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Title: Detection of fluoroquinolones resistance mutations in mycobacterium tuberculosis by hydrolysis probes and RFLP

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Body: A single nucleotide polymorphism in codons 88, 90, 91, 94 of gyrA gene, associated with the resistance to FQ in MTB, can be detected by variety of methods. The aim of the study is to evaluate the method of qPCR with dehydrated reaction mixtures and RFLP for rapid detection of FQ resistant MTB. Results. SNPs in 26 FQ resistant MTB have been detected by GenoType MTBDRsl and by dehydrated qPCR mixtures with primers ($GyrF_{200-218}$+$GyrR_{358-339}$ and $GyrF_{78-97}$+$GyrR_{397-379}$) and hydrolysis probes for SNPs: Ala90Val, Ser91Pro, Asp94Gly/His/Asn/Tyr/Ala. Both methods make it possible to detect the following: one, seven and three isolates of MTB carry mutations in codons 88, 90, 91, respectively; and five, two, one and two isolates of MTB have mutations Asp94Gly, Asp94Ala, Asp94His, Asp94Asn/Tyr, respectively. One MTB carries a double mutation Ser90Pro+Asp94Gly and one MTB has an unidentified SNP in codon 94. Dehydrated qPCR reaction mixtures and PCR plates are a convenient tool for detecting a number of different SNPs in one strain. Rehydration of dried PCR mixtures has restored their activity, resulting in the formation of typical fluorescence curves. Mutations have been confirmed by RLFP analysis with BstUI, TaqI, MluI. Alterations in both codon 90 and 91 can be detected by MluI, resulting in a two-band pattern (320, 160 bp) in mutated MTB versus a three-band pattern (200, 120, and 80 bp) in wild MTB. Restriction with TaqI allowed us to detect mutations in codon 91: mutated and wild MTB have band pattern 220, 100 bp and 120, 100, 80 bp, respectively. BstUI restriction makes it possible to detect MBT with or without SNP in codon 90 (band pattern 320, 160 bp versus 180, 140, 80 bp).