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Comparative proteomic analysis of sequential isolates of *Mycobacterium tuberculosis* sensitive and resistant Beijing type from a patient with pulmonary tuberculosis

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ABSTRACT

Aim & objective: In India, tuberculosis (TB) is a foremost health problem, and the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* (*M. tuberculosis*) has further complicated the situation. Although various mechanisms have been proposed to elucidate the emergence of resistance, our knowledge remains insufficient. The formation of a very complex network and drugs of proteins are countered by their efflux/modification or target over-expression/modification. The analysis of the over-expressed proteins and their qualitative and phenotypic evaluation before and after the development of drug-resistance may be the most appropriate tool to understand the mechanisms of the mechanism of development of drug-resistance. Most studies are performed on distinct strains. Therefore, the objective of this study was to compare the proteomic information of sequential isolates of M. tuberculosis Beijing type from a single patient who developed MDR-TB during the course of anti-tuberculosis therapy.

Methods: In this study, a clinical isolate of *M. tuberculosis* was grown in Middlebrook 7H9 broth medium for 2 weeks, and the cell lysate of isolates was prepared by sonication and centrifugation. We compared and analyzed the whole cell lysate proteins of *M. tuberculosis* sequential clinical isolate from a patient with pulmonary TB before and after the development of drug resistance using two-dimensional gel electrophoresis, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and bioinformatics tools.

Results: The genotypes of both isolates remained homologous, showing no re-infection. The first isolate (before treatment) was sensitive to all the first-line drugs, sequential isolate was found resistant to rifampicin (RIF) and isoniazid (INH) and developed mutations in *rpoB*, *katG* and *inhA*. The concentrations of 17 protein spots were found to be consistently over-expressed in RIF- and INH-resistant isolates. The most prominent and over-expressed

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proteins found during the development of drug resistance were wag31, Rv2714, GarA, SSB, FabG4, Probable lipase, Rv3924c, Rv3204A, Rv2031c, Rv3418c and GroES. The InterProScan and homology searches generated insights into the possible functions and essential domains of the proteins. Rv1827, Rv2626c, Rv2714, Rv2970c, Rv3208A, and Rv3881c showed significant *in silico* interaction with RIF and INH; thus, the over-expression in the drug-resistant isolates could be compensating the inhibited/modulated molecules. Other proteins, which are over-expressed but do not unveil good binding with drug, might be indirectly associated with RIF and INH.

Conclusions: This proteomic study provides an understanding about the proteins that are over-expressed during the development of drug resistance. These over-expressed proteins, identified here, could prove useful as vaccine candidate, immunodiagnostic and possibly drug-resistant or chemotherapeutic markers in future.

Conflicts of interest

The authors have no conflicts of interest to declare.

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