TITLE: ISONIAZID RESISTANCE AND EFFLUX PUMP GENE EXPRESSION IN *Mycobacterium tuberculosis*

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ABSTRACT:

Despite mutations in the *Mycobacterium tuberculosis* genome are the most common mechanisms behind drug resistance, additional mechanisms might be involved. Efflux pump (EP) systems have been shown to play a part in resistance to many anti-tuberculosis drugs. Although some researchers have studied the role of these systems in isoniazid (INH) resistance, much is still unclear. In this sense, the aim of this study was to assess the relative EP gene expression of *M. tuberculosis* exposed to INH and correlate those findings with the susceptibility profile of each strain. Four *M. tuberculosis* strains (H_{37}Rv - reference strain, 47S – susceptible isolate, 14 BRF – INH resistant isolate and 3614 – multi-drug resistant isolate) were selected for the study. Minimal inhibitory concentration of INH was accessed by the means of resazurin microtiter assay plate (REMA). Strains were exposed to ½ MIC of INH for 48h, while one control sample was conducted without INH exposure. Bacteria were lysed and the RNA was extracted with the RNeasy Mini Kit. cDNA was synthetized and relative expression of 10 EPs genes was assayed in the StepOne Real Time PCR system, using the 16s RNA (*rrs)* gene as endogenous control. REMA revealed INH MIC for H_{37}Rv, 47S, 14BRF and 3614 to be: 0.06, 0.06, 32 and 6.25 µg/mL, respectively. Gene expression assays revealed responses against INH pressure that correlated with each resistance profile and presence or absence of *katG* or *inhA* mutations. The susceptible strains presented very distinct profiles upon exposure to INH: no overexpression in H_{37}Rv versus six genes overexpressed in 47S.The isolate 14 BRF harbors *katG* mutations, often related to high INH MIC, which might be the cause of its low response to INH exposure (no gene overexpression). In its turn, 3614 being an MDR strain was able to resist to INH pressure by overexpressing all 10 EPs genes studied. Although both 14 BRF and 3614 have high INH MIC, the second harbors *inhA* mutations, which usually account for MIC lower than those caused by *katG* mutations. To this date, many papers have shown that EPs have some part in resistance to many anti-TB drugs. For isoniazid, these systems possibly allow that susceptible bacillus survive the initial pressure of the drug until a mutation in resistance genes randomly appears.

Keywords: Drug resistance, efflux pump, isoniazid, *Mycobacterium tuberculosis.*

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