Mycobacterial Metabolic Pathways as Drug Targets: A Review

Asaye Moges, Elias Kebede, Yitayew Demessie, Tesema Dumesa, Ayalew Negash and Geda Shendo

Faculty of Veterinary Medicine, University of Gondar, Gondar, Ethiopia, P.O. Box
Email: yitayew455vet@gmail.com

Abstract: *Mycobacterium tuberculosis* (Mtb) is a pathogenic bacteria species in the genus *Mycobacterium* and the causative agent of most cases of tuberculosis. Tuberculosis (TB) is the leading cause of death in the world from a bacterial infectious disease. The emergence of antibiotic resistance strains has raised the need towards the development of new antibiotics or drug molecules which can kill or suppress the growth of *Mycobacterium tuberculosis*. The increasing emergence of drug-resistant tuberculosis along with the HIV pandemic threatens disease control and highlights both the need to understand how our current drugs work and the need to develop new and more effective drugs. Novel efforts in developing drugs that target the intracellular metabolism of *M. tuberculosis* often focus on metabolic pathways that are specific to *M. tuberculosis*. Potential drug targets were also identified from pathways related to lipid metabolism, carbohydrate metabolism, amino acid metabolism, energy metabolism, vitamin and cofactor biosynthetic pathways and nucleotide metabolism. This review provides a brief historical account of tuberculosis drugs, metabolic pathways, examines the problem of current chemotherapy, discusses the targets of current tuberculosis drugs with focuses on some metabolic pathways. Approximately, one-fourth of the *Mycobacterium tuberculosis* genome contains genes that encode proteins directly involved in its metabolism. These represent potential drug targets that can be systematically probed with CB models through the prediction of genes essential (or the combination) for the pathogen to grow.
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1. Introduction

Tuberculosis is caused by *Mycobacterium tuberculosis,* was much more prevalent disease in the past than it is today, and it was responsible for the death of about one billion people during the last two centuries. *Mycobacterium tuberculosis* is a tenacious and remarkably successful pathogen that has latently infected one third of the world population. Each year there are eight million of new tuberculosis (TB) cases and two million deaths (WHO, 2003). The increasing emergencies of drug resistance tuberculosis and HIV infection which compromises host defense and allows latent infection to reactivate or render individual more susceptible to TB pose further challenges for effective control of the disease (Nachega *et al.,* 2003).

Currently, TB chemotherapy is made up of a cocktail of first-line drugs; isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), given for six months (Blumberg, 2003). If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as para-aminosalicylate (PAS), kanamycin (KAN), fluoroquinolones (FQ), capreomycin (CAP), ethionamide (ETA) and cycloserine (CYS), that are generally either less effective or more toxic with serious side effects (Blumberg, 2003). Treatment is made quite difficult by the presence of metabolically silent, persistent or dormant bacteria within host lesions, which are not susceptible to the anti-mycobacterial drugs that usually kill growing bacteria but not persistent bacteria (Zhang, 2004).

As most currently known, anti-bacterials are essentially inhibitors of certain bacterial enzymes, all enzymes specific to bacteria can be considered as potential drug targets. The enzymes in the pathways of *M. tuberculosis*, which do not show similarity to any protein from the host, represent attractive potential drug targets (Michael and Eugene, 1999).

The source of *M. tuberculosis* pathogenecity is due to in large part to its unique metabolism, which provided the capability to survive in the host, resist treatment and resume growth with relapse of disease. Using metabolic pathway information as the starting point for the identification of potential targets has its advantages as each step in the pathway is validated as essential function for the survival of the bacterium (Cole, 2002). It is widely accepted that TB is dynamic disease that result from combination of phenotypically diverse population of bacilli in continually changing host environment. Understanding host-pathogen interactions would give an important clue for developing new drugs, vaccine and diagnostic tests. The release of complete genome sequence of *M. tuberculosis* has facilitated the development of more

rational and specific methods to search for new drug targets and vaccine candidates (Cole *et al*., 1998).

The current rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs. The long duration of TB therapy is a consequence of persistent *M. tuberculosis*, not effectively killed by current anti-TB agents. Recent advances in the knowledge of the biology of the organism and the availability of the genome sequence give an opportunity to explore a wide range of novel targets for drug design. Metabolic studies on mycobacteria have been important areas of the investigation (Zhang, 2005).

Therefore, the objectives of this seminar paper are:

✓ To highlight metabolic pathways used as drug targets in *M. tuberculosis*.

✓ To review potential drug targets in *M. tuberculosis*

2. Metabolic Pathways Used as Drug Targets

Mycobacterial metabolic pathways which do not appear in the host but present in the pathogen are identified as pathways unique to *M. tuberculosis* as compared to the host. Enzymes in these unique pathways as well as enzymes involved in other metabolic pathways under carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism are usually included in studies which try to identify novel drug targets (Kanehisa *et al*., 2002).

2.1. Pathways unique to M. tuberculosis when compared to the host

An important question to be addressed while choosing potential drug targets is whether the biochemical pathway to be targeted is unique to bacteria. These biochemical pathways are; peptidoglycan biosynthesis, mycobactin biosynthesis, d-alanine metabolism, thiamine metabolism and polyketide sugar unit biosynthesis, are all absent in the host and therefore unique to the pathogen *M. tuberculosis* (Tatusov *et al*., 2003).

Among these pathways, peptidoglycan biosynthesis and d-alanine metabolism is common to all bacterial species, and cell wall biosynthetic pathways have long been targeted for anti-microbial discovery. The iron acquisition systems of many pathogenic and saprophytic bacteria rely on the production of small molecules called siderophores. *M. tuberculosis* produces the mycobactin class of siderophore. Therefore, this siderophores used for mycobactin biosynthesis, d-alanine metabolism and peptidoglycan biosynthetic pathways (De Voss *et al*., 2000).

2.2. Mycobactin biosynthesis

Mycobactin G, which catalyzes the hydroxylation of lysine moiety in mycobactin synthesis, is the potential target in this pathway. To overcome iron deficiency imposed by the host defensive system, bacteria have evolved iron acquisition systems where small molecules called siderophores, which bind extracellular iron, are secreted. These get reabsorbed along with the bound iron through specific cell surface receptors (Braun *et al*., 1998; Byers and Arceneaux, 1998). *M. tuberculosis* produces the mycobactin class of siderophore, which contains a salicylic acid derived moiety. A 10-gene clusters spanning 24 kilo bases of the *M. tuberculosis* genome, designated MbtA-J, contains the core components necessary for mycobactin biogenesis. The gene products MbtB, MbtE and MbtF are proposed to be peptide synthetases, MbtC and MbtD polyketide synthases, MbtI an isochorismate synthase that provides a salicylate activated by MbtA, and MbtG a required hydroxylase (Luis *et al*., 1998).

It is obvious that *M. tuberculosis* inhabits one of the most hostile environments, the alveolar macrophage. Among the various defensive mechanisms expressed by the host are a potent burst of oxygen derived radical species and a dramatic restriction of available iron to support microbial growth (Kontoghiorghes and Weinberg, 1995). Disruption of mycobactin biosynthetic pathway may affect the survival of the bacterium under these conditions of iron limitation. It has been shown that siderophore production is also important for the virulence of *M. tuberculosis*(James *et al*., 2000).

2.3. Peptidoglycan biosynthesis

Mycobacterium tuberculosis is surrounded by a lipid-rich outer capsule that protects it from the toxic radicals and hydrolytic enzymes produced as defense by macrophages (Kolattukudy *et al*., 1997). The peptidoglycan layer of the cell wall serves as a base for the lipid-rich capsule. Peptidoglycan or murein is the polymeric mesh of the bacterial cell wall, which plays a critical role in protecting the bacteria against osmotic lysis. The currently used anti-mycobacterial drugs are isoniazid (INH) and ethambutol (EMB). Isoniazid is known to inhibit mycolic acid synthesis (Zhang *et al*., 1992), where as ethambutol inhibits the polymerization step of arabinan biosynthesis of arabinogalactan (Mikusova *et al*., 1995).

2.4. D-Alanine metabolism

The d-alanine–d-alanine ligase (ddlA) and alanine racemase (alr) from this pathway have no similarity to any of the host proteins. D-alanine is a necessary precursor in the bacterial peptidoglycan biosynthetic pathway. The naturally occurring L-isomer is racemized to its D-form through the action

of a class of enzymes called alanine racemases. These enzymes are ubiquitous among prokaryotes and are absent in eukaryotes with a few exceptions making them a logical target for the development of antibiotics. Alanine racemase (alr) has been identified as a target as all the bacteria investigated contained either one or two alanine racemase genes (Strych *et al*., 2001). However, in mycobacteria, there is a single alanine racemase gene. One alanine racemase inhibitor, the structural d-alanine analogue d-cycloserine has been marketed clinically. Although, this is supposed to be an excellent inhibitor of mycobacteria and other pathogenic bacterial species, serious side effects especially CNS toxicity has limited its use (Yew *et al*., 1993).

2.5. Polyketide sugar unit biosynthesis

The rhamnose-GlcNAc disaccharide is a critical linker which connects arabinogalactan to peptidoglycan via a phosphodiester linkage. The biosynthesis of dTDP-rhamnose is catalysed by four enzymes coded by the genes RmlA, RmlB, RmlC and RmlD, and ultimately synthesize dTDP rhamnose from glucose-1-phosphate. Among these genes RmlC has no human homologue. RmlC codes for dTDPd-glucose-3,5-epimerase which is involved in the arabinogalactan biosynthesis. The biosynthesis of arabinogalactan in *M. tuberculosis* begins with the transfer of N-acetylglucosamine-1-phosphate from UDP-N-acetyl glucosamine to prenylphosphate followed by an addition of rhamnose (Rha) from dTDP-Rha, forming a linker region of the arabinogalactan (Mikusova *et al*., 1996, Ma *et al*., 2001).

2.6. Targets from other pathways

Even amongst the pathways shared by the host and the pathogen, there are several proteins from pathways involved in lipid metabolism, carbohydrate metabolism, amino acid metabolism, energy metabolism, vitamin and cofactor biosynthetic pathways and nucleotide metabolism which do not bear similarity to host proteins. While some of them are known to be associated with virulence or important for persistence or vital for mycobacterial metabolism, others should further be investigated for their potential to be drug targets (GIC, 2001).

A significant proportion of the *M. tuberculosis* genome is devoted to lipid metabolism. It possesses more than 250 enzymes involved in lipid metabolism, which includes enzymes for lipid biosynthesis as well as degradation. Degradation of host cell lipids is essential for the intracellular life of the organism. Host cell membranes provide precursors for many metabolic processes. They are also potential precursors of mycobacterial cell wall constituents through the action of beta oxidative enzymes encoded in multiple copies in the genome. Among these secreted proteins of *M. tuberculosis* which could act as virulence factors are a series of phospholipases C, lipases and esterases which might attack cellular or vacuolar membranes. Notable amongst these are phospholipases plcA (Rv2351c), plcB (Rv2350c), plcC (Rv2349c) and serine esterase (Rv2301) (GIC, 2001).

The targets aceARv0467, aceAaRv1915, aceAbRv1916, glcBRv1837, Rv2205 identified, are related to mycobacterial persistence. They are enzymes from the glyoxylate by pass, which is important for mycobacterial persistence (e.g., Isocitrate Lyase and Malate Dehydrogenase). It has been proposed that the enzymes of the glyoxylate cycle are activated during adaptation to the low oxygen environment of the graunuloma (Kumar and Sanyal, 2012). The glyoxylate by pass allows the bacterium to synthesize carbohydrates from fatty acids. Succinate and glyoxylate produced by this cycle are supplied to the TCAcycle and gluconeogenesis. Disrupting this pathway by targeting these enzymes has a potential in the treatment of latent tuberculosis infections (Slayden *et al*., 2013).

It has also been suggested that the bacterium under goes a metabolic down shift (Waynes and Haynes, 1996) in the hostile O_2 limiting environment of the granuloma and switches to anaerobic nitrate respiration. This aids in the persistence of the bacterium under anaerobic conditions (Waynes and Haynes, 1998).

Amongst the new targets from the energy metabolism important ones are cydA cytochrome bdI oxidase sub unit I, qcrC ubiquinol cytochrome c reductase a cytochrome c sub unit, sdhC succinate dehydrogenase cytochrome b-556 sub unit, sdhD succinate dehydrogenase hydrophobic membrane protein anchor, ppk polyphosphate kinase, ppdk pyruvate orthophosphate dikinase and cysE serine O-acetyl transferase. Other important targets include thrB homoserine kinase, serB phosphoserine phosphatase, trpB tryptophan synthase from amino acid metabolism, dnaE1 and dnaE2 DNApolymerase III from nucleotide metabolism, ribH riboflavin synthase, cobD cobalamin synthase from vitamin and cofactor biosynthetic pathways (Mjkusova *et al*., 1996).

2.7. In silico comparative metabolic pathways analysis

As most currently known, anti-bacterials are essentially inhibitors of certain bacterial enzymes; all enzymes specific to bacteria can be considered as potential drug targets. From this adopted a strategy for comparative metabolic pathway analysis to find out some potential targets against *M .tuberculosis* (H37Rv). Only those enzymes which show unique properties than the host were selected as the target. Metabolic genes that are essential for pathogen growth

but are not present in humans constitute actual and potential drug targets (Galperin, 1999).

2.7.1. Identification of unique pathways and potential drug

No new anti-tuberculosis drugs have been developed for well over 20years. In view of the increasing development of resistance to the current leading anti-tuberculosis drugs, novel strategies are desperately needed to avert the "global catastrophe" forecast by the WHO. Therefore, computational approach for drug targets identification, specifically for *M. tuberculosis*, can produce a list of reliable targets very rapidly. These methods have the advantage of speed and low cost and, even more importantly, provide a systems view of the whole microbe at a time. Since it is generally believed that the genomes of bacteria contain genes both with and without homologues to the human host (WHO, 2005).

2.7.2. Identification of essential genes.

Essential genes are those indispensable for the survival of an organism, and their functions are considered as foundation of life. Total 55 enzymes out of all were found to be essential for *M. tuberculosis* life cycle. These targets were found to be potential targets and could be considered for rational drug design. Using metabolic pathway information as the starting point for the identification of potential targets has its advantages as each step in the pathway is validated as the essential function for the survival of the bacterium (Lamichhane *et al.,* 2003).

2.7.3. Identification of drug target's functions using UniProt.

The subcellular localization analysis of all supposed essential and unique enzymes of *M. tuberculosis* were evaluated by UniProt server. As it was suggested that, membrane associated protein could be the better target for developing vaccines. After functional analysis unique enzymes involved in cellular components like cell wall, cytoplasm, extra cellular region, plasma membrane, and so forth, their biological processes and their functions have been retrieved (Asad *et al*., 2014).

3. Possible Drug Targets

In recent years, a number of new genes and their products in *M. tuberculosis* have been identified, which can be possible drug targets for tuberculosis. The gene products that control vital aspects of mycobacterial physiology like, metabolism, persistence, virulence, two component system and cell wall synthesis would be attractive targets for new drugs. A large number of genes are being studied in the search for new drug targets using various approaches (chopra *et al*., 2002).

Because of the drug-resistant TB problem, it is important to develop new drugs that inhibit novel targets that are different from those of currently used drugs. To avoid significant toxicity, the targets of inhibition should be present in bacteria but not in the human host. Although modification of existing drugs for improved half-life, bioavailability, or drug delivery may be of some use, agents obtained by this approach may have a cross-resistance problem. Similarly, targeting existing TB drug targets for drug development may be limited value because of potential cross-resistance (cole *et al*., 1998).

New drugs that inhibit novel targets are needed. In choosing targets for drug development, it is important that they be involved in vital aspects of bacterial growth, metabolism, and viability. Recent developments in mycobacterial molecular genetic tools such as transposon mutagenesis, signature-tagged mutagenesis, gene knockout, and gene transfer will facilitate the identification and validation of new drug targets essential for the survival and persistence of tubercle bacilli not only *in vitro* but also *in vivo*. Below is a list of potential targets where by new drugs may be developed for improved treatment of TB (Zhang *et al*., 2005).

3.1. Targeting Mycobacterial persistence

Mycobacterial persistence refers to the ability of tubercle bacillus to survive in the face of chemotherapy and/or immunity (McDermott, 2000). The nature of the persistent bacteria is unclear but might consist of stationary phase bacteria, post-chemotherapy residual survivors and/or dormant bacteria that do not form colonies upon plating (Zhang, 2004). The presence of such persistent bacteria is considered to be the major reason for lengthy therapy. A lot of research activity is currently aimed at understanding the biology of persistence of the tubercle bacillus and developing new drugs that target the persistent bacteria (GAFTDD, 2001).

Gene products involved in mycobacterial persistence, such as isocitrate lyase (ICL) (Mckinney *et al*., 2000) PcaA (methyl transferase involved in the modification of mycolic acid) (Glickman *et al*., 2000) RelA (ppGpp synthase) (Dahl *et al.*, 2003) and DosR (controlling a 48-gene regulon involved in mycobacterial survival under hypoxic conditions) have been identified and could be good targets for the development of drugs that target persistent bacilli (Park *et al*., 2003).

3.2. Targeting essential Genes

Essential genes are genes whose inactivation leads to non-viability or death of the bacteria. Transposon mutagenesis and signature-tagged mutagenesis have been used to identify genes essential for *M. tuberculosis* growth *in vitro* and survival *in vivo.* In a recent study, 614 genes, about one-sixth of the total number of genes in *M. tuberculosis*, were found to be essential for *in vitro* growth, whereas 194

genes were demonstrated to be essential for *in vivo* survival in mice (Sassetti *et al*., 2003). The genes that are essential for survival *in vitro* and *in vivo* are grouped into the following categories: lipid metabolism; carbohydrate and amino acid transport and metabolism; inorganic ion transport and metabolism; nucleotide transport and metabolism; energy production and conversion; secretion; cell envelope biogenesis; cell division; DNA replication; recombination and repair; transcription and translation; post-translational modification; chaperones; coenzyme metabolism; and signal transduction (Lamichhane *et al.,* 2003).

However, the function of a significant number of essential genes is unknown. Besides systematic analysis of essential genes by transposon mutagenesis, targeted knockout of specific genes is also a valuable approach to identifying essential genes, in other words, those whose disruption leads to non-viability of the bacilli. These essential mycobacterial genes should be good targets for TB drug development (Zhang *et al*., 2003).

3.3. Targeting energy production pathway

All bacteria require energy to remain viable. Although the energy production pathways in *M. tuberculosis* are not well characterized, their importance as drug targets is demonstrated by the recent finding that PZA (a frontline TB drug that is more active against non-growing persistent bacilli than growing bacilli and shortens TB therapy) acts by disrupting membrane potential and depleting energy in *M. tuberculosis*. This study implies that energy production or maintenance is important for the viability of persistent non-growing tubercle bacilli *in vivo*. The recent discovery of the highly effective TB drug diarylquinoline also highlights the importance of energy production pathways for mycobacteria. It is likely that energy production pathways, such as the electron transport chain, glycolytic pathways (like the Embden–Meyerhof pathway) and fermentation pathways, could be good targets for TB drug development (Zhang *et al.,* 2003).

3.4. Targeting virulence factors

A number of genes have been identified, using different techniques like allelic exchange, signature tagged mutagenesis, and anti-sense RNA, that show a role in the virulence of *M. tuberculosis*. Some of these genes include, erp (extracellular repeat protein), which has been shown to be essential for the multiplication of mycobacteria during the acute phase of infection in the mouse model. The most important point is that this gene has no homologues in other organisms, making it an attractive drug target. Recently, two gene clusters were identified and shown to be important for the growth of mycobacteria in the lungs during the early phase of infection. This gene cluster is involved in the synthesis (fadD28) and export (mmpL7) of a complex cell wall associated lipid30, phthiocerol dimycocerosate (Barrett *et al*., 1998).

The approach of targeting virulence factors, like other approaches suffers from some serious drawbacks, like virulence factors may not be necessarily survival genes. Therefore, inhibition of virulence factors may not be lethal to the pathogen. The other very important hurdle in this approach is that drugs that target virulence factors may be of very little or of no use if the disease has already been established. However, inhibitors of these virulence gene products may be used in combination with existing drugs to improve the regime of chemotherapy (Alksne *et al*., 2000).

3.5. Targeting two-component systems

Two-component systems (TCS) are vital components of signal transduction systems in a number of organisms. It consists of a sensor kinase that senses external signals and transmits the signals to the response regulator. The response regulator interacts with transcription factors which in turn will switch on/off a number of genes (Hoch, 2000).

Mycobacterium tuberculosis has shown the presence of at least 12 two-component system homologues with 8 unlinked sensor kinases or response regulators (Cole *et al.,* 1998). However, the exact physiological role of most of these proteins is far from being understood. It has been shown that the inactivation of mtrA (magnesium transporter) component of mtrA-mtrB complex of *M. tuberculosis* H37Rv was possible only in the presence of a functional copy of mtrA, suggesting that this response regulator is essential for the viability of *M. tuberculosis* (Zahrt *et al.,* 2000).

Interestingly, another two-component system, devR-devS, was found to be over expressed in a virulent strain, H37Rv (Dasgupta *et al.,* 2000). Disruption of the phoP component of the PhoP/PhoR in *M. tuberculosis* resulted in a mutant strain with impaired multiplication in the host. This mutant was also found to be attenuated *in vivo* in a mouse model, suggesting that PhoP is required for intracellular growth of *M. tuberculosis*. These observations collectively suggest that TCS in *M. tuberculosis* could be important drug targets (Perez *et al.,* 2001).

3.6. Targeting cell wall synthesis

Mycobacteria including *M. tuberculosis* have a unique cell wall structure. A variety of unique lipids like lipoarabinomannan (LAM), trehalose dimycolate, and phthiocerol dimycocerate which form non covalent anchorage with the cell membrane have been documented to play an important role in the virulence of *M. tuberculosis* (Glickman *et al.,* 2001). Lipids such as cord factor have been suggested to play an important role in the virulence of *M. tuberculosis* by

inducing cytokine mediated events (Devergne *et al.,* 1992). LAM is also a major constituent of the mycobacterial cell wall and has been shown to induce TNF release from the macrophages which plays a significant role in bacterial killing (SchullerLevis *et al*., 1994).

Because of the reasons cited above, genes involved in cell wall synthesis of mycobacteria have been exploited as targets for many anti-mycobacterial drugs. Several important TB drugs such as INH, ETA and EMB target mycobacterial cell wall synthesis. Enzymes involved in this pathway have always been preferred targets in drug development efforts (SchullerLevis *et al.,* 1994).

Thiolactomycin (TLM) targets two β-ketoacyl-acyl-carrier protein synthases, KasA and KasB enzymes that belong to the fatty acid synthase type II system involved in the fatty acid and mycolic acid biosynthesis (Slayden *et al.,* 1996). TLM has also been shown to be active against MDR-TB clinical isolate. Several TLM derivatives have been found to be more potent *in vitro* against fatty acid and mycolic acid biosynthesis (Zhang *et al.,* 2002). Cerulenin, an inhibitor of fatty acid synthesis, has also been shown to inhibit mycobacterial lipid synthesis and is active against *M. tuberculosis in vitro* with an MIC of 1.5-12.5 mg/ml (Parrish *et al.,* 1999).

Octane sulphonyl acetamide (OSA) has recently been identified as an inhibitor of fatty acid and mycolic acid biosynthesis in mycobacteria. The inhibitor was found to be active against both slow growers such as *M. tuberculosis* and also MDR-TB strains with a MIC of about 6.25-12.5mg/ml. These reports clearly suggest that several genes of the cell wall synthesis pathway and enzymes involved in fatty acid and mycolic acid synthesis could be good candidates for further drug development (Jones *et al*., 2000).

3.7. Genes of other metabolic pathways

Genes of some other metabolic pathways can also serve as possible targets for developing drugs against tuberculosis. Some of these genes include, mgtc, which codes for a putative Mg^{+2} transporter protein. This protein has been shown to be essential for the survival of mycobacteria both in macrophages and mice. The Δ-mgtc mutant showed *in vitro* growth defects (Buchmeier *et al*., 2000). Similarly Δ-mbtB mutant deficient in synthesis of siderophores was unable to replicate within the macrophages. Failure of mycobacteria to survive in the absence of specific iron uptake system suggests the scarcity of this important nutrient in phagosomal environment. Members of PE-PGRS family of proteins that are highly expressed within tissue granulomas have been shown to be essential for the virulence of mycobacteria. Therefore, the members of this category of genes also constitute potential drug targets (De Voss *et al.,* 2002).

3.8. TB genomics and drug targets

The first bacterial genome was sequenced by Fleischmann and colleagues at The Institute for Genomic Research (TIGR) in 1995 (Fleischmann *et al.,* 1995). So far, more than 100 bacterial genomes have been sequenced. As bacterial genome sequences become available, there is increasing interest in developing new antibacterial agents using genomics-based approaches (Dougherty *et al.,* 2002). The available genome sequence information, along with molecular genetic tools, allows researchers to identify common essential targets among different bacterial species. The common targets can then be over expressed for biochemical assays in drug screens or structure determination, to be used in the drug design. So far, however, no company has been successful in developing a drug using a genomics approach. The availability of the *M. tuberculosis* genome sequence (Cole *et al.,* 1998) opens up a new opportunity to understand the biology of the organism and provides a range of potential drug targets (Cole, 2002).

The recent developments in microarray technology (Schena *et al.,* 1995), signature tag mutagenesis (Hensel *et al.,* 1995), mycobacterial transposon mutagenesis (Bardarov *et al.,* 1997), and gene knock-out technology (Pelicic *et al.,* 1997) provide important tools to identify new drug targets. Microarray has been used to identify *M. tuberculosis* genes that are induced by INH and ETH (Wilson *et al.,* 1999), and by INH, TLM and triclosan (Betts *et al.,* 2003). Microarray was also used to identify genes that are switched on in the Wayne "dormancy" model under hypoxic and nitric oxide stress conditions (Voskuil *et al.,* 2003), a discovery that led to the identification of a 48-gene "dormancy regulon" controlled by DosR (Voskuil *et al.,* 2003).

A proteomic approach was used to identify potential proteins that are induced in starvation as an *in vitro* model of persistence (Betts *et al.,* 2002). Two unique *M. tuberculosis* proteins with homology to each other were identified: Rv2557 and Rv2558. Rv2557 were also induced in side granulomatous lesions in the human lung. Genes identified by microarray analysis or proteins identified by a proteomic approach should be further validated as potential drug targets by gene knockout and *in vivo* testing in mice before they are selected as targets for drug development (Fenhalls *et al*., 2002).

Table 1: Current tuberculosis drugs and their targets

Drugs	MIC (g/ml)	Mechanism of action	Targets	Gene involved in resistance
Isoniazid	0.01-0.20	Inhibition of cell wall Mycoli acid synthesis	Enoyl acyl carrier protein reductase (InhA)	KatG, inhA
Rifampin	0.05-0.50	Inhibition of RNA synthesis	RNA polymerase, subunit	rpoB
Pyrazinamide	20-100	Depletion of membrane energy	Membrane energy metabolism	pncA
Ethambutol	1-5	Inhibion of cell wall arabinogalactan synthesis	Arabinsyltransferase	embCAB
Streptomycin	2-8	Inhibition of protein synthesis	Ribosomal S12 protein & 16s rRNA	rpcl, rrs
Kanamycin	1-8	Nhibition of protein synthesis	16s rRNA	Rrs
Capreomycin	4	Inhibition of protein synthesis	16s rRNA, 50s ribosome, rRNA Methyl transferase (TlyA)	rrs, tlyAb
Fluoroquinolone	0.2-4.0	Inhibition of DNA synthesis	DNA gyrase	gyrA, gyrB
Ethionamide	0.6-2.5	Inhibition of mycolic acid	Acyl carrier protein reductase (inhA)	inhA, etaA/ethA
PAS	1-8	Inhibition of folate pathway and mycobactin synthesis	Thymidylatesynthase (TlyA)	thyAc

Source; (Maus *et al*., 2005)

4. Anti-tuberculosis Drugs in Current Clinical Practice

As described recently the chemotherapy of tuberculosis has much evolved along the years since it started with the introduction of streptomycin 1946. By 1955, the combination of streptomycin, para-aminosalicylic acid and isoniazid was adopted as a standard treatment by the western world (Datta *et al*., 1993).

More current clinical data suggest using at least five regimens of adequate anti-tuberculosis drugs (Mirsaeidi *et al*., 2005). The choice of these drugs will be driven by the actual or presumed (in view of past failed treatment) resistance characteristic of the strains of *M. tuberculosis* considered. In order of preference they can be chosen from the following.

- In any case, the first line agents still active on the patient: isoniazid, rifampin, pyrazinamide and ethambutol.
- This is followed by the group of injectable drugs: streptomycin, kanamycin, amikacin, capreomycin or viomycin/tuberactinomycin B and the related tuberactinomycins A, N and O.
- One of the many related antibacterial fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin or the more recent sparfloxacin, gatifloxacin, moxifloxacin and sitafloxacin should be included in the regimen. This class of antibiotics has now been proven as indispensable treatment for MDR tuberculosis (Chan *et al*., 2004).
- Second line bacteriostatics, with established clinical efficacy, usually have more important side effects (Sunny *et al*., 2013). They are para-aminosalicylic acid, ethionamide (the propyl analogue prothionamide is also used) and cycloserine.
- Other drugs are also considered. Their use is the subject of debate and only time and proper observations will provide the necessary data. Clofazimine is among these compounds and is also used against *M. leprae*. The combination of amoxicillin and the penicillinase inhibitor clavulanic acid has an anti-mycobacterial effect *in vitro*. The same is true for clarithromycin although its clinical efficacy remains to be established (Forget *et al*., 2006).

4.1. Status of current tuberculosis drug therapy

Current TB therapy, also known as DOTS (directly observed treatment, short-course) consists of , for 2 months daily, followed by treatment with INH and RIF for another 4 months, three times a week(WHO,2000). The targets of these drugs are varied. INH inhibits synthesis of mycolic acid, a cell well component (McMahon *et al*., 2012); PZA targets cell membrane whereas rifampin and streptomycin interferes with the initiation and streptomycin interferes with the initiation of RNA and protein synthesis respectively (Almeida *et al.,* 2009). EMB blocks biosynthesis of arabinogalactan, a major polysaccharide present in the mycobacterial cell wall (Takayama *et al.,* 1989) and kanamycin and capreomycin, like streptomycin, inhibit protein synthesis through modification of ribosomal structures at the 16S rRNA (Zhang *et al.,* 2000). Cycloserine prevents the synthesis of peptidoglycan, a constituent of cell wall (Damtie *et al*., 2014).

4.2. Limitations of current tuberculosis therapy

In the present scenario, due to the emergence of multi drug resistant tuberculosis (MDR-TB) and association between HIV and TB, DOTS is becoming rapidly ineffective in controlling tuberculosis. Recent reports indicate that, areas where there is a high incidence of MDR-TB, DOTS is failing to control the disease. In such circumstances, the second line drugs are prescribed in combination with DOTS. However, this combination of drugs is very expensive, has to be administered for a longer duration and has significant side effects. One major drawback of current TB therapy is that the drugs are administered for at least 6 months (Kimerling *et al*., 1999).

The length of therapy makes patient compliance difficult, and such patients become potent source of drug-resistant strains. The second major and serious

problem of current therapy is that most of the TB drugs available today are ineffective against persistent bacilli, except for RIF and PZA. RIF is active against both actively growing and slow metabolizing non-growing bacilli, whereas PZA is active against semi-dormant non-growing bacilli. However, there are still persistent bacterial populations that are not killed by any of the available TB drugs. Therefore, there is a need to design new drugs that are more active against slowly growing or non-growing persistent bacilli to treat the population at risk (Zhang *et al*., 2002).

4.3. Impact of genome sequence on identification of new drug target

The complete genome sequence of *M. tuberculosis* provides an opportunity for a more focused and planned approach towards the identification of new drug targets. Genome sequence helps in compilation of all the potential gene products encoded by a particular organism, identification of functions (enzymes and pathways) that are missing or unique in a particular organism, and finally identifying the genes that are common to all or most prokaryotes and eukaryotes (Cole *et al*., 1998).

Conclusion and Recommendations

Tuberculosis is still a leading infectious disease worldwide. Along with the socio-economic and host factors that underlie this problem, a fundamental problem that hinders more effective TB control is the tenacious ability of *M. tuberculosis* to persist in the host and to develop drug resistance, often as a consequence of poor compliance to lengthy therapy. Major obstacle in the cure and prevention of tuberculosis is posed by the latent or persistent *M. tuberculosis* infection. This is due to the fact that most of the currently available drugs are ineffective against latent infection. A better understanding on the physiology of mycobacteria during the latent period will help in the identification of new drug targets that can act on the persistent mycobacteria. The list of potential drug targets encoded in the genome of *M. tuberculosis* include genes involved in persistence or latency, cell wall synthesis, virulence, signal transduction, genes encoding transcription factors and enzymes of other metabolic pathways. All these targets should be explored to identify new drugs against tuberculosis that will overcome the limitations of existing drugs such as, prolonged chemotherapy, failure against persistent infection and multidrug resistance.

Based on above conclusion the following recommendations are forwarded.

❖ The lists of potential drug targets encoded in the genome of *M. tuberculosis* should be explored to identify new drug against tuberculosis that will overcome the limitation of existing drugs.

❖ Basic research will have to continually update to prevent the drug-resistant strains from becoming an unmanageable clinical paradigm.

❖ Research should involve testing new or reformulated drug, combination of different drugs to shorten therapy, supplementation and enhancements of existing drugs.

❖ The existing (currently in use) drugs should be modified because of continuous development of drug resistance.

❖ TB drugs should be tested and combined with different drugs to shorten therapy, to reduce toxicity and to enhance its activity.

❖ More research should be conducted on molecular targets of *M. tuberculosis*.

❖ Researcher should actively participate in finding better and more effective drugs that reduce time of treatment and less toxic.

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Corresponding Author:
Dr. Yitayew Demissie
Department of Veterinary Medicine
Tewedros campas, Gondar University
Gondar, Ethiopia
Email: yitayew455vet@gmail.com

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