

Title: Rapid detection of resistance to second-line drugs (fluoroquinolones and amikacin, kanamycin and / or capreomycin) in *Mycobacterium tuberculosis* strains by using the MAS-PCR.

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Abstract:

Tuberculosis remains one of the main causes of death caused by a single infectious agent worldwide. According to the World Health Organization, one third of the world population are infected with *Mycobacterium tuberculosis* strain. Deficiencies in the disease detection process as well as failure of some therapeutic procedures (e.g., due to the dropout during the treatment) has contributed to the emergence of resistant bacteria to one or more antibiotics (MDR - multidrug resistance and XDR - extensively drug-resistant), constituting a serious problem and a real threat to tuberculosis control programs. Because of the need of to develop rapid diagnostic tools as an alternative to traditional approaches, this study aims to quickly identify strains of *Mycobacterium tuberculosis* (Mtb) resistant to fluoroquinolones (FLQs) and amikacin (AMK), kanamycin (KAN) and capreomycin (CAP) (injectable second-line drugs) by point mutations in *gyrA* genes (codon 94) and *rrs* (codon 1045), respectively. The methodology used was the MAS-PCR (Multiplex Allele-Specific - PCR) which is based on PCR amplification and simultaneous detection of point mutations most frequently associated with resistance to FLQs and AMK, KAN and CAP in *M. tuberculosis* strains. One hundred strains of *M. tuberculosis* belonging to the collection of National Reference Laboratory for Tuberculosis / CRPHF / ENSP / FIOCRUZ were analyzed by MAS-PCR method and compared to the results previously obtained by microbiological and automated method BACTEC MGIT 960 in order to evaluate the specificity and sensitivity of this technique. Preliminary results showed a sensitivity of 46.8% in the *gyrA* gene and 35% in *rrs* gene. The low value of sensitivity is due to the fact that some resistant strains showed no mutation at the position analyzed by MAS-PCR, which may mean that the resistant tuberculosis strains present in Brazil have a singularity in the pattern of mutations, other than described in the rest of the world. Discordant results are being elucidated by sequencing analysis to detect mutations in other positions, in an attempt to optimize the MAS-PCR.

Keyword: MAS-PCR, *M. tuberculosis*, fluoroquinolones, amikacin, kanamycin, capreomycin

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