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Title: Detection of fluoroquinolone resistance associated mutations in Mycobacterium tuberculosis by use of sequencing and TaqMan probes

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Body: Mutations conferring resistance of MBT to fluoroquinolones occur in two short discrete segments of gyrA and gyrB genes. The nature of the amino acids at positions 88, 90 and 94 in gyrA plays a crucial role in the acquired resistance to quinolones. The aim of investigations is to evaluate the method of real time PCR with dual TaqMan probes for rapid detection of MBT resistance to fluorquinolones. Methods. Resistance to ofloxacin of MBT was determined by reference technique, gyrA genes were amplified and autosequenced. The real-time PCR with dual TaqMan probes was developed to detect mutations in triplets 90, 94 of the gyrA gene. Results. The gyrA codons 90, 91, 94 were reported to be the most frequently mutated codons worldwide, the same tendency was registered in Belarus: point mutations were predominately localized at codons 90 and 94 and rarer - 91. Mutations occurred at codon 90 resulted in Ala→Val replacements, at triplet 94 – Asp→Gly or Asp→Asn, at codon 91 - Ser→Pro. The designed dual TaqMan probes for real-time PCR allowed detecting mutations in triplets 90, 94 of gyrA gene. Samples with mutations were characterized by above-threshold florescence on JOE-channel and subthreshold florescence on FAM-channel, while samples without mutations displayed above-threshold florescence on FAM-channel and subthreshold florescence on JOE-channel. The results obtained by real-time PCR with dual TaqMan probes showed high level of coincidence with sequencing data (94%). Conclusion. The dominant mutations in 90, 94 triplets gyrA can be rapidly and effectively detected by PCR with dual-probe TaqMan probes.