

A pyrosequencing method for molecular monitoring of regions in the inhA, ahpC and rpoB genes of Mycobacterium tuberculosis.

Garza-González E, González GM, Rentería A, Cruz-Pulido W, Rivera G, Bocanegra-García V.

Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Mexico. elvira_garza_gzz@yahoo.com

Abstract

In this study, a pyrosequencing method for monitoring two genes related to isoniazid (INH)-resistance and a region of the rpoB gene linked to rifampin (RMP)-resistance in Mycobacterium tuberculosis was developed and evaluated. Specifically, a 20-base pair (bp) region of inhA (from -24 to -4), a 35-bp region of ahpC (from -39 to -4), and a 57-bp region of rpoB (from codon 515 to 533) were analysed by pyrosequencing. For the development of the method, selected non-consecutive clinical isolates of M. tuberculosis were analysed, including: 25 isolates susceptible to both INH and RMP, 18 RMP-monoresistant isolates, 17 INH-monoresistant isolates, and 15 multidrug-resistant strains. Our pyrosequencing methodology was further evaluated using 96 M. tuberculosis isolates. Mutations in ahpC were found to be associated with INH resistance ($p < 0.05$). By setting any mutation in ahpC as a marker of resistance, the specificity and the positive predictive value (PPV) were 100%. Similarly, any mutation in the rpoB gene was associated with a RMP resistance phenotype ($p < 0.01$). Using any mutation in rpoB as a marker of RMP resistance, the sensitivity of this assay was 73% and the specificity and PPV were 100%. The use of this pyrosequencing method to analyse the ahpC and rpoB genes allowed us to detect INH- and/or RMP-resistant isolates. Furthermore, this method represents an opportunity to expedite the description of novel mutations related to drug resistance.