High level colistin resistance in *Acinetobacter baumannii* isolate from patient with mediastinitis after coronary artery bypass graft and aortic valve replacement

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**Background:** *Acinetobacter baumannii* is an emerging multidrug-resistant (MDR) nosocomial pathogen of the 21st century, often sensitive only to colistin. However, with increase in use, the resistance to colistin also slowly emerges. The aim of this study was to elucidate the underlying genetic mechanisms of colistin resistance in *A. baumannii* isolates from University Hospital Centre (UHC) Split and find a correlation between clinical data and development of resistance.

**Results:** We tested a total of 1430 *A. baumannii* isolates and 4 showed resistance to colistin. While in three of them minimal inhibitory concentrations (MICs) were slightly above the limit value displaying heteroresistance, one isolate had a high-level colistin resistance with MIC >256 mg/L. Only this isolate showed a presence of mutations in *lpxC* gene resulting in amino-acid changes (alterations of valine to alanine at position 95 and glutamine to glutamic acid at position 184), that possibly introduced modifications in the lipid A component of lipopolysaccharide (LPS) and the initial binding target of polymixin antibiotics. Beside mutations in *lpxC*, there were two mutations in *lpxA* gene (positions 290 and 411), within the first one caused alteration of leucine to serine at position 97, and the second one was silent. The mutations in *lpxD* gene were not found. The same isolate also carried the gene for OXA-23-like carbapenemase that showed 100% sequence identity with already described clinical isolates. All patients with resistant isolates were receiving extended colistin therapy. The patient with high-level resistant isolate underwent coronary artery bypass graft (CABG) surgery and aortic valve replacement (AVR). On day 17th postoperatively, the wound discharge along with temperature rise and increase of inflammatory markers appeared. Mediastinitis required sternectomy, and V.A.C.UTM Negative Pressure Wound Therapy System was set to extract the debris while irrigation of wound with colistin and octenidine dihydrochloride/phenoxyethanol was performed. Furthermore, the omental flap coverage for reconstruction of sternotomy wound was required, but unfortunately the patient passed away.

**Materials/methods:** Consecutive, non-duplicate carbapenem resistant *A. baumannii* isolates from patients treated in UHC Split in period from January 2014 to September 2016 were tested for colistin resistance by gradient test method according The European Committee on Antimicrobial Susceptibility Testing (EUCAST). In colistin-resistant isolates, the mechanisms of resistance were investigated by polymerase chain reaction (PCR) using primers for *lpxA*, *lpxC* and *lpxD* genes involved in lipid A biosynthesis pathway, followed by sequencing of PCR products and subsequent analyses. Additionally, the presence of *blaOXA* genes encoding OXA-type carbapenemases was also investigated by multiplex PCR and sequencing. Clinical data were collected from medical records.

**Conclusions:** Mutations in three genes of the lipid A biosynthesis pathway are known to be connected with fast development of high-level resistance to colistin. In this study, mutations in *lpxC* gene of *A. baumannii* strain with high-level colistin resistance were identified. Amino-acid changes introduced by mutations could alter the lipid A protein and be responsible for reduced or abolished interaction with polymixins. However, other mechanisms of colistin resistance could also be involved, considering a very high level of resistance. Colistin-resistant *A. baumannii* occurred in patients who were receiving prolonged colistin treatment. Such patients should be carefully monitored for early detection of colistin resistance. Furthermore, the presence of OXA-23-like in carbapenem resistant isolate of *A. baumannii* from UHC Split is reported for the first time.