27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: EV026 Resistance surveillance & epidemiology: Gram-negatives

Category: 3b. Resistance surveillance & epidemiology: Gram-negatives

22 April 2017, 08:45 - 15:30
EV0486

Draft plasmid sequence of an XDR Acinetobacter baumannii strain reveals linked dissemination of carbapenem resistance and virulence genes

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Background: *Acinetobacter baumannii* is a threatening opportunistic pathogen in hospital settings and is responsible for various infections, including bacteraemia, urinary tract, skin and wound infections. Carbapenem resistance in *A. baumannii* represents a major threat, often leading to treatment failure and to the persistence of these strains in the hospital environment. Although highly resistant *A. baumannii* strains are increasingly reported in Romania, there are few studies regarding the genetic support of resistance and dissemination in this opportunistic pathogen.

Material/methods: ICUB_Aba1886 *A. baumannii* strain was isolated from a nasal exudates, in a patient hospitalized for surgery in the Institute for Cardiovascular Diseases ”Prof. C.C. Iliescu”,

Bucharest, in May 2016 and selected for further analysis based on its carbapenem resistance phenotype. Antibiotic susceptibility testing was performed by Vitek II system. Carbapenemase genes (OXA-23, OXA-24/40, OXA-58 and OXA-143-like) and plasmid-mediated colistin resistance (*mcr-1*) were inquired for by PCR and sequencing. Transferability of carbapenem resistance was assessed by conjugation (liquid mating) using *Acinetobacter baylei* ADP1 as a recipient strain. The sequence of the plasmid carrying the carbapenemase was determined using a primer-walking approach. Primers were designed considering the common regions of all plasmids from OXA-72 producing *Acinetobacter* sp. with the size of about 10.000 bp deposited in GenBank, that were aligned using Mauve progressive aligner. The amplicons were sequenced using the Sanger method and the corresponding nucleotide sequences were assembled using the Genome ARTIST software. Annotations were performed using the GeneMark software and BLAST tool. Replicon typing was assessed by *in silico* PCR, using Primer Blast.

Results: The selected *A. baumannii* strain exhibited an extended drug resistance (XDR) phenotype including ticarcillin, piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, tobramicin, pefloxacin, ciprofloxacin, colistin and rifampicin. The strain was positive for the OXA-72 carbapenemase, but the *mcr-1* gene was absent. Conjugation assay revealed that the carbapenem resistance is transferable. Whole plasmid sequencing revealed the presence of $bla_{\text{OXA-72}}$ in the 9753 bp plasmid pICUB_Aba1886, belonging to GR2 plasmid family. Adjacent to $bla_{\text{OXA-72}}$, we mapped an IS*Aba31* insertion sequence. Also, two genes with a putative role as virulence factors were noticed: septicolysin (pore forming toxin) and TonB-dependent receptor gene (responsible for iron uptake and virulence, probably involved in the survival of the bacteria in lungs and blood).

Conclusions: Our study reveals the presence of a resistance and virulence transferable platform in an XDR *A. baumannii* strain from Romania. Also, our data support the possible dissemination of this platform in Eastern Europe, as a plasmid with 99% homology (Accession No. KX230793.1) with our draft plasmid sequence was recently described in Serbia. This leads to the necessity to improve the epidemiological control of carbapenem-resistant *A. baumannii* strains, in order to prevent the spreading of such platforms.