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Discovery of Common Putative Drug Targets and Vaccine Candidates for *Mycobacterium tuberculosis* sp.

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ABSTRACT

Mycobacterium tuberculosis is the bacteria that cause tuberculosis (TB), an infection that usually affects the lungs and can be fatal without proper treatment. Combating through available drugs became a difficult task due to drug resistance and lack of appropriate common targets against genetically diverse strains. Since to improve efficacy, the effective targets should be identified and critically assessed. In the study, we aim to predict the potential novel targets against *M. tuberculosis* strains by employing in silico approach. The complete proteomic datasets of 23 *M. tuberculosis* strains was comparatively processed by executing R-scripts and eventually predicted 3906 'conserved gene products'. Further, we performed subtractive proteomic approach in search of promising crucial targets. Consequently, eight enzymes and two membrane proteins were prioritized as new therapeutic and vaccine targets respectively which found to have more interactors in network with high-confidence score, druggability and antigenicity. Therefore, outcomes of the study emphasize the importance of new targets may counteract with false-positive/negatives and facilitate appropriate potential targets for a new insight of reliable therapeutic development.

Keywords: *Mycobacterium tuberculosis,* Multidrug resistance tuberculosis and Extensive drug resistant tuberculosis.

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1. INTRODUCTION

Tuberculosis (TB) is one of the major infectious diseases affecting human beings caused by *Mycobacterium tuberculosis*. Tuberculosis is either latent or active, latent TB means the TB bacteria is in the body, but the body's defenses (immune system) are keeping it static from turning into active TB [1]. Latent TB also called inactive TB or TB infection isn't contagious. It can turn into active TB, so treatment is important for the person with latent TB to prevent its conversion to active form. The symptoms of TB depend on the area of which the body has been infected; primary infection of tuberculosis might affect the lungs, pulmonary tuberculosis (PTB) complications include hemoptysis, pneumothorax, bronchiectasis, malignancy and chronic pulmonary aspergillosis [2]. Symptoms of TB include fatigue, weight loss, and lack of appetite, chills, fever and night sweats. An extra pulmonary (EP) infection can affect any part of the body, the infection spreads through hematogenous route i.e., mainly through blood to the other sites of the body like urinary tract, genital tract, bones and joints etc [3]. Genital TB generally occurs as secondary to pulmonary (commonest) or extrapulmonary TB like gastrointestinal tract, kidneys, skeletal system, meninges and miliary TB through hematogenous and lymphatic route. Over 95 % of new TB cases and deaths occur in developing countries with India accounting for 40 % of the world's TB burden [4]. Co-infection with human immunodeficiency virus (HIV) patients with more liberal immigration from high risk to low-risk areas due to globalization has been responsible for increased incidence all over the world [5].

Globally, 10.4 million new cases of active TB were estimated among which 1.2 million HIV positive people are included, of the 3.5 million were women and 1.0 million were children. About 1,70,000 children have died of TB (excluding children with HIV) [6]. World Health Organization (WHO) recommends the patient should have six months of TB drug treatment (WHO 2015). This consists of a two month "intensive" treatment phase followed by a four-month "continuation" phase [7]. Improper treatment or interruption to medication uptake makes *M. tb* resistance to drugs [8]. Adults with drug-resistant TB should always receive an effective second-line injectable agent as part of their regimen. The emergence of antibiotic resistance *M. tuberculosis* around the world owes as a genetically more diverse form. The resistance mechanisms during evolution described to date are due to

point mutations on the bacterial chromosome considerably results in phenotypic variations in *M. tuberculosis* strains. The prevalence of antibiotic resistance appears to be partly determined by geographical factors. Increase in multi-drug resistance (MDR) and extensively drug-resistance (XDR) were also reported in tuberculosis cases [9].

Therefore, the WHO (2016) changed their recommendations on the use of drugs for the treatment of drug-resistant TB. However, adverse effects need to be carefully monitored while using injectable effective agents. For instance, hearing loss and nephrotoxicity are the most frequent severe side effects [10].

Despite, Bacille Calmette-et-Guerin (BCG) vaccine provides minimal immunity or protection against TB; it is causing side effects like swollen lymph nodes, small red areas at the site of injection, fever, blood in urine, frequent or painful urination, upset stomach and vomiting [11]. To overcome the adverse, side effects and non-efficacious drugs and vaccines in treating and preventing TB caused by MDR and XDR strains, there is an emergent need to discover novel, potential drug targets and vaccine candidates for effective TB therapeutics design. Therefore, in the current study, we aim to predict novel potent drug targets and vaccine candidates against MDR and XDR *M. tuberculosis* strain through *in silico* approaches.

The experimental approaches are tedious and laborious obtain very few results hence computational approaches were used to identify the putative drug targets. Comparative proteomic approach, subtractive proteomic approach, metabolic pathway analysis, non-homologous gut flora analysis, domain search analysis, and protein network analysis are extensively used for the prediction of potential drug targets of the pathogen causing TB. In this scenario, potential targets must be essential for the growth and survival of the pathogen. Further designing of inhibitors should hinder the function exclusively the pathogen and should avoid the undesirable cross-reactivity with the human proteins. The availability of complete proteome sequences of the selected pathogen in combination with Bioinformatics tools and databases is of great importance in reducing the problem of searching for potential drug targets/vaccine candidates in a large pool of proteomes. Therefore, in the current study, we intended to explore potential novel therapeutic and vaccine candidates from numerous genetically diverse *M. tuberculosis* strains inhabitants around the globe for designing new reliable broad-spectrum inhibitors.

2. MATERIALS AND METHODS

2.1. Comparative Proteomic Analysis and Dataset Preparation:

We used proteomes of 23 virulent *M. tb* strains in the current study, among them *M. tb*_H37Rv strain was considered as a reference due to its predominant role in TB [12]. Complete proteomes were retrieved from the National Center for Biotechnology Information (NCBI), an imperative resource for enormous biological information [13]. In comparative proteomic approach, data processing was done using in-house bash and R scripts in Linux environment for downstream dataset preparation and quantification in order to get conserved (common) proteins [14].

2.2. Subtractive Proteomic Approach:

Common protein sequences were subjected for non-human homology [15] by aligning each protein against human proteome for unidentical protein sequences or protein sequences with less than 30 % identity.

2.3. Gene Essentiality and Non-human Homology Analysis:

The derived dataset of conserved proteins was carried to target screening using different channels that optimizes a large number of conserved proteins to few putative targets. In order to screen essential gene products from the dataset, essential gene analysis was performed using the Database of Essential Genes (DEG) accessed at http://tubic.tju.edu.cn/deg/ [15]. Essential proteins were further assigned to non-homology analysis against human proteome [16]. The query sequence that did not match any hit better than the threshold (bit score of >100) were considered as '*M. tuberculosis*-specific' protein. Protein alignments with an expectation value of (E- value) < 10^{-10} were considered as more significant hits. Such proteins were regarded as essential, based on the assumption that similar proteins that are essential in one organism are likely to be essential in another [17].

2.4. Analysis of Non-homology against Host:

The aim of the non-homology analysis is to identify pathogen specific-proteins that are non-homologous to the host. The significance of this step is to minimize undesirable cross-reactivity of the drug thereby preventing its binding to active sites of the host's homologous proteins. Essential proteins were further assigned to non-human homologous analysis, protein alignments E-value >10^{-3} was considered as non-human homologous proteins as described in a previous study [18]. The aim of non-human homology analysis against host is to identify pathogen specific-proteins. The significance of this step is to minimize undesirable cross-reactivity of the inhibitors with host proteins. The essential protein dataset was searched for non-host homology analysis to identify common proteins of *M. tb* strains.

2.5. Host-pathogen Metabolic Pathway Analysis:

KEGG (Kyoto Encyclopaedia of Genes and Genome) accessed at http://www.genome.jp/pathways.html [19] is a pathway database used as a source of metabolic pathway information. Pathways which do not appear in the host but present in the pathogen according to KEGG database have been identified as pathways unique to *M. tuberculosis*. The corresponding protein sequences of enzymes involved in unique pathways were identified [20]. The pathways of drug targets were checked and the drug targets having alternative pathways were not considered because blocking of these drug targets would be ineffective as the product is synthesized by the alternative way.

2.6. Non-homology Analysis against Human Gut-flora and Domains:

All delineated proteins were sought out for similarity search with the proteome of human gut-flora taken from literature reports [21] to evade adverse reactions of chemical inhibitors on host microbiome. Further, the shortlisted enzymes were searched against homologous human domains in Pfam and SMART (Simple Modular Architecture Research Tool) databases to eliminate host's domain level similar protein sequences of MTB, which comprise a large collection of protein families. Finally, the selected enzymes that are un-identical to human proteins/domains were considered as putative targets for validation.

2.7. Protein Interaction Network Analysis:

Analysis of protein-protein networks of the derived target proteins was performed through STRING v10.0, a database providing critical assessment and integration of protein-protein interactions, including direct (physical) as well as indirect (functional) associations [22]. The preferred high

confidence interactions with score ≥ 0.700 and not more than 50 interactors were set to acquire significant target proteins possess with higher interactomes in the networks as well as to avoid false positives and false negatives. The identified targets that are specific and important in various metabolic networks of the pathogen were considered as putative drug targets.

The confidence score of a target =

$$\frac{\text{Number of interactants of target enzyme by used methods}}{\text{Total number of methods used}}$$

2.8. Subcellular Localization Prediction:

Cellular locations of the key target proteins were identified using PSORTb v3.0.2 [24]. Further validated with Subcellular localization of the identified drug targets could be used to obtain information about their potential functions. Subcellular location of the potential target differentiates cytoplasmic, periplasmic or inner membrane proteins that are metabolically important drug targets and outer membrane or extracellular proteins as vaccine candidates. Broad spectrum candidates were classified as either drug targets or vaccine candidates based on their subcellular localization using PSORTb and further validated with CELLO v2.5. The tools that employ four types of sequence coding schemes such as ABC transporter proteins and sequence composition based on physicochemical properties of amino acids. Moreover, the obtained surface exposed proteins were tested for their antigenicity using VaxiJen server for prediction of protective antigens, tumor antigens where antigenic proteins showing score more than the threshold (0.4) were predicted as good antigens and affirmed as subunit vaccines candidates.

3. RESULTS AND DISCUSSION

3.1. Comparative and subtractive proteomic analysis:

As shown in **table (1)**, The common proteins from chromosomal and plasmid DNAs were preferred for target screening as being conserved among several strains could be useful in implementing a common inhibitor. Execution of R-scripts and codes could facilitate the proteome-wide comparison of total 92,325 proteins of 23 *M. tuberculosis* strains obtained 3906 *M. tb* specific common proteins. The dataset derived from the comparative and subtractive proteomic approach has been illustrated in **Figure (1);** composed of conserved proteins with 30–100% identity.

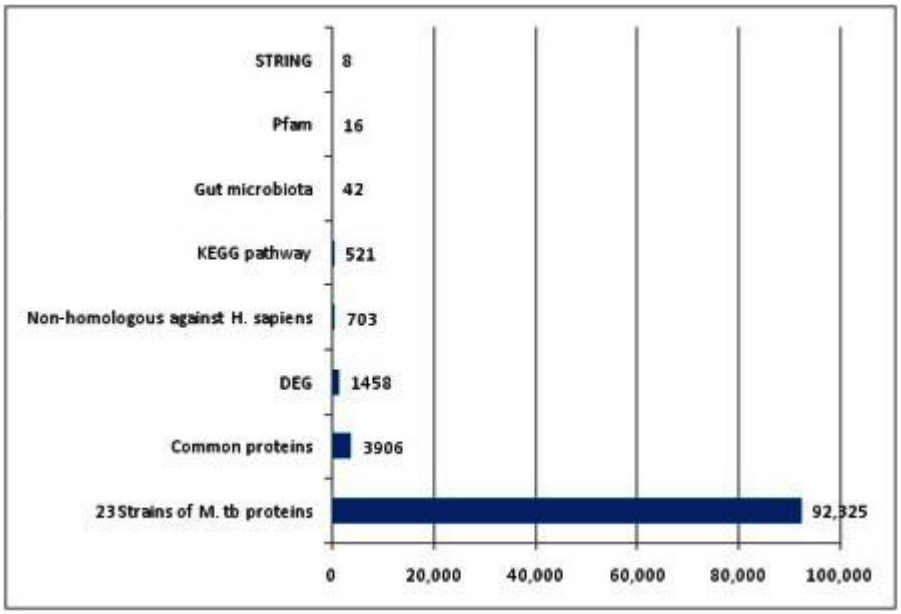

Figure 1: Comparative and subtractive drug target identification approach

3.2. Gene essentiality and non-homology analysis:

As shown in **table (1),** Essential genes are indispensable to maintain cellular life, as they are vital for replication, survival, and viability of the pathogen. Of the common proteins, about 1,458 essential proteins were derived by dint of essentiality analysis. Subsequently, a non-human homologous search of essential proteins had ensued 703 proteins were absent in host (human) and are of great concern in view of designing drugs absolutely specific for the pathogen.

Table 1: Identification of common Drug targets and vaccine candidate of *M.tb* strains

No.	Analyzed criteria	Outcome from the target screening
1.	Proteins in 23 MTB strains	92,325
2.	Common proteins among 23 MTB strains	3906
3.	Predicted essential common genes products	1458
4.	Predicted non-human homologs	703
5.	Pathogen specific unique proteins and enzymes of pathway analysis	521
6.	Unique proteins without alternative pathway analysis	282
7.	Non-homologous to gut microbiota	42
8.	Non- homologous to human domains	12
9.	Proteins with high interaction networks	8
10.	Surface exposed membrane proteins after subcellular location	2

3.3. Metabolic functional association and potential drug targets:

As shown in **table (1)**, Comparative metabolic pathway analysis of non-homologous proteins had unveiled 521 unique proteins comprises 239 enzymes and 282 non-enzymes including membrane proteins, which are absent in *H. sapiens* (distinct pathways). In the present study, metabolic pathways namely porphyrin and chlorophyll, Glycerophospholipid metabolism, arginine and proline metabolism, glyoxylate and dicarboxylate carbon metabolism, phenylalanine, tyrosine and tryptophan metabolism, carotenoid biosynthesis, streptomycin biosynthesis polyketide sugar unit biosynthesis, glutathione metabolism and carbon metabolism contributed to the final drug targets list derived from the utmost analyses .

As shown in **table (2),** Pathways which are not present in the *Homo sapiens* but present in the Mycobacterium are designated as unique pathways. Design and targeting inhibitors against these non-homologous sequences could be the better approach for the generation of new drugs. Thus total 8 unique metabolic pathways have been taken in *M. tuberculosis*.

Table 2: Proposed common drug targets for *M. tb*

No.	Protein Name	Pathway Essential enzymes	Biological Process	Molecular Function
1.	Protoheme IX farnesyl transferase (2.5.1.36)	Porphyrin and chlorophyll	Heme Biosynthesis	Transferase
2.	Phosphatidyl serine decarboxylase proenzyme (4.1.1.36)	Glycerophospholipid metabolism	Lipid biosynthesis, lipid metabolism, Phospholipid biosynthesis, Phospholipid metabolism	Decarboxylase, lyase
3.	1 pyrroline- 5-carboxylate dehydrogenase (1.2.1.88)	Arginine and proline metabolism	Proline catabolic process to glutamate	Nucleotide binding
4.	Putative isocitrate lyase metabolism (4.1.3.1)	Glyoxylate and dicarboxylate carbon	Isocitrate metabolic process	Isocitrate lyase activity
5.	Phospho-2- dehydro-3-deoxyheptonate aldolase (2.5.1.54)	Phenylalanine, tyrosine and tryptophan	Chorismate biosynthetic process	3-deoxy-7-phosphoheptulonate synthase activity
6.	Phytoene synthase (2.5.1.99)	Carotenoid biosynthesis	carotenoid biosynthetic process	Farnesyl-diphosphate farnesyl transferase activity
7.	dTDP-glucose 4, 6-dehydratase (4.2.1.46)	Streptomycin biosynthesis	Polyketide sugar subunit biosynthesis	Coenzyme binding
8.	Isocitrate dehydrogenase (1.1.1.42)	Glutathione metabolism, carbon metabolism	Isocitrate metabolic process	Isocitrate dehydrogenase activity

3.4. Gut microbiota analysis:

As shown in **table (1),** Gut microorganisms benefit the host in many ways such as cleaning the energy from the fermentation of undigested carbohydrates, synthesizing vitamins, subsequent absorption of short-chain fatty acids, degrading xenobiotics *etc*. The human microflora helps in numerous ways which provide resistance to colonization by pathogenic bacteria and indigenous opportunists by influencing the host immune system. Drug designed should neither harm the host enzymes nor hinder the function of enzymes of the gut flora which leads to side effects. Disruption of gut microbiota results in the development of many numerous pathologies in human health. As a result of the human gut-flora analysis, about 42 enzymes out of 239. So, if design a drug for these non-homologous proteins that do not hinder the functioning of the human gut microbiota enzymes.

3.5. Domain search:

The domain similarity search between *M. tuberculosis* and host ensured the elimination of the protein domains which are homologous to host. Domain analysis using the Pfam and SMART databases resulted out 8 enzymes.

3.6. Protein network Analysis:

The selected drug targets were examined by protein-protein interaction network analysis. The network analysis of 8 enzymes and 2 vaccine candidates was determined using the STRING database. Interactions with a score of > 0.400 and interactions with < 50 interactors were regarded as potential drug targets. The analysis of STRING database showed that all the proposed drug targets showed interactions with the high confidence scores of 0.900 and with low confidence interactions less than 50 were incorporated in the interaction network. Each network gives a particular group of proteins. Hovering over each node will display its annotation and full details of the protein, here network nodes represent proteins. Each node in the network has its own importance. Clustering in STRING has two different parameters to cluster the proteins. KMEANS is the parameter which particularly specifies the number of clusters and MCL is the parameter that indirectly related to the precision of the clustering which is mentioned as 'inflation'. The nodes can be deleted and change in the clustering was noted. Upon deletion of each node manually clustering MCL parameter or KMEANS parameter decrease in clustering coefficient was observed and considered as a functionally important protein in the metabolic network. Hence, all the drug targets were considered as potential targets for the pathogen causing tuberculosis.

3.7. Vaccine candidate:

As shown in **table (1),** The subcellular localization prediction determined that 8 enzymes were located in cytoplasmic side and 2 were in the surface exposed membrane (OMP) proteins. From VaxiJen server, the shortlisted OMPs with a score more than the threshold (0.4)

were predicted as good antigens and affirmed as subunit vaccines candidates. Among the common 10 proteins, two vaccine candidates such as Molybdenum transport system permease protein (ModB) and phosphate transport system permease protein (pstc1) was identified as common vaccine candidates for MTB strains causing tuberculosis. Hence, the identification of vaccine candidates would be a promising attempt towards designing T-cell epitope-driven common subunit vaccine for preventing *M. tuberculosis* infections. Similarly, two novel vaccine candidates are membrane components with highly antigenic to host, essential for pathogenesis, cell adhesion and to protect *M. tuberculosis* from osmotic gradients. The implementation of therapeutics such as inhibitor or vaccines against these proposed novel targets may become single or common therapeutic for several *M. tuberculosis* strains and could be effective due to targets involvement in vital pathways. Therefore, the results of the study will highlight the proposed novel targets as more potential therapeutic targets.

4. CONCLUSION

Globally, the emergence of multi-drug resistance among pathogenic *M. tuberculosis* is a growing concern in the medical field. The development of new antimicrobial agents against unexplored drug targets is of major concern to curb these problems. This paper describes and appraises the uncovering set of potential targets that are typically conserved in *M. tuberculosis* strains which present a new opportunity for the experimental biologists to test. We identified the pool of eight common potential drug targets and two surface exposed membrane proteins provides a basis for computer-aided drug and vaccine design strategies lending credence to our approach. Development of powerful drug molecules via *in silico* to *in vivo* pipelines against these putative targets may become potential therapeutics to combat present and upcoming drug-resistant *M. tuberculosis* strains as these are conserved targets critically essential for pathogens, non-homologous to human and gut-flora at sequence to structural level. Therefore, the proposed targets from the study would bring new possibility in obstructing the biological functions essential for the growth and survival of *M. tuberculosis* infections.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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