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GENOME SEQUENCES

Genome Sequences of Clinical Isolates of NDM-1-Producing *Klebsiella quasipneumoniae* subsp. *similipneumoniae* and KPC-2-Producing *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* from Brazil

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ABSTRACT *Klebsiella quasipneumoniae* is an emerging pathogen in human medicine. We report draft genome sequences of NDM-1- and KPC-2-producing *K. quasipneumoniae* strains from inpatients in Brazil. *K. quasipneumoniae* subsp. *quasipneumoniae* and *K. quasipneumoniae* subsp. *similipneumoniae* harbored broad resistomes. These data could contribute to a better understanding of acquired resistance in *K. quasipneumoniae*.

Klebsiella pneumoniae strains of phylogenetic groups Kp1 to Kp7 have been classified as *K. pneumoniae sensu stricto*, *K. quasipneumoniae* subsp. *quasipneumoniae*, *K. variicola* subsp. *variicola*, *K. quasipneumoniae* subsp. *similipneumoniae*, *K. variicola* subsp. *tropicalensis*, *K. quasivariicola*, and *K. africanensis*, respectively (1). Specifically, *K. quasipneumoniae* has been recognized as an opportunistic pathogen that can acquire clinically relevant antibiotic resistance genes (2–5). Here, we report draft genome sequences of two *Klebsiella quasipneumoniae* strains producing KPC-2 and NDM-1 carbapenemases, which confer resistance to all clinically relevant β-lactam antibiotics.

Carbapenem-resistant *K. quasipneumoniae* strains 34H and Kp1345 were isolated in 2014 from perfusion fluid (6) and in 2017 from a rectal swab for surveillance culture (7), respectively, from patients hospitalized in a teaching hospital in Brazil. Species identification was performed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (8), and antimicrobial susceptibility was determined with the Vitek 2 system (bioMérieux, France) according to the manufacturer's instructions. Carbapenemase production was detected by the Blue-Carba test (9) (Fig. 1) and modified Hodge test (10), whereas carbapenemase activity of NDM-1 and KPC-2 β-lactamases was confirmed by EDTA and dipicolinic acid inhibition assays, respectively (11–13). Additionally, $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-2}}$ genes were identified by PCR amplification and direct DNA sequencing of PCR products (14).

For whole-genome sequencing (WGS) analyses, the strains were streaked to single colonies on MacConkey agar plates and then grown for 18 h at 37°C in 3 ml of lysogeny broth. Total genomic DNA was extracted using a PureLink quick gel extraction kit (Life Technologies, CA) and used for library preparation with a Nextera XT kit (Illumina, San Diego, CA). In addition, the DNA was quantified with a double-stranded DNA high-sensitivity assay using a Qubit 2.0 fluorometer (Life Technologies) according to the manufacturer's instructions. Subsequently, sequencing was performed on an Illumina

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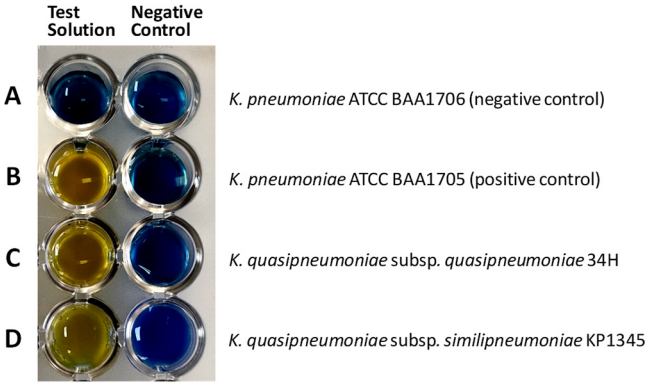

FIG 1 Representative results of the Blue-Carba test for carbapenemase-producing (B, C, and D) and non-carbapenemase-producing (A) bacteria, with test solutions (left) and negative-control solutions (right). (A) *K. pneumoniae* ATCC BAA1706 (carbapenemase-negative control); (B) *K. pneumoniae* ATCC BAA1705 (carbapenemase [KPC]-positive control); (C) *K. quasipneumoniae* subsp. *quasipneumoniae* 34H (this study) (carbapenemase [KPC-2] positive); (D) *K. quasipneumoniae* subsp. *similipneumoniae* Kp1345 (this study) (carbapenemase [NDM-1] positive). The images were obtained after 2 h of incubation. Carbapenemase production was assessed by the Blue-Carba test method (9), which relies on the detection, in a bacterial extract, of hydrolysis of the carbapenem β-lactam ring through the acidification of a bromothymol blue test solution, used as a color indicator. The test solution consists of an aqueous solution of 0.04% bromothymol blue adjusted to pH 6.0, 0.1 mM $ZnSO_4$, and 3 mg/ml imipenem, with a final pH of 7.0. A negative-control solution (0.04% bromothymol blue solution [pH 7.0]) is used to control for the influence of bacterial components or products on the pH of the solution. A loop (approximately 5 μl) of a pure bacterial culture recovered from Mueller-Hinton agar was directly suspended in 100 μl of both test and negative-control solutions in a 96-well microtiter plate and incubated for 2 h at 37°C with agitation (150 rpm). Carbapenemase activity was revealed when the test solution and negative-control wells were yellow and blue, respectively. The non-carbapenemase-producing strain (negative control) remained blue or green with both solutions.

NextSeq PE instrument using a paired-end (150-bp) library. The short reads were handled using FastQC v.0.11.3 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and Trimmomatic v.0.32 (15). *De novo* assembly was performed using SPAdes v.3.9 (16), and draft genome annotations were made using NCBI PGAP v.3.2 (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). Contamination levels were checked using CheckM v.1.0.3 with default settings (17). WGS data were analyzed using PlasmidFinder v.2.0 (18), ResFinder v.3.2 (19), and SpeciesFinder v.2.0 (20) tools (http://www.genomicepidemiology.org). Default parameters were used for all software.

Genome sequence analysis identified *K. quasipneumoniae* subsp. *quasipneumoniae* (strain 34H) and *K. quasipneumoniae* subsp. *similipneumoniae* (strain Kp1345), presenting a total of 16,501,776 and 10,695,728 paired-end reads assembled into 183 and 487 contigs, with 247.0× and 320.0× coverage, respectively. The N_{50} values obtained for strains 34H and Kp1345 were 84,397 and 122,604 bp, with GC contents of 57.6% and 56.8%, respectively. In brief, strain 34H presented a genome size calculated as 5,666,228 bp, with 5,134 protein-coding sequences, 82 tRNAs, 22 rRNAs, 12 noncoding RNAs, and 49 pseudogenes, whereas Kp1345 presented a genome size of 5,921,292 bp, with 5,134 protein-coding sequences, 82 tRNAs, 22 rRNAs, 12 noncoding RNAs, and 49 pseudogenes. CheckM results showed 99.99% and 99.938% completeness and 0.952% and 1.061% contamination for the 34H and KPC1345 genomes, respectively.

In summary, we present the draft genome sequences of two carbapenem-resistant *Klebsiella quasipneumoniae* strains displaying broad resistomes for β-lactams (i.e., bla_{KPC-2}, $bla_{OKP-A-6}$, $bla_{OKP-B-2}$, bla_{NDM-1}, and $bla_{CTX-M-15}$) and other medically important antibiotics. These data could contribute to a better understanding of acquired resistance in *K. quasipneumoniae*.

Data availability. The genome sequences of *K. quasipneumoniae* subsp. *quasipneumoniae* strain 34H and *K. quasipneumoniae* subsp. *similipneumoniae* strain Kp1345 have been deposited in GenBank under accession numbers NZ_VDFT00000000 (SRA number SRR9950479) and NZ_VDFZ00000000 (SRA number SRR9942580), respec-

tively. For a spreadsheet containing details of antibiotic resistance genes, plasmid incompatibility groups, and CheckM and Qubit results, see Table S1 at https://doi.org/10.6084/m9.figshare.11675805.

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