Evaluation of genotype MTBDRplus assay for rapid detection of isoniazid and rifampicin resistance in Mycobacterium tuberculosis clinical isolates from Pakistan

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ABSTRACT

Background: GenoType MTBDRplus is a molecular assay for detection of Mycobacterium tuberculosis resistance to isoniazid (INH) and rifampicin (RMP), the two major anti-tuberculosis (TB) drugs. Identification of INH resistance is largely based on the occurrence of mutations in the katG gene, coding for the catalase-peroxidase, or in the promoter region of the inhA gene, coding for the NADH-dependent enoyl-ACP reductase. For testing the RMP resistance, mutations in the rpoB gene, coding for the RNA polymerase β subunit, particularly in the RMP resistance determining region (RRDR) of the gene are investigated.

The GenoType MTBDRplus assay has been validated in several countries. The aim of this study was to evaluate the ability of the assay to detect INH and RMP resistance among strains of M. tuberculosis, isolated from Pakistani TB patients, and phenotypically identified as multidrug-resistant (MDR), that is resistant to both INH and RMP.

Material/methods: The study included a collection of 100 MDR M. tuberculosis strains isolated from as many Pakistani TB patients over a 9-month period (i.e. between January and September 2014). Drug susceptibility testing was performed using the standard 1% proportion method on Löwenstein-Jensen medium, with INH and RMP critical concentrations of 0.2 mg/L and 40 mg/L, respectively. Genomic DNA was extracted by the cetyl-trimethyl ammonium bromide (CTAB) method. The GenoType MTBDRplus assay (Hain Lifescience, Germany) was done following the manufacturer’s instructions.

Results: In the katG gene, with MTBDRplus assay, a specific mutation associated with INH resistance (i.e. G944C transition, conferring Ser315Thr amino acid change) was detected in 66 (66%) of the strains. Thirty-four (34%) strains did not carry the katG mutation detected by the assay. Mutations in the mabA-inhA promoter region were detected in 10 (10%) strains (C-15T – in 10 strains, and T-8C – in 2 strains). Seventy-seven (77%) strains tested harboured a mutation in rpoB gene. Mutations in the rpoB gene were of four types: C1349T, A1304T, C1333G, and TC1324CA found in 63 (63%), 11 (11%), 8 (8%), and one (1%) strain, respectively. Of the 100 strains designated as MDR by the proportion method, GenoType MTBDRplus confirmed this phenotype in only 62 strains.

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The results of GenoType MTBDRplus and the conventional drug susceptibility method were consistent in 70% (70/100) for INH, and 77% (77/100) for RMP.

Conclusions: As evidenced in this study, the major concern with the GenoType MTBDRplus assay were false negative results. In comparison to conventional drug susceptibility testing, the assay was unable to detect 30 (30/100; 30%) strains resistant to INH and 23 (23/100; 23%) strains resistant to RMP. The GenoType MTBDRplus failed to identify 38 MDR (38/100; 38%) strains. Resistance in those strains probably originate from mutations in other codons and/or genes than those covered by the test. For detecting INH and RMP resistance in TB cases, especially in high TB incidence countries, such as Pakistan, molecular approaches should still be a complement rather than a replacement to conventional drug susceptibility testing.

Conflict of interest

We have no conflict of interest to declare.