Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0405.46**

STRATEGIES FOR DRUG TARGETS SELECTION OF *MYCOBACTERIUM TUBERCULOSIS* H37RV

Zahra M. Al-Khafaji*[1], Aaisha B. Mahmood[2]

1. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

2. Ministry of Agriculture , Veterinary Directorate , Baghdad Veterinary Hospital, Al-Dora Hospital, Iraq.

ABSTRACT: In spite of the availability of effective chemotherapy, tuberculosis still a leading infectious killer worldwide. In general, gene products involved in mycobacterial metabolism, persistence, transcription, cell wall synthesis and virulence would be possible targets for the development of new drugs. The exploitation of host cell especially the outside components of the cell will lead to skip the permeability, the big problem obstacles the effective treatment. Among the strategic targets that can be used is the signaling pathways which could be knocked down and disturbed the cell life cycle. Reversible phosphorylation and dephosphorylation (TCSs) represent good targets due to its vitality and making a good interaction network. Understanding host-pathogen interactions would give an important clues for developing new drugs, vaccine and diagnostic tests. Recent advances in the knowledge of the biology of the *Mycobacterium tuberculosis* and the availability of the genome sequence give an opportunity to explore a wide range of novel targets for drug design. In this study different targets at the out borders were studied for their druggability.

KEYWORDS: *Mycobacterium tuberculosis*, protein druggability, secretory proteins, cell envelope, NTPome.

Corresponding Author: Dr. Zahra M. Al-Khafaji* Ph.D.

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Email Address: zahranasserk@gmail.com

1.INTRODUCTION

Tuberculosis (TB) is the deadliest infectious disease, according to WHO recent reports, 10.4 million TB cases were reported in 2016 [1], this disease characterized by the lack of involvement of classical virulence factors such as toxins or flagella, but it is a dynamic balance between host and pathogen which defines the outcome of the infection [2]. The causative agent *Mycobacterium tuberculosis* (Mtb) strains such as H37Rv, able to modulate several host mechanisms and activities in the host to its favor [3]. Mtb H37Rv is the most studied strain and is the most successful pathogen estimated among Iraqi population [4], its genome has about 4000 genes, with high gene density (0.91gene/kb) as the coding genes are 91.2% [5]. Its success relies on the abilities to utilize macrophages for replication, which should remain viable to host the Mtb [6], these abilities were developed through the cross-talk between the bacterium and the host which create co-evolutionary balances over centuries [6,7]. The stages from the first contact (infection) to disease comprises many stages; adhesion, invasion, intracellular survival, replication and dissemination to other body sites [2] .During the whole cycle the bacterium protects itself by different mechanisms such as formation of granulomas which can preserve the bacteria for decades [8] , this affect the eradication of TB and may causes recurrent infections [9,10]. Adhesion to host cells is a precursor to host colonization and evasion of the immune response, adhesins are microbial cell surface molecules or structures that mediate the attachment of pathogen to host cells, thus represents the first host-pathogen interactions, consequently could be consider as the key player [11] . The first type of host cells encounter in the lung are the epithelial cells, the Mtb-epithelial cell interaction may potentially precede invasion of macrophage and induce subsequent events [12]. The mycobacterial cells are armed against host defenses, first of all, the cell envelope which is unique and performs many processes for the bacterium. It represents a real barrier of any harmful agents or environmental factors. It compromised of 40% lipids of the dry weight, some researches divide it into outer layer (sometimes called capsule), cell wall, and plasma membrane, the two latter separated by periplasm space [13]. In addition, the bacterium is well equipped with secretory proteins (Secretome) which modulate the host responses, some of them confer drug resistance. This has led to appearance of resistant strains at different degree or levels. The resistance generally attributed to low permeability of the cell envelope [14,15,16], in addition to efflux pumps which are overexpressed upon treatment [17,18,19] and other mechanisms to disable the anti-mycobacterial agents. This means that the existing treatment regimen against TB is not adequate and novel therapeutic interventions are required to target the Mtb pathogenesis. In the last decades there have been numerous attempts toward the development of novel approaches to compact TB [20], among these targeting the oxidative phosphorylation as the Mtb an aerobic microorganism, and this can lead to a completely new regimen for drug susceptible and resistant mycobacteria , since the Mtb and related mycobacteria

strains apparently cannot gain enough energy by substrate-level phosphorylation and need oxidative phosphorylation for growth [21,22] , this happens a cross the cell membrane [23]. A major limitation of TB therapy is slow killing of the bacteria which increases the risk for the development of tolerant phenotypes and drug resistance. In general, gene products involved in mycobacterial metabolism, persistence, transcription, cell wall synthesis and virulence would be possible targets for the development of new drugs. Several studies revealed that there are many unexploited Mtb targets may be chemically druggable and could overcome the limitation of existing drugs [24]. There is a need to design new drugs that are more active against slowly growing or non-replicating persistent bacilli to treat the population at risk of developing active disease through reactivation [24]. The aim of this study emphasizes on the strategies to select the drug targets using Bioinformatics approach, since the complete genome sequence of Mtb H37Rv was annotated in 1998, bioinformatics can provide important start point for new targets discovery, and could be a powerful mean for obtaining lists of possible targets for further experimental validation. This study dealt only with genes/proteins at the outer borders of the cell.

2. MATERIALS AND METHODS

Proteins were collected from literatures and databases, using Rv ID for *Mycobacterium tuberculosis* H37Rv strain, the databases used: PATRIC database [25] was used for retrieving different categories of proteins. Uniprot database [26] was used to estimate the subcellular location of proteins and some more characters. TDR v.5 [27] used to find the druggability score of proteins. BLASTp used to find homology of chosen proteins with human proteins.

Methods: The collected proteins from PATRIC database and literatures were checked for their subcellular location and functions using Uniprot database, only those found at the cell out borders such as cell wall, cell membrane, transmembrane proteins, secreted proteins were selected and subjected for further investigations. The selected proteins were checked for their druggability, human protein homology using BLASTp. The pdb structure found in PDB database of druggable proteins were rechecked using Uniprot database.

3. RESULTS AND DISCUSSION

Approximately one-third of global population harbor Mtb but remain asymptomatic as latent TB, and only 5-10% will develop into active TB disease[28].The results of better understanding of physiology of *Mycobacterium* is manifested by fact that the list of targets for TB curing is increasing, as there is about 37% of its proteins are annotated as hypothetical proteins (out of 3989 CDS) for Mtb H37Rv according to PATRIC database 2017 [25] , the utility of mycobacterial proteins/targets , however, cannot be predetermined [24], this means that functional annotation of Mtb genome remains a critical research priority [29] . On the other hand, evidences from bacterial systems indicated that metabolic networks are robust and tolerate to perturbations via compensatory and

regulatory mechanisms that often mediated at multiple levels through complex protein networks [29,30] , these do not need genetic manipulation but depend on metabolites. Cell envelope as a part of the cell has been implicated in the pathogenicity of Mtb, this part is notorious for being several-fold and less permeable to chemotherapeutic agents when compared to similar structure of other bacteria [31] . Therefore, it has been a prime target for the identification and characterization of its attached surface proteins for drug targeting and vaccine development. Proteins used in this study mainly are out border proteins, represented by secreted proteins and those associated with outer cell envelope such as cell wall, cell membrane and cell surface, those were divided into different functional categories as shown in Fig 1

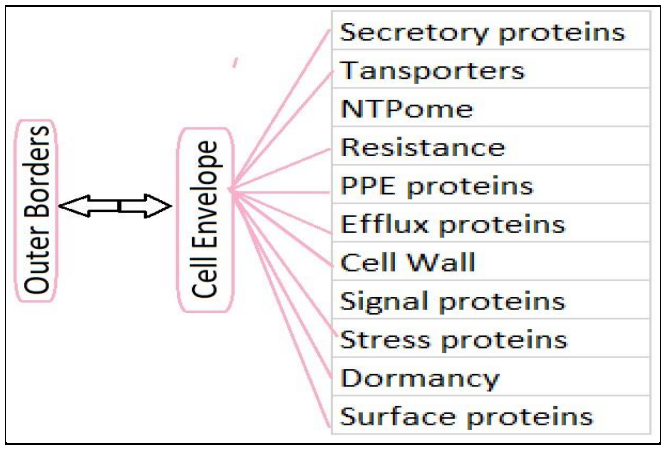

Figure 1: Categorization of proteins used in this study

However, this choice not represent essential criterion, since the latter is by itself insufficient to define a good drug target , as not all essential genes/proteins are equally vulnerable to drug action and essentiality can vary under different conditions , in addition the essential genes in relevant databases such as PATRIC and DEG databases recorded essentiality in cultural media i.e., in vitro which cannot mimic the physiological in vivo conditions [25,32], so other criteria could be taken such as druggability or chemical tractability , and the targets should be with low mutability to reduce the chances for drug resistance [33] . Virulence factors (about 800 found in PATRIC database from different resources) represent a novel strategy for therapeutic intervention [34], but this approach suffers from some serious drawbacks, for example, the virulence factor may be not necessarily survival gene or may not be lethal to the pathogen, and would be of little or no effect if the disease has been already on set [24]. Figure 2 and Figure 3 show the number of proteins surveyed and the percent of the druggable proteins in each group.

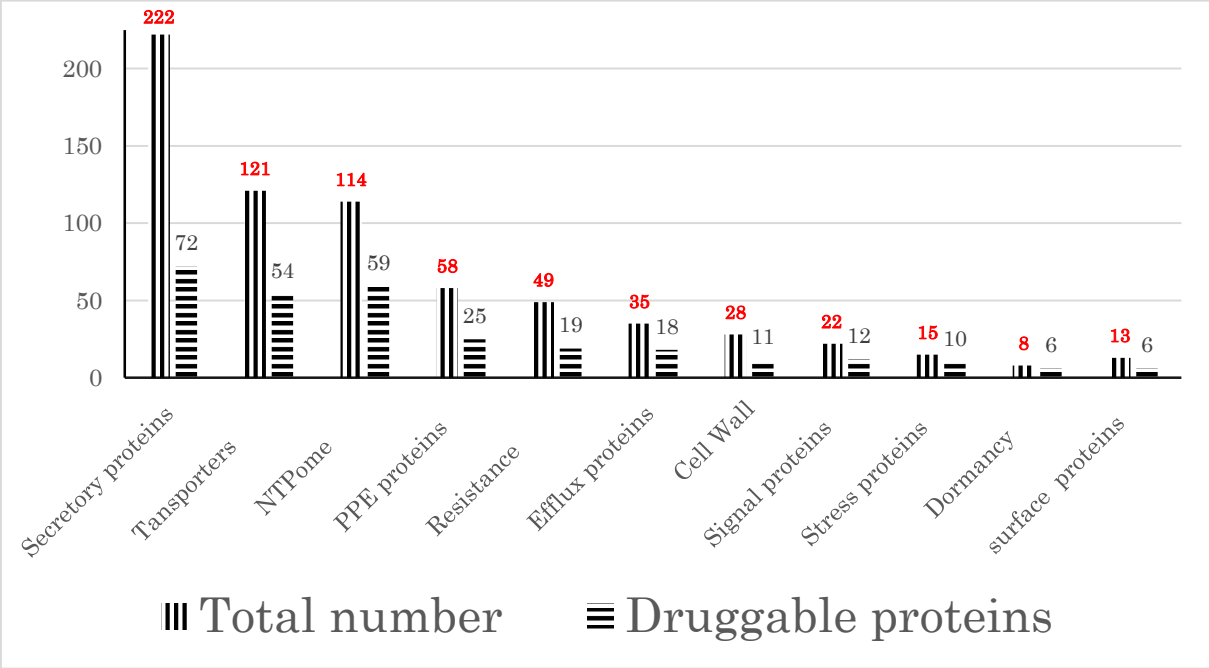

Figure 2: Number of proteins and number of druggable proteins

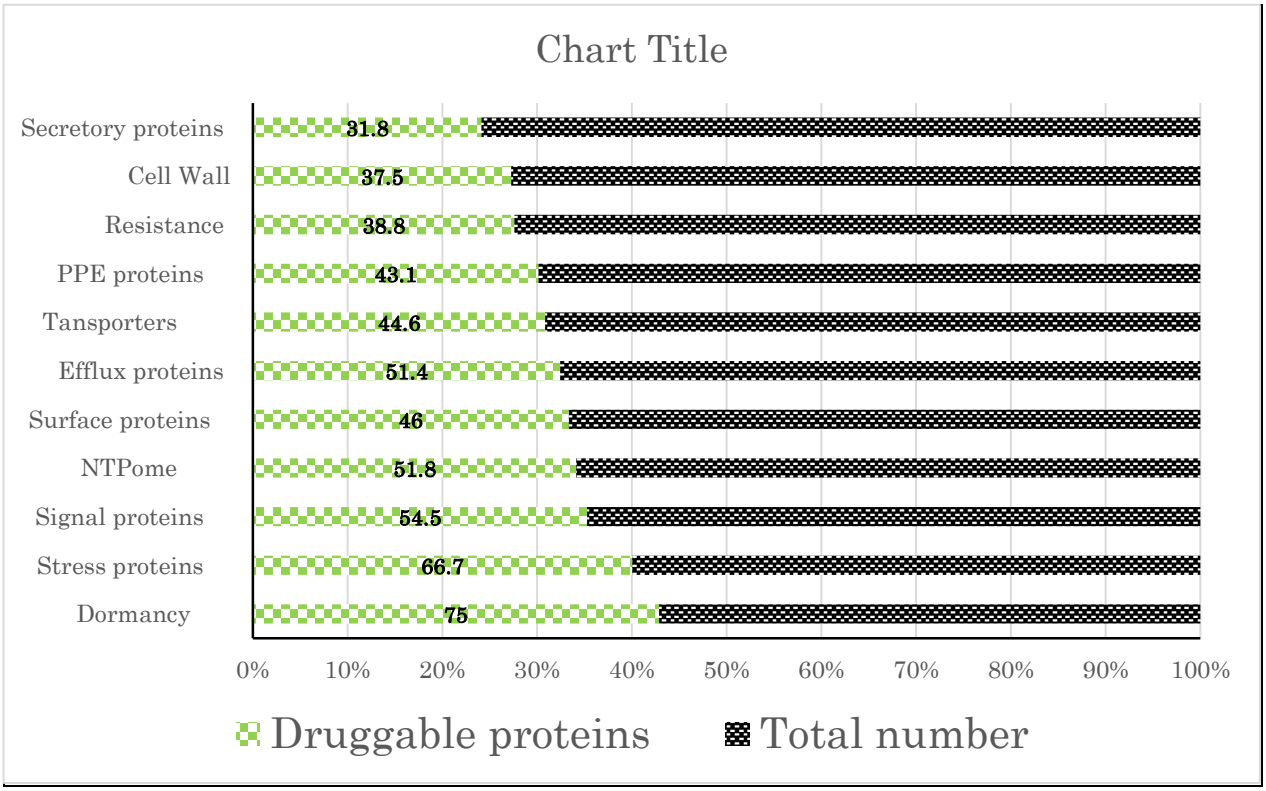

Figure 3: Percentage of druggable proteins

It is well known that the target identification is typically the starting point of the modern drug discovery or design processes, in this study two criteria were used for choosing the targets, subcellular location and druggability. Druggability is effected by some factors, among these geometric configurations of the protein and good drug target should have grooves with sufficient convexity and potential interaction [35]. The reason for selection of outside targets instead of

intracellular targets is beyond the fact that the pathogen in an attempt to survive can shift into different metabolic states upon drug treatment, i.e. using escape pathways associated with changes in bacterial metabolic state [36,37], in addition larger number of proteins can convert into effective target upon disruption of protein interactions by small molecules. According to Figure 2, secretory proteins represented the largest group and dormancy proteins as the smallest group as annotated in Uniprot database.

3.1. Secretory proteins: The selected proteins are shown in Table 1

Table 1: Secretory proteins

Rv ID	Druddabili	Rv0592		Rv1064c		Rv1677	
Rv0016c	D*	Rv0598c		Rv1157c	D	Rv1690	D
Rv0040c		Rv0604		Rv1158c	D	Rv1698	
Rv0063		Rv0671		Rv1166		Rv1796	D
Rv0109	D	Rv0677c		Rv1174c		Rv1799	
Rv0116c		Rv0679c		Rv1184c		Rv1804c	
Rv0125		Rv0680c	D	Rv1214c		Rv1810	
Rv0171	D	Rv0758	D	Rv1235		Rv1813c	
Rv0178		Rv0774c		Rv1244		Rv1815	
Rv0179c		Rv0804		Rv1252c		Rv1821	
Rv0192A		Rv0817c		Rv1268c		Rv1857	
Rv0203		Rv0832		Rv1269c		Rv1860	D
Rv0227c		Rv0835		Rv1270c		Rv1881c	
Rv0291		Rv0838		Rv1271c		Rv1885c	D
Rv0309		Rv0846c		Rv1274		Rv1886c	D
Rv0315		Rv0847		Rv1291c		Rv1906c	
Rv0320		Rv0867c	D	Rv1296		Rv1910c	
Rv0344c		Rv0875c		Rv1352		Rv1911c	
Rv0361		Rv0906		Rv1368		Rv1921c	
Rv0398c		Rv0928	D	Rv1411c		Rv1922	D
Rv0399c	D	Rv0932c				Rv1926c	
Rv0403c	D	Rv0934		Rv1418		Rv1968	
Rv0411c		Rv0962c		Rv1419		Rv1969	
Rv0418	D	Rv0988		Rv1433		Rv1974	
Rv0432		Rv1004c	D	Rv1435c		Rv1975	
Rv0455c		Rv1006		Rv1441c		Rv1980c	
Rv0477		Rv1009		Rv1477	D	Rv1984c	
Rv0526		Rv1016c		Rv1478		Rv1987	
Rv0544c	D	Rv1022		Rv1488		Rv2041c	
Rv0559c		Rv1031		Rv1541c		Rv2068c	D
Rv0583c		Rv1040c		Rv1566c		Rv2075c	

Rv2080	
Rv2138	
Rv2144c	D
Rv2171	
Rv2190c	D
Rv2223c	
Rv2224c	
Rv2253	
Rv2262c	D
Rv2270	
Rv2289	
Rv2290	
Rv2293c	
Rv2301	
Rv2318	
Rv2330c	
Rv2341	
Rv2350c	
Rv2376c	
Rv2396	D
Rv2403c	
Rv2430c	
Rv2450c	
Rv2452c	
Rv2525c	

Rv2532c	
Rv2585c	
Rv2597	
Rv2599	
Rv2668	
Rv2672	
Rv2721c	D
Rv2784c	
Rv2796c	
Rv2833c	
Rv2843	D
Rv2864c	D
Rv2873	
Rv2875	
Rv2891	
Rv2905	
Rv2911	D
Rv2945c	D
Rv2960c	
Rv2972c	
Rv2999	
Rv3004	
Rv3006	
Rv3016	
Rv3033	

Rv3036c	
Rv3044	
Rv3096	
Rv3106	D
Rv3194c	
Rv3244c	
Rv3298c	
Rv3310	
Rv3312A	
Rv3330	D
Rv3333c	
Rv3354	
Rv3390	
Rv3395A	
Rv3449	D
Rv3451	
Rv3452	
Rv3491	
Rv3495c	
Rv3572	
Rv3576	
Rv3584	
Rv3593	
Rv3604c	D
Rv3619c	D

Rv3620c	
Rv3623	
Rv3627c	D
Rv3630	
Rv3654c	
Rv3655c	
Rv3666c	
Rv3668c	
Rv3705c	
Rv3732	D
Rv3759c	
Rv3763	
Rv3803c	D
Rv3804c	D
Rv3810	D
Rv3846	D
Rv3851	D
Rv3872	
Rv3874	D
Rv3875	
Rv3883c	D
Rv3886c	

*D= druggable protein

Secreted proteins play an important role in the interaction of bacteria with each other and with their environments. In Mtb participate in adhesion, cell migration, invasion, signal transduction, virulence and survival inside the host and they are the major source of immunogenic proteins. it compromised about 12% of the mycobacterial proteins. They are secreted by known secretory mechanisms and some secreted by unknown mechanisms, the latter referred as non-classical secretory proteins [38,39].

3.2 Transporters

This group of proteins are essential for bacterial survival and persistence. According to transporter classification system, there are about five classes included in TCDB database adopted by PATRIC database, these are channels, secondary carries, primarily transporters, group translocators and transmembrane [40]. The transporters surveyed in this study are shown in Table 2

Table 2: Transporters

Rv ID	Druggabil
Rv0037c	
Rv0107c	D
Rv0178	
Rv0194	D
Rv0202c	D
Rv0204c	
Rv0205	D
Rv0206c	D
Rv0282	
Rv0283	
Rv0284	
Rv0290	
Rv0292	D
Rv0450c	
Rv0488	
Rv0529	D
Rv0545c	D
Rv0585c	
Rv0638	
Rv0655	D
Rv0676c	
Rv0677c	
Rv0732	D
Rv0820	D
Rv0834c	D
Rv0888	
Rv0899	
Rv0929	
Rv0932c	D
Rv0933	D
Rv0936	
Rv0955	
Rv0985c	
Rv0986	D
Rv0987	
Rv1206	
Rv1235	D
Rv1236	D
Rv1237	D
Rv1238	D
Rv1239c	D
Rv1258c	D
Rv1280c	
Rv1281c	D
Rv1282c	D
Rv1283c	D
Rv1348	D
Rv1349	D
Rv1373	
Rv1410c	D
Rv1411c	
Rv1440	D
Rv1591	
Rv1634	D
Rv1694	
Rv1698	
Rv1704c	D
Rv1707	
Rv1739c	D
Rv1782	
Rv1783	
Rv1784	
Rv1794	
Rv1795	
Rv1796	D
Rv1797	D
Rv1798	
Rv1811	
Rv1819c	D
Rv1821	
Rv1999c	
Rv2154c	
Rv2264c	D
Rv2287	D
Rv2325c	
Rv2326c	D
Rv2333c	D
Rv2397c	D
Rv2398c	
Rv2399c	
Rv2400c	
Rv2434c	
Rv2456c	
Rv2586c	
Rv2587c	D
Rv2588c	
Rv2684	D
Rv2856	
Rv2874	D
Rv2916c	D
Rv2921c	
Rv2942	
Rv2945c	D
Rv3065	D
Rv3240c	D
Rv3270	D
Rv3271c	
Rv3273	D
Rv3493c	
Rv3500c	
Rv3501c	
Rv3614c	
Rv3615c	
Rv3821	
Rv3823c	
Rv3868	
Rv3869	D
Rv3870	D
Rv3871	
Rv3872	
Rv3873	
Rv3874	D
Rv3875	
Rv3876	D
Rv3877	D
Rv3882c	
Rv3884c	
Rv3885c	
Rv3887c	
Rv3895c	
Rv3910	D

These proteins are involved in moving substrates, interestingly, it has been found that exporter: importer proteins is high in Mtb. The influx included sugars, amino acids, metals and anions, while the efflux mainly works with pumping the drugs and deleterious substances to cell such as acetate [41,42,43]. The transporters are overlapped with other categories of bacterial proteins performed other functions (results not shown), about 44.6% are druggable (Figure 2, Figure 3) so can be hit to disturb cellular functions.

3.3 NTPome

Nucleotide tri phosphate binding proteins (NTPome) is the third group of mycobacterial proteome, 114 proteins were surveyed according to the criteria assumed in this study, about half of them are druggable (Table 3).

Table 3: NTPome proteins

Rv ID	Druggabilit
Rv0014c	D
Rv0015c	D
Rv0016c	D
Rv0018c	D
Rv0019c	
Rv0054	
Rv0072	
Rv0285	
Rv0287	
Rv0288	
Rv0384c	D
Rv0410c	D
Rv0440	
Rv0467	D
Rv0490	
Rv0577	
Rv0601c	
Rv0647c	
Rv0820	D
Rv0845	
Rv0859	D
Rv0902c	D
Rv0928	D
Rv0929	
Rv0930	
Rv0931c	D
Rv0932c	D
Rv0933	D
Rv0934	D
Rv0935	
Rv0936	
Rv0938	
Rv0949	
Rv0969	D
Rv0982	
Rv1023	D
Rv1028c	D
Rv1133c	D
Rv1235	D
Rv1236	D
Rv1237	D
Rv1238	D
Rv1266c	D
Rv1267c	D
Rv1270c	
Rv1293	D
Rv1327c	
Rv1340	D
Rv1386	
Rv1391	D
Rv1559	
Rv1626	
Rv1743	D
Rv1746	D
Rv1747	D
Rv1782	
Rv1783	
Rv1818c	D
Rv1821	
Rv1825	
Rv1827	
Rv1843c	D
Rv1859	
Rv1908c	D
Rv2004c	
Rv2031c	
Rv2074	
Rv2088	D
Rv2097c	
Rv2115c	D
Rv2145c	
Rv2176	D
Rv2202c	
Rv2205c	
Rv2215	
Rv2252	
Rv2391	D
Rv2397c	D
Rv2583c	D
Rv2686c	
Rv2687c	
Rv2688c	D
Rv2780	D
Rv2914c	
Rv2936	D
Rv2937	
Rv2938	
Rv2942	
Rv2984	
Rv2996c	D
Rv3028c	
Rv3042c	
Rv3080c	D
Rv3102c	D
Rv3223c	D
Rv3240c	D
Rv3245c	
Rv3270	D
Rv3273	D
Rv3285	D
Rv3331	D
Rv3401	
Rv3417c	D
Rv3425	
Rv3457c	
Rv3610c	D
Rv3709c	
Rv3763	
Rv3764c	
Rv3869	D
Rv3876	D
Rv3910	D

These proteins participate in many cellular processes; it has been found that about 43% of Mtb proteins can theoretically bind to NTP ligands [44]. They regulate signal transduction events, mediate number of transport reactions, importantly they participate in oxidative phosphorylation for gaining energy under aerobic conditions and in other ways for gaining energy [45,46,47]. ATP is abundant molecule serves as phosphate donor for phosphorylation of many proteins and has been found that 72% of NTPome bind to ATP and some of them are ATP dependent such as kinases, some chaperones, transporters and others which play essential roles in Mtb viability, pathogenesis and drug resistance, some of them were exploited as drug targets [48,49,50].

3.4.PE/PPE proteins

This group of proteins play a role in evasion of host immune response, they are divided into several families and are unique to mycobacteria and particularly abundant in pathogenic mycobacteria. It is believed that they are source of antigenic variation which allow the Mtb to escape antigen–specific

host response [51]. Mtb has about 200 putative proteins, they comprised about 6/25 major cell surface proteins [52,53], some are with high molecular weight and may be tightly associated with cell wall and can have limited cleavage sites. Several PE/PPE genes are upregulated upon macrophage infection and host tissues and play important role in subverting innate immune response, it can limit nitric oxide (NO) production. The selected proteins are shown in Table 4

Table 4: PE/PPE proteins

Rv ID	Druggabilit	Rv0151c	D	Rv0285		Rv0652	D
Rv0092	D	Rv0152c		Rv0286	D	Rv0742	D
Rv0096	D	Rv0159c		Rv0305c		Rv0754	D
Rv0103c	D	Rv0256c		Rv0453		Rv0846c	

Rv0878c	D	Rv1450c	D	Rv1881c		Rv3136	
Rv0977	D	Rv1548c		Rv1917c		Rv3425	
Rv1088		Rv1651c	D	Rv1983	D	Rv3426	
Rv1089		Rv1759c	D	Rv2108		Rv3512	D
Rv1195		Rv1782		Rv2328		Rv3539	
Rv1196		Rv1783		Rv2430c		Rv3653	D
Rv1361c		Rv1795		Rv2431c		Rv3738c	D
Rv1386		Rv1797	D	Rv2741	D	Rv3872	
Rv1387		Rv1801	D	Rv2892c		Rv3873	
Rv1430		Rv1818c	D	Rv3043c	D	Rv3874	D
Rv1441c	D	Rv1840c	D	Rv3097c			

More than 40% of the selected proteins are druggable (Figure2, Figure 3). The family PE-PGRS are highly expressed within granulomas and have been shown to be essential for the virulence of Mtb [54], therefore, the members of this category genes/proteins constitute potential drug targets [24] especially the secreted proteins as some associated or be ESX substrates and participate in virulence and pathogenesis mechanisms, they considered as a key comments of cell wall that interact with the host [38,55,56,57].

3.5. Resistance and Efflux pump proteins

The proteins of this selected group are shown in Table 5A for resistance proteins and Table 5B for efflux pumps.

Table 5A: Resistance proteins

Rv ID	Druggability	Rv1484	D	Rv2936	D
Rv0262c	D	Rv1622c	D	Rv2937	
Rv0342		Rv1644		Rv2938	
Rv0343		Rv1694		Rv2984	
Rv0667	D	Rv1854c		Rv3793	D
Rv1267c	D	Rv1908c	D	Rv3794	D
Rv1305	D	Rv2194	D	Rv3795	D
Rv1311		Rv2195		Rv3854c	
Rv1456c		Rv2846c	D		

Table 5B: Efflux pump proteins

Rv ID	Druggability
Rv0191	D
Rv0194	D
Rv0783c	D
Rv0849	D
Rv1006	
Rv1008	
Rv1217	
Rv1218c	D
Rv1235	D
Rv1258c	D
Rv1410c	D
Rv1456C	
Rv1458c	D
Rv1634	D
Rv1686c	
Rv1793	
Rv2209	
Rv2459	D
Rv2589	D
Rv2686c	
Rv2687c	
Rv2688c	D
Rv2858c	D
Rv2936	D
Rv2937	
Rv2938	
Rv2994	D
Rv3044	
Rv3065	D
Rv3106	D
Rv3364c	
Rv3566c	
Rv3728	D
Rv3756c	
Rv3852	

Large number of resistance proteins are druggable (about 39%) and 54% of efflux pump proteins. Drug resistance in Mtb is complicated phenomenon involving both intrinsic and induced mechanisms. The former includes the efflux pumps activation which is regarded as the first step towards high level of resistance [58], and this unfortunately exacerbate the burden of TB and the appearance of MDR-TB and XDR-TB strains worldwide, for example in Angola reached 71% of treated patients and 8% in new cases, and in Ethiopia higher levels were recorded recently [59,60] . At the beginning there was monoresistant TB, then followed by appearance by MDR-TB which is resist to one or more of first line drugs, after that XDR-TB strains appeared as well which are resist one or more of second line of drugs, the situation become worse upon the appearance of XXDR-TB which then named TDR-TB strains, this resists all drugs. TDR stains recorded at the first time in USA, then in Iran, 15 strains were estimated, among them 4.3% in the Iraqi immigrants [61], in India [62], in south Africa [63], and globally there are 10% TDR of all MDR strains [64], these numbers may not reflect the real situation, recent reports pointed that TDR-TB found in 105 countries and is expected to increase in the future [65]. Several strategies are being explored to counter the problem of resistance [66]. Third line of drugs appeared to treat the resistant strains, among these linezolid for treatment of XDR, this drug act by binding to V domain of 16S rRNA in 50S ribosomal subunit, therefore inhibiting the early steps of protein synthesis, but bacteria can develop rapid resistance to this compound by single or multiple mutations in the 16S rRNA as in *Staphylococcus haemolyticus* [67]. The main and essential problem of resistance is attributed to efflux pumps. The pumps involve naturally in removal harmful substances from the interior of cells to exterior environment, a process requires energy [41,42,43]. Efflux pump systems involved in expelling drugs from the bacterial cells leaving low concentrations of drugs which help in development of different mutations, so the drug-tolerant bacteria continue to replicate under the protection of efflux pumps and generate different chromosomal mutations associated with high level of resistance [68]. Mtb presents the largest number of putative efflux pumps and there is about 148

genes coding for membrane transport proteins [69], those belong to ABC (ATP-binding cassette) superfamily, MFS (major facilitator) superfamily and SMR (small multidrug) superfamily [70]. First class use ATP as energy source and are substrate specific, while the rest are not specific and use pmf (proton motive force of H^+ or Na^+) generated across the membrane to derive the extrusion of the drugs from the cell [41]. MDR-TB and XDR-TB overexpress efflux pumps and this may be without involvement of chromosomal mutations leading to treatment failure [71], however , some mutations were recorded in efflux pumps genes but not found in Mtb H37Rv strain [72] .But other genes implicated in drug resistance are mostly practice mutation of drug targets , which differ according to the drug , lineage to which the strain belongs and host [69], and can lead to alteration of cell structures such as the permeability of cell envelope/cell wall [73]. Efflux pumps are becoming an attractive area for research to develop new drugs, and to deal with these pumps may be by blocking ATP energy source, or using inhibitors that restoring the activity of drugs independent of the level of resistance and decreases the concentration of drug expelled by pumps, thus could reduce the duration of TB treatment [58,69]. As chromosomal /gene mutations are the common mechanisms for resistance, so more than one database were built for this reason such as TB Drug Resistance Mutation Database and" MUBII-TB-DB database.

3.6. Cell wall and Cell surface associated proteins

Selected proteins of this categories are shown in Table 6A (Cell wall), Table 6B (Surface proteins)

Table 6A: Cell wall proteins

Rv ID	Druggability
Rv0288	
Rv0311	
Rv0399c	D
Rv0451c	
Rv0556	
Rv0732	D
Rv0817c	
Rv0838	

Rv0875c	
Rv1274	
Rv1303	
Rv1922	D
Rv2068c	D
Rv2150c	D
Rv2153c	D
Rv2553c	
Rv2864c	D

Rv2905	
Rv3019c	
Rv3593	
Rv3717	
Rv3792	
Rv3794	D
Rv3805c	D
Rv3921c	

Table 6B: Cell surface proteins

Rv Id	Druggability
Rv0309	
Rv0440	
Rv0475	

Rv0527	D
Rv1818c	D
Rv1860	D
Rv2416c	
Rv2551c	D

Rv2873	
Rv2599	
Rv3810	D
Rv3763	

For surface proteins there are 46% druggable, while the cell wall proteins only 37.5% are druggable (Figure 2, Figure 3). The cell wall of mycobacteria consists of three main covalently linked polymers, the basal peptidoglycan layer, an intermediate electron-transparent layer and outer dense layer

[73,74], this structure acts as a permeation barrier and for regulation of pathogen-host interactions, and its lipids act as virulence factor during infection [6,7]. Many proteins are associated with this structure can be exploited as drug targets at the outer borders of the bacterium. Concerning the surface proteins, these known to be expressed on the surface of mycobacteria and are the first to come into contact with the host, consequently have significant role in the interactions with the host and initial stages of bacillus invasion, virulence, pathogenesis and survival inside the host. Some proteins may be secreted and detached from the surface, and then accumulated and re-association with bacterial surface where they are able to execute their biological functions including adherence to host and entry [75]. The abundance of cell surface proteins and their functions has led to focusing on these proteins as drug targets and for vaccine development, this has been facilitated by the annotation of the complete genome sequence of Mtb strain H37Rv [39], and some of novel anti-TB agents are in current development and clinical trials.

3.7. Stress and Dormancy protein

Some of the out border proteins are encountered in stress, shown in Table 7A, and the related group, Dormancy proteins are shown in Table 7B

Table 7A: Stress proteins

Rv ID	Druggability
Rv0251c	D
Rv0440	
Rv0928	D
Rv0934	D

Rv1818c	D
Rv2031c	
Rv2429	
Rv2623	D
Rv2710	D
Rv3223c	D

Rv3417c	
Rv3610c	D
Rv3763	
Rv3841	D

Table 7B: Dormancy proteins

Rv ID	Druggability
Rv0569	
Rv0867c	D
Rv1009	D

Rv1739c	D
Rv2389c	D
Rv2450c	
Rv2623	D

Rv2626c	D
Rv3841	D

Proteins in these groups showed the highest druggability scores, 66.7% for stress proteins and 75% for dormancy proteins (Figure 2, Figure 3). Stresses encounter by Mtb with the host can be considered as oxido-reduction stress mainly, these include hypoxia (as in granulomas) , acidity , NO , carbon monoxide (CO) and others such as starvation . Mtb can monitor these factors and response to them in different ways, for example, changing the metabolic pathways or production of redox buffers such as ergothioneine and mycothiol [76,77]. It has been found that under stress of low oxygen tension (hypoxia) the bacterium change the expression of about 230 genes [78]. The most important in this aspect is the superoxide dismutase (Rv Rv3846), which contributes to pathogenicity of many bacterial species [79], the important proteins are those dealing with nitrogen

radicals [80] and others activate transporters to transport metal cations , sulphate, molybdate and peptides and also regulate phosphate homeostasis [81] . Some proteins can be involved in more than one stress such as Rv0014c (pknB) which participate in hypoxia and starvation [82,83], and the universal stress protein Rv2623 participates in more than bacterial activities under different stresses [10]. Facing the different stresses by Mtb leads to dormant states. So dormancy is one of virulence strategy developed by Mtb, and there are 25% of global population are latently infected [76,77] . This state, i.e. built up the success of Mtb to be a good human pathogen, as slowing the growth/arrested growth in responses to immune pressures and other stresses consequently derives drug tolerance, for example the MBC of rifampicin increased to 50-250 times [84], and help the bacterium to escape the activated immune system of the host. Under dormancy state different activities and structures of cell are changed, cell wall undergoes composition alteration and become thicker [84], in addition Mtb exhibiting larger metabolic plasticity, it effects the macrophage metabolism and led the latter to accumulate lipids which provide the bacteria with nutrients required to sustain dormancy for decades [77].

3.8. Signal transduction proteins

Some signal transduction proteins were covered in this study (Table 8)

Table 8: Signal transduction proteins

Rv ID	Druggability
Rv0014c	D
Rv0015c	D
Rv0410c	D
Rv0600c	
Rv0601c	
Rv0724	
Rv0931c	D

Rv1028c	D
Rv1266c	D
Rv1354c	
Rv1357c	
Rv1539	D
Rv1604	D
Rv1743	D
Rv2031c	

Rv2088	D
Rv2093c	
Rv2094c	
Rv2176	D
Rv2234	
Rv2903c	
Rv2914c	
Rv3080c	

more than half are druggable (Figure 2, Figure 3). In general, bacterial cell membrane is in front to sense the environmental factors and initiate a signal transduction which often mediate their biological function through interaction of elements in cascades [10]. Mtb utilizes multiple mechanisms to protect itself from the host antimicrobial assault and evolved systems to sense the stages of infection starting from engulfment within macrophages and activates systems to block host defense pathways [86,87]. The bacterium possesses a wide repertoire of signal transduction systems including 11 two component system (TCS) , 11 eukaryotic-like serine/threonine protein kinases (PknA-PknL) , protein tyrosine phosphatases (PtpA, PtpB and PtkA) .These proteins play key roles in bacterial adaptation and responses to host defense mechanisms through modifying the host activities in a way to support its survival and pathogenesis , in that several studies showed that phosphatases and kinases modify host proteins that help in the establishment of disease , they

reprogram the metabolic activities and inhibition of autophagy by host cell [86,87]. TCSs practice reversible phosphorylation and dephosphorylation which is the key mechanisms by which extracellular signals are translated into cellular responses, and since TCSs of Mtb are quite distinct from regulatory system of mammalian cells, so they represent good drug targets. Other studies demonstrate that serine/threonine protein kinases such as PknB (Rv0014c) is a drug target in all stages of Mtb life cycle [85].

Table 9: Proteins with crystal structure

Rv ID	Pdb ID	Method of Estimation
Secretory proteins		
Rv0016c	3UPN	X-ray
Rv0758	5UKY	X-ray
Rv0928	4LVQ	X-ray
Rv1477	3PBC	X-ray
Rv1885c	2FP1	X-ray
Rv1886c	5TRZ	X-ray
Rv2068c	3DWZ	X-ray
Rv2911	4RYE	X-ray
Rv2945c	2BYO	X-ray
Rv3106	1LQU	X-ray
Rv3330	4PPR	X-ray
Rv3803c	1R88	X-ray
Rv3804c	1SFR	X-ray
Rv3846	1GN4	X-ray
Rv3874	3FAV	X-ray
Transporters		
Rv0202c	4Y0L	X-ray
Rv2874	5CYY	X-ray
Rv2945c	2BYO	X-ray
Rv3273	4YF5	X-ray
Rv3869	4YF5	X-ray
Rv3874	3FAV	X-ray
Rv3877	4KV3	X-ray
Rv3910	3OUN	X-ray
Cell Wall proteins		
Rv2068c	3DWZ	X-ray
Rv2150c	1RQ7	X-ray
Signal proteins		
Rv0014c	2FUM	X-ray
Rv0015c	4X3F	X-ray
Rv0410c	4Y0X	X-ray
Rv0931c	1RWL	X-ray
Rv1266c	4ESQ	X-ray
Rv1743	2H34	X-ray
Dormancy proteins		
Rv1009	4EMN	X-ray
Rv2623	3CIS	X-ray
Rv2626c	1Y5H	X-ray
Rv3841	3QD8	X-ray
PE/ PPE proteins		
Rv0977	4EHC	X-ray
Rv3738c	3CAI	X-ray
Rv3874	3FAV	X-ray
Resistance proteins		
Rv0262c	1M4G	X-ray
Rv0667	5UH5	X-ray
Rv1267c	2FF4	X-ray
Rv1484	1ENZ	X-ray
Rv1908c	2CCA	X-ray
Rv3793	3PTY	X-ray
Stress proteins		
Rv0928	4LVQ	X-ray
Rv0934	1PC3	X-ray
Rv2623	3CIS	X-ray
Rv3841	3QD8	X-ray
Efflux proteins		
Rv3065	2IQ4	model
Rv3106	1LQU	X-ray
NTPome proteins		
Rv0014c	2FUM	X-ray
Rv0015c	4X3F	X-ray
Rv0016c	3UPN	X-ray
Rv0018c	2CM1	X-ray
Rv0410c	4Y0X	X-ray
Rv0467	1F8M	X-ray
Rv0859	4B3I	X-ray
Rv0902c	1YSR	X-ray
Rv0928	4LVQ	X-ray
Rv0931c	1RWL	X-ray
Rv0934	1PC3	X-ray
Rv1266c	4ESQ	X-ray
Rv1267c	2FF4	X-ray
Rv1293	1HKW	X-ray
Rv1340	3B4T	X-ray
Rv1743	2H34	X-ray
Rv1908c	2CCA	X-ray
Rv2115c	3M9B	X-ray
Rv2391	1ZJ9	X-ray
Rv2583c	5XNX	X-ray
Rv2780	2VHX	X-ray
Rv2996c	3DC2	X-ray
Rv3240c	1NL3	X-ray
Rv3285	5MLK	X-ray
Rv3417c	3M6C	X-ray
Rv3869	4KK7	X-ray
Rv3910	3OUN	X-ray

4. CONCLUSION

In all cases and for proteins mentioned through this study the interaction between them could provide another route for effects. It was found that not all the selected proteins could be drug target and they are without druggability characters, as the latter depends on certain criteria, but the bacterial pathogenicity could be attacked through the interactions, since all cellular components are working in network fashion. Many of the studied proteins were found to be overlapped. The designing of drugs could be started with proteins having pdb structures (Table 9), and the other waiting for estimation such structure or using modeling approach. Two druggable proteins (Rv0350, Rv3280) with crystal suture were excluded as they shown human homology.

ACKNOWLEDGEMENT

The authors would like to thank and appreciate the efforts of the lawyer Hafssa Basheer Mahmood and Dr. Natheer Basheer Mahmood for their support and help during the preparation of this manuscript.

CONFLICT OF INTEREST

Nill.

REFERENCES

1. Hameed H, Islam MM, Chhotaray C, Wang C, Liu Y, Tan Y, Li X et al. Molecular Targets Related Drug Resistance Mechanisms in MDR-, XDR-, and TDR-*Mycobacterium tuberculosis* Strains. Front Cell Infect Microbiol. 2018; 8:1-21.
2. Tyagi J, Sharma D. Signal Transduction Systems of Mycobacteria with Special Reference to *M. tuberculosis.* Cur Sci. 2004; 86 :93-102.
3. Diaz G, Wolfe L, Kruh-Garcia N, Dobos K. Changes in the Membrane-Associated Proteins of Exosomes Released from Human Macrophages after *Mycobacterium tuberculosis* Infection. Scientific Reports 2016; 6:1-10.
4. Ali RM. Molecular study and genotyping of *Mycobacterium tuberculosis* complex isolated in respiratory center in Baghdad. PhD thesis University of Baghdad ,2013.
5. Cole S. Learning from the genome sequence of *Mycobacterium tuberculosis* H37Rv. FEBS Letters .1999; 452:7-10.
6. Meena L, Rajn . Survival Mechanisms of Pathogenic *Mycobacterium tuberculosis* H37Rv. FEBS J. 2010 ;277: 2416–2427
7. Dey B, Bishai W. Crosstalk between *Mycobacterium tuberculosis* and the Host Cell. Semin Immunol. 2014; 26: 486–496
8. Ferraris D, Miggiano R, Rossi F, Menico Rizzi M. *Mycobacterium tuberculosis* Molecular Determinants of Infection, Survival Strategies, and Vulnerable Targets. Pathogens 2018; 7:1-16.
9. Murphy D, Brown J. Identification of Gene Targets against Dormant Phase *Mycobacterium*

tuberculosis Infections. BMC Inf Dis. 2007; 7: 1-16.

10. Glass LN, Swapna G, Chavadi SS, Tufariello JM, Mi K, Drumm JE, et al. *Mycobacterium tuberculosis* Universal Stress Protein Rv2623 Interacts with the Putative ATP Binding Cassette (ABC) Transporter Rv1747 to Regulate Mycobacterial Growth. PLoS Pathog. 2017; 13: e1006515
11. da Silva Neto B, de Fa´ tima da Silva J, Mendes-Giannini M, Lenzi H, de Almeida Soares C, Pereira M. The Malate Synthase of *Paracoccidioides brasiliensis* Is a Linked Surface Protein That Behaves as an Anchorless Adhesin. BMC Microbiol, 2009; 9:1-12.
12. Alteri C. Novel pili of Mycobacterium tuberculosis. PhD thesis, The University of Arizona, 2005.
13. Zuber B, Chami M, Houssin C, Dubochet J, Griffiths G, Daffé M. Direct Visualization of the Outer Membrane of Mycobacteria and Corynebacteria in their Native State. J Bacteriol. 2008; 190:5672–5680.
14. Brennan PJ, Nikaido H. The Envelope of Mycobacteria. Annu Rev Biochem. 1995; 64:29–63.
15. Barry CE. Interpreting Cell Wall 'Virulence Factors' of *Mycobacterium tuberculosis*. Trends Microbiol. 2001; 9:237–241.
16. Jarlier V, Nikaido H. Mycobacterial Cell Wall: Structure and Role in Natural Resistance to Antibiotics. FEMS Microbiol Lett, 1994;123: 11–18.
17. Gupta AK, Katoch VM, Chauhan DS, Sharma R, Singh M, Venkatesan K. Microarray Analysis of Efflux Pump Genes in Multidrug-Resistant *Mycobacterium tuberculosis* During Stress Induced by Common Anti-Tuberculosis Drugs. Microb. Drug Resist. 2010; 16:21–28.
18. Jiang X, Zhang W, Zhang Y, Gao F, Lu C, Zhang X, Wang H. Assessment of Efflux Pump Gene Expression in a Clinical Isolate *Mycobacterium tuberculosis* by Real-Time Reverse Transcription PCR. Microb Drug Resist. 2008; 14:7–11.
19. Siddiqi N, Das R, Pathak N, Banerjee S, Ahmed N, Katoch VM, Hasnain SE. *Mycobacterium tuberculosis* Isolate with a Distinct Genomic Identity Overexpresses a Tap-Like Efflux Pump. Infection, 2004; 32:109–111.
20. Mishra A, Mamidi A, Rajmani R, Ray A, Rajanya Roy R, et al. An Allosteric Inhibitor of *Mycobacterium tuberculosis* ArgJ: Implications to a Novel Combinatorial Therapy. EMBO Mol Med, 2018 : e8038 .
21. Sassetti CM, Boyd DH, Rubin EJ. Genes required for Mycobacterial Growth Defined by High Density Mutagenesis. Mol Microbiol . 2003; 48:77– 84.
22. Tran SL, Cook GM. The F1Fo-ATP synthase of *Mycobacterium smegmatis* is essential for growth. J Bacteriol, 2005; 187:5023–5028
23. Bald D, Villellas C, Lu P, Koul A.Targeting Energy Metabolism in *Mycobacterium tuberculosis*, a New Paradigm in Antimycobacterial Drug Discovery. mBio 2017;8: e00272-17.

24. Chopra P, Meena L Singh Y. New Drug Targets for *Mycobacterium tuberculosis*. Indian J Med Res. 2003; 117: 1-9.
25. Wattam A, Davis J, Assaf R, Boisvert S, Brettin T, Bun C, et al. Improvements to PATRIC, the All-Bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res. 2017; 45: Database issue D535–D542.
26. The UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017; 45: D158–D169.
27. Magarin M, Carmona S, Crowther G, Ralph S, Roos D, Shanmugam D et al. TDR Targets: a Hemogenomics Resource for Neglected Diseases. Nucleic Acids Res. 2012; 40: D1118–D1127.
28. Li C-W, Lee Y-L, Chen B-S () Genetic-and-Epigenetic Interspecies Networks for Cross-Talk Mechanisms in Human Macrophages and Dendritic Cells during MTB Infection. Front Cell Infect Microbiol. 2016; 6:1-24.
29. Warner D. *Mycobacterium tuberculosis* Metabolism. Cold Spring Harb Perspect Med.2015; 5:1-23.
30. Ishii N, Nakahigashi K, Baba T, Robert M, Soga T, Kanai A, et al. Multiple High-Throughput Analyses Monitor the Response of *E. coli* to Perturbations. Science, 2007; 316: 593–597.
31. Jarlier V, Nikaido H. Mycobacterial Cell Wall: Structure and Role in Natural Resistance to Antibiotics. FEMS Microbial Lett. 1994; 123: 11–18.
32. Luo H, Lin Y, Gao F, Zhang C, Ren Zhang R. DEG 10, an update of the database of essential genes that includes both protein-coding genes and noncoding genomic elements. Nucleic Acids Res.2014; 42 : D574–D580.
33. Tan Y-T, Tillett DJ, McKay IA. Molecular Strategies for Overcoming Antibiotic Resistance in Bacteria. Mol Med Today 2000; 6:309-314
34. Chandra N. Computational Approaches for Drug Target Identification in Pathogenic Diseases. Expert Opin Drug Discov, 2011; 6: 975-979.
35. Pérot S, Sperandio O, Miteva MA, Camproux AC, Villoutreix BO. Druggable pockets and binding site centric chemical space: a paradigm shifts in drug discovery. Drug Discov Today, 2010; 15:656-667.
36. Danelishvili L, Shulzhenko N, Chinison J, Babrak L, Hu J, Morgun A, et al. *Mycobacterium tuberculosis* Proteome Response to Antituberculosis Compounds Reveals Metabolic "Escape"Pathways That Prolong Bacterial Survival. Antimicrob Agents Chemother . 2017;61: e00430-17.
37. Gashaw I, Ellinghaus P, SommerA, Khusru Asadullah K. What Makes a Good Drug Target? Drug Discov Today, 2012; 17S: S24-S30.
38. Cornejo-Granados F, Zatarain-Barrón ZL,Cantu-Robles VA, Mendoza-Vargas A, Molina-

Romero C, Sánchez F, et al. Secretome Prediction of Two M. tuberculosis Clinical Isolates Reveals Their High Antigenic Density and Potential Drug Targets. Front. Microbiol. 2017; 8:1-12.

39. Vizcaı´no C, Restrepo-Montoya D, Rodrı´guez D, Nin˜o LF, Ocampo M, et al. Computational Prediction and Experimental Assessment of Secreted/Surface Proteins from *Mycobacterium tuberculosis* H37Rv. PLoS Comput Biol. 2010; 6: e1000824.
40. Youm J, Saier Jr M. Comparative Analyses of Transport Proteins Encoded within the Genomes of *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Biochimica et Biophysica Acta, 2012; 1818: 776–797.
41. Gupta AK, Reddy VP, Lavania M, Chauhan DS, Venkatesan K, et al. jefA (Rv2459), a Drug Efflux Gene in *Mycobacterium tuberculosis* Confers Resistance to Isoniazid & Ethambutol. Indian J Med Res. 2010; 132:176-188.
42. da Silva PE, Von Groll A, Martin A, Palomino JC. Efflux as a Mechanism for Drug Resistance in *Mycobacterium tuberculosis*. FEMS Immunol Med Microbiol . 2011; 63:1-9.
43. Kumar A, Schweizer HP. Bacterial Resistance to Antibiotics: Active Efflux and Reduced Uptake. Adv Drug Deliv Rev. 2005; 57:1486-1513.
44. Bhagavat R, Kim H, Kim C, Terwilliger T, Mehta D, Srinivasan N. et al. A Genome-Wide Structure-Based Survey of Nucleotide Binding Proteins in *M. tuberculosis.* Scientific Reports 2017; 7:2-14.
45. Schulz G. Binding of nucleotides by proteins. Curr. Biol. 1992; 2: 61-67.
46. Vetter IR, Wittinghofer A. Nucleoside Triphosphate-Binding Proteins: Different Scaffolds to Achieve Phosphoryl Transfer. Q Rev Biophys. 1999;32: 1–56.
47. Yegutkin G. Nucleotide- and Nucleoside-Converting Ectoenzymes: Important Modulators of Purinergic Signaling Cascade. Biochim. Biophys. Acta , 2008;1783: 673–694 .
48. Magnet S, Hartkoorn RC, Szekely R, Pato J, Triccas JA, et al. Leads for Antitubercular Compounds from Kinase Inhibitor Library Screens. Tuberculosis (Edinb) 2010; 90: 354-360.
49. Schreiber M, Res I, Matter A. Protein Kinases as Antibacterial Targets. Curr Opin Cell Biol. 2009; 21: 325-330.
50. Chene, P. ATPases as Drug Targets: Learning from their Structure. Nat. Rev. Drug Discov. 2002; 1: 665–673.
51. Sampson S.. Mycobacterial PE/PPE Proteins at the Host-Pathogen Interface Clinical and Dev Immunol. 2011; Article ID 497203,:1-11.
52. Cole S, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D. et al. Deciphering the biology of *Mycobacterium tuberculosis* from the Complete Genome Sequence, Nature, 1998; 393: 537–544.

53. Sani M, Houben E, Geurtsen J, Pierson J, Punder K, van Zon M. et al. Direct Visualization by Cryo-EM of the Mycobacterial Capsular Layer: a Labile Structure Containing ESX-1-Secreted Proteins. PLoS Pathogens, 2010; 6: e1000794.
54. Ramakrishnan L, Federspiel NA, Falkow S. Granuloma-Specific Expression of Mycobacterium Virulence Proteins from the Glycine-Rich PE-PGRS Family. Science 2000; 288 : 1436-1439.
55. Chen X, Cheng HF, Zhou J, Chan CY, Lau KF, Tsui SK. Au SW4 Structural Basis of the PE-PPE Protein Interaction in *Mycobacterium tuberculosis*. J Biol Chem. 2017; 13: 16880-16890.
56. Abdallah A, Pittius G, Champion N, Cox P, Luirink J, Vandenbroucke-Grauls C. et al. Type VII secretion – mycobacteria show the way. Nat Rev Microbiol. 2007; 5: 883–891.
57. Fishbein S, Wyk N, Warren R, Sampson S . Phylogeny to Function: PE/PPE Protein Evolution and Impact on *Mycobacterium tuberculosis* Pathogenicity. Mol Microbiol.2015; 96: 901–916.
58. 58.Malinga LA, Stoltz A, Van der Walt M . Efflux Pump Mediated Second-Line Tuberculosis Drug Resistance. Mycobact Dis. 2016; 6:1-9.
59. Rando-Segura A, Aznar M, Moreno M, Espasa M, Sulleiro E, Bocanegra C et al . Drug Resistance of *Mycobacterium tuberculosis* Complex in a Rural Setting, Angola. Emerg Infect Dis. 2018; 24: 569-572.
60. Asgedom S, Teweldemedhin M, Gebreyesus H . Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review J Pathogens, 2018; Article ID 7104921,1-8.
61. Velayati AA, Masjedi MR, Farnia P , Tabarsi P , Ghanavi J , ZiaZarifi AH . et al. Emergence of New Forms of Totally Drug-Resistant Tuberculosis Bacilli: Super Extensively Drug Resistant Tuberculosis or Totally Drug-Resistant Strains in Iran. Chest, 2009 ;136:420-425.
62. Udwadia Z, Amale R, Ajbani K, Rodrigues C. Totally Drug-Resistant Tuberculosis in India. Clin Infect Dis. 2012; 54: 579–581.
63. Klopper M, Warren R, Hayes C, Van Pittius G, Streicher N, Müller E. et al. (2013). Emergence and Spread of Extensively and Totally Drug-Resistant Tuberculosis, South Africa. Emerg Infect Dis. 2013;19: 449–455.
64. World Health Organization (WHO). 'Totally drug-resistant tuberculosis': a WHO consultation on the diagnostic definition and treatment options. Geneva, Switzerland: WHO; 2012 http://www.who.int/tb/challenges/xdr/Report- Meeting_totally drug resistant TB(2018) .
65. Velayatia A, Farniaa P, Sven Hoffnerb S. Drug-Resistant *Mycobacterium tuberculosis*: Epidemiology and Role of Morphological Alterations J Glob Antimicrob Resist. 2018 ;12:192–196.
66. Raman K, Chandra N. *Mycobacterium tuberculosis* Interactome Analysis Unravels Potential Pathways to Drug Resistance. BMC Microbiol 2008; 8:1-13.

67. Al-Heaily MM. Linezolid Resistance Development in *Staphylococcus haemolyticus* Resulted from Changes in rRNA MSc thesis, University of Baghdad, Iraq.
68. Pasipanodya JG, Gumbo T. A New Evolutionary and Pharmacokinetic-Pharmacodynamic Scenario for Rapid Emergence of Resistance to Single and Multiple Anti-Tuberculosis Drugs. Curr Opin Pharmacol . 2011;11: 457-463.
69. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K. Evolution of Drug Resistance in *Mycobacterium tuberculosis*: A Review on the Molecular Determinants of Resistance and Implications for Personalized Care. J Antimicrob Chemother. 2018; 73: 1138–1151
70. Rodrigues L, Parish T, Balganesh M, Ainsa JA.. Antituberculosis Drugs: Reducing Efflux=Increasing Activity. Drug Discov Today, 2017; 22:592-599.
71. Chatterjee A, Saranath D, Bhatter P, Mistry N. Global Transcriptional Profiling of Longitudinal Clinical Isolates of *Mycobacterium tuberculosis* Exhibiting Rapid Accumulation of Drug Resistance. PloS one, 2013; 8: e54717
72. Liu H, Xie J . Comparative Genomics of *Mycobacterium tuberculosis* Drug Efflux Pumps and their Transcriptional Regulators. Crit. Rev. Eukaryot. Gene Expr. 2014; 24:163-180.
73. Hoffmann C, Leis A, Niederweis M, Plitzko JM, Engelhardt H. Disclosure of the Mycobacterial Outer Membrane: Cryo-Electron Tomography and Vitreous Sections Reveal the Lipid Bilayer Structure. Proc Natl Acad Sci USA, 2008;105:3963-3967.
74. da Silva A, Palomino JC. Molecular Basis and Mechanisms of Drug Resistance in *Mycobacterium tuberculosis*: Classical and New Drugs. J Antimicrob Chemother 2011; 66:1417–1430.
75. Bergmann S, Rohde M, Chhatwal G, Hammerschmidt S . a-Enolase of *Streptococcus pneumoniae* Is a Plasmin(ogen)-Binding Protein Displayed on the Bacterial Cell Surface. Mol Microbiol. 2001;40: 1273–1287.
76. Pacl H, Reddy V, Saini V, Chinta K Steyn A. Host-Pathogen Redox Dynamics Modulate *Mycobacterium tuberculosis* Pathogenesis. Pathog Dis. 2018;76: 1-14.
77. Zondervan N, van Dam J, Schaap P, Santos V, Suarez-Diez M. Regulation of Three Virulence Strategies of *Mycobacterium tuberculosis*: A Success Story. Int J Mol Sci. 2018;19: 1-29 .
78. Rustad TR, Harrell MI, Liao R, Sherman DR. The Enduring Hypoxic Response of *Mycobacterium tuberculosis*. PLoS One, 2008; 3: e1502
79. Dussurget O, Stewart G, Neyrolles O, Pescher P, Young D, Marchal G. Role of *Mycobacterium tuberculosis* Copper-Zinc Superoxide Dismutase. Infect Immun. 2001; 69: 529–533.
80. Yuan Y, Lee RE, Besra GS, Belisle JT, Barry CE. Identification of a Gene Involved in the Biosynthesis of Cyclopropanated Mycolic Acids in *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA, 1995; 92: 6630–6634.

81. Muttucumaru D, Roberts G, Hinds J, Stabler R, Parish T. Gene Expression Profile of *Mycobacterium tuberculosis* in a Non-Replicating State. Tuberculosis (Edinb). 2004; 84: 239–246.
82. Sherman D, Voskuil M, Schnappinger D, Liao R, Harrell M, Schoolnik G. Regulation of the *Mycobacterium tuberculosis* Hypoxic Response Gene Encoding A-Crystallin. Proc. Natl. Acad. Sci. USA, 2001; 98: 7534–7539.
83. Betts J, Lukey P, Robb L, McAdam R, Duncan K. Evaluation of a Nutrient Starvation Model of *Mycobacterium tuberculosis* Persistence by Gene and Protein Expression Profiling. Mol. Microbiol. 2002;43: 717–731.
84. Sarathy J, Dartois V, Lee E. The Role of Transport Mechanisms in *Mycobacterium tuberculosis* Drug Resistance and Tolerance. Pharmaceuticals, 2012; 5: 1210-1235.
85. Ortega C. Experimental Characterization of Mycobacterium *tuberculosis* Adenosine Nucleotide Binding and Ser/Thr/Tyr Phosphosignaling . PhD thesis University of Washington, 2013.
86. Stutz M, Clark M , Doerflinger M, Pellegrini M. *Mycobacterium tuberculosis*: Rewiring Host Cell Signaling to Promote Infection . J Leukoc Biol. 2018; 103:259–268.
87. Wong D, Li W, Chao J, Zhou P, Narula G, Tsui Av- GayProtein Tyrosine Kinase, PtkA, Is Required for *Mycobacterium tuberculosis* Growth in Macrophages. Scientific Reports, 2018; 8:1-12.