**M. tuberculosis** Hypothetical Proteins and Proteins of Unknown Function: Hope for Exploring Novel Resistance Mechanisms as well as Future Target of Drug Resistance

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Drug resistance in tuberculosis predominantly, mono-resistance, multi drug resistance, extensively drug resistance and totally drug resistance have emerged as a major problem in the chemotherapy of tuberculosis. Failures of first and second line anti-tuberculosis drugs treatment leads to emergence of resistant *Mycobacterium tuberculosis*. Few genes are reported as the principal targets of the resistance and apart from the primary targets many explanations have been proposed for drug resistance but still some resistance mechanisms are unknown. As proteins involved in most of the biological processes, these are potentially explored the unknown mechanism of drug resistance and attractive targets for diagnostics/future therapeutics against drug resistance. In last decade a panel of studies on expression proteomics of drug resistant *M. tuberculosis* isolates reported the differential expression of uncharacterized proteins and suggested these might be involved in resistance. Here we emphasize that detailed bioinformatics analysis (like molecular docking, pypulation, and proteins-proteins interaction) of these uncharacterized and hypothetical proteins might predict their interactive partners (other proteins) which are involved in various pathways of *M. tuberculosis* system biology and might give a clue for novel mechanism of drug resistance or future drug targets. In future these uncharacterized targets might be open the new resistance mechanism and used as potential drug targets against drug resistant tuberculosis.

**Keywords:** *M. tuberculosis*, proteomics, bioinformatics, hypothetical proteins, mechanism of drug resistance

**INTRODUCTION**

**Current Scenario**

Tuberculosis (TB) still remains one of the deadliest infectious diseases worldwide which is caused by *Mycobacterium tuberculosis*. WHO reported 10.4 million people became ill and that 1.8 million died from TB (WHO, 2016). For controlling this situation the available current tools are vaccine diagnostics and drugs. Over the past 50 years, the *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccine against TB has maintained its position as the world’s most widely used vaccine, despite showing highly variable efficacy (0–80%) in different trials (Andersen and Doherty, 2005).
Sputum smear microscopy is the most common TB diagnostic method worldwide. However, culture remains the gold standard and the use of rapid molecular testing like line probe assay (LPA) is increasing for detection of drug resistant *M. tuberculosis* strains. Recently in India, Revised National TB Control Program (RNTCP) has approved a study for the Validation of second line LPA for detecting resistance to fluoroquinolones, aminoglycosides (kanamycin, amikacin), and cyclic peptides (capreomycin). First and second line drugs are the effective and necessary component of short course chemotherapy. DOTS and DOTS plus program have reduced the incidence of TB caused by susceptible strain but emergence of multidrug-resistant tuberculosis (MDR-TB), extensively drug resistant tuberculosis (XDR-TB), and totally drug resistant tuberculosis (TDR-TB) have worsened the situation and became a major threat to public health. Current tools (vaccines, diagnostics, and therapeutics) are unable to offer the complete protection against these deadly drug resistant situations.

**Mystery behind the Drug Resistance**

Usually interrupted anti-TB drugs treatment (first and second line anti-mycobacterial drugs) leads to emergence of drug resistant *M. tuberculosis* strains. Probably these resistant *M. tuberculosis* strains can resist antibiotic actions by the series of mechanisms such as: mutations in target genes (Beauclerk and Cundliffe, 1987), enzymatic inactivation of antibiotic molecules (Welch et al., 2005), over expression of novel efflux pumps and porin alterations in the cell wall (Magnet et al., 2001; Nikaido, 2003), trapping of drugs and the over expression of proteins involved in neutralizing the effect of drugs (Magnet et al., 2003; Kumar et al., 2013; Lata et al., 2015a; Sharma et al., 2015a, 2016b). Genes involved in these drug resistance mechanisms are tabulated in Table 1 (adopted from Zhang and Yew, 2009 and updated). Figure 1 is the schematic diagram which showed the potential mechanism(s) of action of first line (Rifampicin-inhibition of RNA synthesis, Isoniazid-inhibition of mycolic acid biosynthesis, Ethambutol-inhibition of arabinogalactan synthesis, Pyrazinamide-depletion of membrane energy, and Streptomycin-inhibition of protein synthesis) and second line of anti-TB drugs (Amikacin, Kanamycin, Capreomycin-inhibition of protein synthesis and Quinolones-inhibition of DNA gyrase) and the potential mechanism(s) of drug resistance, respectively. These mechanisms of action of drugs were also tabulated in Table 1. Usually 36–95% resistances in *M. tuberculosis* were contributed by mutations in the target genes, however, remaining 5–64% does not have these mutations and signifying the contribution of some other resistance mechanism(s). Research through expression proteomics (2D gel electrophoresis) and bioinformatic tools (like molecular docking, pupylation, and proteins-proteins interaction) explored the other novel mechanisms of drug resistance discussed in this work we discuss the probable involvement of uncharacterized/hypothetical proteins (differential expression in drug resistance studies were previously reported) in drug resistance via bioinformatics analysis (like molecular docking, pupylation, and proteins-proteins interaction) and suggested that these might be explore novel mechanism of drug resistance or used as potential future drug target/diagnostics against drug resistance.

**Expression Proteomics and Bioinformatics Approaches: A Way for Exploring the Mystery of Drug Resistance**

Advancement in proteomics has explored the mystery behind any complex phenotypes like drug resistance. As proteins manifest most of the biological processes, these are attractive targets for exploring new mechanisms of the drug resistance. Expression proteomics (2-DE coupled with MALDI-TOF-MS) and bioinformatic tools [patch dock and fire dock for molecular docking, GPS-PUP for pupylation, and STRING-10 for protein-protein interaction] have now emerged as major analytical tools for identification and characterization of expression proteome (proteins and its species) (Sharma et al., 2015a; Sharma and Bisht, 2016). In the last decade (2006–2016) few reports on expression proteome of drugs resistant *M. tuberculosis* existed (Jiang et al., 2007; Sharma et al., 2010, 2014, 2015b, 2016b; Kumar et al., 2013; Lata et al., 2015a,b; Singh et al., 2015; Sharma and Bisht, 2017) and suggested that differential expression of functionally known and unknown proteins and their protein-protein interaction might be involved in resistance and could be explore the novel mechanism of resistance.
TABLE 1 | Mechanisms of genes involved in drug resistance in Mycobacterium tuberculosis.

| Drug (Year of discovery) | MIC (µg/ml) | Gene(s) involved in resistance | Gene-product | Mechanism of action | Mutation frequency % |
|--------------------------|-------------|--------------------------------|--------------|---------------------|---------------------|
| Isoniazid, 1952          | 0.02–0.2    | katG inhA                      | Catalase-peroxidase Enoyl ACP reductase | Inhibition of mycolic acid biosynthesis and other multiple effects | 50–95 8–43 |
| Rifampicin, 1966         | 0.05–1      | rpoB                           | β-subunit of RNA polymerase | Inhibition of RNA synthesis | 95 |
| Pyrazinamide, 1952       | 16–50 (pH 5.5) | pncA                          | Nicotinamidase/pyrazinamidase | Depletion of membrane energy | 72–97 |
| Ethambutol, 1961         | 1–5         | embB                           | Arabinosyl transferase | Inhibition of arabinogalactan synthesis | 47–65 |
| Streptomycin, 1944       | 2–8         | rpsL rrs gidB                  | S12 ribosomal protein 16S rRNA rRNA methyltransferase | Inhibition of protein synthesis | 52–59 8–21 ? |
| Amikacin Kanamycin, 1957; Capreomycin, 1960 | 2–4 | Rrs tlyA                        | 16S rRNA 2′-O-methyltransferases | Inhibition of protein synthesis | 76 |
| Quinolones, 1963         | 0.5–2.5     | gyrA gyrB                      | DNA gyrase subunit A DNA gyrase subunit B | Inhibition of DNA gyrase | 75–94 |
| Ethionamide, 1966        | 2.5–10      | ethA                           | Flavin monooxygenase | Inhibition of mycolic acid synthesis | 37 |
| PAS, 1946                | 1–8         | thyA                           | Thymidylate synthase | Inhibition of folic acid synthesis and iron metabolism | 36 |
| Bedaquiline, 2012        | 0.125–0.50  | atpE                           | ATP synthase | Block the proton pump for ATP synthesis | ? |

MIC, minimum inhibitory concentration; ACP, acyl carrier protein; PAS, Para-aminosalicylic acid.

FIGURE 1 | Schematic diagram showed: (A) Potential mechanism(s) of action of first and second line of anti-TB drugs. (B) Potential mechanism(s) of drug resistance in Mycobacterium tuberculosis.
HYPOTHETICAL PROTEINS AND PROTEINS OF UNKNOWN FUNCTION: POTENTIAL TARGETS OF DRUG RESISTANCE OR NOVEL RESISTANCE MECHANISMS

Since last decade, few proteomics and bioinformatics studies of drug resistant *M. tuberculosis* have been accumulated and reported the differential expression of a panel of uncharacterized (proteins of unknown function) and hypothetical proteins. Through *in silico/bioinformatic* (Interproscan and molecular docking) analysis they showed that drugs binds to the conserved domains of hypothetical proteins/uncharacterized proteins and suggested that the over expression of a panel of uncharacterized and hypothetical proteins might neutralize/compensate the effect of drugs. Sharma et al. (2015b, 2016a) reported that inducible over expression of cloned known and unknown proteins (Rv0148 and Rv3841) in *E. coli* makes it two- to threefolds more resistant under drug pressure. Here we emphasize that detailed bioinformatics analysis (like protein-protein interaction) of these uncharacterized and hypothetical proteins might predict their interactive partners (other proteins) which are involved in various pathways of *M. tuberculosis* system biology (exploring/deciphering the *M. tuberculosis* network biology through *in silico/holistic* approaches) and might give a clue for novel mechanism of drug resistance or future target. Research in this direction could prevent the emergence of MDR-TB, XDR-TB, and TDR-TB situation and also these targets may be used to discover the new drug entities as potential drug candidates against drug resistant tuberculosis.

CONCLUSION AND FUTURE PERSPECTIVE

Based on the evidence discussed above related to expression proteomics and bioinformatics studies of drug resistant *M. tuberculosis* isolates by proteomic approach. *Protein Pept. Lett.* 22, 362–371.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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