EV0504

Occurrence of hypervirulent K2 serotype *Klebsiella pneumoniae* ST2398, clonal complex 65, in the context of an invasive liver abscess syndrome

Alexander Mischnik[1], Christine Pichler[2], Martin Büchsel[3], Martina Vavra[1], Winfried V. Kern[1], R. Thimme[2], John W Rossen[4]

[1]*Division of Infectious Diseases, Department of Medicine II, Medical Center – University of Freiburg, Germany*
[2]*Department of Medicine II, Medical Center – University of Freiburg, Germany*
[3]*Institute for Medical Microbiology and Hygiene, Medical Center – University of Freiburg, Germany*
[4]*Department of Medical Microbiology, University Medical Center Groningen, The Netherlands*

Introduction and Purpose

We further analyzed a *Klebsiella pneumoniae* (KP) isolate from a 61-year old male patient with invasive liver abscess syndrome and endophthalmitis without travel history to Asia, but reported a recent journey to Brazil.

Methods

The isolate was sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA) aiming at a coverage of at least 60-fold. Quality trimming of reads was performed with CLC Genomics Workbench 9.0.1 (Qiagen, Hilden, Germany) using a minimum Phred (Q) score of 28. De novo assembly was performed using CLC Genomics Workbench 7.0.4 (Qiagen) with optimal word sizes based on the maximum N50 value. For the assembled genome, the coverage (mean depth) was 85, the number of contigs was also 85, the N50 was 164 351, the maximum contig length was 368 426 nt and the total genome size was 5 459 317 nt. The multilocus sequence typing (MLST) sequence type (ST) was extracted from the assembled genome using Seqsphere+ version 3.0 (Ridom, Muenster, Germany) and appeared to be a new ST that was subsequently submitted to the MLST database (*http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html*). A clonal complex analysis was performed by eBURST (http://eburst.mlst.net/). Genes relating to virulence were detected using the mapping unit of CLC Genomics Workbench to map reads and/or by blasting assembled genomes to a pseudomolecule generated by concatenating a set ofK. Pneumoniae genes. Finally, the genetic similarity of our isolate with other K. pneumoniaestrains [Struve et al., Mbio 2015] was determined by a gene-by-gene comparison using Seqsphere+ version 3.5.0 (Fig. 1).

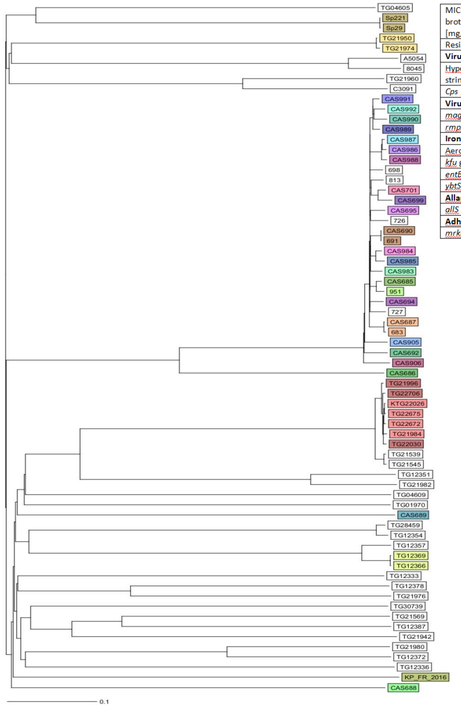

Fig 1 Ridom Seqsphere+ neighbour-joining tree for 68 samples including our strain KP_FR_2016 [*Holt et al.*, PNAS 2015]. Tree is based on 2358 columns, pairwise, ignoring missing values. Distance is based on columns from KP sensu lato cgMLST scheme provided by Seqsphere+.

MICs for selected β-lactams determined by broth microdilution (MICRONAUT system) [mg/L]	Ampicillin/sulbactam 2; amoxicillin/clavulanate 4; piperacillin/tazobactam 1; ceftazidime 0.25; cefotaxime 0.25; cefepime 0.25; meropenem 0.5; ertapenem 0.125
Resistance profile	Negative for ESBL, AmpC, Carbapenemase
Virulence-associated features	
Hypermucoviscosity (defined by positive string test)[2]	Positive
Cps genotype[1]	K2
Virulence gene profiles	
magA gene	-
rmpA gene	Present
Iron acquisition	
Aerobactin (*iutA* gene)	Present
kfu gene	-
entB gene	Present
ybtS gene	Present
Allantoin metabolism	
allS gene	-
Adhesins	
mrkD gene	Present

Table 1 Characteristics KP_FR_2016

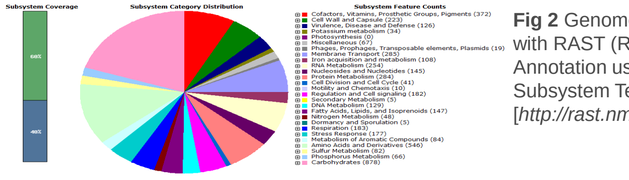

Fig 2 Genome annotation with RAST (Rapid Annotation using Subsystem Technology) [*http://rast.nmpdr.org*]

Fluoroquinolone						
Resistance gene	%Identity	HSP length/Query	Contig	Position in contig	Predicted phenotype	Accession number
oqxB	98.95	3153 / 3153	PIZ18796937_S8_L001_R1_001_(paired)_trimmed_(paired)_contig_17	335838..338990	Quinolone resistance	EU370913
oqxA	99.66	1176 / 1176	PIZ18796937_S8_L001_R1_001_(paired)_trimmed_(paired)_contig_17	339014..340189	Quinolone resistance	EU370913

Fosfomycin						
Resistance gene	%Identity	HSP length/Query	Contig	Position in contig	Predicted phenotype	Accession number
fosA	95.48	420 / 420	PIZ18796937_S8_L001_R1_001_(paired)_trimmed_(paired)_contig_34	14090..14509	Fosfomycin resistance	NZ_AFBO01000747

Fig 3 Resistance genes determined by ResFinder [*https://cge.cbs.dtu.dk/services/ResFinder*]

Discussion

Biofiling of different KP clones revealed several clusters, of which our isolate belonged to clonal complex 65. A recent analysis conducted by *Holt et al.*, PNAS 2015 showed that ST23 and ST65 strains are dominant among hypervirulent KP strains. Our isolate is very close to ST65 and was assigned to ST2398. In Europe several cases have also been described, with many cases in France. Our case illustrates worldwide occurrence of hypervirulent strains. Adequate infection control and antimicrobial stewardship measures must be in place to contain further spread.

Study accession number

Generated raw reads were submitted to the European Nucleotide Archive (ENA) of the European Bioinformatics Institute (EBI) under the study accession number PRJEB19331

Contact

alexander.mischnik@uniklinik-freiburg.de