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Genomic characterisation of a colistin-resistant *Klebsiella pneumoniae* ST11 strain co-producing KPC-2, FloR, CTX-M-55, SHV-12, FosA and RmtB causing a lethal infection

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ABSTRACT

Objectives: The aim of this study was to characterise the genomic and phenotypic characteristics of a colistin-resistant *Klebsiella pneumoniae* isolate causing a lethal infection that was phenotypically resistant to carbapenems and colistin.

Methods: Whole-genome sequencing was performed on an Illumina HiSeq 2500 platform. Genome annotation was performed by the Rapid Annotation using Subsystem Technology (RAST) server. Antimicrobial resistance genes and plasmid replicons were identified using ResFinder 2.1 and PlasmidFinder 1.3, respectively. The isolate was further characterised by plasmid analysis using *S1*-PFGE and Southern blot hybridisation with a digoxigenin-labelled probe specific for bla_{KPC}.

Results: The genome of *K. pneumoniae* LSK16 consists of a 6.02-Mbp chromosome and one plasmid. *Multilocus* sequence typing (MLST) identified the isolate as ST11, a close variant of the international pandemic clone ST258. The isolate was found to harbour bla_{KPC-2}, bla_{SHV-12}, $bla_{CTX-M-55}$, *floR* and *rmtB* genes. Of note, a novel fosfomycin resistance glutathione transferase variant was confirmed by PCR and sequencing, with 98.6% (136/138) identity to *fosA*. Moreover, amino acid substitutions in PmrB (R256G) and PhoQ (D150G) were identified in LSK16, confirming the polymyxin/colistin resistance, although the isolate was negative for *mcr* genes. Southern blotting and plasmid analysis revealed that the bla_{KPC-2} gene was harboured on a non-conjugative IncR plasmid (~165 kb).

Conclusion: Here we identified a colistin-resistant *K. pneumoniae* ST11 strain co-producing KPC-2, FloR, CTX-M-55, SHV-12 and RmtB causing a lethal infection. This study provides new genomic insights into the diversity of *K. pneumoniae* ST11 prevalent in Zhejiang Province, China.

The emergence and dissemination of carbapenemases in Enterobacteriaceae is a global public-health threat [1]. *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-Kp), especially colistin-resistant KPC-Kp, are particularly worrisome as the incidence of such infections has increased dramatically and very few therapeutic options remain. In China, sequence type 11 (ST11) is the most prevalent KPC-Kp clone, contributing to the nationwide spread of KPC-Kp [1]. In the current study, a colistin-resistant ST11 KPC-Kp strain was isolated from a patient with fatal bacteraemia and this strain provides a genomic basis for drug resistance.

In May 2016, a 73-year-old male patient was hospitalised in the Central Hospital of Lishui (Lishui, China) with a history of dyspnoea and chest pain. During his hospitalisation the patient developed a bloodstream infection and an extensively drug-resistant *K. pneumoniae* strain (LSK16) was isolated from blood samples. Despite receiving 2 months of antibiotic therapy with tigecycline and meropenem, no adequate clinical response was noted; the family requested discharge from the hospital and the patient subsequently died.

Strain LSK16 was identified as *K. pneumoniae* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Total DNA of *K. pneumoniae* LSK16 was extracted

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from 2 mL of pure cell culture using a Gentra® Puregene® Yeast/Bact. Kit (QIAGEN, Hilden, Germany). Carbapenemase genes were identified by PCR and Sanger sequencing. Whole-genome sequencing was performed at Novogen Tech. Co. Ltd. (Beijing, China) on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA). Short contigs of <500 nucleotides in length were discarded. Genome annotation was performed by Rapid Annotation using Subsystem Technology (RAST) server, and specific genes were manually curated. Multilocus sequence typing (MLST) was performed by the classical protocol as described previously (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Plasmid sequences and antimicrobial resistance genes were identified using PlasmidFinder 1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/), respectively. The strain was further characterised by plasmid analysis using pulsed-field gel electrophoresis of *S1* nuclease-digested total DNA (*S1*-PFGE) and Southern blot hybridisation with a digoxigenin-labelled probe specific for bla_{KPC} [2].

Strain LSK16 showed a high level of resistance to all antibiotics tested [3] except tigecycline (http://www.eucast.org/clinical_breakpoints/) (Supplementary Table S1). MLST analysis identified the isolate as ST11. PCR and sequencing confirmed that LSK16 harbours bla_{KPC-2} but was negative for *mcr* genes. Plasmid analysis [4] revealed that the bla_{KPC-2} gene was located on an IncR plasmid of ~165 kb in size (Fig. 1A). Repeated attempts at conjugation experiments failed, suggesting that bla_{KPC-2} was located on a non-conjugative plasmid. This is consistent with previous reports indicating that most bla_{KPC-2}-bearing plasmids (75%) detected in *K. pneumoniae* in China are non-conjugative [5].

The genome assembly of *K. pneumoniae* LSK16 has a G+C content of 56.8% and consists of the chromosome of 60 239 kb with 100 contigs. In silico analysis predicted a total of 6176 genes, 5868 protein-coding sequences, 155 pseudogenes, 14 rRNAs, 87 tRNAs and 12 ncRNAs in the whole genome.

Further bioinformatics analysis found that LSK16 harbours genes conferring resistance to multiple antibiotics, including β-lactams (bla_{KPC-2}, bla_{SHV-12}, bla_{TEM-1} and $bla_{CTX-M-55}$), fluoroquinolones (*oqxAB*), macrolides (*mphA*), phenicols (*floR*-like), aminoglycosides (*strA*, *strB*, *rmtB* and *aadA5*), fosfomycin (*fosA*-like), sulfonamides (*sul1*), tetracycline (*tetA*) and trimethoprim (*dfrA17*). A fosfomycin resistance glutathione transferase variant was identified by PCR and sequencing, with 98.6% (136/138) identity to FosA (Supplementary Fig. S1). Moreover, amino acid substitutions in PmrB (R256G) and PhoQ (D150G) were identified in LSK16 compared with *K. pneumoniae* NTUH-K2044 as reference. Interestingly, the substitutions in PmrB and PhoQ were also observed in colistin-resistant *Klebsiella* isolates in previous studies [6,7]. These results may explain the colistin resistance of LSK16, although it was negative for *mcr* genes.

Analysis of the genetic environment of the bla_{KPC-2} gene in LSK16 showed that it was genetically organised in the same manner as the bla_{KPC-2}-bearing plasmids previously reported in China (Fig. 1B). bla_{KPC-2} is flanked upstream by IS*Kpn8* and downstream by IS*Kpn6*-like insertion sequence elements, demonstrating the transmission mechanism of this structure in China. The genetic context of the *floR* gene in LSK16 is identical to chromosomal *floR* in *Escherichia coli* MRY15-117 [8] and the pECY53 plasmid, with the linear structure IS*91*–*virD2*–*floR*–*lysR*–*tetA*–*tetR* (Fig. 1C).

In summary, here we describe a comprehensive characterisation of a colistin-resistant KPC-2-producing *K. pneumoniae* strain causing fatal bacteraemia. This study highlights the risk of infection caused by colistin-resistant KPC-producing *K. pneumoniae* ST11 as well as the difficulty in treating this infection. Moreover, this work also

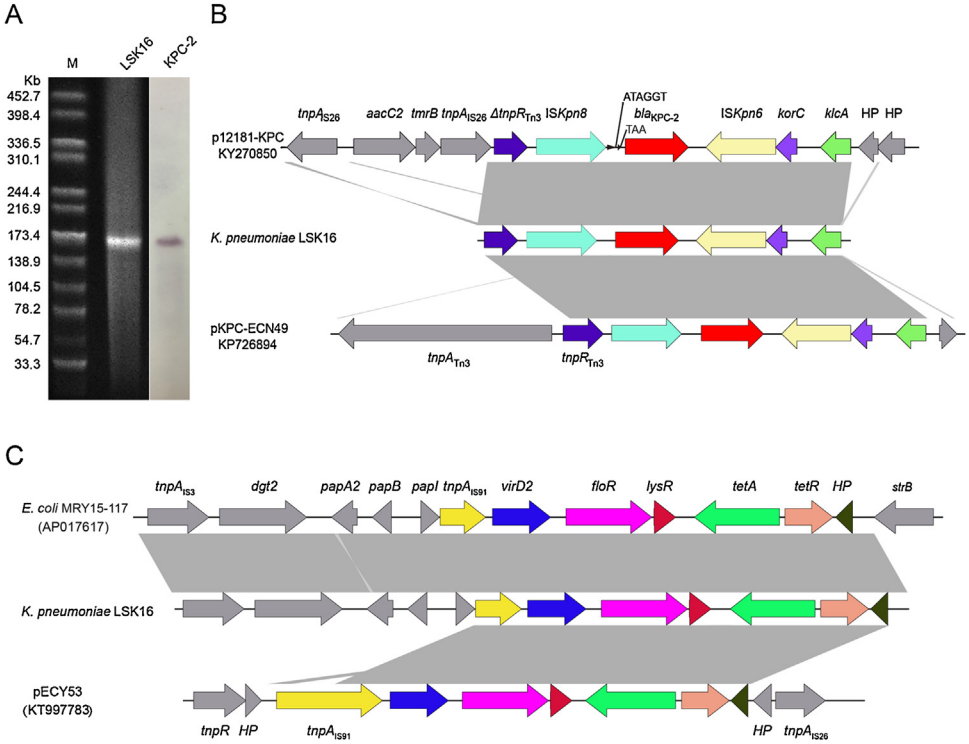

Fig. 1. (A) Pulsed-field gel electrophoresis of *S1* nuclease-digested total DNA from *Klebsiella pneumoniae* LSK16 and Southern blot hybridisation with a bla_{KPC-2}-specific probe. *Salmonella enterica* serovar Braenderup was used as a molecular mass marker. (B) Schematic representation of the genetic environment of the bla_{KPC-2} gene in *K. pneumoniae* strain LSK16 and bla_{KPC-2}-bearing plasmids previously reported in China. (C) Structural features of the *floR* gene in *K. pneumoniae* strain LSK16 and other *floR* genes. Various genes and their directions of transcription are presented as broad arrows. Similar regions are indicated by grey shading.

illustrates a more comprehensive genomic picture of antimicrobial resistance in *K. pneumoniae* ST11.

The Whole Genome Shotgun project of *K. pneumoniae* LSK16 has been deposited at DDBJ/ENA/GenBank under accession no. **NFUI00000000**. The version described in this paper is version **NFUI01000000**.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.08.023.

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