Title: MONITORING MUTATIONS ASSOCIATED WITH ETHIONAMIDE RESISTANCE IN *Mycobacterium tuberculosis*

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Abstract:
Ethionamide (ETH) and isoniazid (INH) are drugs used for treating tuberculosis (TB) that target the InhA enzyme, which participates in the synthesis of mycolic acids, a mycobacterial cell wall component. For these drugs to work they need to be activated by their respective enzymes: EthA activates ETH and KatG activates INH. Mutation in these enzymes is known to confer resistance to each drug. Upregulation of *inhA* by mutation in its promoter region confers low level cross-resistance to ETH and INH. Inactivation of *mhsA*, necessary for mycothiol synthesis, confers ETH^R as ETH activation requires this molecule. Patients taking ETH have experienced previous treatment failure, and often have a complex history of disease and treatment. The sensitivity test for this drug has limited reliability, which makes the diagnosis of resistance difficult. Daily, the Centro de Referência Professor Hélio Fraga receives strains and clinical samples from different regions of Brazil. The samples undergo smear, culture, drug sensitivity testing and identification. In order to improve the monitoring of *M. tuberculosis* resistance to ETH we evaluated resistance profiles and possible correlations with mutations in three regions (*ethA-ethR* region, the *inhA* promoter, and *mshA*). We selected 45 clinical isolates that were analyzed for ETH^R by the Canetti *et al.* susceptibility test using 7H11 medium. DNA was extracted and regions associated with ETH resistance were amplified and sequenced. Several mutations were found in isolates with different ETH resistance profiles, a few of which are novel (EthA: M1K, C131stop, C137W, Q459stop; EthR: F110L, Q143P; *ethA-ethR* region: a-3g; *mshA*: N111S, L188V, ΔP368-A371). The Q459stop mutation results in a deletion of the 30 last amino acids of EthA, possibly affecting protein structure and function. Of five ETH^R and INH^R strains, three bear the c-15t *inhA* promoter mutation, indicating low level cross-resistance. One ETH^R isolate has a deletion in the *mshA* gene, leading to loss of four amino acids that contribute to the protein´s hydrophobic core. Further analyses are in progress to verify the correlation between these mutations and drug resistance and if they influence protein activity. In the future, patients may benefit from molecular techniques that complement sensitivity tests for monitoring resistance to ETH and other drugs.

Keyword: tuberculosis, ethionamide, isoniazid, treatment, mutation
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