

SUMMARY OF THESIS*

CARDOSO, Rosilene Fressatti - **Análise de mutações nos genes relacionados com resistência a isoniazida (INH) em isolados clínicos de *Mycobacterium tuberculosis* de origem brasileira.** São Paulo, 2003. (Tese de Doutorado – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo).

ANALYSIS OF MUTATIONS IN GENES ASSOCIATED WITH RESISTANCE TO ISONIAZID (INH) IN *Mycobacterium tuberculosis* BRAZILIAN CLINICAL ISOLATES

In the present study the mutations frequencies in the *katG*, *kasA*, *inhA* genes and *oxyR-ahpC* intergenic region of 97 resistant and 60 sensitive *Mycobacterium tuberculosis* clinical isolates from Brazil were investigated. The mutations screening were carried out by PCR-SSCP and confirmed by automated sequencing. The minimal inhibitory concentration for INH was done by the microplate Alamar Blue Assay (MABA). The molecular differentiation of INH resistant and sensitive isolates was carried out by Spoligotyping of the locus DR. We found 40 different Spoligotyping patterns when analyzing 97 INH resistant clinical isolates and 30 in 60 INH sensitive isolates. Our data showed no resistant isolates had a complete deletion of the *katG* gene. However, one resistant isolate that had a deletion at region 3' in *katG* gene was found and a high percentage of mutation in this gene (85.6%) mainly at codon 315 (61.9%). Twenty-five new mutations previously not described in the literature, were detected in *katG* gene in all resistant isolates studied and 25.8% had mutation in *inhA* promoter region, 5.82% had mutation in *inhA* structural region and 10.3% at *oxyR-ahpC* intergenic region where one of this is a new mutation (-48) previously not described in the literature.

It was observed in *kasA* gene mutation in resistant and sensitive isolates. The most frequent mutation observed in *kasA* gene was at codon 269 that will cause alteration G269S (23.7%). Other silent mutations were detected only in INH sensitive isolates, however with a low frequency. PCR-SSCP showed good sensibility (90.7%) to detect mutations in INH resistant isolates, analyzing 5 genic regions (315 codon region of *katG* gene, *inhA* promoter and *inhA* structural region and *oxyR-ahpC* intergenic region) by PCR-SSCP. PCR-SSCP showed agreement in 100% when compared to sequencing. We can thus conclude that mutation screening by PCR-SSCP can be used to detect resistance to INH in *M. tuberculosis*.

Rosilene Frenatti Cardoso
Laboratório de Bacteriologia Clínica
Universidade Estadual de Maringá
Bloco J-90 – Sala 05
87000-000 MARINGÁ – PARANÁ
e-mail: rfcardoso@uem.br
mfressatti@uol.com.br

*This thesis is available at the Library of the Instituto de Medicina Tropical de São Paulo