Designing and evaluation of rapid molecular assays for first and second-line anti-tuberculosis drugs


Tuberculosis and Pediatric Infectious Research Center and Department of Microbiology, Arak University of Medical Sciences, Arak, Iran

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ABSTRACT

Introduction: The four basic or “first-line” TB drugs are Isoniazid, Rifampicin, Pyrazinamide and Ethambutol. For the treatment of drug-resistant TB, the current TB drugs are grouped according to their effectiveness and experience of use, such as Streptomycin, pyrazinamide, rifabutin, kanamycin, amikacin, ofloxacin, etc. The drug susceptibility test (DST) is a time-consuming and costly method. Rapid molecular tests may be used by detection of related mutations. The aim of this study is to design and evaluate the quickest methods of detection.

Materials and methods: 120 resistant and susceptible clinical isolates of Mycobacterium tuberculosis (MTB) were evaluated for probable mutations in resistance-related genes. Molecular methods of polymerase chain reaction (PCR)-RFLP, AS-PCR and MAS-PCR as nested or semi-nested forms were used for mutation detection in katG, rpoB, pncA, rpsL, gyrA, inh, rrs, inhA and embA. Evaluation of ethA and pncA genes in the isolates was accomplished by sequencing. Furthermore, the sequencing method was used for all the genes as the golden standard.

Results: 88% prevalence of the katG315 mutation was detected in INH-resistant isolates by AS-PCR and 95.6% by PCR-RFLP. In 93% of rifampin-resistant isolates point mutation at codons 516, 526 or 531 were detected by MAS-PCR method and 75% by AS-PCR method. In rapid detection of resistance to injectable drugs, the sensitivity and specificity of PCR-RFLP method for mutation detection in rrs gene by BSTFNI enzyme were 95/65% and 70/83%, by AJII enzyme were 60% and 90/62% and by MAS-PCR method were 50% and 70/58%, respectively. Ofloxacin resistance was detected in 84.6% of resistant isolates by 4 endonuclease enzymes in PCR-RFLP method and sensitivity and specificity of a MAS-PCR method were 86/11% and 100%, respectively. Sensitivity and specificity of a MAS-PCR method for pncA were 66% and 90% and for PCR-RFLP method for rpsL were 90% and 95%, 36.5% (CI:0.09–0.45) and 100% only for one resistance-related codon for emb gene, respectively. Ethionamide and Pyrazinamide resistance in resistant isolates was proved by 100% sensitivity by the sequence method.

Conclusion: Molecular methods of PCR-RFLP, MAS-PCR and sequencing were successfully used for rapid detection of first- and second-line antimycobacterial drugs.

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