

TITLE: Efflux pumps as a possible mechanism of resistance of a clinical isolate XDR of *Mycobacterium tuberculosis*

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ABSTRACT:

Mycobacterium tuberculosis is the main infectious agent of tuberculosis and currently the 10th leading cause of death in the world. The arise of multidrug resistant strains (MDR) and/or extensively drug-resistant strains (XDR) are an important contributing factor to this position. Among the mechanisms of resistance of *M. tuberculosis*, it is possible to emphasize the efflux pumps (EP), which are membrane-carrying proteins capable of expelling several substances of their metabolism and antimicrobials out of the mycobacteria. Considering this, the objective of this work was searching for the possible mechanism of resistance to aminoglycosides (AMI) and fluoroquinolones (FLQ) of a XDR clinical isolate that did not shows previously mutations in *rrs* and *gyrA* genes, main genes related to resistance to AMI and FLQ, respectively. The clinical isolate was exposed to subinhibitory concentrations (1/2 MIC) of ofloxacin (OFL) and streptomycin (STP) for 48 hours and also was withdrawn this exposure for an equal period to use the ethidium bromide (EtBr) accumulation methodology. EtBr has a higher fluorescence when it is inside the bacterium, once intercalated in its DNA. In addition to the exposures, efflux pumps inhibitors (EPI) were also part of the experiment and subinhibitory concentrations of verapamil (VP), reserpine (RES) and carbonyl m-chlorophenylhydrazon cyanide (CCCP) were used. In 48 hours, the basal EP with and without EPI were measured. Later, the strain was challenged with OFL and STP in separated experiments, with and without EPI for 48 hours. The last experiment was withdraw the treatment with the antibiotics, incubated for 48 hours and then, EtBr accumulation was measured. In the first experiment CCCP inhibited more than RES and VP, respectively. The literature shows ABC and MFS as the main EP family inhibitors of OFL and STP. Besides that, VP and CCCP are described as inhibitors of these families. Our second experiment shows VP better EPI when challenged with STP confirming the literature. However RES was the best inhibitor of the strain EP when challenged with OFL, contradicting the literature. When the antibiotics were withdrawing, the results were the same than the first experiment. Thus, the mechanism of resistance of this clinical isolate may be related to the increase in EP activity of families ABC, MFS and RND (family that has RES as the main EPI) and this activity increasement is induced by the antibiotic's presence.

Keywords: Efflux pump, *Mycobacterium tuberculosis*, XDR, efflux pump inhibitors, mechanism of resistance

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