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Draft Genomes Sequences of KPC-2, OXA-1, OXA-9, SHV-28, TEM-1 and CTX-M-15 Producing *Klebsiella pneumoniae* ST15 and KPC-2, OXA-1, TEM-1 and LEN-2 Producing *Klebsiella variicola* ST32 from Clinical Samples in Rio de Janeiro, Brazil

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Abstract:
KPC-producing bacteria are considered one of the most worrisome multidrug-resistant micro-organisms in nosocomial infections. Here we report the draft genomes sequences of two KPC-2 and CTX-M-15 producing *Klebsiella pneumoniae* sequence type 15 (ST15) and *Klebsiella variicola* sequence type 32 (ST32) isolates obtained from clinical samples of patients in Rio de Janeiro. A genomic library was constructed using a Nextera XT Kit. An Illumina platform was used to perform whole-genome sequencing (WGS). WGS of isolates *K. pneumoniae* and *K. variicola* resulted in estimated genome sizes of 5 662 554 and 5 868 756 bp, respectively. Resistome analysis of the clinical strains revealed the presence of resistance genes to the following antimicrobials in *K. pneumoniae*: aminoglycosides [*aadA1*], [*aph (3')*-Ia], [aph(3'')-Ib], [aac(6')-Ib3], [aadA2] and [aph(6)-Id]; beta-lactams (bla_{OXA-1}, bla_{oxa-9}, $bla_{CTX-M-15}$, bla_{TEM-1}, and bla_{SHV-28}); carbapenem (bla_{KPC-2}); fluoroquinolones [*aac(60)-Ib*-cr], [*oqxA*] and [*oqxB*]; fosfomycin [*fosA*]; macrolides [*mph*(A)] and [erm(B)]; phenicols [*catB3*]; sulfonamides [*sul1*]; trimethoprim [*dfrA12*] and tetracycline [tetA] and to *K. variicola*: aminoglycosides [*aadA1*], [*aph (3)*-IIa], [aac(6')-Ib-cr] and [aph(6)-Id]; beta lactams (bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{TEM-1B} and bla_{LEN-2}); carbapenem (bla_{KPC-2}); fluoroquinolones [*aac(60)-Ib*-cr], [qnrB1], [*oqxA*] and [*oqxB*]; fosfomycin [*fosA*]; phenicols [*catB3*]; sulfonamides [*sul2*]; trimethoprim [*dfrA14*] and tetracycline [tetA]. The resistome revealed by this study might be a useful tool to elucidate the dissemination of nosocomial resistance genes in Rio de Janeiro.

Keywords: Genome, antibiotic resistance, infection, *Klebsiella pneumonia*, *Klebsiella variicola*.

INTRODUCTION

K. pneumoniae is a Gram-negative pathogen causing a wide spectrum of hospital and community-acquired infections, reaching mortality rates of more than 20%. Recently it has emerged as an increasingly resistant pathogen, frequently resistant to clinically critical antibiotic classes. In the case of carbapenemase-producing *K. pneumoniae* (KPC-Kp), the mortality can reach alarmingly more than 40% of patients (Ramos-Castañeda *et al.*, 2018; Xu *et al.*, 2017). A meta-analysis review showing the KPC-KP infection-related mortality points out that this rate varies according to several factors including the country. In Brazil, this rate is higher than the average reaching, alarmingly, 51,3% of mortality. Moreover, despite the high mortality ratio, Brazil was also the country that had the lowest number of studies considering more than 11 countries from 5 continents (Ramos-Castañeda *et al.*, 2018). In this scenario, understanding pathogen epidemiology is imperative for tracking outbreaks and developing therapeutics.

High mortality is also associated with other members of the *Klebsiella* genus, that possess overlapping in biochemical and phenotypic features and are frequently misidentified (Potter *et al.*, 2018).

The underestimated plant-associated *K. variicola* that fix nitrogen and promote plant growth (Yang, *et al.*, 2020) is being isolated not only from plants, vegetables, and animals but from human clinical samples (Martínez-Romero *et al.*, 2017). The inaccurate identification has limited the study of *K. variicola* particularly important as it has been related to diseases and can even be associated with higher mortality compared to K. pneumoniae (Maatallah *et al.*, 2014).

Here we report the draft genome sequences of two strains: a carbapenemase-producing *K. pneumoniae* and a carbapenemase-producing *K. variicola* isolated from clinical samples obtained from patients in Rio de Janeiro.

MATERIALS AND METHODS

In 2017 and 2018, *K. pneumoniae* and *K. variicola* isolates were obtained from a rectal swab of the patients in *Gaffrée and Guinle University Hospital* in Rio de Janeiro.

The genomic DNA was extracted by an EasyPure Genomic DNA Kit (Transgen Biotech Company, Beijing, China) and a genomic library was constructed using a Nextera XT Kit (Illumina Inc., San Diego, C) by Genone Company. Total genomic DNA of both strains was sequenced using a MiSeq platform from Illumina Inc. (Genone Company). The sequence reads were assembled and the draft genome using Prokka (Seemann, 2014) in the Galaxy site https://www.usegalaxy.org and RAST (Brettin *et al.*, 2015) in the site http://rast.nmpdr.org/rast.cgi. The drafts genomes sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP).

Multilocus sequence typing (MLST) were identified using the services of Institute Pasteur (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Antimicrobial resistance genes were identified using ResFinder, KmerResistance available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) and the Comprehensive Antibiotic Resistance Database (CARD) (Zankari *et al.*, 2012). Plasmid replicons and species identification were identified using the services of PathogenFinder and PlasmidFinder, respectively, available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).

RESULTS

The *K. pneumoniae* isolate showed resistance to β-lactams (ceftazidime, cefepime, cefuroxime, cefuroxime axetil, cefoxitin, ceftriaxone, ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, ertapenem, imipenem and meropenem), amikacin, ciprofloxacin, tigecycline and gentamicin, and susceptibility to polymyxin B. The *K. variicola* isolate showed resistance to β-lactams (ceftazidime, cefepime, cefuroxime, cefuroxime axetil, cefoxitin, ceftriaxone, ampicillin, ampicillin-sulbactam, piperacillin-tazobactam ertapenem, imipenem and meropenem), ciprofloxacin, tigecycline and gentamicin, and susceptibility to amikacin and colistin.

For clinical isolate, *K. pneumoniae*, a total of 5 662 554 bp were generated with 89.16 coverage. A total of 115 contigs were annotated, resulting in 5471 protein-coding genes, 92 RNA-encoding genes (77 tRNAs, 4 rRNAs and 11 ncRNAs) and 137 pseudogenes, with a G+C content of 57.04%. For clinical isolate *K. variicola* generated a total of 5 872 496 bp, assembled into 128 contigs, with 91.69 coverage. Annotation resulted in 5586 protein-coding genes, 89 RNA-encoding genes (76 tRNAs, 3 rRNAs and 10 ncRNAs) and 201 pseudogenes, with a G+C content of 56.80%.

Resistome analysis revealed the presence of resistance genes to the following antimicrobial agents in *K. pneumoniae* strain: aminoglycosides [aadA1], [aph (3')-Ia],[aph(3")-Ib], [aac(6')-Ib3], [aadA2] and [aph(6)-Id]; β-lactams (blaOXA-1, blaoxa-9, blaCTX-M-15, blaTEM-1, blaSHV-28 and blaKPC-2); fluoroquinolones [aac(60)-Ib-cr, oqxA and oqxB]; fosfomycin (fosA); macrolides [mph(A)] and [erm(B)]; phenicols (catB3); sulfonamides (sul1); trimethoprim (dfrA12) and tetracycline [tetA] and in *K. variicola* strain: aminoglycosides [aadA1], [aph (3)-IIa],[aac(6')-Ib-cr] and [aph(6)-Id]; β-lactams (blaOXA-1, blaCTX-M-15, blaTEM-1B, blaLEN-2 and blaKPC-2); fluoroquinolones [aac(60)-Ib-cr, qnrB1, oqxA and oqxB]; fosfomycin (fosA); phenicols (catB3); sulfonamides (sul2); trimethoprim (dfrA14) and tetracycline [tetA].

The *K. pneumoniae* strain was confirmed as ST15 and *K. variicola* strain was confirmed as ST32 according to MLST. PlasmidFinder identified three plasmids belonging to the IncFIB(pKPHS1), IncFII (K) and IncFIB (K) in *K. pneumoniae* and IncFII (K) and IncR in *K. variicola* (Seemann, 2014).

DISCUSSION

K. pneumoniae carbapenemase (KPC) enzyme, encoded by alleles of the blaKPC gene, represents one of the five major carbapenemase families, others being the VIM, IMP and NDM metallo- β-lactamases, and the OXA-48-like oxacillinases (Sadek *et al*., 2020).

The spread of KPC-type carbapenemase-producing strains is evident in the countries of Europe, Asia, North America, South America and Africa, being endemic in Greece and Israel. In Brazil, outbreaks are evident in hospitals in several states (Miranda *et al.*, 2018).

Many Brazilian researchers have been identified KPC-Kp in transplanted, oncology patients or with bacteremia or directly from outbreaks of KPC-Kp in intensive care units (Bergamasco *et al*., 2012; Freire *et al*., 2015; Rossi Gonçalves *et al*., 2016; Tuon *et al*., 2012). Alarmingly, the very same isolate has been responsible for lethal nosocomial outbreaks (Snitkin *et al.*, 2012).

A major challenge of *K. pneumoniae* resistant to multiple antibiotics involves the lack of treatment options, particularly in immunocompromised patients (Russo and Marr, 2019).

The present sample of K. pneumoniae, in addition to the KPC-2 gene, demonstrated a wide redundancy of β-lactamases resistance

genes (blaOXA-1, blaCTX-M-15, blaTEM-1B, and blaLEN-2). Even though several cases of resistance redundancy have been reported in form of articles or draft genomes reports (Cerdeira *et al*., 2017; Girlich *et al*., 2013; Nielsen *et al*., 2011), but still, this exact redundancy never mentioned in the available reports except a general mentioning of redundancy of resistance β-lactamases without mentioning each gene.

The redundancy was either evident in genes presenting resistance to aminoglycosides, and the results are comparable to other clinical *K. pneumoniae* data (Mbelle *et al.*, 2020). Particularly important as that it demonstrated that sub-lethal concentrations of some aminoglycosides could promote the transfer of resistance genetic elements in *K. pneumoniae* (Acosta *et al.*, 2020).

Underreported in literature, the *K.variicola* can be associated with higher mortality compared to *K. Pneumoniae* (Maatallah *et al.*, 2014). Researching clinical *K. variicola*, the β-lactamases genes were the most abundant resistance genes, ratifying our results. In this cohort study, the blaLEN-2 was found in 99,3% of the isolates, the chromosomal blaLEN-2 genes had been directly associated and generally conserved in *K. variicola* (Potter *et al.*, 2018). This ubiquitous gene is considered a constitutive β-lactamase in *K. variicola* and has been proposed to be used as a PCR strategy specifically targeting, that properly distinguish *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae* (Fonseca *et al.*, 2017). However, chromosomal recombination has already been demonstrated (Holt *et al.*, 2015; Long *et al.*, 2017).

Notably, the carbapenemase blaKPC-2 was identified in *K. variicola* isolates, originally misidentified as *K. pneumoniae*, recovered from human infections in Houston Methodist Hospital System (USA). Considering our study, the proportion of resistance genes found in each isolate was significantly lower, a maximum of three (Long *et al.*, 2017). It was also identified in clinical isolates of *K. variicola* in 80% in a China Hospital (Liu *et al.*, 2018) and 2,8% in a hospital in the USA (Potter *et al.*, 2018).

In addition to the β-lactamase genes, the blaCTX M-15 has already been identified in clinical isolates (Long *et al.*, 2017; Potter *et al.*, 2018), including hypervirulent *K. variicola* (Rodríguez-Medina *et al.*, 2019).

The redundancy of β-lactamase genes observed in the *K. variicola* investigated, named blaKPC-2, blaOXA-1, blaCTX-M-15, blaTEM-1B, blaLEN-2, was either observed in nosocomial isolates of *K.variicola* named blaKPC-2, blaLEN-2 e blaCTX-M-14 (Potter *et al.*, 2018). This redundancy was again observed in aminoglycosides resistance gene (aac(6=)lb-cr, aadA16) resembling our results (Potter *et al.*, 2018).

The *K. variicola* harbor the oqxA and oqxB encoding the OqxAB efflux pump genes that may confer resistance to fluoroquinolone such as ciprofloxacin and to other substances as the quinoxaline olaquindox. The latter has been used extensively as a growth promoter for pigs (Hansen *et al.*, 2005). Although widespread in *K. variicola* and *K. pneumoniae*, it is not necessarily involved in quinolone resistance, depending on the high expression of the oqxAB (Potter *et al.*, 2018; Rodriguez-Martinez *et al.*, 2013).

Plasmids play an important role in the dissemination of antimicrobial resistance. The IncF plasmid group, found in both *K. variicola* and *K. pneumoniae,* are known carriers of antibiotic resistance genes, including blaCTX-M and blaOXA β-lactamases (Carattoli, 2009; Potter *et al.,* 2018).

The chromosomal recombination and exchange of plasmids between *K. variicola* and *K. pneumoniae* enable them to alter the repertoire of virulence factors and antimicrobial resistance genes, causing an additional complexity in the antibiotic resistance (Holt *et al.*, 2015; Long *et al.*, 2017).

Taking into account the individual clinical importance that each of these genes has, it highlights the importance of the present report. The draft genomes sequences of these strains can be used for further comparisons of *K. pneumoniae* and *K. variicola*, helping to elucidate the antibiotic resistance mechanisms of these strains that can persist over time, inside and outside nosocomial environment.

This Whole genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers **SZNE00000000** and the version **SUB5533100** and under the accession numbers **SZND00000000** and the version **SUB5541117**, for *K. pneumoniae* and *K. variicola*, respectively.

AUTHORS CONTRIBUTION

VAM designed the study. CRS, RMM, VHLMM, MAOS, KROMR contributed to the acquisition of data: (laboratory or clinical). CRS and VAM performed data analysis. All authors contributed in the drafting of article and/or critical revision and final approval of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not required.

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