



Genome Note

Draft genome sequences of KPC-2- and CTX-M-15-producing *Klebsiella pneumoniae* ST437 isolated from a clinical sample and urban rivers in Sao Paulo, Brazil



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ABSTRACT

Objectives: KPC-producing *Klebsiella pneumoniae* is considered one of the most worrisome multidrug-resistant micro-organisms in nosocomial infections. It has also been reported in wastewater and urban rivers in the city of Sao Paulo, Brazil. Here we report the draft genome sequences of three KPC-2- and CTX-M-15-producing *K. pneumoniae* sequence type 437 (ST437) isolates obtained from two urban rivers and from a clinical sample of a patient in Sao Paulo.

Methods: A genomic library was constructed using a Nextera XT Kit. An Illumina platform was used to perform whole-genome sequencing (WGS).

Results: WGS of environmental isolates Kp148/PINH-4900 and Kp196/TIET-4200 and clinical isolate Kp314/11 resulted in estimated genome sizes of 5 464 058, 5 437 723 and 5 319 218 bp, respectively. Resistome analysis of the environmental and clinical strains revealed the presence of resistance genes to the following antimicrobials in all strains: aminoglycosides [*aac(6′)-Ib-cr*]; β-lactams (*bla_{OXA-1}*, *bla_{SHV-11}*, *bla_{CTX-M-15}* and *bla_{KPC-2}*); fluoroquinolones [*aac(6′)-Ib-cr*, *oqxA* and *oqxB*]; fosfomycin (*fosA^{KP}*); macrolides [*mph(A)*]; phenicols (*catB4*); sulfonamides (*sul1*); and trimethoprim (*dhfrA30*). The tetracycline resistance gene *tetA* was identified in Kp148/PINH-4900 and Kp314/11 only; the aminoglycoside resistance gene *aph(3′)-Ia* was found only in environmental isolates, and *aadA2* only in Kp314/11; and the phenicol resistance gene *catA1* was identified only in Kp148/PINH-4900.

Conclusions: The draft genome sequences of these strains help us to elucidate the dissemination of resistance genes in micro-organisms inside and outside the hospital and are useful for further comparisons between clinical and environmental strains.

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1. Introduction

KPC-producing *Klebsiella pneumoniae* of clonal complex 258 (CC258), comprising sequence types (STs) ST11, ST258, ST340 and ST437, are disseminated worldwide and are one of the most worrisome multidrug-resistant (MDR) micro-organisms in nosocomial infections. Isolates of ST437 and ST11 are predominant in Brazil [1,2].

The presence of KPC-2-producing *K. pneumoniae* ST437 has also been reported in urban rivers in the city of Sao Paulo, Brazil [3]. Here we report the draft genome sequences of three strains of KPC-2- and CTX-M-15-producing *K. pneumoniae* ST437 isolated from two urban rivers and from a clinical sample obtained from a patient in Sao Paulo.

2. Materials and methods

A local study to monitor the occurrence of carbapenemase-producing Gram-negative bacteria from urban rivers in Southeast Brazil was conducted from January to June 2011 in which two

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strains of *K. pneumoniae* ST437 positive for both *bla*_{KPC-2} and *bla*_{CTX-M-15} were isolated [3]. The first environmental isolate (Kp148/PINH-4900) was isolated from the Pinheiros River and showed resistance to β -lactams (ceftazidime, cefotaxime, cefepime, aztreonam, ticarcillin/clavulanate, ertapenem, imipenem and meropenem), quinolones, trimethoprim/sulfamethoxazole, chloramphenicol, fosfomycin and polymyxin B, and susceptibility to aminoglycosides (amikacin and gentamicin) and tigecycline. The second environmental isolate (Kp196/TIET-4200), was isolated from the Tietê River and showed the same resistance profile as Kp148/PINH-4900, except for susceptibility to chloramphenicol and polymyxin B [3]. In the same year, a *K. pneumoniae* ST437 isolate positive for both *bla*_{KPC-2} and *bla*_{CTX-M-15} (Kp314/11) was obtained from a blood culture of a patient in a hospital located 4 miles from these rivers.

The three abovementioned isolates were selected for whole-genome sequencing. A PureLink™ Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA) was used to extract genomic DNA, and a genomic library was constructed using a Nextera XT Kit (Illumina Inc., San Diego, CA). Total genomic DNA of *K. pneumoniae* Kp148/PINH-4900 and Kp196/TIET-4200 was sequenced using a MiSeq platform (Illumina Inc.), whilst strain Kp314/11 was sequenced using a NextSeq platform (Illumina Inc.). In brief, sequence reads were initially assembled de novo using the A5-miseq pipeline [4] or Velvet v.1.2.10 (<https://www.ebi.ac.uk/~zerbino/velvet/>), and contigs were curated using Geneious v.R9 (Biomatters Ltd., Auckland, New Zealand). The draft genome sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.3.2.

Multilocus sequence typing (MLST), antimicrobial resistance genes and plasmid replicons were identified using the services MLST, ResFinder and PlasmidFinder, respectively, available from the Center for Genomic Epidemiology (<http://genomicepidemiology.org/>).

3. Results and discussion

For environmental isolate Kp148/PINH-4900, a total of 1463504 paired-end reads (estimated size 5464058 bp) were generated with 78 \times coverage. A total of 53 contigs were annotated, resulting in 5549 protein-coding genes, 121 RNA-encoding genes (81 tRNAs, 28 rRNAs and 12 ncRNAs) and 159 pseudogenes, with a G+C content of 57.4%. For environmental isolate Kp196/TIET-4200, 1024364 paired-end reads (estimated size 5437723 bp) were generated with 54 \times coverage. A total of 95 contigs were generated. Annotation resulted in 5506 protein-coding genes, 121 RNA-encoding genes (80 tRNAs, 29 rRNAs and 12 ncRNAs) and 154 pseudogenes, with a G+C content of 57.4%. Finally, clinical isolate Kp314/11 generated 11865174 paired-end reads (estimated size 5319218 bp), assembled into 375 contigs, with 635.6 \times coverage. Annotation resulted in 5525 protein-coding genes, 75 RNA-encoding genes (59 tRNAs, 3 rRNAs and 13 ncRNAs) and 272 pseudogenes, with a G+C content of 57.3%.

Resistome analysis both of environmental and clinical strains revealed the presence of resistance genes to the following antimicrobial agents in all strains: aminoglycosides [*aac*(6′)-Ib-cr]; β -lactams (*bla*_{OXA-1}, *bla*_{SHV-11}, *bla*_{CTX-M-15} and *bla*_{KPC-2});

fluoroquinolones [*aac*(6′)-Ib-cr, *oqxA* and *oqxB*]; fosfomycin (*fosA*^{KP}); macrolides [*mph*(A)]; phenicols (*catB4*); sulfonamides (*sul1*); and trimethoprim (*dfpA30*). The tetracycline resistance gene *tetA* was identified in Kp148/PINH-4900 and Kp314/11 only; the aminoglycoside resistance gene *aph*(3′)-Ia was found only in environmental isolates, and *aadA2* only in Kp314/11; and the phenicol resistance gene *catA1* occurred only in strain Kp148/PINH-4900.

All strains were confirmed as ST437 according to MLST. PlasmidFinder identified four plasmids belonging to the IncFIB (pQil), IncFII (K), IncFIB (K) and IncN in all strains [4].

Surveillance of resistance genes outside of the hospital is becoming critical, mainly in regions where domestic sewage and hospital wastewater are directly discharged into rivers.

The draft genome sequences of these strains can be used for further comparisons of *K. pneumoniae* genome sequences both between clinical and environmental isolates, helping to elucidate the dissemination of antimicrobial resistance genes occurring in micro-organisms inside and outside the hospital environment. This information can also be used for public-health strategies to reduce the environmental spread of MDR micro-organisms.

These Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under accession nos. **LZCY00000000**, **LZCZ00000000** and **MAOP00000000** for strains Kp148, Kp196 and Kp314, respectively. The versions described in this paper are versions **LZCY01000000**, **LZCZ01000000** and **MAOP01000000**.

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Competing interests

None declared.

Ethical approval

Not required.

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