Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(1)

Resistance Mechanisms to First Line Drug in *Mycobacterium tuberculosis: A Review*

Jabbar S. Hassan[1] *PhD*, **Bushra J. Al-Tamimi**[2] *PhD*, **Thanaa R. Abdulrahman**[1] *PhD,* **Qudas W. Jamal**[1] *PhD*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, University of Babylon, Iraq

Abstract

The emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis (Mtb)* strains, identified as resistant to at least isoniazid and rifampin, these two drugs form the backbone of the first line drug which is used for tuberculosis (TB) treatment, unresponsive has hampered TB control. As a result of the nearly universal calculation of with half a million new cases of MDR/first line drug TB per year, it is important to keep the database up to date awareness of the processes that contribute to the emergence of MDR *Mtb*. This resistance is produced for a variety of reasons, including genetic, microbiological factors, non-adherence to treatment by patients and/or failures in therapy administration by some referrer medical centre for TB. This review offers a detailed summary of genetic mechanisms that lead to resistant to first line drug therapy used in management of TB as well as up-to-date information on some new aspects lead to such problem.

Keywords *Mycobacterium tuberculosis*, first line drug, anti-TB treatment, World Health Organization, isoniazid and rifampin

Citation Hassan JS, Al-Tamimi BJ, Abdulrahman TR, Jamal QW. Resistance mechanisms to first line drug in *Mycobacterium tuberculosis*: A review. Iraqi JMS. 2022; 20(1): 35-43. doi: 10.22578/IJMS.20.1.5

List of abbreviations: DST = Drug susceptibility testing, XDR-TB = Extensively drug-resistant tuberculosis, HGT = Horizontal gene transfer, MDR = Multidrug-resistant, *Mtb* = *Mycobacterium tuberculosis*, PZA = Pyrazinamide, TB = Tuberculosis

Introduction

Tuberculosis (TB), one of the ancient infections known to affect humans, the main target organ of this disease is the lung, but can be disseminated to involve any tissue through the body. The mortality rate increases annually [(1)]. World Health Organization (WHO) reported that around 1/4 of the world's population can presented with latent TB, in which tuberculin skin test give positive, which can progress to active TB [(2)].

Treatment regimen involved streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide (PZA), which referred as first-line anti- TB drug, while the second line of anti-TB composed from fluoroquinolones, amikacin, kanamycin, capreomycin, ethionamide, prothionamide, cycloserine, and para-amino salicylic acid [(3)].

One of the most treatment challenged is the appears of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strain, which is including either un-respond to first-line therapy mainly isoniazid and rifampicin and it is called multidrug-resistant TB (MDR-TB), or if resistant to isoniazid and rifampicin plus resistance to fluroquinolone such as levofloxacin or

moxifloxacin in addition to that it resistant to at least one of the three injectable second-line drugs (amikacin, capreomycin or kanamycin), it refers to extensively drug-resistant TB (XDR-TB) [3].

The burden of the drug resistances TB strain will be growing in last years in the TB endemic countries, many fundamental risk factors may be led to this troubled situation, such as misused of anti TB drug, therapy regimen failure, tuberculosis relapse, difficulty getting a drug susceptibility testing (DST) and in some country's patients did not get drug supply easily [4].

Unluckily, conventional culture methods using egg-yolk-enriched media till know considered the most utilized methods for TB-DST, long time required to get result for this conventional method displease doctors in the management of TB cases, this forces doctors to give treatment to newly diagnosed patients without resorting to TB-DST, despite the presence of newer methods for studying TB-DST, unfortunately the high cost of such newly methods in low and middle-income countries determine their use [4].

Cell wall structure of *Mtb* is complex and diverse it composed from high lipid content, the fraction lipid of this cell wall including cord factor, mycolic lipid, wax D in addition to that the cell envelope of *Mtb* is extraordinarily hydrophobic and forms a remarkably strong permeability barrier, rendering *Mtb* naturally not respond to a broad variety of anti-TB therapy [5].

The resistance of TB bacteria to various treatment mainly to isoniazid and rifampicin, the most effective anti-TB drugs has become one of the global problems and lead to increase morbidity and mortality of this disease [6].

From an evolving viewpoint, *Mtb* use two main genetic strategies to avoid and escape from attack of anti-TB treatment, these genetic methods including mutations in gene(s), which often related to the mechanism of action of the compound, and second strategies is acquisition of DNA coding for resistance determinants by means of horizontal gene transfer (HGT) [7].

Genetic structure of *Mycobacterium tuberculosis*

The chromosomal characteristic of *Mtb* equal to 4,200,000 nucleotides long and the G to C ration is about 65%, this genome comprised from 4000 gene, and one of the essential gene in this genome is genes that code for lipid metabolism which is Which constitutes roughly about 8% of the total of this genome makeup [8].

DNA homology between all *Mtb* complex show 95-100% connections, while in the case of the 16S rRNA gene, it is found in all types of these bacteria, for this reason some researchers suggest this species grouped as a single species [9].

From other hand, l6S-23S rRNA internal transcript spacer (ITS), which is spacer DNA well used for species determination of mycobacteria that due to elevated of variation between species, both in their base length and in their sequence the data that suggest mycobacterium species contain alleles in the ribosomal operon in their genome enhance the potential that a considerable quantity sequence variation exists in these spacer regions, even among strains of the same species [10].

First-line drugs used in the fight against tuberculosis

The battle against TB started since ancient times and is still going on. Various types of drugs have been introduced into the battlefield, and the first-line drugs remain among the most important and ancient weapons in this confrontation for various reasons, including ease of processing and access to them, and that is their ability to deal with disease under different TB pathogenesis process [11].

Effective TB treatment introduce in 1952 and since, through all this time *Mtb* have acquired unresponsiveness to various type of drugs

effect both treatment and control programs. MDR and XDR-TB have emerged due to inappropriate use of anti-TB medications, incorrect prescriptions, poor quality drugs and ending treatment prematurely [12].

The manner to treatment the TB is completely distinct from that for other bacterial infections, *Mtb* required long generation time and have capability for latency which led to decrease the metabolic processing makes it a problematic in therapeutic target [13].

Furthermore, one of most effective scape mechanisms in *Mtb* is granuloma formations, which composed of solid caseous material make the penetration of anti-TB is difficulty and the environmental pH is adequately low to prevent the activity of anti- tuberculosis therapy [14]. Particularly when treatment by using mono-therapy anti- TB, for instance, isoniazid is vital in the initiation of therapy; its bactericidal activity promptly diminishing the viable organism in sputum because it is active mostly toward the TB growing in pulmonary cavities [15]. PZA is only working at low pH, making it preferably suitable for destroying the tuberculosis inside caseous necrotic foci [16]. Rifampin has a great role in destroying the bacteria that are metabolizing slowly in constant, and lead to reduce the infectious agent in the target tissue [17].

The consequent descriptions of first-line anti-TB therapy gave the medical center the basic methods for TB management and control. The subsequent series of trials performed under the supervision of the U.S. Public Health Service, WHO guideline and others produced data lead to that cure rates of over 95% with limited relapse rates were practicable in as little as 6 months, using the first line with the new drug against TB, many countries have seen the virtual eradication of TB [18].

Drug resistance in *Mtb*

One of the important methods lead to developing drug resistance in *Mtb* is mutation in genes, which leads to the influence or disruption of the work drug-activating enzymes, these mutations can take form of single nucleotide polymorphisms (SNPs), insertions or deletions and to lower level, large deletions. *Mtb* differ from other bacteria in that, un-responses to drug are not gained by HGT by mobile genetic elements [17].

There are two major mechanisms lead to drug resistance in *Mtb*, which is primary drug resistance in which bacteria acquired when it is transmitted to a new host, the second mechanisms are secondary drug resistance in which *Mtb* obtainment drug resistance mutations to one or more drugs [19].

Many researchers according to whole genome sequencing (WGS) reported that *Mtb* drug resistance firstly to isoniazid then resistance to rifampicin or ethambutol followed by resistance to PZA and lastly, resistance to second- and third-line drugs. These studies give valuable perception into the evolution of the *Mtb* [20-22].

Mechanisms of resistance to first-line drugs

Isoniazid

Isoniazid belongs to a group of bioreversible derivatives therapy called prodrug, in which must be experience enzymatic and/or chemical conversion in vivo to release the active origin drug, so in the isoniazid drug an activating process takes place through catalase/peroxidase enzyme encoded by the katG gene, after triggered isoniazid lead to prevent formation of mycolic acid by the NADH-dependent enoyl-acyl carrier protein reductase, which is a key enzyme in fatty acid synthesis, this enzyme is encoded by gene called inhA gene [23,24].

The mechanisms that lead to developed unresponsive to isoniazid is mediated by mutation that occur in the katG, inhA gene, the widespread resistance process has been detected is the katG S315T mutation, this mutation causes ineffective isoniazid–NAD product inhibiting the antimicrobial action of isoniazid which is consequently lead to high-rate isoniazid resistance in MDR isolates [25,26].

Machado et al. stated that mutations that take place in the inhA regulatory region and coding region generated high-ranking isoniazid resistance and it's a key step in resistance to ethionamide [27].

In addition to katG, inhA gene mutation, other mutation has been recorded and involved in resistance to isoniazid such as dfrA gene mutation which encodes a thymidylate synthase, furthermore alkyl hydroperoxide reductase C is a type of the peroxiredoxin family possess peroxinitrite reductase activity as well as peroxidase activity hat diminish organic peroxides to their corresponding organic alcohols which encoded by the ahpC gene in mycobacteria [28].

Mutations in the promoter region of the ahpC gene were proposed as representative markers for isoniazid unresponsive. Studies have also reported a variety of mutations were identified in the kasA, oxyR-ahpC and furA-katG in isoniazid-resistant isolates of *Mtb* [29].

However, systematic review reported that mutations in katG and inhA is the play important mechanisms associated with isoniazid resistance, respectively. These common mutations, in association with frequently occurring mutations in the ahpC-oxyR, account for 84% of worldwide phenotypic isoniazid resistance [30].

WGS display proof that isoniazid resistance predates rifampicin resistance, associated with the katG S315T mutation. This carries out this mutation a perfect marker of the pre-MDR phenotype. Globally, the case rate of isoniazid resistance is over growing and is associated with worse outcomes [31].

Rifampicin

When *Mtb* sensitive to anti-TB therapy, rifampicin concerned one of the most effective drugs in first line anti-TB drug, its act actively in both metabolizing and low-metabolizing TB [32,33].

The mechanism of action is initiating when rifampin inhibits elongation of mRNA by binding to the b subunit of the RNA polymerase, which lead to gradual accumulation of mutations in 81-bp rifampicin resistance-determining region, which is found in the gene rpoB (codons 507 to 533), this process caused transcription failure in bacterial cell. The widespread mutations in the rifampicin resistance determining region (RRDR) are reported in codons 526 and 531 and its estimated 62.5% - 81.1% of *Mycobacterium* rifampin-resistant [34].

Cross-resistance to all class of rifamycin antibiotics such as rifabutin and rifalazil have been reported with rpoB mutations and attributable to mutations within the hotspot region, early regions of the rpoB gene and double mutations in codons 516 and 529 [35].

Some investigations reported absence of alteration in the rpoB gene in *Mycobacterium* rifampicin-resistant isolates suggesting other mechanisms of rifampicin resistance [36]. Minh et al. suggested that at external RRDR, rare mutations were reported in *Mycobacterium* isolates [37].

Mtb that resistant to only rifampicin without other agent in first line anti-TB drug such as isoniazid refer as rifampicin monoresistance, and it's not commonly reported strain [38].

Rifampicin mono-resistant TB led to unsuccessful treatment of TB depend on first line anti-TB drugs and any TB regimen without rifampin lead to bad prognosis outcome [39]. The American Thoracic Society, Centers for Disease Control and Infectious Diseases Society of America advisable a 12–18-month regimen for rifampicin mono-resistant Tb with isoniazid, ethambutol, and a fluoroquinolone, with the addition of PZA for the first 2 months [40].

Pyrazinamide

Pyrazinamide (PZA) is a major member of first line anti TB regimen and sometime used as a key component in treatment of rifampicin mono-resistant TB, it is considered as a pro-drug required conversation into pyrazinoic acid (POA) by the pyrazinamidase/nicotinamidase (PZase) enzyme, encoded by the pncA gene [41].

The activity of PZA is thought to be more at an acidic pH (within macrophages) and its effective against dormant or non-replicating (TB lesion) and little activity toward *Mtb* [42,43].
PZA enters bacilli through passive diffusion and is converted into POA by the cytoplasmic PZase encoded by pncA. POA then gets out of the cell through passive diffusion and a deficient efflux mechanism in *Mtb*, upon activated, POA inhibiting membrane transport through break up the bacterial membrane, in an acidic environment, POA is protonated allowing for reabsorption into the cell, resulting in cellular damage [44].
Resistance to PZA is associated with many mutations such as mutations in the rpsA (ribosomal protein I) gene and mutation in aspartate decarboxylase (PanD) However, the mutation in the pncA gene and its promoter region remains the most important one in these mutations and commonest mechanism-initiated pyrazinamide resistance [45].

Ethambutol

Since its introduction as a treatment for TB in 1966 until now, this treatment is still the backbone agent in first line drug used in the management of TB. The anti-TB activity of this agent relating to the ability to prevent formation of mycobacterial cell wall by interaction with arabinogalactan and lipoarabinomannan (LAM) through membrane-embedded arabinosyltransferases — EmbA, EmbB, and EmbC [46].
Some mutant proteins from the Emb family (ABC) have been isolated in ethambutol-resistant *Mtb* which suggest that resistance to ethambutol is mediated via mutations in the Emb family [47]. Safi et al reported that mutations in ubiA gene which encodes for decaprenyl-phosphate 5-phosphoribosyltransferase synthase cause high-level ethambutol resistance when they occur with embB mutations [48].

Streptomycin

Streptomycin, an injectable aminoglycoside therapy, it's one of the first anti-TB agent that has a distinct repressive effect on tuberculous infections. As a result, to initial broad use as anti-TB led to the early emergence of streptomycin resistance [49].
This drug is active against slow-growing bacilli and it have direct effect on bacterial ribosome particularly 30S subunit, when it interacts with S12 and 16S rRNA in form of irreversibly binding via this interaction, streptomycin blocking translation consequently inhibiting protein synthesis [50].
The leading mechanism of resistance to streptomycin is occur through the mutations in the rpsL and rrs genes, encoding the ribosomal protein S12 and the 16S rRNA [51].
There are some studies mentioned that gidB gene which encoding a 7- methylguanosine methyltransferase specific for methylation of the G527 in loop of the 16S rRNA may be undergo a mutation lead to low-level streptomycin resistance [52,53].

Epistasis in *Mtb* drug resistance

Epistasis or interactions between genes, is a situation in genetics in which the impact of a gene mutation is dependent on the presence or absence of mutations in at least one different gene [54]. Epistasis occurs when several mutations interact with each other to express new advantageous traits for an organism and are often necessary for bacteria to modify their fitness cost (fitness in microbiology is the ability of microbes to thrive in a competitive environment. It is often determined by comparing the growth rate in a given environment of a mutant strain with that of its non-mutant isogenic relative [55].
During epistatic interactions, the effect of multiple mutations is greater or less than the effect of the individual mutation and can lead to either beneficial or deleterious phenotypes. According to this concept epistasis is classified as positive epistasis (antagonistic), negative epistasis (synergistic), and sign epistasis [56]

(Figure 1). A study by Borrell et al. has reported the role of positive epistasis in drug resistance development in *Mtb* and they reported that a particular combination of mutations in rpoB and gyrA that conferred resistance to RIF and ofloxacin (OFX) [57].

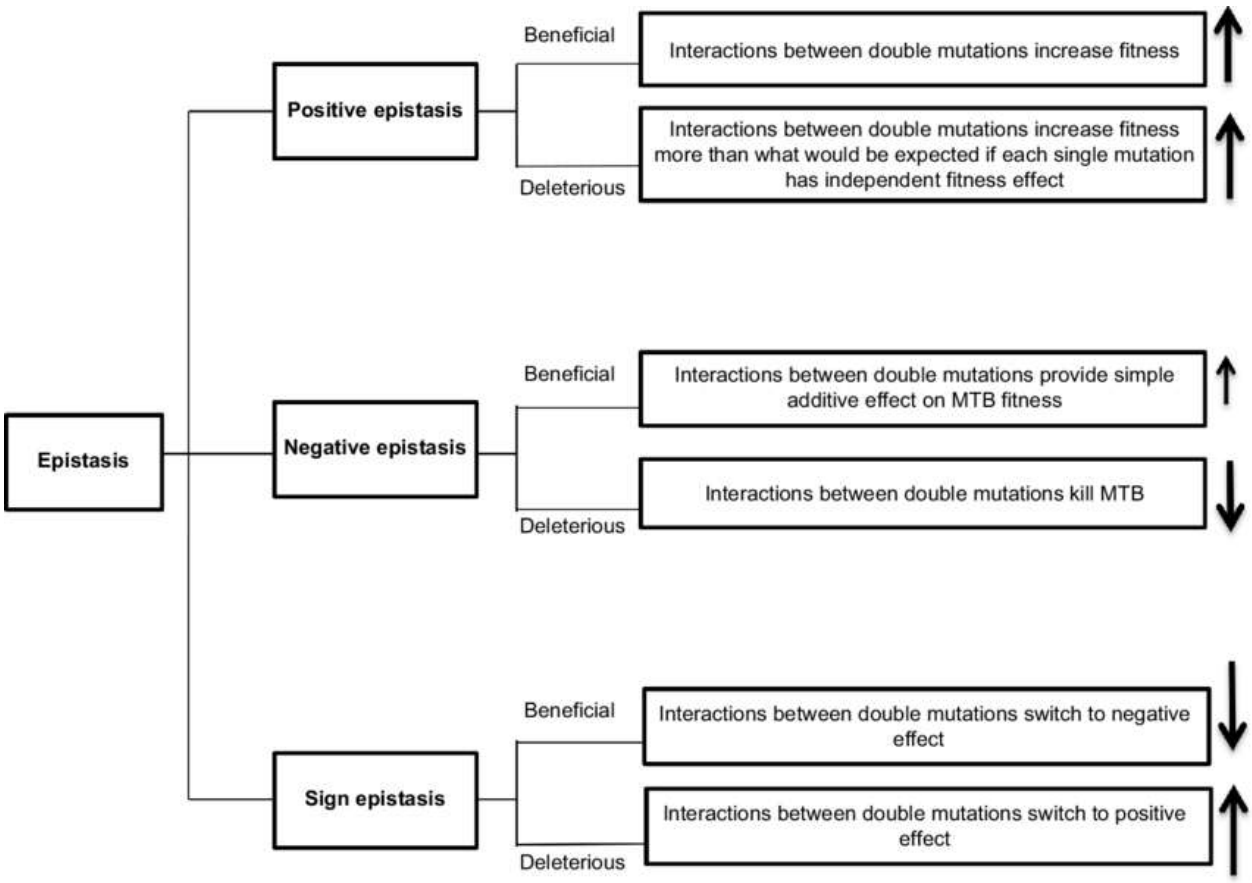

Figure 1. Forms of epistatic interaction between mutations [56]. (↑) indicated high MTB fitness and (↓) indicated low MTB fitness

Concluding remarks

TB is one of the deadly diseases that have led to increase morbidity and mortality through the world, after the initiation of drugs, which lead to great effect in reducing the devastating effects of this infection, especially used of the first-line drugs, but unfortunately, another major problem appeared in recent years that scientists and doctors faced which is drug resistant TB. It is a serious and growing problem in modern medicine and it is emerging as an outstanding public health threat particularly to first line drug, which is used for the treatment of new diagnosis patients. Many genetic and phenotypic mechanisms involved in development of resistance to therapy by *Mtb*. Understanding of these mechanisms allow scientists to initiate effective drug.

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Correspondence to Dr. Jabbar S. Hassan
E-mail: jabbarsalman30@yahoo.com
Jabbarsalman30@nahrainuniv.edu.iq
Received Apr. 4th 2021
Accepted Feb. 7th 2022