TITLE: Genetic polimorphisms as determinants of ethionamide resistance in *Mycobacterium tuberculosis.*

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ABSTRACT:

In *Mycobacterium tuberculosis*, *ethA* encodes the enzyme EthA, responsible for the activation of the antimycobacterial drug ethionamide (ETA). Adjacent to *ethA*, *ethR* encodes a transcriptional repressor, whose overexpression inhibits transcription of *ethA*, resulting in high levels of resistance to ETA. Mutations in *ethA* are associated with resistance to ETA; however, clinical isolates of *M. tuberculosis* that are resistant to ETA but do not bear mutations in *ethA* have been described. The role of EthR in the transcriptional control of *ethA* and the identification of an isolate resistant to ETA but without mutations in this gene (but with a mutation in *ethR*) suggest that mutations in *ethR* could be associated with resistance to ETA. Therefore, the goal of this work was to investigate if mutations in *ethR* could be determinants of resistance to ETA, and to evaluate if the detection of mutations in regions associated with resistance to ETA could complement the results of phenotypical drug susceptibility tests. To do this, 69 M. tuberculosis clinical isolates, from 45 patients that received ETA as part of their treatment, were reactivated from our strain collection at the National Reference Laboratory for Tuberculosis, at the Oswaldo Cruz Foundation. ETA, as well as isoniazid, resistance profiles were determined, and genomic DNA was obtained from all strains. In the present study, the possibility of using mutations in specific loci as markers of resistance of *M. tuberculosis* to the antibiotic ethionamide was evaluated. Such loci include the *ethA* and *ethR* genes, as well as their intergenic region, the *inhA* gene promoter and the *mshA* gene. It was possible to identify seventeen mutations in these loci, among which twelve were possibly related to resistance to ETA, three of them already described in the literature. The rapid detection of antimicrobial resistance is a critical factor in the treatment of tuberculosis patients, and is crucial for the control of the transmission of this disease. The results of this study may lead to the identification of molecular markers associated with resistance to ETA. In the future, such markers may be used to determine resistance profiles more quickly than by using phenotypical methods, thereby aiding in the control of tuberculosis.

KEYWORDS: *Mycobacterium tuberculosis*, ethionamide, *ethA*, *ethR*, resistance, transcriptional regulation.

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