An in silico Analysis of Carbapenem-Resistant Klebsiella pneumoniae

Lauren Elam, Krishnan Chittur, Tatyana A. Sysoeva, Department of Biological Sciences

Overview

Carbapenems are broad-spectrum antibiotics in the beta-lactam family. They are used against severe infections, often as “last resort” antibiotics. Patients infected with carbapenem-resistant bacteria have high mortality rates. Our objective was to assemble whole genome sequences of clinical isolates from Duke University Hospital that tested positive for carbapenem resistance through antibiotic susceptibility testing and to identify the genetic mechanisms of carbapenem resistance and determine their mobility.

Methods

Sequencing data collection was sponsored by the NIH K12 DK100024 award to TAS. All assemblies were performed using services provided by the Alabama Supercomputer Authority. Thank you to the offices of the President, Provost, and Vice President for Research and Economic Development at UAH, as well as the College of Science for funding this project.

Analysis

ResFinder
CARD rgl
PlasmidFinder
oriT Finder
BLAST

Analyses of assembled sequences:
Numerous antibiotic resistance genes were discovered in the genomes of each sample. Each genome included the gene blaKPC-3, which encodes for the carbapenemase KPC-3. Plasmids were then characterized by replicon type and presence of mechanisms for conjugation to determine mobility (oriT, relaxase, T4SS, T4CP).

Isolates CRE 12 and 24:
More detailed analyses of CRE 12 and 24 isolates revealed highly similar genomes. Plasmid p1CRE24 aligns with p1CRE12 but contains an insertion including three additional resistance genes. Plasmid p2CRE24 aligns with 2aCRE12 and 2bCRE12 combined. p3CRE12 and p3CRE24 have identical sequences.

Additional findings showed that while the CRE 12 and CRE 24 samples were collected over two months apart, the later sample seems to have acquired three new antibiotic resistance genes. Additional in vitro testing can be utilized to compare the genomes of the isolates and to confirm the nature and relationship between p2CRE24 and p2a/2bCRE12.

Conclusions

A gene known to cause carbapenem resistance, blaKPC-3, was identified in each of the genomes of the three clinical isolates studied. The genes are located on plasmids currently not thought to be mobile, but further testing should be done for confirmation. There were, however, two plasmids that appear to have conjugative capabilities which contain multiple other types of antibiotic resistance genes.

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References

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