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Genome Note

Genomic analysis of the first isolate of KPC-2-producing *Klebsiella pneumoniae* from Uruguay

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ABSTRACT

Objectives: Since KPC-2-producing *Klebsiella pneumoniae* are associated with successful dissemination of a major clone, defined as sequence type 258 (ST258), the aim of this study was to perform whole-genome sequencing (WGS) of the first colistin-resistant *K. pneumoniae* strain (Kpn666) carrying bla_{KPC-2} identified in Uruguay in 2011 in order to identify genomic and phylogenetic traits.
Methods: WGS of strain Kpn666 isolated from an asymptomatic urinary tract infection was performed using Illumina MiSeq, and de novo assembly was performed using SPADES v.3.11. Contigs were re-ordered using the ST258 reference genome NJST258_1 (GenBank **CP006923**) and were oriented with the MAUVE Contig Mover. Twenty complete genomes of *K. pneumoniae* identified as ST258 using the Pasteur MLST site were downloaded from GenBank (May 2017). A maximum-likelihood tree was created using MEGA7 based on core single nucleotide polymorphisms (SNPs) from whole-genome alignment obtained with SNP sites (https://github.com/sanger-pathogens/snp-sites).
Results: WGS analysis revealed a genome of 5 448 179 bp (5232 CDS, 108 RNAs). Phylogenetic analysis identified that Kpn666 belonged to clade I lineage of ST258. Further studies also identified IncR, IncFIB(K) and IncFII(K) plasmid replicons and 11 transferable associated antimicrobial resistance genes (ARGs) comprising four drug classes. The *mgrB* gene involved in colistin resistance was shown to be disrupted by insertion of an IS*5*-like element.
Conclusions: The first isolate of KPC-2-producing *K. pneumoniae* detected in Uruguay was sequenced and the results confirm the ability of this bacterium to capture several ARGs. The KPC-2 carbapenemase in Uruguay is likely to have been introduced by the high-risk clone ST258.

The main clinical impact of *Klebsiella pneumoniae* is as a nosocomial pathogen of urinary tract infections, respiratory tract infections and bloodstream-associated infections [1]. *K. pneumoniae* sequence type 258 (ST258) is a high-risk clone with pandemic dissemination that emerged during the early 2000s in the USA [2], usually associated with isolates producing the *K. pneumoniae* carbapenemase (KPC) enzyme. Phylogenetic analysis of single nucleotide polymorphisms (SNPs) of the core genome of ST258 *K. pneumoniae* strains revealed that there are two distinct genetic clades (ST258 clades I and II), largely due to an ca. 215-kb region of divergence that includes genes involved in capsular polysaccharide synthesis (*cps*), the capsule repeat unit polymerase gene (*wzy*) and the capsule assembly protein gene (*wzi*) [3,4]. KPC-2-producing *K. pneumoniae* was reported for the first time in Uruguay in 2011 associated with a *K. pneumoniae* outbreak in a general hospital in a tourist area [5]. Isolates from the outbreak exhibited an extensively drug-resistant phenotype including resistance to piperacillin/tazobactam, amikacin, gentamicin, ciprofloxacin, ceftazidime, cefepime, cefotaxime, imipenem, meropenem, trimethoprim/sulfamethoxazole and colistin [5]. Genomic DNA of one *K. pneumoniae* isolate from the outbreak (strain Kpn666) was sequenced using Illumina MiSeq-I with Nextera XT libraries for

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sample preparation. Reads were assembled with SPADES v.3.11 [6] using *k*-mers 21, 33, 55, 77, 99, 127 with 'careful' option turned on and cut-offs for final assemblies: minimum contig/scaffold size = 500 bp; minimum contig/scaffold average Nt coverage = 10-fold. Contigs were re-ordered using the ST258 reference genome NJST258_1 (GenBank **CP006923**) and were oriented with the MAUVE Contig Mover. The draft genome sequence of Kpn666 consisted of 95 contigs, a total sequence of 5 448 179 bp with an N_{50} contig size of 203 938 bp. The GC% average was 57.4%. Using the Rapid Annotations using Subsystems Technology (RAST) server [7] to detect coding regions, 5232 coding sequences (CDS) and 108 RNAs were identified. Analysis of Kpn666 contigs with plasmidSPAdes (http://cab.spbu.ru/software/plasmid-spades/) and PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) with a threshold of ID = 95% revealed the presence of IncR, IncFIB(K) and IncFII(K) plasmid replicons with 100% identity with references (**DQ449578**, **CP000648** and **JN233704,** respectively) in three complete contigs. Multilocus sequence typing (MLST) was performed in silico using the MLST database and schema for *K. pneumoniae* at the Pasteur MLST website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The MLST technique classified Kpn666 as ST258, which clusters in the clonal group 258 (CG258). All *K. pneumoniae* complete genomes available in GenBank until May 2017 were downloaded and MLST of each genome was identified as previously described. A maximum-likelihood tree of the 20 complete genomes of *K. pneumoniae* ST258 downloaded, including the Kpn666 genome, was created using MEGA7 based on core genome SNPs from whole-genome alignment obtained with SNP sites (https://github.com/sanger-pathogens/snp-sites) using genome NJST258_1 as reference. The substitution model used in MEGA7 for creating the tree was HKY with 100 bootstraps. The phylogenetic tree obtained in the present work recreated the two genetic clades previously described (ST258 clades I and II) and Kpn666 clustered in clade I lineage. The full length of the *wzy* gene and the neighbouring *wzi* gene in Kpn666 revealed that these genes had 100% identity with the ST258 cps 1 genes. Using the Basic Local Alignment Search Tool (BLAST) [8], the integrated conjugative element IC*EKp258* region that harbours a type IV pilus gene cluster and a type III restriction–modification system was also detected, confirming the association of IC*EKp258* with ST258 described elsewhere [9]. Antimicrobial resistance genes (ARGs) for resistance to aminoglycosides [*aac(6′)-Ib*, *aadA2*, *aadA1*, *aph(3′)-Ia*, *aac(3)-IVa*, *aph(4)-Ia*], β-lactams ($bla_{\text{KPC-2}}$, $bla_{\text{OXA-9}}$, $bla_{\text{TEM-1A}}$), chloramphenicol (*cmlA1*) and sulphonamides (*sul3*) were identified using ResFinder. The $bla_{\text{SHV-11}}$ and *fosA* genes were located on the chromosome. Alteration in the *mgrB* gene has been reported as a mechanism of colistin resistance in *K. pneumoniae* isolates. Using BLAST, the *mgrB* gene in Kpn666 was shown to be disrupted by insertion of an IS*5*-like element in the same position as previously reported [10]. Further investigations are required to confirm the role of altered *mgrB* in Kpn666 as the mechanism of colistin resistance. These results showed that the KPC-2 carbapenemase in Uruguay was introduced by the high-risk clone ST258, evidencing again how clones with epidemic behaviour are one of the global threats for public health. A more exhaustive analysis of the locations and contexts of the ARGs harboured by strain Kpn666, as well as experimental data regarding ARG maintenance, will be included in a future publication. The possibility of analysing the complete genome sequence of this strain and comparison with those obtained in the rest of the world could shed more light on this important nosocomial pathogen. These findings could accelerate research on novel diagnostic, therapeutic and vaccine strategies designed to prevent and/or treat infections caused by multidrug-resistant *K. pneumoniae*.

Nucleotide sequence accession nos.

This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number **PVNV00000000**. The version described in this paper is the first version (**PVNV01000**).

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Competing interests

None declared.

Ethical approval

Not required.

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