

TITLE: Persister cells in *Acinetobacter baumannii* after ciprofloxacin exposure

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ABSTRACT: *Acinetobacter baumannii* is an important opportunist pathogen responsible for several healthcare-associated infections, which may be aggravated by increased levels of antimicrobial resistance and the ability of them to form persister cells. Persisters comprise a small population formed stochastically and/or in response to inducers as a strategy to survive different environmental conditions, including high doses of antimicrobials, even to which they are genetically susceptible. This phenotypic variant is closely related to recalcitrance and therapy failure of several chronic infections. In this context, the aim of this study was to evaluate the formation of persister cells in *A. baumannii* after exposure to ciprofloxacin. Five clinical *A. baumannii* isolates (tracheal aspirate, blood and wound secretion) from a university hospital in Porto Alegre, South Brazil, were characterized as susceptible to ciprofloxacin by the assessment of Minimum Inhibitory Concentration (MIC), presenting MIC values that ranged from 0.125 to 0.5 µg/mL. The isolates were grown in LB broth at 37°C until the late exponential growth phase and exposed to 100-fold the MIC for ciprofloxacin of each isolate for 48 h. In order to determine the number of colony-forming units per milliliter (CFU/mL) of surviving cells, aliquots were removed after 3, 6, 24 and 48 h of antimicrobial treatment, followed by serial dilutions and spotted onto nutrient agar. The experiments were performed in three biological replicates. All isolates formed persister cells after 48-h ciprofloxacin exposure, but at different fractions, ranging from 0.0182% to 0.7514% of the original population, and presented classical killing curves consistent with persistence phenotype. Moreover, surviving cells obtained after 48 h of treatment remained susceptible when regrown in LB supplemented with the same concentration of ciprofloxacin used in the assay, confirming the drug-tolerance phenotype. To the best of our knowledge, this is the first report of persister cells in *A. baumannii* after fluoroquinolone exposure. Moreover, our data demonstrate that this phenotype is distributed among *A. baumannii* from healthcare-associated infections and may contribute to ineffectiveness of the antimicrobial therapy.

Keywords: *Acinetobacter baumannii*, persistence, ciprofloxacin, antimicrobial therapy failure

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