Study of Primer Competition in Rapid Detection of Resistance to Ofloxacine by MAS-PCR in Clinical Isolates of Mycobacterium tuberculosis

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Background & Objectives: Mutations in QRDR (Quinolon Region of Drug Resistance) of gyrA gene is related with resistance to Ofloxacine. A molecular technique has been designed for fast detection of resistant isolates, when a kind of competition in PCR reaction was observed.

Methods: In this study 40 isolates of *Mycobacterium tuberculosis* resistant to Ofloxacine were investigated. Multiplex-Allele specific-PCR (MAS-PCR) methods was designed by F and R primers amplifying 194bp fragment of gyrA gene, and by internal specific primers for codons of 90, 91 and two alleles of 94 codons. Sequence methods was used to evaluate the result.

Results: This methods could detect mutations related with the resistance to Ofloxacine in 31 samples from 36 phenotypic resistant strains. Sequence methods confirmed the results. Interestingly, a phenomenon was observed in many samples where in MAS PCR reaction with R, F and internal primers, the main band of 194 had been deleted. Experiments were repeated, even though the same result was obtained. Internal primers were designed on the basis of bonding or not bonding of their 3 to nucleotide place where the mutation had been occurred. Deleting of 194 band may be explained with amounts of Tm and ΔG formation of secondary dimer structures and hairpin in R, 90 and 91 primers. Assuming a kind of competition between R and 90 primers, it can be concluded that by deletion of a few R primers in the volume unit (due to temperature difference of its Tm compared to 90 primer), the rest were deleted because of more negative ΔG or higher possibility of secondary structures formation with other primers. The R primer was consequently defeated in competition for bonding to DNA template with 90 primer, therefore 194 band was omitted.

Conclusion: The disappearance of main band in MAC-PCR in this study is due to the competition of primers which must be attended in the interpretation of the results, although correction of primers sequence is usually impossible for exclusiveness of the mutation point.

Keywords: *Mycobacterium tuberculosis*; Multiplex-Allele Specific-PCR; Ofloxacine Resistance; Primer Competition