

Title: ANALYSIS OF *Acinetobacter* spp. PERSISTENT CELLS FORMATION EXPOSED TO TOBRAMYCIN

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Abstract:

Acinetobacter spp. are opportunistic human pathogens causing several healthcare-associated infections, which have increasingly presented complications in the therapy due to the ability of these bacteria to express and acquire resistance to various antimicrobial agents. Furthermore, the antimicrobial-tolerant phenotype, in which a small population of susceptible bacteria survives to high doses of antimicrobial treatment, was already reported in *Acinetobacter baumannii*. The carbapenems used to be the choice treatment for *A. baumannii* – *calcoaceticus* (ACB) complex infections; otherwise, some aminoglycosides such as tobramycin are widely used in nosocomial pneumonia due its better penetration into the lungs. In this study, the purpose was to evaluate the persistence phenotype in *A. baumannii* – *calcoaceticus* complex isolates, once it was exposed to different tobramycin concentrations. A total of 10 nosocomial isolates of ACB complex were analyzed, which showed minimal inhibitory concentrations (MIC) ranging from <0.25 µg/mL to 2 µg/mL. The logarithmic-phase cultures were exposed to 20 µg/mL or 10X its MIC of tobramycin for 48 h, and then, in order to determine bacterial survival rates, we proceeded serial dilutions and colony counting. Different fractions of persister cells were found among the clinical ACB complex isolates after tobramycin exposure. When exposed to 10X its MIC to tobramycin, the isolates presenting MIC of <0.25 (one isolate), 0.5 (four isolates), 1 (four isolates) and 2 µg/mL (one isolate), exhibited, respectively, a survival cells fraction of 0.0593%, 0.0022% to 0.0726%, 0.4231% to 0.0032% and 0.1072%. However, when exposed to 20 µg/mL of tobramycin, the isolates presenting MIC of <0.25, 0.5 and 1 µg/mL exhibited, respectively, a survival cells fraction not detectable, a range of 0.000037% to 0.001075% and 0.00049% to 0.00785%. Thus, the results suggest that persister cells formation can be dose-dependent, once the isolates with lower MICs to tobramycin presented reduced survival cells fractions comparing to isolates with higher MICs, when exposed to 20 µg/mL of tobramycin. Assuming that the tolerant phenotype is related to treatment difficulties and recalcitrant infections, it is important to know how different concentrations can influence the formation, or even eradication, of persister cells, thus helping the choice of treatment of infections caused by ACB complex isolates.

Keywords: *Acinetobacter* spp., persistence, tobramycin

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