



## Genome Note

Unravelling the genome sequence of a pandrug-resistant *Klebsiella pneumoniae* isolate with sequence type 11 and capsular serotype KL64 from China

Huiqiong Jia, Hangfei Chen, Zhi Ruan\*

Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, China

## ARTICLE INFO

## Article history:

Received 7 August 2019

Received in revised form 13 August 2019

Accepted 17 August 2019

Available online 23 August 2019

## Keywords:

Whole-genome sequencing

*Klebsiella pneumoniae*

Pandrug resistance

KL64

## ABSTRACT

**Objectives:** *Klebsiella pneumoniae* has emerged worldwide as a major cause of severe infections owing to the rising prevalence of multidrug-resistant strains in clinical settings. This study aimed to investigate the genomic features of pandrug-resistant *K. pneumoniae* strain KP2 with high colistin and tigecycline resistance isolated from a patient in China.

**Methods:** The antimicrobial susceptibility of *K. pneumoniae* KP2 was determined by microdilution broth assay. Whole genomic DNA was extracted and was sequenced using an Illumina HiSeq X10 platform. *De novo* genome assembly was performed using Unicycler, and the draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The sequence type (ST), capsular type, antimicrobial resistance and virulence-related genes were identified from the genome sequence. Core genome multilocus sequence typing (cgMLST) analysis was performed by BacWGSTdb server.

**Results:** *Klebsiella pneumoniae* KP2 was resistant to all antimicrobial agents tested, including colistin and tigecycline. The genome size was calculated as 5 729 339 bp, with 5772 protein-coding sequences and a G + C content of 57.0%. The isolate was assigned to ST11 with capsular serotype KL64. Several antimicrobial resistance genes and virulence genes as well as genomic islands and multiple insertion sequences were identified in the genome sequence. The closest relative of *K. pneumoniae* KP2 was another isolate from Hangzhou that differed by only 45 cgMLST loci.

**Conclusion:** The genome sequence data presented in this study can serve as an important reference sequence for further understanding of the antimicrobial resistance mechanisms and virulence potential of this bacterial species.

© 2019 International Society for Antimicrobial Chemotherapy. Published by Elsevier Ltd. All rights reserved.

*Klebsiella pneumoniae* has gained notoriety as a major opportunistic bacterial pathogen causing a wide range of hospital-acquired infections [1]. The rapid emergence and global spread of multidrug-resistant *K. pneumoniae* strains, which hamper the effective treatment of infections, has resulted in extensive public-health concern [2]. Carbapenem-resistant *K. pneumoniae* was listed as one of the most urgent antibiotic resistance threats and as the top priority organism requiring new antimicrobials by the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) [3]. Here we describe the genomic features of a pandrug-resistant *K. pneumoniae* isolate also

exhibiting resistance to colistin and tetracycline recovered in China.

*Klebsiella pneumoniae* strain KP2 was cultured from a blood sample of a 66-year-old female patient hospitalised with symptoms of pneumonia and fever. The purified isolate was grown overnight at 37 °C in Mueller–Hinton broth (Oxoid Ltd., Basingstoke, UK). The bacterial species was identified using a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and 16S rRNA gene sequencing. *Klebsiella pneumoniae* KP2 was subjected to antimicrobial susceptibility testing by the microdilution broth method for the following antimicrobial agents: amikacin; aztreonam; cefepime; cefotaxime; cefoxitin; ceftazidime; ciprofloxacin; colistin; ertapenem; fosfomycin; gentamicin; imipenem; levofloxacin; meropenem; tetracycline; and tigecycline. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, except for tigecycline and colistin that

\* Corresponding author.

E-mail address: [r\\_z@zju.edu.cn](mailto:r_z@zju.edu.cn) (Z. Ruan).

were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Genomic DNA was extracted using a QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the protocol recommended by the manufacturer. The quality of the extracted DNA was examined using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit v.2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). A DNA library was subsequently prepared using a Nextera™ DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA), and whole-genome sequencing was performed using a HiSeq X10 platform (Illumina Inc.) with a 150-bp paired-end protocol. After sequencing, all sequence reads were pre-processed to remove low-quality or artefactual bases. FastQC v.0.11.8 was used to assess the quality of the raw data, and Trimmomatic v.0.39 was used to trim the raw sequence reads. The trimmed reads were *de novo* assembled using Unicycler v.0.4.7 with the Pilon v.1.23 option for modification of the assembled reads [4].

The genome sequence was automatically annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The multiple online web servers ResFinder 3.2, Comprehensive Antibiotic Resistance Database (CARD) 2019, Virulence Factors Database (VFDB) 2019 and Kaptive were used to identify the acquired antimicrobial resistance genes, virulence genes and capsular serotype. *In silico* multilocus sequence typing (MLST) and bacterial source tracking using a core genome multilocus sequence typing (cgMLST) strategy were performed by BacWGSTdb server [5]. Further bioinformatics analysis, such as identification of genomic islands, insertion sequence (IS) elements, prophage sequences, clustered regularly interspaced short palindromic repeat (CRISPR) sequences and secondary metabolite gene clusters, were predicted by application of IslandViewer 4, ISfinder 1.0, PHASTER 2016, CRISPRCasFinder 1.0 and antiSMASH 5.0.0, respectively, with default parameters.

The draft genome sequence of *K. pneumoniae* KP2 consists of 165 contigs comprising 5 729 339 bases, and the PGAP server predicted a total of 5772 protein-coding sequences. The overall G + C content of this strain amounts to 57.0%. In total, 78 tRNA genes and 12 rRNA operons were identified. Antimicrobial susceptibility testing showed that the isolate was resistant to all of the tested antimicrobial agents, including colistin [minimum inhibitory concentration (MIC) =64 mg/L] and tigecycline (MIC = 8 mg/L). The resistome of KP2 consists of genes responsible for resistance to aminoglycosides (*aadA2* and *rmtB*),  $\beta$ -lactams (*bla*<sub>CTX-M-65</sub>, *bla*<sub>KPC-2</sub>

and *bla*<sub>TEM-1B</sub>), fluoroquinolones (*qnrS1*), fosfomycin (*fosA*), phenicols (*catA2*), sulfonamides (*sullI*), tetracyclines [*tet(A)*] and trimethoprim (*dfrA14*) (Table 1). The isolate also possesses target alterations in the proteins GyrA (D87G) and ParC (S80I) conferring resistance to fluoroquinolones. No *mcr* genes were detected but the isolate carried a known colistin-resistant Q30Stop deleterious mutation in MgrB, a negative regulator of the PhoP/PhoQ two-component system. The genome also contains at least 15 genomic islands and several IS elements, the majority belonging to the IS3, IS5 and IS6 families. Similarly, one prophage sequence and two CRISPR sequences can be predicted in the genome. The presence of four putative secondary metabolite gene clusters, including the aerobactin, enterobactin, O-antigen and yersiniabactin biosynthetic gene clusters, can also be predicted. Several virulence-associated genes, including aerobactin (*iutA*, *iucA*, *iucB*, *iucC* and *iucD*), hypermucoviscosity (*rmpA* and *rmpA2*) and yersiniabactin (*ybtA*, *ybtE*, *ybtP*, *ybtQ*, *ybtU*, *ybtT* and *ybtX*) were identified in the genome. This strain can be classified as sequence type 11 (ST11) according to the MLST scheme developed by the Institut Pasteur and has the *wzi* allele 64 assigned to K-loci-type KL64. The phylogenetic relationship between *K. pneumoniae* KP2 and a total of 7484 *K. pneumoniae* strains currently deposited in the NCBI GenBank database was analysed. Data from the current study suggest that the closest relative of *K. pneumoniae* KP2, another ST11 strain (*K. pneumoniae* L20) recovered from a human faecal specimen in Hangzhou in March 2016, differed by only 45 cgMLST loci. Compared with *K. pneumoniae* KP2, no insertion or deletion event was found in the *mgrB* gene, and the regulator of the mucoid phenotype gene (*rmpA*) was also undetectable in these classic ST11 KPC-2-producing strains, constituting a different antimicrobial resistance and virulence potential between these strains.

In summary, here we report the genomic characteristics of a pandrug-resistant *K. pneumoniae* isolate from China. Various antimicrobial resistance genes and virulence genes, such as those encoding aerobactin, yersiniabactin and RmpA/RmpA2, the hallmarks of hypermucoviscous *K. pneumoniae*, were detected in the genome. These data can facilitate unravelling of the multidrug resistance mechanisms and virulence potential of this species, which may guide further development of new therapeutic strategies and ultimately lead to successful treatment of *K. pneumoniae* infections.

#### Nucleotide sequence accession no.

The genome sequence of *K. pneumoniae* KP2 (BioSample ID **SAMN12389481**) can be accessed at DDBJ/ENA/GenBank under the

**Table 1**  
Antimicrobial resistance genes in *Klebsiella pneumoniae* strain KP2.

Antimicrobial agent/gene	% identity	HSP length/query	Contig	Position in contig	Predicted phenotype	GenBank accession no.
<b>Aminoglycosides</b>						
<i>aadA2</i>	100	780/780	contig_101	1165–1944	Aminoglycoside resistance	<b>D43625</b>
<i>rmtB</i>	100	756/756	contig_99	498–1253	Aminoglycoside resistance	<b>AB103506</b>
<b><math>\beta</math>-Lactams</b>						
<i>bla</i> <sub>CTX-M-65</sub>	100	876/876	contig_116	102–977	$\beta$ -Lactam resistance	<b>EF418608</b>
<i>bla</i> <sub>KPC-2</sub>	100	882/882	contig_72	1461–2342	$\beta$ -Lactam resistance	<b>AY034847</b>
<i>bla</i> <sub>TEM-1B</sub>	100	861/861	contig_99	1423–2283	$\beta$ -Lactam resistance	<b>AY458016</b>
<b>Fluoroquinolones</b>						
<i>qnrS1</i>	100	657/657	contig_31	3697–4353	Quinolone resistance	<b>AB187515</b>
<b>Fosfomycin</b>						
<i>fosA</i>	99.27	412/420	contig_49	18901–9312	Fosfomycin resistance	<b>ACW001000079</b>
<b>Phenicols</b>						
<i>catA2</i>	96.11	642/642	contig_108	191–832	Phenicol resistance	<b>X53796</b>
<b>Sulfonamides</b>						
<i>sullI</i>	100	816/816	contig_82	512–1327	Sulfonamide resistance	<b>AY034138</b>
<b>Tetracyclines</b>						
<i>tet(A)</i>	100	1200/1200	contig_90	971–2170	Tetracycline resistance	<b>AJ517790</b>
<b>Trimethoprim</b>						
<i>dfrA14</i>	100	474/474	contig_123	282–755	Trimethoprim resistance	<b>KF921535</b>

accession no. VONW00000000. The version described in this paper is the first version (VONW01000000).

#### **Funding**

None.

#### **Competing interests**

None declared.

#### **Ethical approval**

Not required.

#### **References**

- [1] Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013;13:785–96.
- [2] Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol* 2014;22:686–96.
- [3] World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva, Switzerland: WHO; 2017. . [Accessed 28 July 2019] <https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en>.
- [4] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:e1005595.
- [5] Ruan Z, Feng Y. BacWGSTdb, a database for genotyping and source tracking bacterial pathogens. *Nucleic Acids Res* 2016;44:D682–7.