O018

2-hour Oral Session

Whole genome sequencing analysis of a *Klebsiella pneumoniae* strain from Turkey carrying NDM-1, VIM-1 and OXA-244 carbapenamase encoding genes

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Objectives: To evaluate the genetic characteristics, resistance genes and indole elements associated with potent carbapenamase-encoding genes detected in a *K. pneumoniae* isolate as part of the SENTRY Antimicrobial Surveillance Program using whole genome sequencing analysis (WGS).

Methods: Isolate was tested for susceptibility by CLSI methods. Total genomic DNA was used as input material for library construction prepared following the Nextera™ protocol. DNA sequencing was performed on a MiSeq Sequencer (Illumina, CA, USA). Sequencing coverage depth was approximately 30x and of the DNA sequence reads were *de novo* assembled using the CLCBio suite. Final DNA analysis used DNAStar NGen 12. Plasmid assignments of the carbapenamase genes was confirmed by SI nuclease digests followed by hybridization with specific probes.

Results: A 69 y/o female was admitted to a hospital ICU in Istanbul, Turkey after multiple cardiac surgeries in a different institution and presented with sepsis and pneumonia. The patient was treated with meropenem (12 days), teicoplanin and daptomycin. Blood cultures grew *A. baumannii.* A *K. pneumoniae* was recovered from a urine specimen after a prolonged stay in the ICU and was submitted to the SENTRY Program. This isolate was resistant to all beta-lactams, including carbapenems. The NDM-1 encoding gene was carried in a 120-Kb plasmid Tn*3*-like transposon, followed by *bleoR,* PRAI (phosphoanthranilate isomerase involved in tryptophan biosysnthesis), a hypotetical protein, *CutA/DsbC*, *groES*, *groEL* (and another copy of PRAI in this other). The VIM-1 gene was located in the first position of a class 1 integron followed by *aacA27* and *qacEDelta/sul1* which were located in a 190-Kb plasmid. The carbapenemase gene encoding OXA-244 was embedded in a 70-Kb plasmid and followed by IS*1999* inverted repeat (IR), *tnpA,* IS*1999* IR, *tir* (disrupted by Tn*1999*) and plasmid toxin-antitoxin genes. Other resistance genes identified were bla_{TEM-1}, bla_{SHV-11}, bla_{OXA-10}, *dfrA1*, *aadB*, *cml-15*, *catA2*, *rmtF*, *aacA4*. Outer membrane protein sequences displayed stop codons (*ompK35*), multiple insertions and deletions of 4-7 amino acids (*ompK36* and *ompK37*). *K. pneumoniae* isolate belonged to ST525 (alleles 4-4-1-1-48-1-22).

Conclusions: Various carbapenemase-encoding genes seem to be circulating among Enterobacteriaceae and other Gram-negative pathogens in Turkish hospitals, but in this instance three of these genes have been detected in a single *K. pneumoniae* clinical isolate. All three genes were located in different plasmid structures suggesting separate acquisition events. Furthermore, this strain belonged to ST525 that was recently reported as a cause of a large multi-year outbreak in Hungary and further investigations should focus on the dissemination of this organism and it potential to acquire resistance determinants in other European countries. Highlights its potential of *K. pneumoniae* isolates to acquire multiple resistance mechanisms that could lead to clinical failure.