Co-occurrence of Carbapenemases genes in Acinetobacter baumannii - a myth or a reality?

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Background: Acinetobacter baumannii is an important opportunistic pathogen that is rapidly evolving towards multidrug resistance and is responsible for life-threatening infections. Carbapenems are commonly used to treat A. baumannii infections but emergence of carbapenemase encoding genes, such as blaOXA23-like, blaOXA24-like, blaOXA58-like, and blaNDM has been recently reported. Moreover, several studies have reported the co-occurrence of two distinct carbapenemases in some isolates. The aim of the present study was to study such isolates to demonstrate that this phenomenon was in fact due to the existence of different bacterial clones harboring different genes.

Material/methods: We studied 10 strains of A. baumannii: 2 containing blaOXA23-like and blaOXA24-like genes, and 8 with blaOXA23-like and blaNDM genes. Each strain was subcultured 10 fold in limit dilution in water. Every dilution was cultivated on Trypticase Soy agar plates for 24h at 37°C and isolated bacteria was analysed. Antibacterial susceptibility testing, real time PCR for detection of antibiotic resistance genes and multilocus sequence typing were performed on strains before and after limit dilution.

Results: For each strain with co-occurrence of carbapenemases, 10 colonies were selected after limit dilutions so a total of 100 clones were studied. By example, from the strain AH35 containing blaOXA23-like and blaOXA24-like genes, we isolated after limit dilution 6 colonies containing only blaOXA23-like and 4 with only blaOXA24-like. Each population has the same ST type (ST 2) but different antibiotic susceptibility testing. For the strain 924 containing blaOXA23-like and blaNDM, we differentiate 2 populations with different carbapenemase encoding genes, different resistance phenotypes and also different STs i.e 5 clones with blaOXA23-like (ST 2) and 5 clones with blaNDM gene (ST 25).

For each strain tested, we always separated 2 different populations of A. baumannii, each of them with a different carbapenemase, also a different antibiotic susceptibility testing and often different clonal types. Moreover the 2 different populations had a different aspect on agar plate.

Conclusions: Here we report that the coexistence of two carbapenemases in single isolates of A. baumannii is likely due to the existence of different clones harboring different carbapenemases encoding genes. This result is the first to our knowledge to demonstrate that A. baumannii infections could be linked to multiple clones.