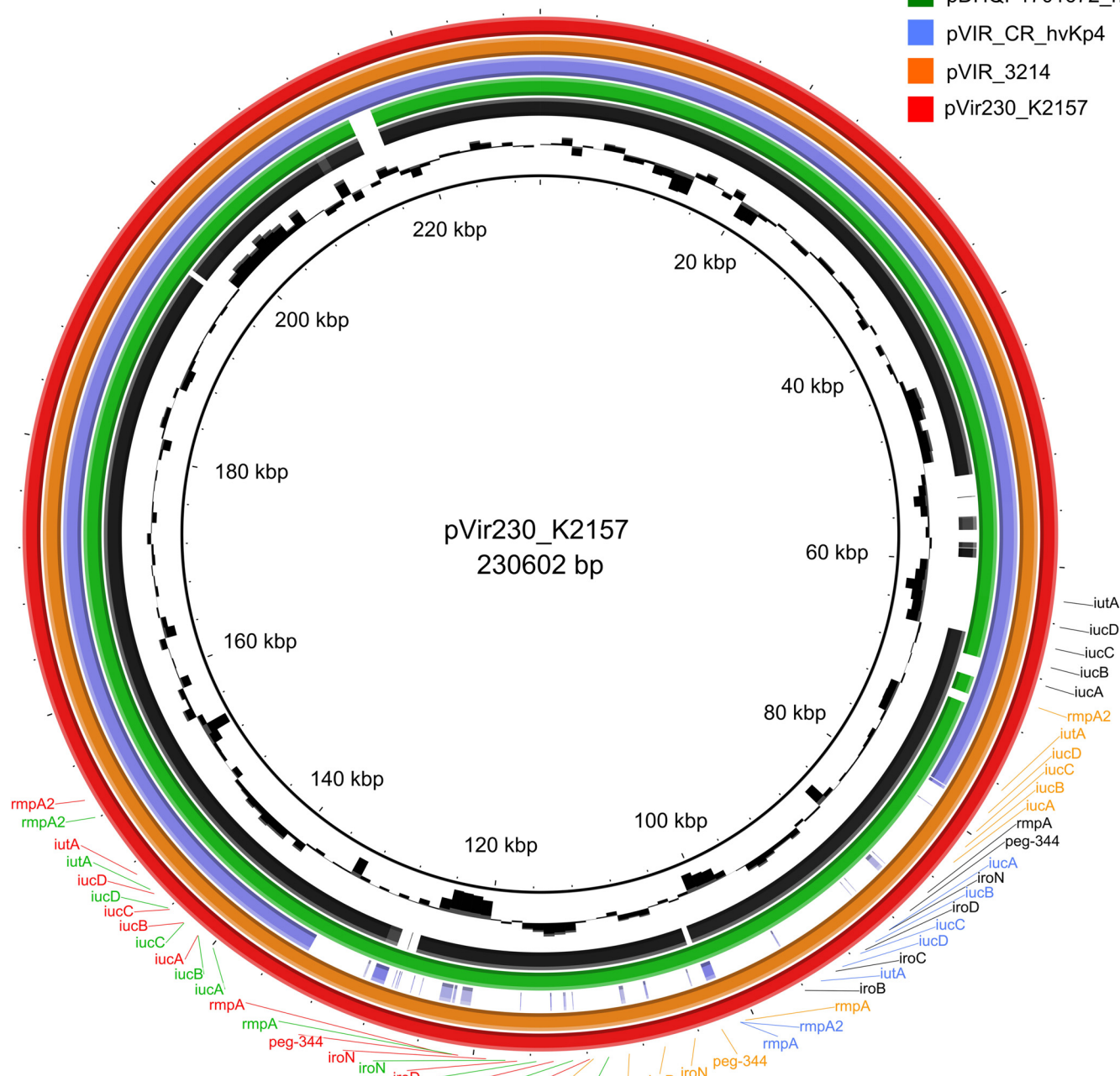


FIG 1 (Continued)

In conclusion, our results represent the first genomic characterization of a hypervirulent and carbapenem-resistant *K. pneumoniae* isolate from Chile, revealing that this isolate belongs to the virulent K1-ST23 lineage harboring a KPC plasmid. These findings highlight the relevance of performing genomic surveillance to detect strains that represent a serious threat to public health.

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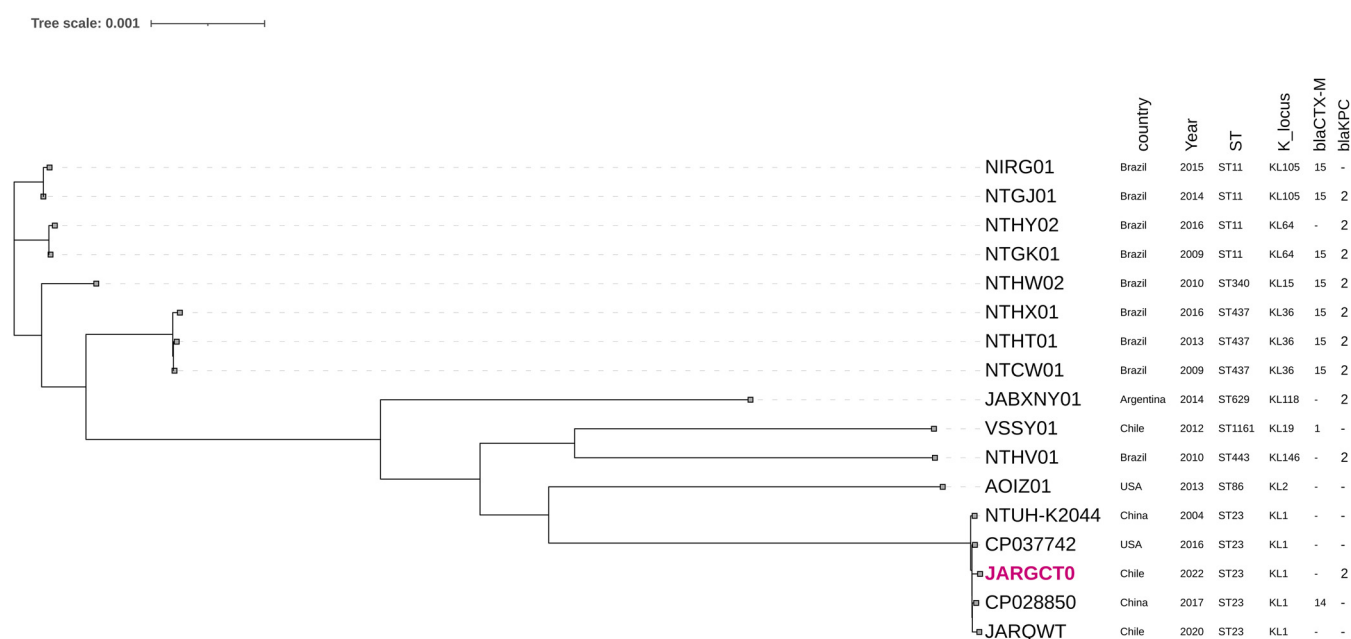


FIG 2 Maximum likelihood phylogenetic tree of the core genomes of 17 hypervirulent *K. pneumoniae* isolates. The tree was constructed using Roary and FastTree based on core genome SNPs. The country and year of isolation, sequence type (ST), K locus, CTX-M-type extended-spectrum β -lactamases (ESBLs), and KPC carbapenemases are included. The genome of K-2157 is indicated in red.

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REFERENCES

- Paczosa MK, Meccas J. 2016. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol Mol Biol Rev 80:629–661. <https://doi.org/10.1128/MMBR.00078-15>.
- Wyres KL, Lam MMC, Holt KE. 2020. Population genomics of Klebsiella pneumoniae. Nat Rev Microbiol 18:344–359. <https://doi.org/10.1038/s41579-019-0315-1>.
- de Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. 2020. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 33:e00181-19. <https://doi.org/10.1128/CMR.00181-19>.
- World Health Organization. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.
- Antimicrobial Resistance Collaborators. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399:629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0).
- Choby JE, Howard-Anderson J, Weiss DS. 2020. Hypervirulent Klebsiella pneumoniae—clinical and molecular perspectives. J Intern Med 287:283–300. <https://doi.org/10.1111/joim.13007>.
- Catalán-Nájera JC, Garza-Ramos U, Barrios-Camacho H. 2017. Hypervirulence and hypermucoviscosity: two different but complementary Klebsiella spp. phenotypes? Virulence 8:1111–1123. <https://doi.org/10.1080/21505594.2017.1317412>.
- Li Y, Hu D, Ma X, Li D, Tian D, Gong Y, Jiang X. 2021. Convergence of carbapenem resistance and hypervirulence leads to high mortality in patients with postoperative Klebsiella pneumoniae meningitis. J Glob Antimicrob Resist 27: 95–100. <https://doi.org/10.1016/j.jgar.2021.02.035>.
- Chen L, Kreiswirth BN. 2018. Convergence of carbapenem-resistance and hypervirulence in Klebsiella pneumoniae. Lancet Infect Dis 18:2–3. [https://doi.org/10.1016/S1473-3099\(17\)30517-0](https://doi.org/10.1016/S1473-3099(17)30517-0).
- Clinical and Laboratory Standards Institute. 2021. Performance standards for antimicrobial susceptibility testing, 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
- Ono R, Kitagawa I. 2022. Positive string test in a patient with hypermucoviscous Klebsiella pneumoniae infection. QJM 114:815. <https://doi.org/10.1093/qjmed/hcab215>.
- Park S, Lee H, Shin D, Ko KS. 2020. Change of hypermucoviscosity in the development of tigecycline resistance in hypervirulent klebsiella pneumoniae sequence type 23 strains. Microorganisms 8:1562. <https://doi.org/10.3390/microorganisms8101562>.
- Pires J, Novais A, Peixe L. 2013. Blue-Carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol 51:4281–4283. <https://doi.org/10.1128/JCM.01634-13>.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119–123. <https://doi.org/10.1016/j.diagmicrobio.2010.12.002>.
- Russo TA, Olson R, Fang C-T, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR, Hypervirulent Klebsiella pneumoniae Investigator Group (HVKPIG). 2018. Identification of biomarkers for differentiation of hypervirulent Klebsiella pneumoniae from classical K. pneumoniae. J Clin Microbiol 56:e00776-18. <https://doi.org/10.1128/JCM.00776-18>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics 31:3350–3352. <https://doi.org/10.1093/bioinformatics/btv383>.
- Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. 2021. A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nat Commun 12:4188. <https://doi.org/10.1038/s41467-021-24448-3>.
- Robertson J, Nash JHE. 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb Genom 4:e000206. <https://doi.org/10.1099/mgen.0.000206>.
- Galata V, Fehlmann T, Backes C, Keller A. 2019. PLSDb: a resource of complete bacterial plasmids. Nucleic Acids Res 47:D195–D202. <https://doi.org/10.1093/nar/gky1050>.

21. Arcari G, Carattoli A. 2022. Global spread and evolutionary convergence of multidrug-resistant and hypervirulent *Klebsiella pneumoniae* high-risk clones. *Pathog Glob Health* <https://doi.org/10.1080/20477724.2022.2121362>.
22. Morales-León F, Opazo-Capurro A, Caro C, Lincopan N, Cardenas-Arias A, Esposito F, Illesca V, Riosco ML, Domínguez-Yévenes M, Lima CA, Bello-Toledo H, González-Rocha G. 2021. Hypervirulent and hypermucoviscous extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* and *Klebsiella variicola* in Chile. *Virulence* 12:35–44. <https://doi.org/10.1080/21505594.2020.1859274>.
23. European Centre for Disease Prevention and Control. 2021. Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries. European Centre for Disease Prevention and Control, Stockholm, Sweden.
24. Zhu J, Wang T, Chen L, Du H. 2021. Virulence factors in hypervirulent *Klebsiella pneumoniae*. *Front Microbiol* 12:642484. <https://doi.org/10.3389/fmicb.2021.642484>.
25. Hernández M, López-Urrutia L, Abad D, Serna MDF, Ocampo-Sosa AA, Eiros JM. 2021. First report of an extensively drug-resistant ST23 *Klebsiella pneumoniae* of capsular serotype K1 co-producing CTX-M-15, OXA-48 and ArmA in Spain. *Antibiotics (Basel)* 10:157. <https://doi.org/10.3390/antibiotics10020157>.
26. Falcone M, Tiseo G, Arcari G, Leonildi A, Giordano C, Tempini S, Bibbolino G, Mozzo R, Barnini S, Carattoli A, Menichetti F. 2022. Spread of hypervirulent multidrug-resistant ST147 *Klebsiella pneumoniae* in patients with severe COVID-19: an observational study from Italy, 2020–21. *J Antimicrob Chemother* 77:1140–1145. <https://doi.org/10.1093/jac/dkab495>.