Chapter

Mechanisms and Action of Drug Resistance on *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) remains the most challenging infection to treat worldwide. The contemporary TB regimens consist of 6–9 months of daily doses of four drugs in the existing regimen that is extremely toxic to patients. The purpose of these longer treatments is to eliminate *Mycobacterium tuberculosis*, notorious for its ability to resist most antimycobacterial drugs, thereby preventing the formation of drug-resistant clinical strains. On the contrary, prolonged therapies have led to impoverished patient adherence. Furthermore, the severe limitations of drug choices have resulted in the emergence of drug-resistant strains. Unfortunately, the lack of great lethargy toward developing effective antituberculosis regimens with a large-scale prevalence rate is a tremendous challenge to controlling the pandemic. In fact, the current improvement in genomic studies for early diagnosis and understanding of drug resistance mechanisms, and the identification of newer drug targets, is remarkable and promising. Identifying genetic factors, chromosomal mutations, and associated pathways give new hope to current antituberculosis drug discovery. This focused review renders insights into understanding molecular mechanisms underlying the profound drug resistance. This knowledge is essential for developing effective, potent antibiotics against drug-resistant strains and helps shorten the current treatment courses required for drug-susceptible tuberculosis.

Keywords: chromosomal mutations, tuberculosis, arabinogalactan, peptidoglycan, mycolicacids

1. Introduction

Tuberculosis (TB) is caused by the grievous pathogen *Mycobacterium tuberculosis*, which has infected about one-third of the world's population. Although the TB incidence has declined over the past decades, there were an estimated 10.4 million new cases in 2021, of which 0.48 million were caused by *M.tuberculosis* strains classified as multidrug-resistant [1, 2]. Multidrug-resistant TB (MDR-TB) is a major global public

health problem that has threatened progress in TB care and prevention in recent decades. Two mechanisms make it possible to develop drug-resistant tuberculosis (DR-TB). Firstly, acquired drug-resistant tuberculosis occurs when TB treatment is suboptimal due to insufficient policies and failures of health systems and care provision, lousy quality of tuberculosis drugs, bad prescription practices, patient nonadherence, or a combination of the above [3]. Secondly, primary drug resistant-tuberculosis results from the direct transmission of drug resistant-tuberculosis from one person to another. About 3.6% of new cases and 17.0% of previously treated tuberculosis cases were estimated to have had multi-drug resistant tuberculosis (MDR-TB) or rifampicin-resistant tuberculosis (RR-TB) in 2021. Each year MDR-TB or RR-TB accounts for about 580,000 new cases and approximately 230,000 deaths worldwide. The global burden of multi-drug or rifampicin-resistant TB (MDR/RR-TB) remains stable, and it is estimated that 18% of previously treated cases and 3.3% of new TB patients will have MDR/RR-TB in 2021 [4].

The prevalence of drug-resistant tuberculosis (DR-TB) is becoming a global challenge to tuberculosis (TB) control programs. Nowadays, global TB control activities are challenged by the emergence of drug resistance to more antituberculosis drugs resulting in multi-drug resistance tuberculosis (MDR-TB), pre-extensively drug-resistant (Pre-XDR-TB), and extensively drug-resistant (XDR-TB) tuberculosis. Tuberculosis is caused by *M. tuberculosis* strains resistant to at least one antituberculosis drug, mono-resistance. In addition, tuberculosis is resistant to at least two or more antituberculosis drugs other than rifampicin, known as poly-tuberculosis resistance. Multi-drug resistance tuberculosis is resistant to at least two potent anti-TB drugs, rifampicin and isoniazid. Pre-extensively drug-resistant tuberculosis is MDR tuberculosis plus resistant to any one fluoroquinolone drug or any one of the second-line injectable antituberculosis drugs. Finally, extensively drug-resistant tuberculosis is MDR tuberculosis plus resistant to anyone fluoroquinolone drug-resistant and at least one of three injectable second-line drugs resistant (i.e., amikacin, kanamycin, or capreomycin). More recently, a more worrying situation has emerged with the description of resistance to all antituberculosis drugs available for testing, a condition labeled as totally drug-resistant (TDR-TB) tuberculosis [5, 6].

2. Mechanisms of drug resistance

M.tuberculosis has natural defenses against some drugs and can acquire drug resistance through genetic mutations. This is because the bacterium cannot transfer genes for resistance between organisms through plasmids. Some mechanisms of drug resistance include:

1. Cell wall: The complex lipid molecules in the cell wall of *M.tuberculosis* act as a barrier to stop drugs from entering the cell.

2. Drug modifying and inactivating enzymes: The target gene encodes for enzymes (proteins) that deactivate drug molecules. These enzymes are usually phosphorylated, adenylate or acetylate drug compounds.

3. Drug efflux systems: The cell wall of *M. tuberculosis* contains molecular systems that actively expel the drug molecules out of the cell.

4. Mutations: Spontaneous mutations in the genome can make to change the drug target proteins, making drug-resistant strains.

The mycobacterial cell wall consists of arabinogalactan polysaccharide, peptidoglycan, and long-chain mycolic acids. This cell wall-based permeability barrier contributes to the resistance of mycobacteria to many antibiotics, and defects in lipids would damage the function of the cell wall as a barrier and increase the sensitivity to various antimycobacterial drugs. The cell wall enzymes involved play an essential role in developing drug resistance in mycobacterium. The intrinsic resistance of mycobacterial species to most antibiotics is generally attributed to the low permeability of the cell wall. Certain antibiotics may be cleaved or altered structurally to render them ineffective after penetrating the cell envelope [7].

3. Intrinsic drug resistance in *Mycobacterium tuberculosis*

The inherent resistance mechanism of *M.tuberculosis* is not only to the existing tuberculosis drugs but also to recently introduced drugs (**Figure 1**). The drug-resistant mechanisms of *M.tuberculosis* are broadly classified into two categories, such as inherent and acquired for passively neutralizing the activity of already applied antituberculosis drugs. Besides obtaining new resistance through chromosomal mutations, *M. tuberculosis* is endowed with an array of inherent resistance mechanisms that allow

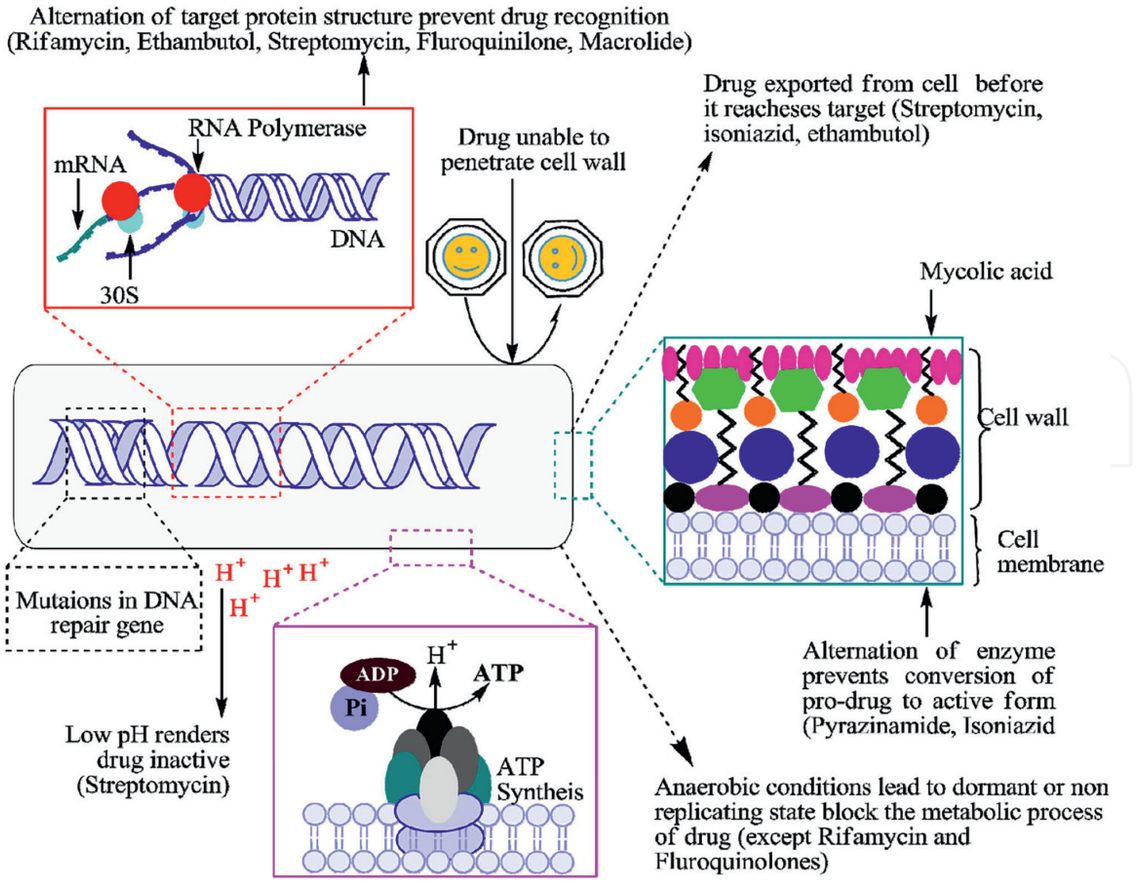

Figure 1.
A diagrammatic representation of the drug-resistant mechanism of M.tuberculosis [3].

active neutralization of drug actions. However, these resistance mechanisms provide a high degree of resistance background that limits the application of available drugs to tuberculosis treatment and hampers the development of new drugs. Therefore, the inherent drug resistance of *M.tuberculosis* can be classified into passive resistance and specialized resistance mechanisms [3].

3.1 Mycobacterial cell wall and drug penetration

The intrinsic resistance of mycobacteria against various classes of antibiotics has commonly been assigned to the unusual structural composition of the mycobacterial cell envelope. The cell wall of the mycobacterium genus is much thicker and more hydrophobic due to the presence of a wide array of lipids, including mycolic acids, than the other gram-negative bacteria. Many studies performed in different mycobacterial species demonstrated that the composition of the cell envelope and the low numbers of porins contribute significantly to the cell envelope's low compound permeability. The lipid layer of the cell wall constituent in mycobacterium organism is linked covalently to the peptidoglycan layer by way of arabinogalactan [8]. Furthermore, the cell wall of mycobacterium contains "extractable" immunogenic glycolipids. The lipid-rich nature of the mycobacterium cell wall renders it extremely hydrophobic and prevents the permeation of hydrophilic drug compounds. Therefore, tiny hydrophilic drug compounds, including active drugs against *M. tuberculosis*, can only traverse the cell wall through water-filled porins. Indeed the heterologous expression of the *M.smegmatis* porin MspA in *M.tuberculosis* decreases the minimal inhibitory concentration (MIC) for various hydrophilic drugs, indicating that porins might play an indispensable role in the diffusion of hydrophilic drugs across the cell wall. However, reports on the presence of porins in *M. tuberculosis* were still lacking. The protein CpnT in the outer membrane channel was demonstrated to be involved in the nutrient uptake in *tuberculosis* and *M.bovis* BCG, mediating susceptibility to nitric oxide and antibiotics in *M.bovis* BCG. The presence of CpnT protein in the *M.tuberculosis* clinically isolates under positive selection is demonstrated by the over-representation of non-synonymous mutations in the gene encoding CpnT protein (Rv3903c). However, the crucial role of CpnT protein in mediating drug susceptibility to the hydrophilic drug in *M.tuberculosis* needs further investigation, as deletion mutation in CpnT protein does not demonstrate drug resistance phenotypes in vitro. However, the studies confirmed the existence of porins in the outer membrane of *M.tuberculosis* and their role in the uptake of tiny hydrophilic drug compounds. Furthermore, the cell wall lipids' physical nature limits the membrane's fluidity [9]. The cell wall of mycobacterium targeting all antituberculosis drugs has been recorded to be ineffective due to the development of resistance by mutation with individual genetic factors, as depicted in **Tables 1–3**.

3.2 Drug inactivation by *M. tuberculosis*

After penetrating the cell wall of mycobacterium, antibiotics may be cleaved enzymatically to make them inefficient in functions. Besides drug cleavage, antibiotics may be inactivated by modification (methylation or acetylation). Until today the crucial drug inactivation mechanism by chemical/enzymatic modification in *M. tuberculosis* is the acetylation of aminoglycoside/cyclic peptide by the enhanced intracellular survival protein (Eis). Various promotor mutations identified in clinical *M. tuberculosis* isolates lead to overexpression of Eis. This confers low-level resistance

Drug	Class(activity type)	Inhibition target (associated action)	Genetic factor	Frequency (%)	Associated function	Drug resistance
Streptomycin	Aminoglycoside (bacteriostatic)	30S, 16S ribosomal protein and 7-methyl guanosine methyltransferase (inhibition of arabinogalactan and protein synthesis)	*gpsI*, *rpsL*, *rrs*, *gidB*	~6 <10 To be determined	Involved in mRNA degradation Translation initiation step Synthesis of stable RNAs probable glucose-inhibited division protein B	Variation in drug target binding site due to mutations
Isoniazid	Isonicotinic acid (bactericidal)	Enoyl-(acyl-carrier-protein) reductase including, catalase peroxidase, NADH-dependent enoyl ACP, 3-Oxoacy ACP, βKetoacyl ACP) (inhibition of cell wall synthesis)	*katG*, *inhA*, *kasA*, *Ndh* *AhpC*, *niA*, *FadE24*, *FabG1*	70 ~10 ~10	Intracellular survival Mycolic acid biosynthesis Fatty acid biosynthesis Electron transference from NADH to the respiratory chain Defense from oxidative stress Associated with efflux pump Degradation of lipid and fatty acid Fatty acid biosynthesis pathway	Modification overexpression of drug target due to mutations and altered efflux pump activity and pro-drug conversion
Rifampicin	Rifamycin (bactericidal)	RNA polymerase, β-subunit (inhibition of RNA synthesis)	*rpoB* *rpoA* *rpoC*	95	Catalyze the transcription for DNA into RNA synthesis	Modification in drug target due to mutations
Pyrazinamide	Pyrazine (bactericidal/ bacteriostatic)	Pyrazinamidase; ribosomal protein 1 30S ribosomal subunit, cytoplasm	*pncA* *rpsA* *panD* *clpC1*	~99 No evidence No evidence	To converts amides into acid Translate mRNA with a shine dalgarno purine-rich sequence Pantothenate biosynthesis Protein degradation, hydrolyses proteins in the presence of ATP	Abolition of pro-drug conversion mechanism
Ethambutol	Ethylenediamine (bacteriostatic)	Arabinosyl transferase (inhibition of arabinogalactan synthesis)	*embA* *embB* *embC* *embR* *rmLD* *iniA* *ubiA*	~70 ~45 occurs with *embB*	Associated with biosynthesis of the mycobacterial cell wall embCAB operon synthesis regulator dTDP-L-rhamnose biosynthesis Associated with efflux pump	Change and overexpression of drug target; and altered efflux pump activity

Table 1.
Genetic factors involved in first-line anti-TB drug resistance in Mycobacterium tuberculosis *[3, 10, 11].*

Drug	Class (activity type)	Inhibition target (associated action)	Genetic factor	Frequency (%)	Associated function	Drug resistance
Moxifloxacin Gatifloxacin	Quinolones/8-methoxy fluroquinolone (bactericidal)	DNA gyrase and DNA topoisomerase (Inhibits DNA synthesis)	*gyrA*, *gyrB*	~90 <5	Negatively supercoils closed circular double-stranded DNA	Alteration of drug target due to mutation
Kanamycin Amikacin	Amino-glycosides (bactericidal)	Inhibition of RNA-dependent synthesis by binding to 30S subunit (inhibition of protein synthesis)	*rrs* *eis* *whiB7* *tlyA*	60–70 ~80 (Low level KAN) ~3(CAP)	Synthesis of stable RNAs Acetylation, intracellular survival Associated with transcription Methylates 16S and 23S rRNA	Mutations on 16S rRNA and overexpression machines
Capreomycin	Cyclic polypeptide (bactericidal)	Inhibition of 50S subunit (inhibition of protein synthesis)	*rrs* *tlyA*	60–70 ~3(CAP)	Synthesis of stable RNAs Acetylation, intracellular survival Methylates 16S and 23S rRNA	Mutation alteration drug target
Ethionamide	Isoconitic acid derivative (bacteriostatic)	Inhibition of mycolic acid synthesis by binding to the ACP reductase InhA (disrupts cell wall biosynthesis)	*ethA* *ethR* *KasA* *inhA* *inhA pro*	mutations occurring in various combinations in these genes account for 96% of ethionamide resistance	Activates the prodrug ethionamide Regulates transcriptional repressor protein EthR Involved in fatty acid biosynthesis Mycolic acid biosynthesis Regulation of expression of inhA	alteration and over-expression drug target due mutation
Para-amino salicylic acid	Para-amino salicylic acid bacteriostatic	Dihydropteroate synthase (inhibits folate and thymine nucleotide metabolism biosynthesis)	*thyA* *folC* *dfrA* *ribD*	~40 To be determined ~90	Deoxyribo-nucleotide biosynthesis Regulate folates to polyglutamate conversion De novo glycine and purine synthesis Involved in riboflavin biosynthesis	Removal of pro-drug conversion procedure
Cycloserine	Serine derivative (bacteriostatic)	Inhibition of peptide-glycan synthesis by blocking dalanine racemase enzyme (inhibition of cell wall synthesis)	*Alr* *Ddl* *Ald* *cycA*		Associated with d-alanine required for cell wall biosynthesis Involved in cell wall formation Associated with cell wall synthesis Transport across the cytoplasmic membrane	Overexpression of resistance gene

Table 2.
Genetic factors involved in second-line anti-TB drug resistance in Mycobacterium tuberculosis *[3, 10, 11].*

Drug	Class (activity type)	Inhibition target (associated action)	Genetic factor		Associated function	Drug resistance
Clofazimine	Iminophenazine derivative (bacteriostatic)	Produces reactive oxygen, inhibits energy production, potassium transporter (inhibition of mycobacterial growth targeting mycobacterial DNA)	rv0678 rv1979c rv2535c *ndh* *pepQ*	~80 (cross Res with BDQ) ~20 To be determined	Transcription repressor for efflux pump MmpL5 Role in the transportation of amino acid Associated/encodes a putative peptidase *pepQ* Associated with oxidation and reduction reaction Possibly hydrolyse peptides	Up-regulation of MmpL5, efflux pump due to mutation
Bedaquiline	Quinoline (bactericidal/ bacteriostatic)	Inhibits the adenosine 5′-triphosphate synthase (inhibition of ATP synthase)	*rv0678* *atpE* *pepQ*	To be determined	Transcription of efflux pump MmpL5 Encodes the c part of the F0 subunit of the ATP synthase Possibly hydrolyze peptides	Mutations on binding site and co-infection
Delamanid Pretomanid	Nitroimidazole (bactericidal)	Obstructs the synthesis of mycolic acid	*fgd1* *fbiC* *fbiA* *fbiB* *ddn*	To be determined	Catalyzes oxidation of glucose-6- phosphate to 6- phosphogluconolactone Participates in a portion of the F420 biosynthetic pathway Required for coenzyme F420 production from FO Required for coenzyme F420 production from FO Converts bicyclic nitroimidazole drug candidate pa-824–3 metabolites	Mutations on reductive activating gene
Linezolid	Oxazolidinone (bactericidal)	50S, 23S ribosomal subunit (inhibition of protein synthesis)	*rplC* *rrl*	~90 1.9–11	Formation of ribosomal peptidyl transferase Formation of stable RNAs	Mutation in 50S ribosomal L3 protein

Table 3.
Genetic factors involved in third-line anti-TB drug resistance in Mycobacterium tuberculosis *[3, 10, 11].*

against kanamycin A but not amikacin. However, it is unclear whether the overexpression of enhanced intracellular survival (Eis) protein alone leads to clinically relevant levels of capreomycin resistance or not [12]. The enhanced intracellular survival (Eis) protein over-expression might be a stepping stone for the evolution of high degree-level aminoglycoside/cyclic peptide resistance. Recently, a novel mechanism of drug deactivation was discovered in *M.tuberculosis* clinical isolates. The pyridobenzimidazole compound "14" is N-methylated, and it is encoded by gene Rv0560c. It has powerful antimycobactericidal activity against *M. tuberculosis* [13]. However, the methylated compound 14 cannot hamper its target, the decaprenylphosphoryl-β-D ribose 2-oxidase (DprE1), involved in synthesizing arabinogalactan [13]. Although this is a novel drug-resistance mechanism in *M. tuberculosis* and bacteria in general, it has unknown clinical relevance to date.

3.3 Drug efflux in *M.tuberculosis*

Efflux systems are essential constituents of bacterial and eukaryotic physiology. The drug efflux mechanism might explain why about 30% of isoniazid and 3% of rifampicin-resistant *M. tuberculosis* isolates do not show resistance due to mutation. However, this unexplained resistance is potentially perplexed by not all mutational known targets of drug resistance. An array of different resistance mechanisms for certain antibiotics, such as isoniazid, is already known. On the other hand, rifampicin resistance is thought to be conferred by mutations in the gene encoding of the drug target, contributing to efflux pumps to unexplained resistance phenotypes more likely.

Furthermore, efflux systems are essential in *M.tuberculosis* for intracellular growth in macrophages. Mycobacterial efflux pumps can expel nearly all first and second-line antituberculous drugs [10]. The expression of efflux pumps can be viewed as a plastic trait, meaning that expression levels are modified via non-mutational processes upon environmental changes. The efflux pumps are induced or upregulated when specific antibiotics or the intracellular environments of a macrophage are present. It has been demonstrated that efflux pumps in *M.tuberculosis* are generated upon the macrophage infections, which concur with increased minimal inhibitory concentrations for isoniazid. A subclass of the strains was resistant to the higher dose of isoniazid at the pinnacle serum concentrations [14]. The expression of the efflux systems is continuous even after the mycobacterial cells have been liberated from the macrophages.

Furthermore, multidrug-resistant *M. tuberculosis* isolates have been shown to express genes involved in drug efflux. The majority of drug-resistant *M.tuberculosis* strains harbor chromosomal mutations linked to drug resistance. However, there are examples of clinically relevant doses of resistance conferred by the "over-expression" of efflux pumps. Mutations in *the MmpR* gene lead to over-expression of the multi-substrate efflux pump Mmpl5, which coincides with cross-resistance to clofazimine and bedaquiline drugs. *MmpR* mutants are likely resistant to isoniazid as *Mmpl5* is also involved in isoniazid extrusion. However, efflux systems might act as a stepping stone for the evolution of higher-level resistance, as convincingly demonstrated by *in vitro* studies. In addition, the efflux pump inhibitors might inhibit bacterial growth and lower the minimal inhibitory concentrations for certain drugs, as efflux pumps seem essential for macrophage infection [15].

4. Acquired resistance mechanisms

Acquired drug resistance is facilitated by the horizontal relocation of mobile genetic elements such as plasmids, transposons, integrons, and phages. Acquired drug resistance mutations occur in chromosomal genes and extrachromosomal or gene transfer tools. However, no horizontal transfer of drug resistance in mobile genetic elements has been reported in *M.tuberculosis*. Instead, drug resistance in *M.tuberculosis* primarily emerges due to mutations in the chromosome, genes encoding drug targets, or drug-activating enzymes, in response to the selection pressure of antibiotics [16]. Drug-resistant mutants evolve due to continuous drug exposure during extended treatment regimens and noncompliance with the drug regimen. Therefore, drug concentration is a significant determinant of mutations associated with resistance. Apart from the innate resistance mechanisms mentioned above, the most clinically relevant drug resistance in *M.tuberculosis* is conferred by chromosomal mutations. These chromosomal mutations confer drug resistance via many grant different resistance levels. *M. tuberculosis* also pays a physiological cost for drug resistance against antituberculosis drugs. As a result, the degree of mutation rate in the base pair is inversely genome size close to 0.0033 per replication in prokaryotes. However, in *M.tuberculosis* cases, uttermost of the lead antituberculosis drugs occur at 10^{-9} mutations per cell division. Thus, the nature of drug selection is also directly related to the mutation rate, which is the foremost ideal approach behind the combined formulation of antituberculosis drugs in every single dose. The degree of the fitness cost of individual *M. tuberculosis* depends on their growth and virulence transmission capacity from one host to another host. For example, rpoB gene mutation in *M.tuberculosis* clinical isolates manages rifampicin resistance; but sometimes the identical isolates have less fitness cost *in vitro.* Thus, it may depend on mutations associated with some minor or significant cost of the fitness of a strain. Therefore, fitness cost depends on the genetic circumstances and the specific resistance mutation of the strains [17].

The degree of the fitness cost of diverse chromosomal mutations is directly proportional to antibiotic resistance. Still, limited data on the drug resistance and degree of fitness cost in *M.tuberculosis*. However, chromosomal mutation is the uttermost well-known drug-resistance mechanism. Mutation in S315T on the catalase-peroxidase enzyme (*katG*) is the uttermost pervasive mutation, and nearly 40–94% of resistance is associated with isoniazid-resistant *M.tuberculosis* clinical isolates. Mutations can reduce the ability of *katG* to convert isoniazid into iso-nicotinic acid, a precursor for the development of the isoniazid-NAD adduct in mycolic acid synthesis. However, rifampicin-resistant clinical isolates have been compared with their susceptible parental strain, four out of five strains with the mutation S531L, and no fitness cost for this mutation [18, 19].

4.1 Drug target alteration

The most familiar mechanism of drug resistance in *M. tuberculosis* is the alteration of the drug target. Among these, the interactions of drug and drug target moieties are precise. Changes in the drug–drug target interaction sites might reduce or altogether abolish drug binding and grant resistance to the drug. Alternatively, nucleotide substitutions in the operon encoding the ribosomal RNA are observed frequently to confer

resistance to the drug in *M.tuberculosis* as in the case of resistance against all antituberculosis drugs. The mutations in the DNA-dependent RNA polymerase, corresponding to the rifampicin resistant determination region (81 bp) of rpoB, render resistance to the rifampicin drug by decreasing the affinity of rifampicin for the drug target gene. The crucial cellular functions of drugs and the drug targets performing these functions are mainly predicted. The extremely preserved drug target nature limits the mutational target size as the resistance mutation has to succeed in two things: first, it has to prevent the antibiotic from inhibiting the drug target, and second, it can still perform the indispensable drug target functions. In many cases, but not all, this leads to a decrease in the degree of fitness of bacterial cells in the absence of the drug [12].

4.2 Abrogation of prodrug activation

Most antituberculosis drugs are prodrugs, and annulment of the drug-activating mechanisms leads to resistance to the drugs, as in pyrazinamide, isoniazid, para-aminosalicylic acid, ethionamide, delamanid, and pretomanid. In some instances, the prodrug-activating enzyme is not essential for mycobacterial growth and survival. The drug target size for drug resistance-conferring chromosomal mutations is remarkable for any-point mutations, insertions/deletions, mobile genetic elements, etc., which will interrupt the prodrug-activating gene product without compromising mycobacterial survival. Furthermore, mutations in the promoter gene might lead to lower transcript and enzyme levels activating the prodrug. Lower levels of the prodrug-activating enzyme will then, in turn, lead to higher minimal inhibitory concentrations (MIC) for the drug in question. For example, in pyrazinamide-resistant *M.tuberculosis* clinical isolates, a broad array of different mutations in the pncA gene encodes the enzyme metabolizing pyrazinamide to its active form, pyrazinoic acid. On the other hand, the mutational drug target size for delamanid/pretomanid resistance is enormously larger as multiple enzymes and cofactors are involved in prodrug metabolism to their active forms. It suggests that resistance to the latter two drugs may develop swiftly due to the sizeable mutational target size [20].

Besides, the gene *katG* encoding a catalase/peroxidase enzyme activating isoniazid is required to replicate *M. tuberculosis* in macrophages. The mutational target size of isoniazid drug resistance is tiny compared to pyrazinamide or delamanid/pretomanid drug. Therefore, the mutation in the *katG* gene ought to preserve the essential function of the catalase/peroxidase-detoxification enzyme and prevent the activation of the isoniazid drug. Most of the *M.tuberculosis* clinical isolates harbor, the point mutation at codon S315T in the *katG* gene, which retains most catalase/peroxidase functions and confers high-level isoniazid drug-resistance. On the other hand, the *katG* gene is not essential for *in vitro* replication. This dramatically enlarges the mutational drug target size for *in vitro* resistance, as any mutation disrupting the function of *katG* will lead to resistance [18].

4.3 Over-expression of drug targets

Over-expression of the drug target gene may overcome the inhibition by the drug in question due to an overabundance of the target. Mutations in transcriptional repressors or the drug target's promoter may cause overexpression, as in the case of ethambutol, isoniazid, and cycloserine. The over-expression in the drug target gene confers lower-level resistance to isoniazid or cycloserine. Usually, it can be overcome by increasing the administration of drug doses. Drugs administered at fixed doses are often adjusted for

the patient depending on weight and age. In general, it is done to achieve the maximum effectiveness of the drug while minimizing the adverse effects of administered drugs. As certain antibiotics show dramatic adverse effects, the lower dose is given to patients as much as possible, which means there is little chance of increasing the drug doses to overcome resistance due to drug target over-expression. On the other hand, over-expression of drug targets might serve as a stepping stone to high-dose resistance, either by alteration in drug target or abrogation of prodrug activation [21].

5. Resistance to first-line TB drugs

Any drug used in the antituberculosis regimen is supposed to have an effective sterilizing activity capable of shortening the duration of treatment. Four-drug regimen (HRZE) consisting of isoniazid, rifampicin, pyrazinamide, and ethambutol is currently practiced for tuberculosis treatment. Resistance to all first-line drugs has been linked to mutations in eleven genes; *katG, inhA, ahpC, kasA,* and *ndh* for isoniazid resistance, *rpoB* for rifampicin resistance, *embCAB* for ethambutol resistance, *pncA* for pyrazinamide resistance, and *rpsL* and *rrs* for streptomycin resistance [3].

5.1 Mechanism and action of streptomycin drug

Streptomycin is an active drug against slow-growing mycobacterial species and acts by irreversibly attaching to the S12 ribosomal protein and 16S rRNA.The S12 ribosomal protein and 16S rRNA are the main components of the 30S subunit of the mycobacterial ribosome. Through this interconnection, streptomycin blocks the translation process, inhibiting protein synthesis. The primary resistance mechanism of streptomycin is mediated through mutations in the *rrs* and *rpsL* genes, encoding the 16S rRNA and ribosomal protein S12, respectively. It accounts for about 60–70% of streptomycin drug resistance. Recently, mutations in the gene *gidB*, encoding a 7-methylguanosine methyltransferase specific for methylation of G527 in the loop of the 16S rRNA, have been implicated in lower-level streptomycin drug resistance. In addition, whole-genome sequence (WGS) analysis has also proved a deletion of 130 bp within the *gidB* gene, possibly mediating streptomycin drug resistance [11].

5.2 Mechanism and action of isoniazid drug

Isoniazid is a prodrug consisting of a pyridine ring and a hydrazide group. The prodrug enters the cytoplasm of *M. tuberculosis* cell through simple passive diffusion. Therefore, it can act only on microorganisms in the active process of replication, not during the stationary phase of growth or not acting during growth under anaerobic conditions. Because isoniazid drug is a prodrug, it is activated by the enzyme catalase-peroxidase encoded by the *katG* gene. Once it gets activated, it interferes with the synthesis of essential mycolic acids by inhibiting NADH-enoyl-ACP-reductase encoded by the *inhA* gene [22]. Mutations in the *katG* gene play a crucial role in mediating resistance to isoniazid as it develops the inactivation of this nonessential enzyme. The two main molecular mechanisms of isoniazid drug resistance are gene mutations in *inhA* or its promoter region and *katG.* Indeed, numerous studies have found that 75% of mutations in these two genes are most commonly associated with isoniazid resistance. Among these, the mutation at codon S315T in the *katG* gene *is* most prevalent, resulting in an isoniazid dose deficient in forming the isoniazid-NAD

adduct needed to exert its antimicrobial activity. In addition, the mutation at codon S315T in the *katG* gene has been consistently associated with high-dose resistance (MIC >1 μg/mL) to isoniazid and occurs more frequently in MDR strains [18].

5.3 Mechanism and action of rifampicin drug

Rifampicin diffuses freely through the cell wall of *M.tuberculosis* and, once inside the cell, inhibits gene transcription by blocking the RNA polymerase enzyme, preventing the transcription of messenger RNA. Therefore, rifampicin drug resistance in *M. tuberculosis* can be attributed mainly to β-subunit modifications of RNA due to mutations in the *rpoB* gene. Rifampicin is a potent first-line antituberculosis drug for determining the effectiveness of treatment regimens. Because >90% of rifampicin-resistant *M.tuberculosis* clinical isolates are also resistant to isoniazid, rifampicin resistance can be a valuable surrogate marker for MDR-tuberculosis. The rifampicin drug mechanism arrests DNA-directed RNA synthesis of *M.tuberculosis* by interacting with the β subunit of RNA polymerase (RNAP). Approximately 95% *of rpoB* gene mutations in M.tuberculosis clinical isolates with rifampicin resistance and are more likely located in the 81-bp region (codons 424–452) called the rifampicin resistance-determining region (RRDR). Inside the 81-bp RRDR, mutations within codons 435, 445, and 450 are responsible for up to 90% of rifampicin-resistant strains [23]. However, not all mutations within the RRDR display the same loss of rifampicin susceptibility. The amino acid alterations of codon 445 or codon 450 cause high-level resistance to rifampicin, the minimal inhibitory concentration (MIC) greater than 32 μg/ml.

5.4 Mechanism and action of pyrazinamide drug

Pyrazinamide drug is a nicotinamide analog that has significantly reduced the treatment duration of drug-sensitive tuberculosis (DS-TB) to 6 months. A vital characteristic of pyrazinamide drug is its ability to inhibit semi-dormant mycobacterium bacilli in acidic environments, such as tuberculosis lesion. Pyrazinamide (PZA) drug is a prodrug activated by the enzyme pyrazinamidase/nicotinamidase (PZase), encoded by the *pncA* gene. Once activated, pyrazinoic acid interrupts the mycobacterial membrane energetics, thereby inhibiting the membrane transport system. Pyrazinamide drug enters into the mycobacterial cell by passive diffusion, and then it is converted into pyrazinoic acid. The pyrazinoic acid is expelled from the mycobacterial cell by an efflux mechanism. In an acidic environment condition, the pyrazinoic acid is protonated, allowing cell reabsorption, and resulting in cellular damage. Pyrazinoic acid and its n-propyl ester have also been actively implicated in inhibiting fatty acid synthase I in *M.tuberculosis*. In addition, it has been recently reported that pyrazinoic acid is actively involved in inhibiting trans-translation in *M.tuberculosis*. Mutations in the gene *pncA* and its promoter region remain the common uttermost mechanism mediating pyrazinamide drug resistance. The mutations identified within the gene *pncA* are diverse, with 600 distinctive mutations in 400 positions reported to date, accounting for about 72–99% of pyrazinamide drug resistance [24].

5.5 Mechanism and action of ethambutol drug

Ethambutol drug targets the mycobacterial cell wall through interaction with arabinosyl transferases involved in the biosynthesis of arabinogalactan (AG) and lipoarabinomannan (LAM). It specifically inhibits the polymerization of cell-wall arabinan, thereby

leading to the accumulation of b-D-arabinofuranosyl-1-monophosphoryldecaprenol (DPA). Three genes designated *embCAB* to encode homologous arabinosyl transferase enzymes involved in ethambutol drug resistance. Once the ethambutol drug diffuses into mycobacterium cells, it inhibits the synthesis of arabinosyl transferases, preventing the formation of lipoarabinomannan cell wall components lipoarabinomannan and arabinogalactan and preventing cell division. In addition, the ethambutol drug is active against actively multiplying mycobacterium bacilli, interrupting the arabinogalactan biosynthesis in the cell wall of Mycobacterium species. The *embC,embA*, and *embB* operons encode the mycobacterial arabinosyl transferase enzyme. Resistance to ethambutol is mediated through mutations in the *embB* gene. The most common resistance mechanism is the alteration in codon 306 of the *embB* gene [25].

Further, this mutation predisposes the clinical isolate to create resistance to other tuberculosis drugs and is not necessarily involved in ethambutol resistance. Only specific amino acid substitutions led to ethambutol drug resistance, not any other gene mutation that caused resistance to ethambutol drug. The mutations in the decaprenylphosphoryl-b-D-arabinose biosynthetic and utilization pathway genes (Rv3806c and Rv379) co-occur with mutations in *embB* and *embC*, resulting in a variable minimal inhibitory concentration (MIC) range for ethambutol drug. This is based on the type of mutation present in *M.tuberculosis*. Furthermore, this suggests that the *embB306* mutation results in differing degrees of ethambutol drug resistance but does not cause higher-level ethambutol drug resistance. About 30% of ethambutol drug-resistant isolates lack alteration in *embB*, suggesting a different resistance mechanism. Additive mutations in the *ubiA* gene have been reported to cause higher-level ethambutol resistance when they occur with *embB* gene mutations [26]. The gene *ubiA* encodes decaprenyl-phosphate 5-phosphoribosyltransferase synthase, which is actively involved in the synthesis of cell walls. Alteration in the *ubiA gene* is reported to be lineage-specific and is predominant in the African isolates.

6. Resistance to Second-Line TB Drugs

6.1 Mechanisms of quinolone resistance

The action of quinolones drug is inhibited by the activity of DNA gyrase and topoisomerase IV—two essential bacterial enzymes that modulate the chromosomal supercoiling required for critical nucleic acid processes. Both targets are type II topoisomerases, having distinctive functions within the mycobacterial cell. DNA gyrase enzyme introduces negative supercoils into DNA during replication, which helps relieve torsional isolates caused by the introduction of positive supercoils while replicating. These negative supercoils are essential for promoting transcription initiation and chromosome condensation. It comprises two A subunits and two B subunits, of which the A subunits appear to be the fluoroquinolone target. In addition to the relaxation of positive supercoils functions, Bacterial topoisomerase IV is crucial for DNA replication at terminal stages and functions to "unlink" newly replicated chromosomes to complete bacterial cell division [27]. Inhibition of these enzymes by levofloxacin results in a blockade of DNA replication, thus inhibiting cell division and resulting in cell death.

The main mechanism of developing fluoroquinolone (FLQ) resistance in M. tuberculosis is chromosomal mutations within the quinolone resistance-determining region (QRDR) of *gyrA* or *gyrB* genes. However, only 60–70% of *M. tuberculosis*

clinical isolates with fluoroquinolone drug resistance can be accounted for by these mutations within QRDR. Most mutations are in codons at 90, 91, and 94 in the *gyrA* gene. In addition, novel mutations at codons, such as Met81Thr, Leu109Pro, and Gln113Leu substitutions, have been detected in fluoroquinolone drug-resistant isolates within the gyrA gene. In addition, other factors, such as active efflux mechanisms, could contribute to the rest of fluoroquinolone drug resistance [28].

6.2 Mechanisms of second-line injectable drugs resistance

Aminoglycosides (i.e., amikacin, kanamycin) and cyclic peptides (i.e., capreomycin) group referred to as injectable inhibits protein synthesis through modification of ribosomal structures at 16S rRNA and formation of 30S ribosomal subunit respectively. Aminoglycosides are pseudo-polysaccharides, consisting of amino sugars, and can therefore be considered polycationic species to understand their biological interactions. Since aminoglycosides are positively charged at physiological pH values, they strongly bind to nucleic acids, especially for some prokaryotic ribosomal RNA (rRNA) portions. Aminoglycoside uptake by bacterial cells has been shown to occur in three phases. The initial step involves electrostatic interactions between the antibiotic and the gram-negative outer membrane's negatively charged lipopolysaccharide (LPS). Then, the polycationic drugs competitively displace essential divalent cations (magnesium) that cross-bridge and stabilize adjacent lipopolysaccharide (LPS) molecules. Due to this, the rupture of the cell's outer membrane has been proposed to enhance permeability and initiate aminoglycoside uptake. Aminoglycoside transport across the cytoplasmic membrane involves an initial lag phase followed by a log phase in which the drug is swiftly taken up. This transportation across the cytoplasmic membrane needs energy from the electron transport system in an oxygen-dependent process [28]. The inherent resistance of anaerobic bacteria to aminoglycosides can be explained by the failure to transport the drug inside the cell. Once inside the cell, the drug attaches to the 30S ribosomal subunit at the aminoacyl-tRNA (aa-tRNA) acceptor site (A) on the 16S ribosomal RNA (rRNA), affecting protein synthesis by induction of codon misreading and inhibition of translocation.

Therefore, the appropriate use of second-line injectable drugs of amikacin, kanamycin, and/or capreomycin is critical for the effective treatment of multi-drug resistant- tuberculosis and the prevention of extensively drug-resistant—tuberculosis. Point mutations at codons of A1401G and G1484T within the 16S rRNA gene (*rrs*) are responsible for higher-level resistance to amaikacin and kanamycin drugs. Mutations further cause kanamycin drug resistance at codons C14T, G37T, and G10A found within the promoter region of the enhanced intracellular survival (*Eis*) gene. While mutations mainly cause capreomycin resistance at codons C1402T in the *rrs* gene and *tlyA* gene, they have been detected with lower frequency. Therefore, only about 70–80% of global *M.tuberculosis* isolates with injectable resistance harbor mutations in the *rrs, eis*, and *tlyA* genes [29]. The remaining resistance cannot be explained based on these target site mutations, and other mechanisms could be involved.

6.3 Mechanisms of Ethionamide drugs resistance

Ethionamide drug is a derivative of isonicotinic acid, and it is a structural analogue of isoniazid drug. The bactericidal action of ethionamide drug depends on the concentration of drugs at the site of infection and the susceptibility of the infecting

microorganism. Ethionamide, such as prothionamide and pyrazinamide, is a nicotinic acid derivative related to isoniazid. The ethionamide drug undergoes intracellular modification and acts similarly to the isoniazid drug. Like the isoniazid drug, the ethionamide drug also inhibits the synthesis of mycolic acids, which is an essential component of the mycobacterial cell wall. Specifically, the isoniazid drug inhibits *inhA*, the enoyl reductase from *M.tuberculosis*, by forming a covalent adduct with the NAD cofactor. The INH-NAD adduct acts as a slow, tight-binding competitive inhibitor of *inhA*. Ethionamide drug is a prodrug activated by the mono-oxygenase enzyme, encoded by the *ethA* gene. Once it gets activated, the ethionamide drug inhibits the synthesis of mycolic acid during cell wall biosynthesis by inhibiting the enzyme enoyl-acyl carrier protein reductase. Regulatory control of the *ethA* gene occurs through the transcriptional repressor, the *EthR* gene. Mutations mediate resistance to ethionamide drug in the *etaA/ethA, ethR*, and *inhA* genes. Mutations in the gene *inhA* mediate co-resistance to isoniazid and ethionamide [30].

6.4 Mechanisms of para-aminosalicylic acid resistance

Para-aminosalicylic acid is the analogue of para-aminobenzoic acid. It was one of the first antibiotics to treat tuberculosis, with isoniazid and streptomycin. Para-aminosalicylic acid is now part of second-line treatment regimens applied to treat drug-resistant tuberculosis. Two mechanisms are responsible for the bacteriostatic action of para-aminosalicylic acid against *M. tuberculosis*. Firstly, para-aminosalicylic acid inhibits folic acid synthesis (without potentiation with antifolic compounds). Binding para-aminobenzoic acid to pteridine synthetase is the first step in folic acid synthesis [31]. Para-aminosalicylic acid attaches pteridine synthetase with greater affinity than para-aminobenzoic acid. Therefore, it effectively inhibits the synthesis of folic acid as mycobacteria cannot use external sources of folic acid, and cell growth and multiplication are slow. Secondly, para-aminosalicylic acid may inhibit the synthesis of mycobactin, a cell wall component, thus reducing iron uptake by *M. tuberculosis*. The primary resistance mechanism mediating para-aminosalicylic acid has been identified as mutations occurring in the *gene*, which accounts for 40% of total para-aminosalicylic acid resistance [32]. The mutation at codon T202A in the gene *thyA*, initially associated with para-aminosalicylic acid resistance, was a phylogenetic marker related to the Latin American isolates families rather than resistance to para-aminosalicylic acid.

6.5 Mechanisms of D-Cycloserine resistance

D-Cycloserine (DCS) is a broad-spectrum antibiotic classified as a second-line drug for treating tuberculosis. The bactericidal/bacteriostatic action of D-Cycloserine is based on its drug concentration at the infection site and the susceptibility of the microorganism. D-Cycloserine is an analog of the amino acid D-alanine. Cycloserine drug works by blocking the formation of these peptidoglycans. D-Cycloserine inhibiting cell-wall biosynthesis in mycobacteria. As a cyclic analog of D-alanine, cycloserine acts against two crucial enzymes:L-alanine racemase forms D-alanine from L-alanine, and D-alanylalanine synthetase incorporates D-alanine into the pentapeptide necessary for peptidoglycan formation and bacterial cell wall synthesis [33]. If both enzymes are inhibited, then D-alanine residues cannot form, and previously created D-alanine molecules cannot be joined together. This effectively leads to the inhibition of peptidoglycan synthesis.

7. Mechanisms of resistance to new and repurposed drugs

7.1 Mechanisms of Clofazimine resistance

Clofazimine drug is now a part of the newly standardized short-course regimen proposed by the World Health Organization for treating drug-resistant tuberculosis. Although the exact mechanism(s) of action of clofazimine has not been explained, the antimicrobial activity of clofazimine appears to be membrane-directed. A recent theory involves clofazimine interacting with bacterial membrane phospholipids to create antimicrobial lysophospholipids—bactericidal efficacy may emerge from the combined membrane-destabilizing effects of clofazimine and lysophospholipids, which interfere with K+ uptake and, ultimately, ATP production. Resistance to clofazimine drug has been attributed to nontarget mutations in *the rv0678* gene, leading to the efflux of the drug. In addition, resistance to clofazimine drug has been linked to cross-resistance with bedaquiline drug [34]. The *pepQ* and the *rv1979c* genes have been recently reported as additional mechanisms associated with clofazimine drug resistance.

7.2 Mechanisms of Bedaquiline resistance

Bedaquiline acts by targeting mycobacterial ATP synthase, inhibiting bacterial respiration. The drug is, therefore, active against dormant bacilli, an invaluable characteristic for *M.tuberculosis* infection. Bedaquiline, in combination with pyrazinamide, has acted with remarkable sterilizing activity in a mouse model. Target-based mutations in the atpE gene in strains selected in vitro have been associated with higher-level resistance to bedaquiline, with up to a 4-fold increase in minimal inhibitory concentration. The gene encodes the mycobacterial F1F0 proton ATP synthase, a crucial enzyme in ATP synthesis and membrane potential generation. The binding of bedaquiline to ATP synthase "leading" and "lagging" sites of subunit *c* and, to a lesser degree, subunit *a*, resulting in significant changes in mycobacterial ATP synthase. BDQ-mediated inhibition of ATP synthesis also has downstream effects on many metabolic processes, including the inhibition of glutamine synthetase [35], and the change of mycobacterial metabolism that increases confidence in glycolysis and substrate-level phosphorylation to generate cellular ATP. Inhibitors of *MenA*, which catalyzes the final step in menaquinone biosynthesis, had a synergistic killing interaction with bedaquiline, resulting in complete sterilization of *M. tuberculosis* cultures within 14–21 days of treatment [36]. Zimenkov et al. [37] recently described the first occurrence of *atpE* D28N and A63V mutations in two clinical isolates of *M.tuberculosis* associated with a MIC of 0.12 and 1.00 mg/L, respectively. Mutations in the second nontarget mechanism, *pepQ*, were reported with the association of lower-level bedaquiline resistance and cross-resistance to clofazimine. Similar to *rv0678*, mutations in the gene *pepQ* result in modest increases of bedaquiline and clofazimine minimal inhibitory concentrations.

7.3 Mechanisms of delamanid and pretomanid resistance

Delamanid (DLM) renders mycobactericidal activity by inhibiting the production of methoxy and keto mycolic acid through the mycobacteria F420 system generating nitrous oxide. Delamanid and isoniazid (INH) act by preventing the synthesis of mycolic acids, which plays a crucial role in the mycobacterial genus survival.

Compared with DLM, INH, as the inhibitor of a-mycolic acid synthesis, has a different strategy for impeding cell wall synthesis. Enoyl-acyl carrier protein reductase encodes the *InhA* gene, a specific factor for the function of the isoniazid drug. In contrast, the delamanid drug needs a mycobacterial F420 system to activate. The analog of flavin mononucleotide complex consists of two enzymes, deazaflavin-dependent nitroreductase encodes *Ddn*, Rv3547, and F420- dependent glucose-6-phosphate dehydrogenase encodes G6PD; FGD1, Rv0407 and four coenzymes, *FbiA*, *FbiB*, *FbiC*, and Rv0132c. Mutations in enzymes and one of the four coenzymes, F420 genes, *fgd, Rv3547, fbiA, fbiB,* and *fbiC*, have been proposed as the mechanism of resistance to delamanid drug [38]. Upon activation, the radical intermediate formed between delamanid drug and desnitro-imidazooxazole derivative is mediated antimycobacterial actions through the inhibition of methoxy-mycolic and keto-mycolic acid synthesis, leading to the dwindling of mycobacterial cell wall components and destruction of the mycobacteria cell. In addition, nitroimidazooxazole derivative is generated to reactive nitrogen species, including nitrogen oxide. However, unlike the isoniazid drug, the delamanid drug does not have alpha-mycolic acid. Resistance to the pretomanid drug has been linked to mutations in the genes related to prodrug activation (*ddn* and *fgd1*) or genes associated with the F420 biosynthetic pathway. Rifat et al. [39] recently reported mutations at codon D49Y in the *fbiA* gene, and a frameshift mutation at codon 49 of the *fdg1* gene resembled increasing phenotypic delamanid drug resistance. Similar to a pretomanid drug, it is a prodrug that requires activation through the same pathway, and thus, resistance to a delamanid drug is related to mutations in one of the five genes. Thus, any mutations in this pathway result in the reduction of mycobacterium bacilli to metabolize prodrug and lower- to higher-level delamanid resistance.

7.4 Mechanisms of linezolid resistance

Linezolid wields its antibacterial effects by interfering with mycobacterial protein translation. It binds to a site on the mycobacterial 23S ribosomal RNA of the 50S subunit. It prevents the genesis of a functional 70S initiation complex, essential for mycobacterial reproduction, thereby preventing bacteria from dividing. In addition, linezolid acts by binding to the V domain of the 50S ribosomal subunit, thereby inhibiting an early step in protein synthesis. Resistance to linezolid has been associated with mutations in the 23S rRNA (*rrl*) gene. Du et al. [40] detected the mutations at codons G2576T and A2572C in the gene *rrl* in a patient with corresponding phenotypic linezolid resistance. The mutations at codons G2061T and G2572T in the *rrl* gene were associated with higher-level resistance in the range of 16–32 mg/L, and mutants bearing lower-level resistance of 4–8 mg/L had no alteration in the *rrl* gene. More recently, a mutation in the *rplC* gene, encoding the 50S ribosomal L3 proteins, in *in vitro* selected mutants and clinical isolates has been described as a mechanism of linezolid drug resistance. Zimenkov et al. [37] reported on acquired linezolid drug resistance in 10 patients in a cohort of 27. The most frequent resistance mutation is at codon C154R in the *rplC* gene, and seven of the ten patients had alterations in the *rplC* gene only. The mechanism resulting in resistance to linezolid drug is yet to be elucidated.

8. Conclusion

Drug-resistant tuberculosis (DR-TB) remains a critical public health challenge of modern times. The basic drug resistance mechanisms in *M. tuberculosis* and their

implications for possible clinical outcomes are increasingly skilfully understood. However, contemporary diagnostic techniques for monitoring resistance mutations are limited to our current knowledge of mutation patterns. Large-scale analyses conducted on whole genome sequences (WGS) have assisted in cataloging diverse causative to compensatory or adaptive mutations and their differing roles in mediating drug resistance in the microorganism. The dynamics of resistance development and the factors that facilitate resistance development within a patient are still severely understood and require further elucidation. Resistance-conferring mutations in mycobacteria can develop dynamically over time under drug pressure in patients. The relationship between drug resistance, virulence factor, and microorganism fitness requires further study. Although, rapid molecular diagnostics, such as Xpert MTB rifampicin assay and Hains line probe assays, are limited in their ability to produce a spacious resistance profile. Rapid whole genome sequences (WGS) are the most promising utility for the personalized care of patients with drug-resistant tuberculosis. Personalized treatment can improve treatment outcomes by limiting the therapeutic regimen to effective drugs only, reducing the unnecessary pill burden, and significantly reducing the harmful resulting from the debilitating adverse side effects of contemporary treatment. This is the only remaining strategy for managing the drug resistance catastrophe as our existing antimicrobial repertoire quickly diminishes.

Conflict of interest

The authors declare no conflict of interest.

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