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Short Research Communication

Draft Genome of Multidrug-Resistant *Klebsiella pneumoniae* 223/14 Carrying KPC-6, Isolated from a General Hospital in Malaysia

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Abstract

We performed whole genome sequencing on a clinical multidrug-resistant *Klebsiella pneumoniae* strain 223/14. Investigation into its draft genome revealed the presence of KPC-6 variant, suggesting carbapenemase is present in this isolate. We found a plasmid-borne KPC gene (882 bp) inserted between two transposase genes in the genome of *K. pneumoniae* 223/14.

Key words: *Klebsiella pneumoniae* Carbapenemase (KPC), multidrug resistance, whole genome sequencing

The antibiotic Carbapenem is considered a last option to treat nosocomial infections caused by extended spectrum β-lactamases (ESBL)-producing multidrug-drug-resistant Gram-negative bacteria (1). However, the emergence and widespread of carbapenem resistant pathogens pose a major threat in global healthcare (2). Among the documented resistant mechanisms, increasing worldwide reports of hospital outbreaks caused by *Klebsiella pneumoniae* Carbapenemase (KPC) producing *Enterobacteriaceae* are of great concern (3). Known to be the most common carbapenemase, KPC is mostly found in *Klebsiella pneumoniae* with increasing identification in other *Enterobacteriaceae* that display carbapenem resistance (4). KPCs are categorized into eleven known variants (KPC-2 to KPC-12) differing by changes in a few amino acids (5). In this study, the genetic determinants for multidrug-resistant of a *K. pneumoniae* strain 223/14, isolated from infected laparotomy wound of a patient were investigated by whole-genome sequencing.

The genome sequence of *K. pneumoniae* strain 223/14 was generated using Single Molecule Real-Time (SMRT) RSII sequencing platform (Pacific Biosciences, Inc., CA). Prior to sequencing analysis, the genomic DNA was isolated using MasterPure™ DNA purification kit (Epicentre, USA) followed by shearing of the DNA to fragment size targeted at 10kb. The SMRTbell library preparation was subsequently performed using Template Preparation Kit (Pacific Biosciences, Inc., CA) and P4/C2 chemistry was employed for the sequencing analysis. A total of 3 SMRT cells were used for sequence collection and the duration was set at 180 minutes per cell with stage

start option. After acquisition of the sequence data, quality filtering and assembly was subsequently performed using the Hierarchical Genome-Assembly Process (HGAP) module available from the Pacific Biosciences's SMRT portal (6).

A total of 106,678 polymerase reads with average read length of 5,218 bp, amounting to 556,747,120 bases were generated after quality filtering process. The polished assembly size of the draft genome was at 6,165,397 bp with average G+C content of 57%. The assembly has yielded 13 contigs with average sequencing depth of 75.73× and the largest contig size was at 5,584,553 bp. Using Rapid Annotation Subsystems Technology (RAST) server (7), prediction and annotation of genes in the assembled sequences was performed, identifying 5,973 open reading frames and 112 coding sequences of rRNAs and tRNAs. Validation of ORFs associated with antibiotics resistance was subsequently performed via sequence comparison with NCBI-NR database using BLAST.

A plasmid-borne KPC gene (882 bp) inserted between two transposase genes was identified in the genome of *K. pneumoniae* 223/14. Sequence alignment using ClustalW (8) with eleven known KPC variants has verified that the gene belongs to KPC-6 variant based on nucleotide variations at position 147, 308, 502, 716, and 814 of the gene (5). Such finding has represented the emergence of pathogens harboring KPC-6 in Malaysia since the initial detection of this KPC variant from Puerto Rico in 2008 (9).

Nucleotide sequence accession number

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession JRTV00000000. The version described in this paper is the first version, JRTV01000000.

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Competing Interests

The authors have declared that no competing interest exists.

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