

Review on Molecular mechanism of first line antibiotic resistance in *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) is among the most common infectious diseases and frequent causes of death worldwide claiming many of lives annually. The problem of tuberculosis is hampered by the emergence of multi drug resistant (MDR) and extensively drug resistant tuberculosis. Anti-tuberculosis drugs are a two-edged sword. While they destroy pathogenic *Mycobacterium tuberculosis* they also select for drug resistant bacteria against which those drugs are then ineffective.

In contrast to other bacteria, resistance of *M. tuberculosis* is exclusively associated with chromosomal mutations. Globally, the emergence of multidrug-resistant strains of *M. tuberculosis* is an increasing problem which adversely affects patient care and public health. The objective of this review is therefore to compile available literatures about the drug resistance mechanisms of *M. tuberculosis* which gives insight understanding for the development of new therapeutic and diagnostic methods for the management of MDR and XDR tuberculosis infections.

Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; *katG*, *inhA*, *ahpC*, *kasA* and *ndh* for INH resistance; *rpoB* for RIF resistance, *embB* for EMB resistance, *pncA* for PZA resistance and *rpsL* and *rrs* for STR resistance. The search for new anti-tuberculosis drugs shall consider new targets which are less susceptible for mutation.

Keywords: Antibiotic resistance, Mechanism, *M. tuberculosis*

Introduction

Tuberculosis (TB) is among the most common infectious diseases and frequent causes of death worldwide [1]. An estimated one third of the world's population is infected with *Mycobacterium tuberculosis*, and nearly 9 million persons develop disease caused by *M. tuberculosis* each year [2]. In 2009, there were an estimated 14 million prevalent and 9.4 million incident cases. Among 5.8 million notified cases of TB patients in 2009, an estimated 250 000 had multidrug resistant TB (MDR-TB) [3]. Ethiopia ranks seventh among the world's 22 high-burden tuberculosis (TB) countries. According to the World Health Organization's (WHO's) Global TB Report 2009, the country had an estimated 314,267 TB cases in 2007, with an estimated incidence rate of 378 cases per 100,000 population [4].

Anti-tuberculosis drugs are a two-edged sword. While they destroy pathogenic *M. tuberculosis* they also select for drug resistant bacteria against which those drugs are then ineffective. Global surveillance has shown that drug resistant Tuberculosis is widespread and is now a threat to tuberculosis control programs in many countries [5]. Globally, it is estimated that 3.3% of all new TB cases had multi drug resistant tuberculosis (MDR-TB) in 2009 each year, about 440,000 MDR-TB cases are estimated to emerge, and 150,000 people die [6]. MDR-TB is much more difficult and costly to treat than drug susceptible TB, but recent work has shown that it is feasible and cost-

effective even in settings of limited resources. Extensively drug resistant tuberculosis (XDR-TB) raises the possibility that the current TB epidemic of mostly drug susceptible TB will be replaced with a form of TB with severely restricted treatment options [7]. Multidrug resistance (MDR) epitomizes the increasing health problem of tuberculosis (TB) in the world. According to the fourth report on the Global Project on Anti- Tuberculosis Drug Resistance Surveillance, the world's highest rate of MDR-TB (60%) was observed in Tashkent, Uzbekistan [8].

Globally, the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* is an increasing problem which adversely affects patient care and public health. In contrast to other bacteria, resistance of *M. tuberculosis* is exclusively associated with chromosomal mutations. Recently developed molecular biological techniques have significantly helped in understanding the basis of drug action and resistance mechanisms in this organism. The information gained at the molecular level is helpful to develop efficient diagnostic strategies and create novel drugs, both of which will ultimately have a direct impact on treatment programs [9]. The objective of this review is therefore to compile available literatures about the antibiotic resistance mechanisms of *Mycobacterium tuberculosis* which provide researchers an insight understanding about the opportunities of developing new therapeutic and diagnostic methods for the management of MDR and XDR tuberculosis infections.

Mechanisms of drug resistance in *M. tuberculosis*

Resistance of *Mycobacterium tuberculosis* to anti-TB drugs is man-made. Wild isolates of *M. tuberculosis* that have never been exposed to anti-TB drugs are virtually never clinically resistant [10]. There are a few exceptions, but these exceptions are not thought to contribute greatly to the overall burden of resistance. For instance, isolates of *M. tuberculosis* from Madras (Chennai), India, have been found to have a higher average level of resistance to para-aminosalicylic acid (PAS) than isolates from patients in the United Kingdom. This resistance is called natural resistance [11].

Resistance due to exposure to a single drug, whether as a result of poor adherence to treatment, inappropriate prescription, irregular drug supply, or poor drug quality, suppresses the growth of bacilli susceptible to that drug but permits the multiplication of pre-existing drug-resistant mutants-acquired resistance /resistance among previously treated cases/. Subsequent transmission of such bacilli to other persons may lead to disease that is drug-resistant from the outset, an occurrence known as primary resistance/ resistance among new cases [12,13].

Drug resistance in *M. tuberculosis* occurs by random, single step, spontaneous mutation at a low but predictable frequency in large bacterial populations. The possibility of incidence of drug resistant mutants is 3.1×10^{-8} for rifampicin, while for isoniazide and some of other commonly used drugs is 3.5×10^{-6} [14]. An environment containing selective antibiotics promotes the occurrence of mutations in the pathogen's genome [15]. Consistent with these findings is the occurrence of drug-resistant strains of MTB, which have been divided into two main groups. Multi-drug resistant (MDR) strains are resistant to two front line drugs, isoniazid and rifampicin, and these strains have been estimated to cover 5.3% of all new cases of TB around the world [16].

Extensively drug resistant (XDR) strains are strains which are resistant to isoniazid and rifampicin plus one of the fluorquinolones and at least one second-line injectable drug (capreomycin, kanamycin or amikacin). Even though it is still to be established whether *Mycobacterium tuberculosis* (MTB) possess plasmids and if there is a functional conjugational apparatus in MTB up to now all drug-resistance determinants are thought to be chromosomally encoded and to be a result of spontaneous nucleoid mutations or gene inactivation by a mobile genetic element [17]. Mutations are acquired mainly by deletions, insertions or single nucleotide alterations [18].

Resistance to first line drugs

Any drug used in the anti-TB regiment is supposed to have an effective sterilizing activity that is capable of shortening the duration of treatment. Currently, a four-drug regiment is used consisting of isoniazide (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) [19]. Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; katG, inhA, ahpC, kasA and ndh for INH resistance; rpoB for RIF resistance, embB for EMB resistance, pncA for PZA resistance and rpsL and rrs for STR resistance [14].

Isoniazid

INH or isonicotinic acid hydrazide, was synthesized in the early 1900s but its anti-TB action was first detected in 1951. INH enters the cell as a prodrug that is activated by a catalase peroxidase encoded by

katG and the peroxidase activity of the enzyme is necessary to activate INH to a toxic substance which subsequently affects intracellular targets such as mycolic acid biosynthesis [20]. A study conducted in Italy to identify gene mutation responsible for isoniazide resistance identified katG, ahpC, inhA promoter and kasA genes as responsible genes for isoniazide resistance [20]. Mutations in katG315 were significantly more common in the multidrug-resistant isolates while mutations in the inhA promoter were significantly more common in isoniazid-mono-resistant isolates [21]. Of the total 50 INH resistant isolates in China, 82% had a katG315 mutation and 18% had an inhA mutation [22]. A study conducted on the comprehensive evaluation of INH resistant *M. tuberculosis* strains (n=224) from three South American countries with high burden of drug resistant TB characterize mutations in katG, ahpC and inhA gene loci and correlate with minimal inhibitory concentrations (MIC) levels. Mutations in katG were observed in 181 (80.8%) of the isolates of which 178 (98.3%) was contributed by the katG S315T mutation. Additional mutations seen included oxyR-ahpC; inhA regulatory region and inhA structural gene. The S315T katG mutation was significantly more likely to be associated with MIC for INH $\geq 2 \mu\text{g}/\text{mL}$ [23]. In the absence of an exogenously added oxidant, the rate of INH-NADH formation as catalyzed by WT KatG was found to be 10-fold higher than that for KatG (S315T), representing the first observed correlation between suppressed INH-NADH adduct formation and INH resistance by a mutant KatG. This finding supports the theory that InhA inhibition is the primary means by which KatG activated INH confers anti tubercular activity [24].

Rifampicin

RIF was first introduced in 1972 as an anti-TB drug and has excellent sterilizing activity. RIF in combination with INH forms the backbone of short-course chemotherapy. It is interesting to note that mono resistance to INH is common but mono resistance to RIF is quite rare. It has thus been proposed that resistance to RIF can be used as a surrogate marker for MDR-TB as nearly 90% of RIF resistant strains are also INH resistant. RIF interferes with transcription by the DNA-dependent RNA polymerase by binding to the β -subunit hindering transcription and thereby killing the organism [20].

RIF resistance is associated with a hotspot (codon 507 to 533) core region called rifampicin resistance determining region (RRDR), for "rifampicine resistance determining region" (81 bp) of the rpoB gene with more than 95% of RIF resistant *M. tuberculosis* has a mutation in this specific zone [20]. The most common codons changes occur in Ser531Leu, His526Tyr and Asp516Val. These changes occur in more than 70% of RIF resistant isolates [25]. The microarray study result in China indicated that 100% of rifampicin-resistant *M. tuberculosis* strains isolated in Chongqing had rpoB mutations, with 531-Ser and 526-His being the most common positions substituted [23].

The mutational analysis in Kazakhstan revealed that the most frequent mutations associated with rifampicin resistance in *M. tuberculosis* are the substitutions at codons 531 (82.7%) in the rpoB followed by mutations with lower frequency at codons 526 (8.4%) and in 6.2% of the isolates, no mutations were found in the rpoB gene [26]. A study in Western Poland indicated that proB gene mutations associated with nucleotide replacements in codons 526 (His-Asp) and 531 (Ser-Leu) were associated with a high percentage of RMP resistance, whereas mutations in codons 516 (Asp-Val) and 526 (His-Tyr) were observed in a low percentage of RMP-resistance [27]. A study conducted in Turkey depicted that among 48 strains, 46 (95.8%)

were found to have rpo gene mutations with 13 different types while in two (4.2%) of the 48 strains, no mutations were detected. Point mutations at the 531st (52.1%) and 526th (18.9%) codons were frequent. The most frequent point mutation was Ser531Leu, and it was found in 21 (43.8 %) of 48 strains [28].

Pyrazinamide

PZA, a nicotinamide analog, was first discovered to have anti-TB activity in 1952. PZA targets an enzyme involved in fatty-acid synthesis and is responsible for killing persistent tubercle bacilli in the initial intensive phase of chemotherapy. PZA is a prodrug which is converted to its active form, pyrazinoic acid (POA) by the pyrazinamidase (PZase) encoded by pncA. Accumulation of POA results in the lowering of intracellular pH to a level that inactivates a vital fatty acid synthase. Therefore resistance to pyrazinamide in *Mycobacterium tuberculosis* is usually associated mutations in pncA, the pyrazinamidase coding gene [29].

To better understand the relationship between pncA mutations and pyrazinamide-resistance, it is necessary to analyze the distribution of pncA mutations from pyrazinamide resistant strains [30]. A study done in china revealed that among 47 pyrazinamide resistant *M. tuberculosis* clinical isolates 44[93.6%] exhibited 29 different changes in the pncA gene compared with wild type [H37rv] sequence and lacks pyrazinamidase activity. Of the mutant isolates the predominant mutation was a point mutation which results in an amino acid substitution [33/44] [31]. Similarly a study done in South Africa on clinical isolates of pyrazinamide resistant *M. tuberculosis* 25 unique mutations in pncA gene were detected [32]. Another study done in Brazil among 31 isolates resistant to pyrazinamide, 26 (83.9%) showed at least one mutation in the pncA gene or in its putative regulatory region. Three pyrazinamide-resistant isolates, confirmed by MIC varying from 800 to 1600 mg/L, carried the wild-type pncA sequence and retained PZase activity from this one can understand that there are other mechanisms of resistance to pyrazinamide which needs farther study [33].

Ethambutol

EMB targets the mycobacterial cell wall through interaction with arabinosyl transferases involved in arabinogalactan (AG) and lipoarabinomannan (LAM) biosynthesis. It specifically inhibits polymerization of cell-wall arabinan, thereby leading to accumulation of b-D-arabinofuranosyl-1-monophosphoryldecaprenol (DPA) [34]. Three genes, designated embCAB, that encode homologous arabinosyl transferase enzymes involved in EMB resistance. A study in Germany depicted 18 non-synonymous mutations in 15 distinct codons of the embCAB operon from 34 ethambutol-resistant strains. The majority occurred in the embB gene (10 distinct codons), in a 570 bp region also encompassing embB306. Mutations in embC and embA were found rarely and in most cases in combination with polymorphisms in embB [35]. In a study done by Johnson et al. it was shown that genotypic analysis identified mutations at codon 306 of the embB gene rendering resistance to EMB [36]. Among 37 isolates from different parts of India 30 isolates showed embB gene mutation with the most common mutation observed at codon 306 in 9/22 isolates followed by 299 in 6/22 isolates [37]. Recent study revealed that 66% of spontaneous mutants contained a single point mutation in embB, with 55% of these occurring at embB 306 [38]. Multidrug-resistant (MDR) strains had a higher proportion of embB306 mutants than non-MDR

strains thus; embB306 locus is a candidate marker for rapid detection of MDR and extremely drug resistant tuberculosis [39].

Streptomycin

STR, an aminocyclitol glycoside, is an alternative first line anti-TB drug recommended by the WHO. STR is therefore used in the retreatment of TB cases together with the four drug regimen that includes INH, RIF, PZA and EMB. The effect of STR has been demonstrated to take place at the ribosomal level. STR interacts with the 16S rRNA and S12 ribosomal protein (rrs and rpsL) inducing ribosomal changes, which cause misreading of the mRNA and inhibition of protein synthesis [40]. In certain strains of STR-R *M. tuberculosis*, the rpsL gene is a wild type and resistance is attributed to mutations of the rrs gene coding for 16S ribosomal RNA : 8 to 21% of STRR strains have at least one mutation in the rrs gene associated with an intermediary resistance level [41]. Of the 98 SM-resistant isolates in China, 78 (79.6%) had missense mutations in codon 43 or 88 of rpsL resulting in a Lys to Arg substitution, 6 (6.1%) had mutations of the rrs gene at positions 513 A to C or T or 516 C to T, and 14 (14.3%) had the wild-type sequence [42]. Mutations in gidB appeared in 27% of streptomycin-resistant strains that contained no mutations in the rpsL or rrs genes, and they were associated with low-level streptomycin resistance. However, the association of certain mutations in gidB with streptomycin resistance needs to be further investigated, as mutations in gidB also found in streptomycin-susceptible strains [43].

Conclusion

Emergence of MDR-TB and XDR-TB is the great challenge to the world. Majority of the drug resistance in *M. tuberculosis* are made that leads to mutation of genes which confer drug resistance to the *Mycobacterium tuberculosis*. Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; katG, inhA, ahpC, kasA and ndh for INH resistance; rpoB for RIF resistance, embB for EMB resistance, pncA for PZA resistance and rpsL and rrs for STR resistance. The search for new anti tuberculosis drugs should be encouraged considering new targets which are less susceptible for mutation.

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