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Genome Note

Draft genome sequence of a metallo-β-lactamase (bla_{AIM-1})-producing *Klebsiella pneumoniae* ST1916 isolated from a patient with chronic diarrhoea

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ABSTRACT

Objectives: *Klebsiella pneumoniae* colonisation of the human gastrointestinal tract is a significant risk factor for extraintestinal infections in severely ill patients. Recent reports have indicated the high rate of *K. pneumoniae* infection resulting from the patient's own gut microbiota. Here we report the draft genome sequence of a multidrug-resistant (MDR) *K. pneumoniae* strain (SM32) harbouring the bla_{AIM-1} gene isolated from a patient with chronic diarrhoea in China.

Methods: Whole genomic DNA was sequenced using an Illumina MiSeq platform. The generated reads were de novo assembled using SOAPdenovo v.2.04. All probable coding sequences were predicted by Glimmer v.3.02 and were annotated using information from GenBank, Pfam, Nr, COG, String, GO and KEGG. Resistance-related genes were also further identified.

Results: The draft genome of *K. pneumoniae* SM32, belonging to sequence type (ST) 1916, was assembled into 165 contigs comprising 5 238 542 bp. A total of 5013 protein-coding sequences and several genes associated with resistance to β-lactams, tetracycline, aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole were preliminarily identified.

Conclusions: MDR *K. pneumoniae* colonising the human gastrointestinal tract provides a potential reservoir for extraintestinal infections. The genome sequence of *K. pneumoniae* SM32 will be helpful to reveal the key role of mobile genetic elements in the adaptive translocation and spread of antimicrobial resistance.

Klebsiella pneumoniae is one of the most important emerging opportunistic pathogens causing severe nosocomial infections such as urinary tract, respiratory tract and bloodstream infections, particularly in patients with serious illnesses [1]. In the past few decades, easy accessibility and abuse of antibiotics has led to the emergence of multidrug-resistant (MDR) *K. pneumoniae* as a common intestinal symbiotic micro-organism colonising the human gastrointestinal tract in China, providing a potential reservoir for extraintestinal infections in severely ill patients [2,3]. Recent reports also indicated that colonisation of the gastrointestinal tract with MDR *K. pneumoniae* is strongly linked to subsequent infections, many of which result from the patient's own microbiota [1,2]. This phenomenon has been exacerbated by the spread of metallo-β-lactamases (MBLs) owing to their typically broad-spectrum activities encompassing carbapenems and conferring resistance to nearly all β-lactams [4]. To date, many MBL genes (e.g. bla_{NDM-1}, bla_{IMP}, bla_{VIM} and bla_{KMH}) have been found in *K. pneumoniae*, but very little is known about the bla_{AIM-1} gene, belonging to subgroup B3 MBLs, that is mobilisable. The bla_{AIM-1} gene was first discovered in three *Pseudomonas aeruginosa* isolates from Australia [5].

K. pneumoniae strain SM32 was isolated from a faecal sample of a 34-year-old male patient with long-term diarrhoeal disease. It

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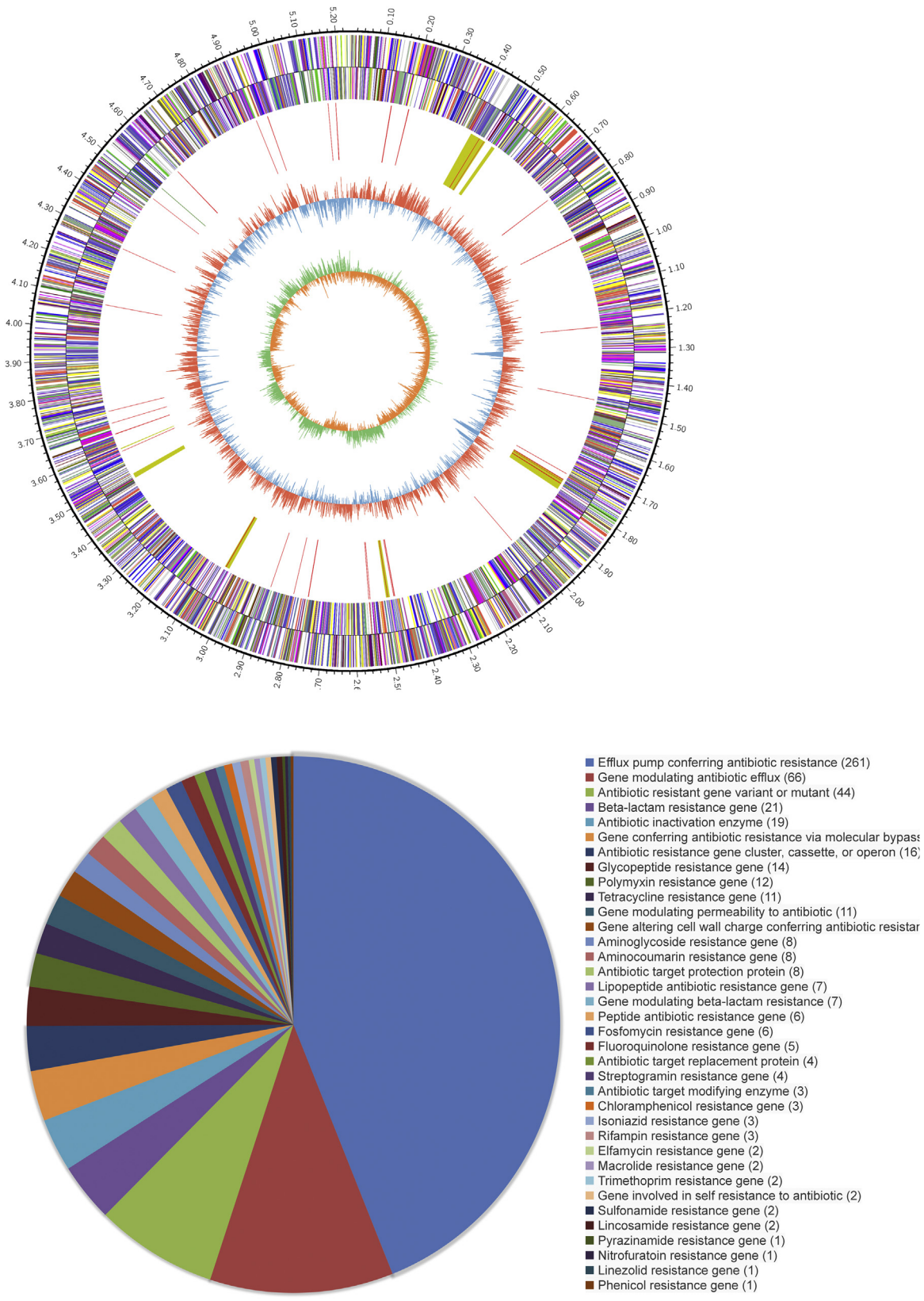

Fig. 1. Overview of the genome of ST1916 *Klebsiella pneumoniae* strain SM32. (a) Circular map of the draft genome. Features from the outside to inside: (i) DNA base position (black); (ii and iii) forward and reverse strand coding sequences, respectively [different colours represent different functional categories of Clusters of Orthologous Groups (COG)]; (iv) tRNA (red), rRNA (green) and genomic islands (black–yellow); (v) G + C content (positive, red; negative, blue); and (vi) GC skew (positive, green; negative, orange). (b) Resistance-related genes based on Antibiotic Resistance Ontology (ARO) category.

was identified as *K. pneumoniae* according both to Bruker Biotyper MALDI-TOF/MS system (Bruker Daltonik GmbH, Bremen, Germany) and 16S rRNA gene sequencing analysis. Antimicrobial susceptibility testing and interpretation were performed using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. *Klebsiella pneumoniae* SM32 displayed resistance to several antibiotics including ampicillin, ampicillin/sulbactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole and tetracycline but remained susceptible to piperacillin, amikacin, aztreonam, levofloxacin and chloramphenicol.

K. pneumoniae strain SM32 was cultured overnight in liquid Luria–Bertani medium at 37 °C and its genomic DNA was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Valencia, CA). A TruSeq™ DNA Sample Preparation Kit (Illumina Inc., San Diego, CA) was used to construct the DNA library (ca. 400 bp library), and paired-end sequencing was performed using a MiSeq platform (Illumina Inc.) at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions. Following quality control, the generated reads were de novo assembled using SOAPdenovo v.2.04 (http://soap.genomics.org.cn/). Glimmer v.3.02 (http://ccb.jhu.edu/software/glimmer/index.shtml) was used to predict genes, and Barrnap 0.4.2 and tRNAscan-SE v.2.0 were used to forecast rRNAs and tRNAs, respectively.

The predicted protein-coding sequences were blasted with each function database (GenBank, Pfam, Nr, COG, String, GO and KEGG). Further bioinformatics analysis, such as for genomic islands (GIs), insertion sequence (IS) elements, prophage sequences, clustered regularly interspaced short palindromic repeats (CRISPRs) and secondary metabolite gene clusters, were predicted by IslandViewer (http://www.pathogenomics.sfu.ca/islandviewer/), ISfinder (https://www-is.biotoul.fr/), PHAST (http://phast.wishartlab.com/), CRISPRFinder (http://crispr.i2bc.paris-saclay.fr/Server/) and antiSMASH (https://antismash.secondarymetabolites.org/) tools, respectively. In addition, multilocus sequence typing (MLST) and resistance-related genes of *K. pneumoniae* SM32 were analysed using the *K. pneumoniae* MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) and the Resistance Gene Identifier from the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/genomes).

The final assembled draft genome sequence of *K. pneumoniae* SM32 consists of 165 contigs comprising 5 238 542 bp, with an average of 198-fold coverage and 57.44% G + C content (Fig. 1a). A total of 5013 protein-coding sequences, 83 predicted tRNAs and 3 rRNAs were identified in the genome (Fig. 1a). The genome also contains nine GIs and several IS elements, with the majority belonging to the IS*21*, IS*5*, IS*3* and IS*4* families. Six prophage regions (of which two are intact and two incomplete), 131 repeats and 16 CRISPR-Cas sequences have been identified in the genome, and three putative secondary metabolite gene clusters, including a non-ribosomal peptide synthetase (NRPS), bacteriocin and thiopeptide biosynthetic gene cluster, can also be predicted. MLST analysis showed that isolate SM32 belongs to ST1916.

Based on annotation from CARD, the resistance-related genes of *K. pneumoniae* SM32 are presented in Fig. 1b. Interestingly, the genome harbours a large number of efflux pump genes conferring antimicrobial resistance (*mdtD*, *macB*, *emrB*, *bcrA*, *acrA*, *vgaC*, etc.), which may contribute to the development of multidrug resistance in this strain. The following resistance genes were identified: bla_{AIM-1}, *mecC*, *nmcR* and *ompF* (β-lactam resistance); *kdpE* and *acc (6′)-Iy* (aminoglycoside resistance); *fyuA*, *tetT*, *tetB*(P), *tetS*, *otrA* and *rpsJ* (tetracycline resistance); *oqxA*, *oqxB*, *gyrA* and *mfd* (fluoroquinolone resistance); *vgaA*, *vatB* and *erm*(34) (streptogramin resistance); *cmlv* (chloramphenicol resistance); *macA* and *macB* (macrolide resistance); *fosA5* and *murA* (fosfomycin resistance); *leuO* and *folP* (sulfonamide resistance); *arnA*, *pmrC* and *lpxA* (polymyxin resistance); and *dfrA3* and *dfrE* (trimethoprim resistance).

In summary, here we report the draft genome sequence of a clinical MDR *K. pneumoniae* SM32 (ST1916) strain harbouring the bla_{AIM-1} gene. Antimicrobial susceptibility testing revealed that the strain was resistant to most usual antibiotics and may eventually become pandrug-resistant. Future studies on comparative analysis of this and other MDR *K. pneumoniae* from different sources are currently underway, which may help our understanding of the genomic diversity and molecular mechanisms of antimicrobial resistance in this bacterial pathogen.

Nucleotide sequence accession no.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession no. **QUOX00000000**. The version described in this paper is the first version (**QUOX00000000.1**). Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (accession no. **SRR7445582**).

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Competing interests

None declared.

Ethical approval

Not required.

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