Journal of Scientific and Innovative Research 2022; 11(2): 36-42
Available online at: www.jsirjournal.com

Review Article

ISSN 2320-4818
JSIR 2022; 11(2): 36-42

Received: 28-05-2022
Accepted: 25-06-2022

Sushil Giri
Govt. Medical College Haldwani, Rampur Road Haldwani, Nainital, Uttarakhand-263139, India

Kailash Chandra Semwal
Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Shruti Agarwal
Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Gautam Singh Bhandari
Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Amit Kumar
Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Akash
Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Sandhya Dogra
Head, Department of Microbiology, Sai Institute of Paramedical & Allied Sciences, Dehradun, Uttarakhand- 248001, India

Correspondence:
Sushil Giri
Govt. Medical College Haldwani, Rampur Road Haldwani, Nainital, Uttarakhand-263139, India
Email: sushilgiri256@gmail.com

Resistance acquiring *Mycobacterium tuberculosis* in human body during drug therapy: resistance mechanism and future anticipations

Sushil Giri, Kailash Chandra Semwal, Shruti Agarwal, Gautam Singh Bhandari, Amit Kumar, Akash, Sandhya Dogra

Abstract

Acquiring of the resistance to the variant line of the drugs used in drug therapy for M. tuberculosis is becoming a crucial problem for the entire globe. Mutation in cord factor led to the bacterium resistant against antibiotics therapy. These changes drive the chromosomal mutations resultant, the drugs which are sensitive against the M. tuberculosis becomes the resistant via overexpression or modification of the drug target. Essential for viability and virulence, enzyme involved in the biosynthesis of mycolic acid represents novel target for drug development. This is particularly relevant to the impact on global health given the rise of MDR and XDR strains of M. tuberculosis. According to the intrinsic drug resistance mechanism the unusual composition and structure of the bacterial cell envelop and the low numbers of the porins assign notably to the envelope's low compound permeability. For better diffusion of antibiotics across the cell envelope there are require a high membrane fluidity. Though, the lipid-rich nature builds the cell wall exceedingly hydrophobic and prevents the permeation of hydrophilic compounds. Acquired resistance accomplish when a bacterium has the ability to resist the activity of an antimicrobial agent to which it was previously susceptible. The acquisition of the acquired resistance follows up the case of successful gene mutations. Although M. tuberculosis has low genetic diversity as compare to the other pathogens but the genetic diversity of the M. tuberculosis can influence multiple aspects in therapy of drug resistance tuberculosis. From mono drug resistant to MDR and XDR, is threatening to make TB once again an untreatable disease if new therapeutic option does not soon become available.

Keywords: Drug Resistance Tuberculosis (DR-TB), Multiple Drug Resistance (MDR), Membrane Fluidity, Cord Factor, Antibiotics Therapy.

INTRODUCTION

Tuberculosis (TB), is an airborne, communicable but curable disease when diagnosis at time which is major threat for the entire health sector and classified as a very crucial public health concern by the World Health Organization in 1993. The most affected part are human's lungs refers to the pulmonary tuberculosis, on the other hand the extra pulmonary tuberculosis affects the other part of the body like brain, spinal cord, lymph node, heart etc., infection of other organ causes a wide range of symptoms [1]. People those, don't have any symptoms represents a latent stage of the tuberculosis called latent tuberculosis [2]. In untreated cases about 10% of latent tuberculosis progress to active stage of tuberculosis. Active TB is an illness in which the bacterium rapidly multiply and invades into different organs of the body [3].

The etiological bacterium called as Koch's bacillus named after Robert Koch who first discovered it in 1882 [4]. M. tuberculosis have presence of waxy coating in cell wall that provide it acid-fast property with mycolic acid, play a major role as virulence factor. This coating makes the cell impervious to gram stain, so acid-fast stain is used instead of gram stain [5].

One of the deadliest infectious diseases, with an estimated 1.3 million deaths globally in 2016, down from 1.7 million in 2000. In 2016, 10.4 million cases of tuberculosis were reported. Cases no. of 0.49 million were reported for MDR TB, which is a major global health problem this requires second line antibiotics treatment, that are less effective, more toxic and more expensive.

Drug resistant-TB can develop in 1° and 2° drug resistant TB. Primary DR-TB occurs in person who are initially infected with resistant organism; while secondary DR-TB or acquired resistance TB, develops during tuberculosis therapy, either because the patient was treated with an inadequate regimen, did not take prescribed regimen appropriately or because of other condition such as drug mal-absorption or drug-drug interactions that lead to low serum level [6,7]. Circumstances in which an exposed person is at an increased risk of infection with drug resistant tuberculosis includes, exposure to a person who has known drug-resistant TB disease, exposure to a person with TB disease from an area in which there is a high prevalence of drug resistance or travel to one of these areas, exposure to a person who had been took drugs irregularly and incorrectly. encephalopathy, meningitis/encephalitis, and acute Guillain-Barre Syndrome are some of the neurological complications reported with COVID-19 [10,11]. This review is aimed at the study of Covid-19 infection and its neurological manifestation. Before starting the neurological effect, we will discuss what COVID-19 is, from which family it belongs and how it is spread and what are general effects of COVID-19 are.

MDR-TB is caused by strain, that resistant to the 1st line antibiotics includes, isoniazid (INH), rifampicin (RIF), and ethambutol (EMB), and in case of XDR-TB, strain is resistant to 1st line antibiotics with any fluoroquinolone and at least 2 or 3 injectable second-line antibiotics such as, amikacin, kanamycin, capreomycin [8,10].

The 1st anti-TB drug was streptomycin, discovered in 1994 but it was soon understood the evolution of acquiring resistance rendering streptomycin ineffective [11,12]. The first combined therapy was introduced by the British Medical Research Council, in which streptomycin with para-amino salicylic acid used for treatment of pulmonary tuberculosis [13,14]. Discoveries of anti-tuberculous antibiotics changed the scenario to treat the tuberculosis disease. Direct Observed Therapy Short Course (DOTS) a short period antibiotics treatment which started by World Health Organization for 6 months or 18 months course duration according to the strain which is responsible for tuberculosis disease in patient [15,16]. Significantly mutation in TB bacteria genome also effects the treatment with the cellular changes within human body. The mutation effects on drugs makes the drug resistant to the bacterium by inhibiting the mechanism of the drugs, e.g. the bacterium acquiring mutation in *rpoB*, *katG*, *inhA* gene which codes for respectively β subunit of RNA polymerase, catalase/peroxidase enzyme and NADH dependent enoyl-acyl carrier protein (ACP) reductase enzyme responsible for synthesis of mycolic acid present in the cell wall of bacterium [17-20].

Table 1: Pathogenesis of M. *tuberculosis* in human body

S. No.	Pathogenesis
1.1	Determinants of pathogenicity: - Cord factor [glycolipid derivatives of mycolic acid a virulent factor] responsible for inhibiting phago-lysosome formation and allowing intracellular survival of bacilli after ingestion by macrophages. Inhibits the migration of polymorph nuclear leucocytes and elect's granuloma formation.
1.2	Immunological aspects: - Delayed or type IV hypersensitivity recognized. granuloma formation occurs with subsequent decreases in the number of bacilli. Some remain for viable or dormant for many years in viable or dormant stages. This stage is called latent TB infection; an asymptomatic, and radiological undetected stage. Pathogen associated molecular patterns [PAMPs] binds to pattern recognition receptors [PRRs] on defense cells like macrophages, dendritic cell, B lymphocytes, T lymphocytes and fibroblast cells. Defense cell of body secrets proteins called cytokines like interleukins, interferon; promotes innate immune defense such as inflammation, phagocytosis, activation of complement pathways. Inflammatory effects from excessive cytokines along with release of toxic lysosomal component of macrophages tries to kill the bacilli.

Table 2: The nature of immune response following infection changes with time so that human tuberculosis is divided into primary and post primary tuberculosis with different pathologic features

S.No.	Type
2.1	PRIMARY TUBERCULOSIS Primary infection in lungs, tonsils, intestine or skin. Bacilli invade and replicate within endosomes of alveolar macrophages after get entry through respiratory tract. Initial lesion has shown in the primary site of infection in the lungs, called ghon focus. Dendritic cells picked bacilli which are transport them to local mediastinal lymph node. The ghon focus together with enlarged lymph nodes forms a primary complex, which is asymptomatic and undergoes fibrosis (spontaneous healing) or calcification which lead to hypercreativity called tuberculin allergy against tuberculous- protein. The primary ghon focus complex may be in the skin with development of regional lymph nodes. "Prosecutor warts" term given by anatomist with pathologist and it is an occupational disease form of tuberculosis. Form granuloma formation within 10 days around the infection which involves T lymphocytes releases cytokines and interferon with active macrophages, fibroblasts and B lymphocytes. Formation of granuloma causes to prevent dissemination of the mycobacteria, and provides an environment to communicate, cells with the immune system. The granuloma contains a mixture of necrotic tissues and dead macrophages which form it cheesy like appearance and consistency is referred to as caseous necrosis. And is responsible for cell death called necrosis. activated T lymphocytes $CD8^+$ can directly kill infected cells. Whether activated macrophages in granuloma inhibit the replication of bacilli and consume O_2 resulting anoxia and acidosis probably kills most of bacilli. But some bacilli remain for dormant stage, resulting post primary infection.
2.2	POST PRIMARY INFECTION necrotic elements of the reaction cause tissue destruction and formation of large area caseous ion term "tuberculoma". Protease liberated by activated macrophages cause softening and liquefaction if this caseous material and excess of tumor necrosis factor and other immunological mediators causes the fever characteristics of the disease. The dissemination of bacilli to lymph nodes and other organ causes infection and sac lesions are develops in lower lobes of the lungs. Reactivation TB is more severe in immunocompromised patients or the old age having low immune system.
2.3	PEOPLE MAY EXPERIENCE Pain area: - chest Pain circumstances: - can occur while breathing Cough: - can be chronic or with blood Whole body: - loss of appetite, malaise, night sweat, fever Common condition: - loss of muscles, weight loss, shortness of breath, swollen lymph nodes

MECHANISMS OF DRUG RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

The genus Mycobacterium have a wide range of species from which *M. tuberculosis* complex (MTC) is most well-known member to infection the human lungs. This species has been noted for their intrinsic and acquired resistance to a wide array of antibiotics. The lipid rich cell well, low compound permeability, membrane fluidity, water filled porins, enzymatic actions and mutations in nucleoid shows the intrinsic and acquired antibiotics resistance mechanisms respectively [21-23]. Antibiotics injected into the body through oral route primary, which are able to penetrate cell wall but they are destroyed by enzyme or bypass that make them ineffective. Many studies have been done on a different species of genus Mycobacterium such as *M. smegmatis* an used because it's genome (roughly 1.5 times the size) equal to *M. tuberculosis*, with this lower pathogenicity, biosafety requirements and faster growth properties provide it another advantages to research to use this strain. Therefore, results of these studies directly correlated to *M. tuberculosis.*

Intrinsic drug resistance mechanism

Several classes of antibiotics have been attributed to the intrinsic mechanism of resistance. The intrinsic resistance mechanism provides the TB bacillus with a high background of drug resistance which makes the development of new drugs more difficult.

cell permeability for drug penetration

The cell wall of *M. tuberculosis* is much thicker and lipid-rich in nature due to the presence of unique fatty acid i.e., mycolic acid, renders the cell extremely hydrophobic and prevents the permeation of hydrophilic compounds or antibiotics. Also, low number of porins significantly makes the cell envelop less permeable which functions as effective barrier for drug penetration. The constituents of the cell structure of the M. tuberculosis are peptidoglycan the innermost layer is covered by a layer of arabinogalactan both are covalently linked to the mycolic acid and forms a mycolic-arabinogalactan-peptidoglycan (mAGP) complex is essential for the viability of mycobacterium tuberculosis and maintain the robust basal structure supporting the upper myco-membrane [24]. This complex also makes a hydrophobic barrier restricting the entry of hydrophilic molecules. It is thought that some small hydrophilic compounds or antibiotics can only transverse via water filled porins. These porins might play a role in diffusion of hydrophilic antibiotics across the cell wall of *M. tuberculosis.* The major porin MspA of *M. smegmatis* was expressed in *M. tuberculosis* play a role in transport of beta-lactamase and hydrophilic antibiotics such as norfloxacin and chloramphenicol etc. susceptibility of *M. tuberculosis* to theses antibiotics enhanced by the MspA, decreasing the minimal inhibitory concentration. This study provides the first experimental evidence that porins are important for drug susceptibility of *M. tuberculosis.* Bioinformatic analysis has identified Rv1698 (outer membrane protein, Omp), a porin in *M. tuberculosis* having a same function as MspA in participating to intrinsic resistance to hydrophilic compound [25,26].

Membrane's Fluidity

Lipid bilayer's viscosity in a cell membrane called membrane fluidity. With including cording factor and mycolic acid in which both are composed of longer beta-hydroxy chain as well as shorter alpha-alkyl side chain. Mycolic acid provide advantage to organism against antibiotics and dehydration. The low permeability of the mycobacterial cell wall is responsible for the resistance of mycobacterium to the drugs. A study has demonstrated that, comparing to other actinobacteria, *M. smegmatis* (has lowest membrane fluidity), demonstrated by a study. Allows less influx of lipophilic drugs i.e., norfloxacin, chenodeoxycholate when grown at high temperature. Exposure of *M. smegmatis* to subinhibitory concentration of ethambutol increases the rate of diffusion of compounds across the cell membrane, demonstrated in study [27]. This provides the circumstance for novel drug combination therapies, as using the ethambutol with the M. smegmatis can render M. tuberculosis susceptible against drugs [28].

Enzymatic Action to Inactivate Drugs in Mycobacterium tuberculosis

The mode of resistance involves enzyme that retarded the activity of the drugs. Several enzymes were coded by bacteria that degrades or modified the effect of antibiotics and targeting such enzymes is novel approach that can help therapy effectiveness to combat against resistance problem. The most prominent enzyme is β-lactamase which is encoded by the *Blac* gene, localise to the periplasmic space, or anchored in the outer layer of the plasma membrane as a lipoprotein or unbound; enzyme causes the degradation of β-lactam antibiotic i.e., penicillin, ampicillin, cefazolin, cefotaxime, imipenem, ceftazidime. Beta lactamase enzyme hydrolyse amide group of Beta lactam ring [29]. The *M. tuberculosis* β-lactamase also show broad spectrum substrate specificity even against new antibiotics like carbapenem (including imipenem and meropenem). The activity of the β-lactamase is inhibited by some agents such as clavulanate sulbactam and m-amino-phenylpyruvate [30]. Combining this agent with a beta-lactamase susceptible antibacterial use to tackle infections causing organisms which producing this lactamase enzyme, decreased turnover rate is result of the beta-lactamase sensitive antibiotic and enhances its antibacterial activity. However, some isolates causing MDR and XDR tuberculosis shows resistant to these agents. This hypothesis still needs further assessments [31].

Methylation, acetylation of aminoglycoside or cyclic peptide group's antibiotics are chemical methods which uses to tackle MDR TB by the modify intracellular survival protein (Eis). The mycobacterium has strategies to evade the killing mechanisms applied by the macrophages and acquires enclosed environment within its host cell to avoid the humoral and cell mediated immune response. The Eis (Rv2416c) gene has been identified to code a secretary protein which enhances intracellular survival of *Mycobacterium smegmatis* in the macrophage cell line. 4 proteins; adenosyl homocysteinase, aspartate carboxyltransferase, putative thiosulfate sulfotransferase and universal stress protein present in resistant as well as sensitive strains of tuberculosis. MALDI-MS used for identification of the intracellular MDR and sensitive isolates revealed that majority protein are common which expressed in the extracellular state belonged to intermediary metabolism and respiration category. Hydrolysis of S-adenosylhomocysteine (SAH) into free adenosine and L-homocysteine catalysed by adenosyl homocysteinase (SAHH) in which SAH is by-product of SAM-dependent methyltransferase reactions. Methylation plays a role in cellular process including DNA replication and repair system, metabolism of methionine and phospholipid biosynthesis [32]. Aspartate carboxyltransferase or transcarboxylase (AT Case) catalyses the pyrimidine biosynthesis. Putative thiosulfate sulfotransferase Rhodanese-like protein and much more functional information is not available. Proteomic and transcriptomic analysis of these proteins are significantly upregulated under hypoxic condition and in response to nitric oxide and carbon monoxide, as well as during *M. tuberculosis* infection of macrophage cell lines suggesting their probable role in persistence or intracellular survival [33].

During the intracellular state MDR tuberculosis shows the adenosine kinase activity which catalysis the phosphorylation of adenosine is essential for the cellular level regulation of adenosine and its nucleotides because during the intracellular state bacilli are not metabolically inactive but maintain a low-level metabolism to tide over the unfavourable condition [34]. Glucose-6-phosphate isomerase (PGI) have essential role in glycolysis and gluconeogenesis. Glucose auxo-trophy results in interruption of PGI gene [35]. During the intracellular state, significantly glycolytic enzymes level increase due to the metabolic shifting from the strict aerobic mode to anaerobic metabolism. During this state about 70% energy is derived from the glycolysis. Thus, these enzymes being central to the bacilli survival, and attractive target for the drug designs. Eis has been demonstrated to acetylate and methylate to inactivate the clinically relevant second line injectable aminoglycoside and cyclic peptide drug kanamycin and capreomycin [36,37].

Drug Efflux System

Both the pathogenic and non-pathogenic proposed a several types of drug efflux pump system to move lipophilic drugs out of the cell to prevent

being killed by the drugs. Efflux system play a key role in surviving the bacterium in intracellular macrophage state [38]. These system results in low intracellular regimen of the drug that makes the drug ineffective. Some early study shows that the mycobacterium has a multitude of different efflux system belonging to the ATP-binding cassette [39,41]. Mycobacterial efflux systems are able to extrude nearly all ant tuberculous drugs including, streptomycin, rifampicin, isoniazid, clofazimine, ethambutol etc [42].

Modification of Antimicrobial Targets

Some organisms (e.g., *Streptomyces spp.*) produce a product (Streptomycin; macrolides, clinolamides, and streptogramins) is naturally used antibiotic in treatment of tuberculosis. The resistant strain of mycobacterium has a reprogramming camouflaging critical target sites to avoid recognition. Therefore, presence of antimicrobial compound led to no binding with inhibition take place. This strategy has been observed in *Mycobacterium spp.* against streptomycin (modification of ribosomal proteins or of 16s rRNA) [43,44]. Resistance in *Streptomyces spp.* against antibiotics through methyltransferase which methylate the adenosine residue 2058 of the 23S rRNA. This modification prevents aforesaid products from binding to ribosome and inhibiting translation. Resistance to various macrolide antibiotics confers monomethylating resistance. The genome of *M. tuberculosis* encodes the methyltransferase Erm37 is able to monomethylate residues 2057-2059 of the 23S rRNA [45].

Acquired Drug Resistance Mechanism

Obtaining an ability to resist the activity of a particular drug by the bacterium to which it was previously sensitive causes the mutation of genes involved in normal physiological and cellular process and structure. The majority of acquired resistance in *M. tuberculosis* is governed by mutation in chromosome through various mode of horizontal gene transfer which prevalent among in bacterial biofilms. Changes in bacterial genome through mutation or horizontal gene acquisition or change in the nature of proteins expressed by the organism may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic, known as acquired resistance.

The following headings details the mutations mediating resistance to each of the anti-TB drugs

Isoniazid

Isoniazid or iso-nicotinic acid hydrazide activated by the catalase/peroxidase enzyme which is coded by the *KatG* gene. This antibiotic inhibits the synthesis of mycolic acid through the NADH-dependent enoyl-acyl carrier protein (ACP) reductase enzyme encoded by *inhA* gene [46,47]. The isoniazid resistance is acquired by the *katG* or *inhA* gene. Most common resistance mechanism is identified as the katG S315T leads to decrease isoniazid-NADH substrate affinity. Mutation occurring in *inhA* gene is cross resistance to ethionamide. This mechanism is associated with (Drug target overexpression) high-level isoniazid resistance in MDR isolates [48-50].

Rifampicin

Rifampicin act on fast growing bacilli through slowing their metabolism. Mechanically this antibiotic bind to the β-subunit of RNA polymerase, inhibiting the elongation of m-RNA in bacterium. The bacilli acquire mutation in 507-533 (rifampicin resistance-determining region) of the *rpoB* gene that codes for β-subunit of RNA polymerase [51]. Resultant conformational changes (Drug targ*et al*teration) decrease the affinity for the drug and result in development of resistance. Rifampicin resistance always occurs in conjugation with other drugs most commonly isoniazid making rifampicin targets a surrogate marker of the MDR phenotype [52].

Ethambutol

Ethambutol acts as bacteriostatic agent against multiplying bacilli interfering with the biosynthesis of arabinogalactan in the cell wall of bacterium. Mutation in codon 306 of *embB* gene lead to emergence of resistant mechanism to ethambutol in tubercle bacilli. *embLAB* is a specific operon system which recognized for coding of arabinose transferase enzyme which involve in arabinogalactan synthesis [53-56]. Experimental study shows certain amino acid substitutions lead to ethambutol resistance [57]. Decaprenyl phosphoryl-β-D-arabinose biosynthetic pathway and utilization pathway are disturbed by the mutation occurring in *embB* and *embC* causes variable MIC range for ethambutol. Alteration in embB gene does not cause high level of ethambutol resistance. Mutation in *ubiA* gene that encodes for decaprenyl-phosphate 5- phospho- ribosyl transferase synthase (which involved in cell wall synthesis). A embB gene reported, that cause ethambutol high resistance [58,59].

Fluoroquinolones

In *M. tuberculosis* type 2 topoisomerase (DNA gyrase) is formed by two α and β subunits, coded by *gyrA* and *gyrB*, which catalyzes the supercoiling of DNA. The fluoroquinolone group includes, old generation drugs ciprofloxacin and ofloxacin are synthetic derivatives of nalidixic acid governs the resistance by chromosomal mutation associated with quinolone resistance-determining region of *gyrA* and *gyrB* gene. Both the antibiotics prevents the transcription during cell replication [60,62]. A recent analysis revealed low level resistance to new generation fluoroquinolones [63].

Kanamycin, Amikacin and Capreomycin, Viomycin

In *M. tuberculosis* both, aminoglycosides group include kanamycin, amikacin and cyclic peptide with capreomycin and viomycin inhibits the protein synthesis by their mode of action on 16S rRNA. These drugs are use as second line drugs in treatment of drug resistant TB [64]. 1400bp region of Rs gene mutation is common, found against kanamycin and amikacin resistance mechanism. However, capreomycin and viomycin binds with the same site of ribosomes. These antibiotic shows the resistance mechanism by the mutation in *tlyA* gene that codes rRNA methyltransferase that show specificity for the 2'-O-methylation of ribose in rRNA. Mutation in *tlyA* loses the methylation activity [65]. Cross-resistance in kanamycin, amikacin and capreomycin has also reported. Inhibition of translation by drugs lead to cross resistance between them is occur likely. The bacterium conserves an Eis gene which codes for aminoglycoside acetyltransferase, signifies a genetic alteration in the promotor of this gene. Enzyme, aminoglycoside acetyltransferase produces an overexpression of protein and figure out the low-level of resistance to kanamycin but not amikacin. About 80% clinical isolates evaluated and having low-level resistance to kanamycin had mutations in the Eis promoter [66,67].

Pyrazinamide

Pyrazinamide converted to pyrazino acid an active form by an enzyme pyrazinamide or nicotinamides (PZase) [68]. This drug has ability to inhibit dormant bacilli in acidic environment such as TB lesions [69,70]. Pyrazinamide is analogy of nicotinamide and used for treatment that can reduce therapy length from 9 to 6 months. Pyrazinamide enters the bacterium via passive diffusion and then converted into pyrazino acid by PZase, which mainly inhibit the membrane transportation by disrupting membrane energetics. Previous studies have shown that pyrazino acid excreted by the efflux pump. Under acidic condition the protonated pyrazino acid is reabsorbed and accumulate inside the cell due to an inefficient efflux pump, resulting cellular damage [71]. PZase is encoded by the gene pncA in bacterium and the mutations occur in a 561bp region of open reading frame or in an 82bp region of its promotor region [72-74]. However, mutation in pncA or its promotor region didn't show in some Paz resistant strain [75].

P-Amino Salicylic Acid

Para-amino salicylic acid, an analogue of para-amino benzoic acid, was first used in treatment of tuberculosis in combination with the isoniazid and streptomycin [76]. P-amino salicylic acid may compete with P-amino benzoic acid for dihydropteroate synthase, an enzyme required for folate

biosynthesis. mutations occurring in the *tlyA* gene, accounting for 40% of para-amino salicylic acid resistance resulting in decreased enzyme activity. A recent study demonstrated that mutations in *folC*, which encodes dihydrofolate synthase, conferred resistance in clinical isolates [77]

Table 3: List of most common targets of chromosomal mutation conferring drug resistance in *M. tuberculosis*

Antibiotics	Target gene	Resistance mechanism	References
isoniazid	katG	Prodrug activation	78
	inhA	Drug target alteration	79,80,81
	inhA promotor	Drug target overexpression	80,81
Rifampicin	rpoB	Drug target alteration	82
Ethambutol	embB	Drug target alteration	83
Fluoroquinolones	gyrA/B	Drug target alteration	84,85
Kanamycin A	rrs	Drug target alteration	86
	eis promotor	Overexpression of drug and inactivating enzyme	87
Amikacin	rrs	Drug target alteration	86,88
Capreomycin	rrs	Drug target alteration	88
	tlyA	Drug target methylation	89,90
Pyrazinamide	pncA	Abrogated prodrug activation	91
P-aminosalicylic acid	tlyA	Drug target bypassing	92,93
	folC	Abrogated prodrug activation	92,93
Linezolid	rplC	Drug target alteration	94
	rrl	Drug target alteration	95
Clofazimine	Promotor/mmpR	overexpression of efflux pump Mmpl5	96,97
Bedaquiline	Promotor/mmpR	overexpression of efflux pump Mmpl5	96,97
	atpE	Drug target alteration	98

CONCLUSION

To tackle resistance in Mycobacteria, we need to developed new antibiotics for world health sector because antimicrobial resistance emergence, challenge the antibiotics therapy which is given to patient at a time of treatment. The problem of resistant mutants is a challenging and increasing the rate of death is a miserable for the entire globe. The resistant mechanism can reduce by the improving techniques or by initiating the drug designing which can be used in treating of those mutation that acquired by the bacterium. Now various methods like GENE MAPPING, NGS (Next Generation Sequencing) are available to diagnose of tuberculosis disease in the patient. Amino Acid Sequencing technique is useful tool to detect the mutated gene in codons. The problem of resistant mechanism can be avoided by the Chromatography by separating the protein molecules or amino acid from the mutant genes that acquired by the bacterium. Detection of proteins from a muted gene can be treated with the adjuvants for emphasizing the production of immunoglobulins, to prevent the immune system because the patient does not die due to the tuberculosis but mainly the rate of death becomes more higher in case of immunodeficiency disease like AIDS due to poor immune system. Developing a new formulation of drugs that can actually works on mutant gene can be the solution to avoid the resistance mechanism in therapy of tuberculosis.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. "Tuberculosis (TB)". www.who.int. Retrieved 8 May 2020.
2. "Basic TB Facts". Centers for Disease Control and Prevention (CDC). 13 March 2012. Archived from the original on 6 February 2016. Retrieved 11 February 2016.
3. MacPherson P, Lebina L, Motsomi K, Bosch Z, Milovanovic M, Ratsela A, *et al*. Prevalence and risk factors for latent tuberculosis infection among household contacts of index cases in two South African provinces: Analysis of baseline data from a cluster-randomized trial. Plops One. 2020;15(3):e0230376.
4. Madigan, Michael T., *et al*. Brock Biology of Microorganisms: Thirteenth edition. Benjamin Cummings: Boston, 2012. Print.
5. Fu LM, Fu-Liu CS. Is Mycobacterium tuberculosis a closer relative to Gram-positive or Gram–negative bacterial pathogens? Tuberculosis. 2002;82(2-3):85-90.
6. World Health Organization. Global Tuberculosis Report 2016. Geneva, 2016.
7. CDC, Management of person exposed to multidrug- resistant tuberculosis. MMWR1992:41[No. RR-11]:59-71.
8. Wright A, Bai G, Barrera L, Boilable F, Martin-Casaubon N, Gilpin C, *et al*. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs--worldwide, 2000-2004. Morbidity and Mortality Weekly Report. 2006;55(11):301-6.
9. World Health Organization. Multidrug and extensively drug-resistant TB (M. World Health Organization. 2010.
10. Shah NS, Richardson J, Moodley P, Moodley S, Babaria P, Ramathan M, *et al*. Increasing drug resistance in extensively drug-resistant tuberculosis, South Africa. Emerging infectious diseases. 2011;17(3):510-13.
11. Schatz A, Bugie E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. 1944 [published correction appears in Clin Orthop Relat Res. 2005 Dec;441:379]. *Clin Orthop Relat Res*. 2005;(437):3-6.
12. Grofton J, Mitchison DA. Brit. Med. J. 1948(4588):1009.
13. Lehmann J. Para-amino salicylic acid in the treatment of tuberculosis. Lancet. 1946:15-6.
14. Medical Research Council. Treatment of pulmonary tuberculosis with streptomycin and para-amino-salicylic acid: a Medical Research Council Investigation. BMJ 1950;2:1073–85.
15. British Thoracic and Tuberculosis Association. Short-course chemotherapy in pulmonary tuberculosis. Lancet 1975;305:119–24.
16. 16. World Health Organization. TB: WHO Report on the Tuberculosis Epidemic 1997. Geneva, 1997.

17. Comas I, Borrell S, Roetzer A, Rose G, Malla B, Kato-Maeda M, Galagan J, Niemann S, Gagneux S. Whole-genome sequencing of rifampicin-resistant Mycobacterium tuberculosis strains identifies compensatory mutations in RNA polymerase genes. Nature genetics. 2012;44(1):106-10.
18. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature. 1992;358(6387):591-93.
19. Rawat R, Whitty A, Tonge PJ. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the Mycobacterium tuberculosis enoyl reductase: adduct affinity and drug resistance. Proceedings of the National Academy of Sciences. 2003;100(24):13881-6.
20. Heym B, Alzari PM, Honore N, Cole ST. Missense mutations in the catalase-peroxidase gene, katG, are associated with isoniazid resistance in Mycobacterium tuberculosis. Molecular microbiology. 1995;15(2):235-45.
21. Brennan PJ, Nikaido H. The envelope of mycobacteria. Annu Rev Biochem. 1995;64:29-63.
22. Jarlier V, Nikaido H. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. FEMS Microbial Lett. 1994;123:11–8.
23. Janardhan S, Ram Vivek M, Narhari Sastry G. Modeling the permeability of drug-like molecules through the cell wall of Mycobacterium tuberculosis: an analogue based approach. Mol BioSyst. 2016;12:3377–84.
24. Marrakech H, Lanelle MA, Daffé M. Mycolic acids: structures, biosynthesis, and beyond. Chemistry & biology. 2014;21(1):67-85.
25. Siroy A, Mailaender C, Harder D, Koerber S, Wolschendorf F, Danilchanka O, *et al*. Rv1698 of Mycobacterium tuberculosis represents a new class of channel-forming outer membrane proteins. Journal of Biological Chemistry. 2008;283(26):17827-37.
26. Song H, Sandie R, Wang Y, Andrade-Navarro MA, Niederweis M. Identification of outer membrane proteins of Mycobacterium tuberculosis. Tuberculosis. 2008;88(6):526-44.
27. Abate G, Hoffner SE. Synergistic antimycobacterial activity between ethambutol and the beta-lactam drug cefepime. Diagn Microbiol Infect Dis. 1997;28(3):119-22.
28. Bosne-David S. Intrinsic resistance of Mycobacterium tuberculosis to clarithromycin is effectively reversed by subinhibitory concentrations of cell wall inhibitors. J Antimicrobe Che moth. 2000;46:3915.
29. Abraham EP, Chain E, Fletcher CM, Florey HW, Gardner AD, Heatley NG, *et al*. Further observations on penicillin. 1941. European journal of clinical pharmacology. 1992;42(1):3-9.
30. Wang F, Cassidy C, Schettino JC. Crystal structure and activity studies of the Mycobacterium tuberculosis -lactamase reveal its critical role in resistance to -lactam antibiotics. Antimicrobe Agents Ch. 2006;50:2762–71.
31. Cohen KA, El-Hay T, Wires KL, Weisbord O, Munsamy V, Yanover C, *et al*. Paradoxical Hyper susceptibility of Drug-resistant Mycobacterium tuberculosis to β-lactam Antibiotics. Biomedicine. 2016;9:170-9.
32. Wolfe MS, Borchardt RT. S-adenosyl-L-homocysteine hydrolase as a target for antiviral chemotherapy. J Med Chem. 1991;34:1521–30.
33. Schnapp Inger D, Ehrt S, Voskuhl MI, Liu Y, Mangan JA, Monahan IM, *et al*. Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: insights into the phagosome environment. The Journal of experimental medicine. 2003;198(5):693-704.
34. Hotter B, Dick T. Increased alanine dehydrogenase activity during dormancy in Mycobacterium smegmatis. FEMS Microbiol Lett. 1998;167(1):7-11.
35. Tuckman D, Donnelly RJ, Zhao FX, Jacobs WR Jr, Connell ND. Interruption of the phosphoglucose isomerase gene results in glucose auxotroph in Mycobacterium smegmatis. J Bacterial. 1997;179(8):2724-30.
36. Zaunbrecher MA, Sikes Jr RD, met chock B, Shin nick TM, Posey JE. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences. 2009;106(47):20004-9.
37. Houghton JL, Green KD, Pricer RE, Mayhoub AS, Garneau-Tsodikova S. Unexpected N-acetylation of capreomycin by mycobacterial Eid's enzymes. Journal of Antimicrobial Chemotherapy. 2013;68(4):800-5.
38. LaMichael G, Tyagi S, Bisha WR. Designer arrays for defined mutant analysis to detect genes essential for survival of Mycobacterium tuberculosis in mooselings. Infect Immun. 2005;73:2533–40.
39. Louw GE, Warren RM, Gey van Pittius NC, McEvoy CR, Van Halden PD, Victor TC, *et al*. A balancing act: efflux/influx in mycobacterial drug resistance. Antimicrobe Agents Che's mother. 2009;53(8):3181-89.
40. 40. Louw GE, Warren RM, Gey van Pittius NC, *et al*. Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant Mycobacterium tuberculosis through efflux. Am J Respir Crit Care Med. 2011;184(2):269-276. doi:10.1164/rccm.201011-1924OC
41. Paulsen IT, Chen J, Nelson KE, Saier Jr MH. Comparative genomics of microbial drug efflux systems. Journal of molecular microbiology and biotechnology. 2001;3(2):145-50.
42. Anthony Malinga L, Stoltz A. Efflux pump mediated second-line tuberculosis drug resistance. Mycobacterial Dis. 2016;6:2161-1068.
43. Berger-Bächi B. Resistance Mechanisms of Gram-Positive Bacteria. International Journal of Medical Microbiology. 2002.292:27-35.
44. Lambert PA. Bacterial resistance to antibiotics: modified target sites. Adv Drug Deliv Rev. 2005;57(10):1471-85.
45. Baranova K, Doucet-Populaire F, Dorson O, Gondar A, Ganassi JC, Weiser J, *et al*. Molecular basis of intrinsic macrolide resistance in the Mycobacterium tuberculosis complex. Antimicrobial agents and chemotherapy. 2004;48(1):143-50.
46. Palomino JC, Martin A. Drug Resistance Mechanisms in Mycobacterium tuberculosis. Antibiotics (Basel). 2014;3(3):317-40.
47. Almeida Da Silva PE, Palomino JC. Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. J Antimicrobe Che mother. 2011;66(7):1417-30.
48. Ramaswamy SV, Reich R, Dou SJ, Jaspers L, Pan X, Wanger A, *et al*. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2003;47(4):1241-50.
49. Hazbón MH, Francombe M, Bobadilla del Valle M, Cava tore M, Guerrero MI, Varma-Basil M, *et al*. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2006;50(8):2640-9.
50. Banerjee A, Dubna E, Quemada A, Balasubramanian V, Um KS, Wilson T, *et al*. InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263(5144):227-30.
51. Telenti A, Imboden P, Marchesi F, Schmid Heini T, Bodner T. Direct, automated detection of rifampin-resistant Mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrobial agents and chemotherapy. 1993;37(10):2054-8.
52. Traore H, Fossette K, Bastian I, Devleeschouwer M, Portales F. Detection of rifampicin resistance in Mycobacterium tuberculosis isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. The international journal of tuberculosis and lung disease. 2000;4(5):481-4.
53. Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. Antimicrobe Agents Che's mother. 1989;33(9):1493-99.
54. Makarova K, Slay den RA, Basra GS, Brennan PJ. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrobial agents and chemotherapy. 1995;39(11):2484-9.
55. Telenti A, Philipp WJ, Srivatsa S. The ebb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. Nat Med. 1997;3(5):567-70.
56. Srivatsa S, Stock Bauer KE, Pan XI, Kreis Wirth BN, Mogwase SL, Jacobs Jr WR, *et al*. Ethambutol resistance in Mycobacterium tuberculosis: critical role of ebb mutations. Antimicrobial agents and chemotherapy. 1997;41(8):1677-81.
57. Safi H, Sayers B, Hazbón MH, Aland D. Transfer of ebb codon 306 mutations into clinical Mycobacterium tuberculosis strains alters susceptibility to ethambutol, isoniazid, and rifampin. Antimicrobial agents and chemotherapy. 2008;52(6):2027-34.
58. Safi H, Linga Raju S, Amin A, Kim S, Jones M, Holmes M, *et al*. Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenyl phosphoryl-β-D-arabinose biosynthetic and utilization pathway genes. Nature genetics. 2013;45(10):1190-7.
59. He L, Wang X, Cui P, Jinn J, Chen J, Zhang W, *et al*. ubiA (Rv3806c) encoding DPPR synthase involved in cell wall synthesis is associated with ethambutol resistance in Mycobacterium tuberculosis. Tuberculosis. 2015;95(2):149-54.
60. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis. World Health Organization; 2016.
61. Fabregas A, Madura S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. Microbial biotechnology. 2009;2(1):40-61.
62. Aubrey A, Pan XS, Fisher LM, Jarlier V, Cambium E. Mycobacterium tuberculosis DNA gyrase: interaction with quinolones and correlation with antimycobacterial drug activity. Antimicrobial agents and chemotherapy. 2004;48(4):1281-63.
63. Zignol M, Dean AS, Alikhanova N, Andres S, Cabibbe AM, Cirillo DM, *et al*. Population-based resistance of Mycobacterium tuberculosis isolates to pyrazinamide and fluoroquinolones: results from a

multicountry surveillance project. The Lancet infectious diseases. 2016;16(10):1185-92.
64. Palomino JC, Martin A. Drug Resistance Mechanisms in Mycobacterium tuberculosis. Antibiotics (Basel). 2014;3(3):317-40.
65. Monshupanee T, Johansen SK, Dahlberg AE, Douthwaite S. Capreomycin susceptibility is increased by TlyA-directed 2'-O-methylation on both ribosomal subunits. Mol Microbiol. 2012;85(6):1194-03.
66. Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, *et al*. Molecular detection of mutations associated with first-and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2011;55(5):2032-41.
67. Zaunbrecher MA, Sikes Jr RD, Metchock B, Shin nick TM, Posey JE. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences. 2009;106(47):20004-9.
68. Konno K, Feldmann FM, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am Rev Respir Dis. 1967;95(3):461-69.
69. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. Tubercle. 1985;66:219–25.
70. Mitchison DA. Basic mechanisms of chemotherapy. Chest J. 1979;76(6):771–81.
71. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuber Lung Dis. 2003;7(1):6-21.
72. Scorpio A, Zhang Y. Mutations in pence, a gene encoding pyrazinamide/nicotinamide, cause resistance to the ant tuberculous drug pyrazinamide in tubercle bacillus. Nat Med. 1996;2:662–7.
73. Heifetz SA, Gilman R, Siddiqi S, Cinnamon M, Zhang Y. Characterization of pence mutations in pyrazinamide-resistant Mycobacterium tuberculosis. Antimicrobe Agents Che's mother. 1997;41:540-3.
74. Jurien P, Wenger J, Toro JC, Hoffner S. Pyrazinamide resistance and pence gene mutations in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2008;52(5):1852-4.
75. Cheng SJ, Hibbert L, Sanchez T, Heifets L, Zhang Y. pence mutations as a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis: spread of a nonresistant strain in Quebec, Canada. Antimicrobial agents and chemotherapy. 2000;44(3):528-32.
76. Lehmann J. Para-amino salicylic acid in the treatment of tuberculosis. Lancet. 1946:15-6.
77. Zhao F, Wang XD, Erber LN, Luo M, Guo AZ, Yang SS, *et al*. Binding pock*et al*terations in dihydrofolate synthase confer resistance to para-amino salicylic acid in clinical isolates of Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2014;58(3):1479-87.
78. Heym B, Alzari PM, Honore N, Cole ST. Missense mutations in the catalase-peroxidase gene, katG, are associated with isoniazid resistance in Mycobacterium tuberculosis. Molecular microbiology. 1995;15(2):235-45.
79. Banerjee A, Dubnau E, Quemada A, Balasubramanian V, Um KS, Wilson T, *et al*. InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263(5144):227-30.
80. Morlock GP, Metchock B, Sikes D, Crawford JT, Cooksey RC. ether, InhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. Antimicrobial agents and chemotherapy. 2003;47(12):3799-805.
81. Hazbón MH, Francombe M, Bobadilla del Valle M. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrobe Agents Che's mother. 2006;50(8):2640-49.
82. Telenti A, Imboden P, Marchesi F, Matter L, Schafer K, Bodner T, *et al*. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. The Lancet. 1993;341(8846):647-51.
83. Srivatsa S, Stock Bauer KE, Pan XI, Kreis Wirth BN, Mogwase SL, Jacobs Jr WR, *et al*. Ethambutol resistance in Mycobacterium tuberculosis: critical role of ebb mutations. Antimicrobial agents and chemotherapy. 1997;41(8):1677-81.
84. Tariff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreis Wirth B, *et al*. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrate and garb genes and detection of quinolone resistance mutations. Antimicrobial agents and chemotherapy. 1994;38(4):773-80.
85. Malik S, Will by M, Sikes D, Tsodikova OV, Posey JE. New insights into fluoroquinolone resistance in Mycobacterium tuberculosis: functional genetic analysis of gyrA and gyrB mutations. Plops one. 2012;7(6):e39754.
86. Analgene GJ, Kreis Wirth BN, Aoudad A, Khetarpal M, Igno FR, Mogwase SL, *et al*. Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 1998;42(5):1295-97.
87. Kambili P, Ajani K, Nika C, Samdani M, Shetty A, Wadia Z, *et al*. Correlating errs and eis promoter mutations in clinical isolates of Mycobacterium tuberculosis with phenotypic susceptibility levels to the second-line injectables. International journal of mycobacteriology. 2016;5(1):1-6.
88. Maus CE, Plicates BB, Shin nick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrobe Agents Ch. 2005;49:3192–7.
89. Maus CE, Plicates BB, Shin nick TM. Mutation of TlyA confers capreomycin resistance in Mycobacterium tuberculosis. Antimicrobe Agents Ch. 2005;49:571–7.
90. Monshupanee T, Johansen SK, Dahlberg AE, Douthwaite S. Capreomycin susceptibility is increased by TlyA-directed 2'-O-methylation on both ribosomal subunits. Mol Microbiol. 2012;85(6):1194-03.
91. Scorpio A, Zhang Y. Mutations in pence, a gene encoding pyrazinamide/nicotinamide, cause resistance to the antituberculousdrug drug pyrazinamide in tubercle bacillus. Nature medicine. 1996;2(6):662-7.
92. Zhao F, Wang XD, Erber LN, Luo M, Guo AZ, Yang SS, *et al*. Binding pock*et al*terations in dihydrofolate synthase confer resistance to para-amino salicylic acid in clinical isolates of Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2014;58(3):1479-87.
93. Minato Y, Thieve JM, Korus SL, McIlveen EJ, Turman BJ, Baugh AD, *et al*. Mycobacterium tuberculosis folate metabolism and the mechanistic basis for para-amino salicylic acid susceptibility and resistance. Antimicrobial agents and chemotherapy. 2015;59(9):5097-106.
94. Becker P, Hilleman D, Kohl TA, Kalinowski J, Richter E, Niemann S, *et al*. plc. T460C identified as a dominant mutation in linezolid-resistant Mycobacterium tuberculosis strains. Antimicrobial agents and chemotherapy. 2012;56(5):2743-5.
95. Hilleman D, Rusch-Gerdes S, Richter E. In Vitro-Selected linezolid-resistant Mycobacterium tuberculosis mutants. Antimicrobe Agents Ch 2008;52:800–1.
96. Harthorne RC, Uplekar S, Cole ST. Cross-resistance between clofazimine and bed aquiline through upregulation of MmpL5 in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2014;58(5):2979-81.
97. Bloomberg GV, Gagneux S, Böttger EC. Acquired resistance to bed aquiline and dexaminid in therapy for tuberculosis. The New England journal of medicine. 2015;373(20):1986.
98. Huitric E, Ver Hasselt P, Koul A, Andries K, Hoffner S, Andersson DI, *et al*. Rates and mechanisms of resistance development in Mycobacterium tuberculosis to a novel diarylquinoline ATP synthase inhibitor. Antimicrobial agents and chemotherapy. 2010;54(3):1022-8.