Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

A Comprehensive Review of Genetic Mechanisms of *Mycobacterium tuberculosis* Resistance to Injectable Agents, Second-Line Drugs, and Novel Therapeutics

Mehdi Roshdi Maleki

Department of Microbiology, Male.C., Islamic Azad University, Malekan, Iran

Correspondence: Mehdi Roshdi Maleki, Department of Microbiology, Male.C., Islamic Azad University, Malekan, Iran, Tel +989143200846, Email Me.roshdi@iau.ac.ir

Abstract: Tuberculosis (TB) has been one of the deadliest infectious diseases since ancient times and remains a major threat to public health, causing significant morbidity and mortality. The global TB outbreak in 1993 prompted the World Health Organization to declare the disease an emergency priority. However, public neglect and the ability of *M. tuberculosis* to develop drug resistance have led to the emergence of multidrug-resistant, extensively drug-resistant, and totally drug-resistant strains. These resistances are mainly caused by genetic mutations in antibiotic target genes, which reduce the effectiveness of drugs and make it more difficult to treat patients. In this review article, focusing on molecular and genetic mechanisms, the resistance of *M. tuberculosis* to injectable agents, second-line drugs, and novel anti-TB agents is investigated. Relevant studies were identified through a comprehensive search of major scientific databases, including PubMed, Scopus, and Web of Science. Articles were selected based on relevance to the molecular and genetic mechanisms of drug resistance in *M. tuberculosis*. The evidence was synthesized narratively to highlight current insights into resistance to anti-TB drugs.

Keywords: *M. tuberculosis*, drug resistance, injectable agents, second-line drugs, novel agents, genetic mechanisms

Introduction

Tuberculosis (TB) is a major infectious disease that threatens human health and kills millions of people annually.[1] In 1993, the World Health Organization (WHO) declared a global emergency due to the widespread occurrence of TB, emphasizing the importance of controlling this disease. However, public neglect and the ability of *M. tuberculosis* to develop drug resistance have led to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and finally, totally drug-resistant (TDR) strains; drug-resistant TB kills about 200,000 people annually. These resistances are mainly caused by genetic mutations in antibiotic target genes, which reduce the effectiveness of drugs and make it more difficult to treat patients.[2] According to the WHO 2024 report, nearly 11 million people worldwide were infected with TB in 2023, and 1.25 million people died from the disease.[3] The report shows that TB remains the deadliest infectious disease, being the second leading cause of death from infectious diseases[4] and one of the top 10 causes of death worldwide.[5] Along with the increasing number of deaths from TB, the emergence of drug-resistant strains has posed a major challenge to the treatment of the disease and is a serious threat to its control. About a quarter of TB-related deaths are attributed to antimicrobial drug resistance.[6] In 2021, about 450,000 cases of MDR-TB were reported, an increase compared to previous years.[7]

Drug-resistant strains of *M. tuberculosis* pose a major challenge to global efforts to control TB. MDR strains, which are resistant to rifampicin (RIF) and isoniazid (INH), are difficult to detect and treat, making treatment complex and expensive. Furthermore, fewer treatment options are available for patients infected with XDR strains. XDR-TB is defined

https://doi.org/10.2147/IDR.S568121
Received: 16 September 2025
Accepted: 12 January 2026
Published: 30 January 2026

as a strain that, in addition to being MDR, is also resistant to a fluoroquinolone and at least one second-line parenteral antibiotic, such as kanamycin (KAN), amikacin (AMK), or capreomycin (CAP).[8]

Strains of *M. tuberculosis* that are resistant to all first-line anti-TB drugs (INH, RIF, ethambutol (EMB), streptomycin (STR), pyrazinamide (PZA), and second-line drugs (ofloxacin (OFX), ciprofloxacin (CIP), cycloserine (CS), prothionamide (PTH), AMK, KAN, ethionamide (ETH), para-aminosalicylic acid (PAS), and CAP) are known as XDR-TB. Finally, strains of *M. tuberculosis* that are resistant to all available drugs for the treatment of TB, designated XXDR-TB, have recently received attention.[9,10]

Mycobacteria use several molecular mechanisms to acquire antimicrobial resistance (AMR), including: (1) use of barrier mechanisms, such as reduction of cell wall permeability or reverse drug transport by expression of efflux transporters (ETs); (2) use of degradation or inactivation enzymes; (3) modification of pathways involved in drug activation or metabolism; and (4) changes in drug targets or gene amplification.[11,12]

M. tuberculosis uses all of these mechanisms to develop drug resistance. However, in this article, only resistance resulting from genetic changes is reviewed. Therefore, the molecular basis of this bacterium's resistance to injectable agents, second-line drugs, and novel agents is analyzed from a molecular genetic perspective and provides current knowledge about the molecular mechanisms of *M. tuberculosis* resistance to anti-TB agents. A detailed understanding of these mechanisms and the molecular basis of antibiotic resistance can open new horizons in the rapid and accurate identification of resistant strains, the design of effective treatment regimens, and the overcoming of drug resistance.

The injectable agents and second-line anti-TB drugs, together with their associated resistance genes and major resistance mechanisms, are summarized in Table 1, and the overall schematic representation of these relationships is illustrated in Figure 1.

Search Strategy and Study Selection

In this review, the primary focus was to explore the molecular and genetic mechanisms underlying *M. tuberculosis* resistance to injectable agents, second-line drugs, and emerging therapeutics. To identify relevant literature, a comprehensive search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, Embase, and ScienceDirect.

The search strategy incorporated keywords related to *M. tuberculosis*, drug resistance, MDR-TB, XDR-TB, and TDR-TB, as well as names of specific drug classes and therapeutic agents, including aminoglycosides, fluoroquinolones, ethionamide, linezolid, clofazimine, bedaquiline, and para-aminosalicylic acid. These terms were combined using the Boolean operators (AND) and (OR) to ensure broad and comprehensive retrieval of relevant studies.

Inclusion criteria consisted of English-language original research and review articles with full-text availability, published in journals indexed in the Journal Citation Reports. Study screening was conducted in multiple stages based on titles, abstracts, and full texts. Exclusion criteria included articles outside the scope of this review and lack of access to the full text. All retrieved references were imported into EndNote™ 20 (Clarivate Analytics, Philadelphia, PA, USA) reference management software, and duplicate records were subsequently removed.

Injectable Agents

Aminoglycosides

Since the discovery of STR's bactericidal activity against *M. tuberculosis*, aminoglycosides have been used to treat TB. Today, the aminoglycosides AMK and KAN are used to treat MDR-TB, and resistance to any of the second-line injectable antibiotics, including KAN, AMK, or CAP, is a major feature of XDR-TB.[8] STR has played a historic role in the human victory over TB and paved the way for the development of aminoglycoside antibiotics. Its efficacy for the treatment of various types of TB was demonstrated in 1944, and it is still considered a first-line drug. It is a broad-spectrum antibiotic. However, its use is limited today due to increasing resistance and the need for parenteral administration. STR is a rapid bactericidal agent, but its efficacy is reduced at acidic pH. AMK and KAN are other important aminoglycosides used in the treatment of TB. These drugs are administered intravenously or intramuscularly and are used as second-line drugs for the treatment of MDR-TB or XDR-TB. Aminoglycosides are bactericidal drugs and cause rapid

Table 1 List of Injectable and Second-Line Drugs Used in the Treatment of Tuberculosis and the Most Common Mutations That Confer Drug Resistance in *M. tuberculosis*

Drug(s)	Resistance Gene(s)	Gene Function	Mechanism of Drug Resistance	Ref
Streptomycin (STR)	*rpsL*	Encodes ribosomal protein S12	Alteration of drug binding site	[13]
	rrs	Encodes 16S rRNA	Alteration of drug binding site	
	gidB	Encodes methyltransferase	Loss of rRNA methylation (low-level resistance)	
Amikacin/Kanamycin (AMK/KAN)	*rrs*	Encodes 16S rRNA	Alteration of drug binding site	[13]
	eis	Aminoglycoside acetyltransferase	Increased drug inactivation (acetylation)	[14]
Capreomycin (CAP)	*rrs*	Encodes 16S rRNA	Alteration of drug binding site	[13]
	tlyA	rRNA methyltransferase	Loss of rRNA methylation	[15]
Quinolones (Qs)	*gyrA*	Encodes A subunit of DNA gyrase	Alteration of drug binding site	[16]
	gyrB	Encodes B subunit of DNA gyrase	Alteration of drug binding site	
Ethionamide (ETH)	*ethA*	Encodes monooxygenase enzyme	Reduced drug activation	[17]
	ethR	TetR-family transcriptional repressor protein	Reduced drug activation	
	inhA	NADH-dependent enoyl-acyl carrier protein reductase	Increased target expression or alteration	
Para-aminosalicylic acid (PAS)	*thyA*	Thymidylate synthase pathway	Alteration of drug binding site	[18]
	folC	Folate biosynthesis enzyme	Reduced drug activation	[18]
	ribD	Riboflavin synthesis enzyme	Altered folate pathway (reduced activation)	[19]
D-Cycloserine (DCS)	*alr*	Encodes alanine racemase	Alteration of drug binding site	[20]
	ddl	D-alanine D-alanine ligase	Alteration of drug binding site	
	cycA	Transport protein	Reduced drug uptake	[21]
Bedaquiline (BDQ)	*atpE*	ATP synthase subunit c	Alteration of drug binding site	[22]
Linezolid (LZD)	*rrl*	23S rRNA	Alteration of drug binding site	[23]
	rplC	50S ribosomal protein L3	Alteration of drug binding site	
Delamanid (DLM)	*ddn*	Encodes F_{420}-dependent nitroreductase	Reduced drug activation	[24]
	fbiA/B/C/D	F_{420} biosynthesis proteins	Reduced drug activation	

Abbreviations: *rpsL*, Ribosomal protein S12 gene; *rrs*, 16S rRNA gene; *gidB*, Glucose-inhibited division protein B; *eis*, enhanced intracellular survival protein; *tlyA*, rRNA methyltransferase; *gyrA*, DNA gyrase subunit A; *gyrB*, DNA gyrase subunit B; *ethA*, monooxygenase EthA; *ethR*, transcriptional regulator EthR; *inhA*, enoyl-ACP reductase; *thyA*, thymidylate synthase A; *folC*, folylpolyglutamate synthetase; *ribD*, riboflavin biosynthesis protein RibD; *alr*, alanine racemase; *ddl*, D-alanine–D-alanine ligase; *cycA*, D-serine/D-alanine/glycine transporter; *atpE*, ATP synthase subunit c; *rrl*, 23S ribosomal RNA; *rplC*, 50S ribosomal protein L3; *ddn*, deazaflavin-dependent nitroreductase; *fbiA/B/C/D*, F_{420} biosynthesis proteins.

reduction of bacterial populations in the initial phase of treatment. However, these drugs have poor sterilizing activity and must be administered in combination with drugs such as rifamycins and PZA to achieve sustained and effective therapy.[11,25,26]

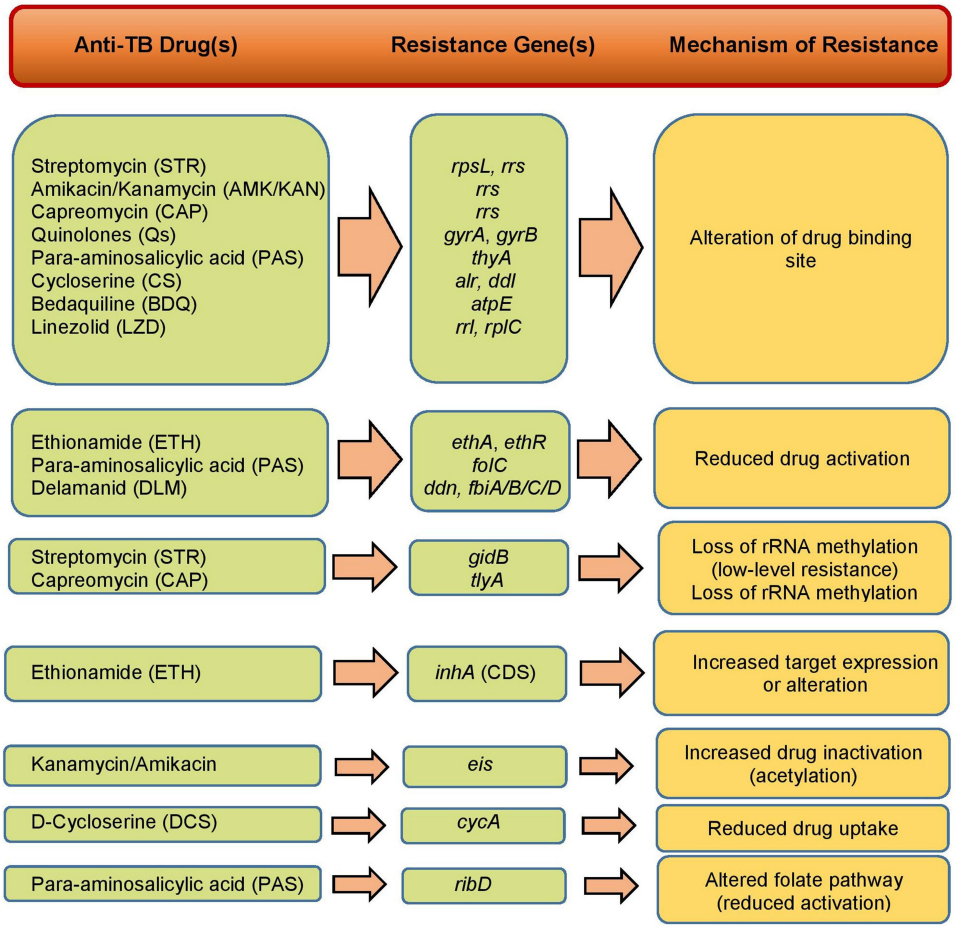

Figure 1 Schematic overview of the major injectable and second-line anti-tuberculosis drugs, their associated resistance genes, and the corresponding molecular mechanisms of drug resistance.

Mechanism of Action

Aminoglycosides exert their effect by attaching to the 30S subunit of the mycobacterial ribosome, leading to inhibition of protein synthesis. Most mutations that confer resistance to these drugs directly or indirectly alter their binding sites on the ribosome. These alterations prevent drug binding but preserve ribosome function, as seen in mutations in the *rpsL, rrs*, and *gidB* genes. A secondary mechanism of aminoglycoside resistance is chemical modification of the drug, exemplified by acetylation of the drug by the Eis protein. Enhanced intracellular survival proteins (Eis), a type of acetyltransferase enzyme, enhance the intracellular survival of mycobacteria in macrophages by acetylating amines present in aminoglycoside antibiotics to confer resistance to these antibiotics and by acetylating Lys55 in the phosphatase DUSP16/MPK-7 to suppress host innate immune defense.[27,28]

STR is a streptidine aminoglycoside that inhibits translation initiation by binding to the 30S ribosomal subunit and may cause errors in mRNA reading. In contrast, AMK and KAN are deoxystreptamine aminoglycosides that bind to a different site on the 30S ribosome. Due to the difference in the ribosomal binding site between STR and AMK/KAN, the mutations associated with drug resistance in these two drug classes are different. In contrast, due to the structural and functional similarity between AMK and KAN, there is extensive cross-resistance between the two.[11] Their precise mechanism of action is that STR disrupts the decoding process by binding near the codon recognition site on the 30S ribosomal subunit. This binding causes a conformational change in the 16S rRNA, specifically bases A1492 and A1493, which are involved in codon recognition. As a result, STR makes near-cognate codon-anticodon mispairing more stable while destabilizing cognate codon-anticodon recognition. This disruption in decoding accuracy can lead to the production of defective and inactive proteins in the bacterial cell.[29,30]

Mechanisms of Resistance

Mutations Associated with STR Resistance (*gidB*, *rrs*, *rpsL*)

High-level resistance to STR is mainly caused by mutations in the *rpsL* (approximately 50% of STR-resistant strains) and *rrs* (approximately 15% of STR-resistant strains) genes. Important *rrs* mutations associated with STR resistance include *rrs* A514C and A908C. While the *rrs* A514C mutation is associated with high-level resistance to STR, the most common *rrs* mutation that confers resistance to AMK and KAN is the *rrs* A1401G mutation. However, this mutation does not confer cross-resistance to STR. *gidB* mutations are less common among STR-resistant strains (approximately 20% of strains) and are associated with low-level resistance to STR. Low-level resistance mechanisms are not well understood, but are likely to involve efflux.[8,31]

gidB Gene

The *gidB* gene (Rv3919c; homologous to *rsmG*) encodes an S-adenosylmethionine-dependent methyltransferase that catalyzes the conserved N7-methylation of G527 in the 530 stem-loop of 16S rRNA in *M. tuberculosis*. Although nonessential for bacterial viability, *gidB* ensures accurate ribosomal decoding. Loss-of-function mutations—such as nonsense, frameshift, or deletions—abolish m^7G527 formation, producing undermethylated ribosomes with reduced STR binding at the A-site.[13] Consequently, these strains display modest increases in STR minimum inhibitory concentration (MIC) of 10–20 μg/mL, reflecting the low-level resistance observed in multiple bacterial taxa. Diverse *gidB* variants, including premature terminations and missense changes, are associated with decreased susceptibility to STR and other aminoglycosides, indicating a loss-of-function-driven resistance mechanism. Notably, similar polymorphisms occur in drug-sensitive strains, highlighting that *gidB* mutations alone are insufficient to predict resistance. Moreover, *gidB* inactivation synergizes with high-impact mutations in *rpsL* (eg, K43 substitutions) or *rrs* (530-loop alterations), markedly increasing STR resistance and complicating TB treatment in polypharmacy-based regimens.[13,31]

rrs Gene

The *rrs* gene directly codes for the 16S ribosomal RNA molecule. Within this rRNA, critical regions such as the 530 stem-loop and the 915 helix turn serve as primary binding sites for aminoglycoside antibiotics. Similar to *rpsL, rrs* is an essential gene required for bacterial viability. Missense mutations in *rrs* decrease the affinity of aminoglycosides for the ribosome while maintaining near-normal ribosomal activity.[11,32] Because most bacterial species carry several copies of the *rrs* gene, a mutation in only one copy typically produces low-level resistance. In sharp contrast, *M. tuberculosis* possesses a single *rrs* copy; therefore, mutations in this gene almost always lead to high-level aminoglycoside resistance.[32] To date, more than 20 distinct *rrs* mutations have been linked to aminoglycoside resistance, although the most frequently encountered are A1401G, A514C, and C517T. Several of these variants also cause cross-resistance to the cyclic peptide CAP. A1401G is by far the most common mutation associated with resistance to second-line injectable drugs (SLIDs). In a large systematic review, this substitution was detected in 78%, 56%, and 76% of *M. tuberculosis* isolates resistant to AMK, KAN, and CAP, respectively.[32]

rpsL Gene

Genetic analyses of clinical *M. tuberculosis* isolates show that resistance to STR often arises from mutations in the *rpsL* gene, which encodes ribosomal protein S12, essential for maintaining the 30S subunit architecture. Certain amino-acid–substituting mutations can occur without disrupting basic ribosomal function but subtly alter the drug-binding site, reducing aminoglycoside affinity. Studies in Korea and Japan have consistently identified K43R (lysine to arginine at codon 43) and K88R (lysine to arginine at codon 88) as the main contributors to STR resistance in circulating strains, highlighting their central role in target-based resistance mechanisms.[33,34]

Mutations Conferring Resistance to AMK and KAN: *rrs* and *eis*

The *rrs* A1401G mutation (an adenine to guanine change at position 1401 of the *rrs* gene) is the most common mutation conferring resistance to AMK and KAN, occurring in approximately 85% or more of strains resistant to AMK or KAN. The presence of the A1401G mutation appears to be 100% specific for simultaneous resistance to AMK and KAN. Of the

strains resistant to AMK and KAN, 10–15% have *eis* mutations, suggesting that *eis* is a minor factor in resistance to AMK and KAN.[14,33]

Increased *whiB7* Gene Expression

Recently, mutations in the promoter region of the *whiB7* gene, a transcriptional activator, have been identified in clinical isolates. These mutations, which increase *whiB7* expression, in turn lead to increased *eis* expression and thus resistance to KAN. In addition to *eis*, the *whiB7* regulon also includes Rv1258c, which encodes the Tap efflux pump. This pump is activated in conjunction with *eis* and confers resistance to STR. Therefore, promoter mutations in *whiB7* can lead to changes in drug targeting as well as increased efflux from the cell, ultimately leading to cross-resistance to 2 groups of aminoglycoside antibiotics.[8,35]

Inactivating Mutations of *eis*

The *eis* gene (Rv2416c/MT2489) encodes an aminoglycoside acetyltransferase enzyme that has a high affinity for KAN. Acetylation of KAN by this enzyme inactivates it by preventing KAN from binding to the 30S ribosomal subunit. Regulatory enhancer mutations in the 5′ untranslated region (5′UTR) of the *eis* gene are associated with clinical resistance of *M. tuberculosis* to KAN. While the Eis protein is also capable of acetylating AMK, its affinity for AMK is low. Studies of clinical isolates harboring mutations in the *eis* promoter region have shown that these mutations confer selective resistance to KAN, while maintaining sensitivity to AMK to a large extent. However, clinical isolates with *eis* mutations that are resistant to KAN and AMK have also been reported, so the specificity of *eis* mutations for KAN resistance remains unclear.[33,36]

Cyclic Peptide Antibiotics: Capreomycin and Viomycin

Capreomycin and viomycin (VIO) are drugs used in combination with AMK and KAN for the treatment of MDR-TB. All of these drugs inhibit translation, and cross-resistance to them during treatment is a major concern.[37] CAP and VIO are structurally similar cyclic peptide antibiotics that are active primarily against mycobacteria. Both drugs show significant activity against *M. tuberculosis* and act by inhibiting bacterial growth by blocking protein synthesis at the ribosome. These drugs have similar cross-resistance in *M. tuberculosis* and appear to have a similar mechanism of action. CAP and VIO bind to the B2a intersubunit bridge in the bacterial ribosome, which is formed by the interaction of helix 69 of the 23S rRNA (large subunit) and helix 44 of the 16S rRNA (small subunit), preventing the ribosome from functioning properly in the translation process.[38]

While VIO is rarely used due to its high toxicity, CAP is an injectable drug that is commonly used as a second-line drug in the treatment of MDR-TB and XDR-TB that are resistant to aminoglycosides. Like structurally unrelated aminoglycosides, CAP and VIO are bactericidal drugs that inhibit protein synthesis. VIO has been shown to bind to the 30S and 50S subunits of the ribosome and inhibit ribosomal transport by disrupting the peptidyl-tRNA acceptor site. Due to the overlap in the binding region between CAP and aminoglycosides, some mutations confer cross-resistance to CAP and AMK/KAN. In contrast, cross-resistance between CAP and STR is rare. The main mechanisms of CAP resistance involve mutations that lead to changes in the ribosome, particularly in the *rrs* and *tlyA* genes. Interestingly, *tlyA* mutations uniquely affect CAP resistance and do not appear to play a role in aminoglycoside resistance.[39,40]

The ribosomal proteins L12 and L10 in *M. tuberculosis* interact with each other and form the backbone of the 50S ribosomal subunit. These proteins play a critical role in the recruitment of initiation and elongation factors during translation. Therefore, the interaction between L12 and L10 is considered essential for ribosome function and protein synthesis. Studies have shown that CAP inhibits the interaction between these proteins, thereby disrupting translation and protein synthesis in bacteria. Resistance to CAP in *M. tuberculosis* usually occurs through increased expression of L12 or L10 proteins, which increases the MIC of the drug. It also inhibits the activity of the G-dependent GTPase and ribosomal protein synthesis. Finally, resistance to CAP may occur by preventing the disruption of the L12-L10 interaction and inhibiting protein synthesis in bacterial cells.[41]

Genetic Basis of CAP Resistance

Mutations Affecting Drug Binding and rRNA Methylation

rrs Gene

Point mutations in the *rrs* gene, which encodes the 16S rRNA subunit, are strongly associated with resistance to injectable drugs such as CAP, AMK, and KAN, and are predominantly located in the region between nucleotides 1400 and 1500.[42] Among these, the A1401G substitution plays a pivotal role in the development of CAP resistance.[43] The *rrs* A1401G mutation is detected in approximately 85% of CAP-resistant XDR-TB strains. Other *rrs* mutations, such as C1402T, occur less frequently, contributing to <3% of cases.[33] Collectively, *rrs* mutations account for ~88% of CAP resistance in this XDR-TB cohort, consistent with global reviews reporting 76–77% prevalence of *rrs* mutations among broader CAP-resistant populations.[14,32] Rare variants, including C1402T and G1484T, have also been implicated in CAP resistance.[32,33]

tlyA Gene

Mutations in the *tlyA* gene are another mechanism by which *M. tuberculosis* develops resistance to CAP. The *tlyA* gene (Rv1694/MT1733) is a non-essential locus and is also present in several other bacterial species.[11] It encodes an rRNA methyltransferase enzyme, mutations in which reduce ribosomal methylation and thus reduce sensitivity to CAP. This mechanism is similar to that of the *eis* gene, which confers resistance to KAN. Several mutations in *tlyA* have been reported, including L180R, S265T, S64W, frameshift mutations at 218L, N236K, and L150P.[15] Some CAP-resistant mutants have transposon insertions or point mutations in *tlyA*, which result in the lack of inhibition of transcription-translation by CAP. Also, overexpression of the efflux transporter Tap (Rv1258c) has been reported in some resistant isolates, which increases drug efflux from the bacterial cell and reduces its efficacy. These mechanisms, independently or in combination, contribute to resistance to CAP and may contribute to cross-resistance with other aminoglycoside antibiotics such as VIO.[37, 34]

Second-Line Drugs

Quinolones and Fluoroquinolones

Quinolones (Qs) are one of the most important classes of antibiotics, having been identified for more than 60 years. This group of antibiotics was first introduced with the discovery of nalidixic acid in 1962s, which was used to treat Gram-negative bacterial infections. However, nalidixic acid had limited activity and was not effective in treating tissue infections or more serious infections.[44,45] Gradually, fluoroquinolones (FQs) were developed, in which a fluoro group was added to the quinolone chemical structure, increasing their efficacy. The discovery of FQs in the 1980s was a major breakthrough. Fluoroquinolones are broad-spectrum antibiotics that are effective against a wide range of bacteria, including *Mycoplasma, Chlamydia, Chlamydophila* species, and *Mycobacterium*, and play an important role in the treatment of serious bacterial infections, especially nosocomial infections and other infections where resistance to older classes of antibiotics is likely.[46] Fluoroquinolones readily enter cells through purines and, therefore, are often used to treat intracellular pathogens. Quinolone antimicrobial agents exert their antibacterial effects by inhibiting type II topoisomerase enzymes, including DNA gyrase and DNA topoisomerase IV.[45]

Important FQs include CIP, Gatifloxacin (GAT), Moxifloxacin (MOX), and Levofloxacin (LFX), which are widely used to treat various infections. Fluoroquinolones are currently second-line anti-TB drugs and form the mainstay of MDR-TB therapy. In addition to their use in the treatment of drug-resistant TB, they are also prescribed for the treatment of drug-susceptible TB in patients who are intolerant to components of first-line therapies.[46]

Mechanism of Action

Quinolones exert their antibacterial effect by inhibiting bacterial topoisomerase enzymes, which regulate DNA supercoiling and are essential for the processes of DNA replication, transcription, and recombination. While many bacterial species possess both type II topoisomerase enzymes (DNA gyrase and topoisomerase IV), sequencing of the *M. tuberculosis* genome has shown that this bacterium possesses a type I topoisomerase, TopA, and only a type II

topoisomerase, DNA gyrase, and lacks a topoisomerase IV analogue.[47] Although bacterial type I topoisomerase has not yet been used as a target for clinical antibiotics, DNA gyrase has been widely targeted. Thus, in *M. tuberculosis*, the only target of quinolone activity is DNA gyrase.[48] The observed differences in clinical efficacy of quinolones may be due to their specificity for different topoisomerases. Ciprofloxacin, which is less effective against *M. tuberculosis*, primarily targets topoisomerase IV, which is absent in this bacterium. In contrast, newer generation FQs such as MOX and LFX, which have also shown better efficacy, preferentially target DNA gyrase.[16]

DNA gyrase is an ATP-dependent enzyme that cuts and joins double-stranded DNA, allowing the insertion of negative supercoils into the DNA. DNA gyrase is a 4-subunit protein (tetramer) composed of 2 α-subunits and 2 β-subunits, encoded by the *gyrA* (Rv0006/MT0006) and *gyrB* (Rv0005/MT0005) genes, respectively.[11]

Resistance to FQs

Although FQs are currently used to treat TB mainly in cases where first-line anti-TB drugs are resistant or difficult to tolerate, these drugs have the potential to become first-line drugs and are being studied for this use. However, there are concerns about the development of resistance to FQs in *M. tuberculosis*, especially when administered as monotherapy or as the only active agent in a failed multidrug anti-TB regimen. In these circumstances, treatment failures and relapses have been documented.[16] With the increasing number of FQs prescribed and the widespread use of these broad-spectrum drugs for the treatment of many infections, the selective pressure resulting from the use of FQs has led to the rapid emergence of resistance to these drugs in various species of organisms, including *M. tuberculosis*. Resistance is increasing among *M. tuberculosis* and may pose a serious threat to the long-term clinical use of FQs in the future. Discussion and education on the appropriate use of these drugs are essential to maintain the effectiveness of this class of antibiotics in the face of the growing risk of resistance.[16]

Mutations Altering DNA Gyrase Drug Binding

Within the *gyrA* and *gyrB* genes is a region called the quinolone-resistance-determining region (QRDR), which is highly conserved, and mutations in these regions are known to be the main cause of resistance to FQs in many bacterial species, including *M. tuberculosis*. However, some resistant isolates have been found without these mutations, suggesting the existence of other resistance mechanisms such as efflux pumps. For example, the efflux pump Rv1258c in *M. tuberculosis*, which is homologous to the Tap efflux pump in *M. fortuitum*, is involved in *M. tuberculosis* resistance to aminoglycosides. However, there is no evidence that this pump is involved in resistance to FQs.[49]

In many studies, more than 90% of quinolone-resistant *M. tuberculosis* strains have mutations in the *gyrA* or *gyrB* genes.[50] Classically, mutations in codons 90, 91, and 94 of the *gyrA* gene are most associated with drug resistance, and the A90V, D94G, and D94H mutations have been frequently observed in clinical isolates.[51] The most common and major cause of resistance to FQs in *M. tuberculosis* is mutation in the *gyrA* gene. Mutations in the *gyrB* gene are less common, but can confer resistance alone or in combination with mutations in *gyrA*. Among quinolone-resistant isolates, double mutations in *gyrA* or in addition to *gyrB* have been observed, leading to increased MICs.[50,51]

Another possible mechanism of quinolone resistance is DNA mimicry. In the DNA mimicry mechanism, the MfpA protein directly binds to DNA gyrase and inhibits its activity. MfpA is one of the important proteins in *M. tuberculosis*, whose 3D structure is a right-handed quadrilateral β-helix, which has similar size, shape, and electrical charge characteristics to form B-DNA, and plays a key role in conferring resistance to FQs.[52,53] This protein belongs to the pentapeptide repeat family of proteins. MfpA inhibits the activity of DNA gyrase, an important enzyme in various processes such as DNA replication and repair. Here, resistance to FQs is achieved through a direct inhibition of the enzyme, rather than through changes in gene structure. Therefore, this protein is still considered a defense mechanism for *M. tuberculosis* against FQs.[52,53]

Ethionamide and Prothionamide

Ethionamide (ETH) and its propyl analogue PTH are both thionamide drugs that are structurally similar to INH.[54] These drugs are highly effective in treating *M. tuberculosis, M. leprae*, and *M. avium* infections and are used in the treatment of MDR-TB and XDR-TB. ETH and PTH are considered second-line drugs, and their use is rapidly increasing with the

increasing incidence of drug resistance.[55] Due to cross-resistance between ETH and PTH, these drugs are used interchangeably and can be considered functionally equivalent.[11] ETH and PTH are prodrugs that exert their effects after enzymatic activation in mycobacteria. They are commonly prescribed as adjunctive or alternative therapy in patients who cannot tolerate mainstream drugs such as INH.[56] These drugs inhibit the enzyme [enoyl-acyl carrier protein (ACP) reductase], which is responsible for mycolic acid synthesis in mycobacteria, through a specific effect on the *inhA* product; therefore, they can be classified as bactericidal drugs.[54]

Activation and Mechanism of Action of ETH

ETH and INH have the same mechanism of action. Both are prodrugs that inactivate mycolic acid synthesis in mycobacteria (see Figure 2), but their activating enzymes are different. Unlike INH, which is activated by the enzyme KatG (catalase-peroxidase), ETH is activated by the monooxygenase enzyme encoded by the *ethA* gene. Mutations in the *katG* gene confer only high-level specific resistance to INH, whereas mutations in the common target of ETH and INH, *inhA*, confer low-level cross-resistance to both drugs.[11]

The enzyme EthA is a flavin-dependent monooxygenase (FAD-dependent monooxygenase) in *M. tuberculosis*. This enzyme is specific for the activation of ETH and does not play a role in the activation of INH. After activation by EthA, ETH combines with NAD^+ to form an adduct, which then inhibits the NADH-dependent enoyl-ACP reductase, InhA, as does the INH-NAD complex. This enzyme catalyzes one of the steps of the FAS-II cycle (fatty acid synthesis cycle type II), which is involved in the synthesis of mycolic acids in the cell wall of *M. tuberculosis*. Inhibition of this enzyme disrupts the production of mycolic acids and prevents bacterial growth.[11,57]

It has long been known that strains with low-level resistance to INH are also resistant to ETH, but strains with high-level resistance to INH may still remain susceptible to ETH.[11] Rueda et al have shown that among INH-resistant isolates, ETH resistance can be both independent and cross-resistant. Therefore, it has been emphasized that ETH susceptibility should be independently tested before this drug is used in the treatment of patients with MDR-TB.[60] In addition to EthA, five other Baeyer-Villiger monooxygenases (BVMOs) are predicted to be present in the *M. tuberculosis* genome: Rv0892, Rv0565c, Rv1393c, Rv3049c, and MymA (Rv3083). Ethionamide activation has also been demonstrated for MymA and Rv0565c. Mutations in these regions have been observed in clinical isolates, but their clinical significance and their co-occurrence with *ethA* mutations are still unclear.[61] In another report, deletion of the *mymA* gene in clinical isolates only resulted in a slight increase in MIC, which is still below the clinical threshold.[62]

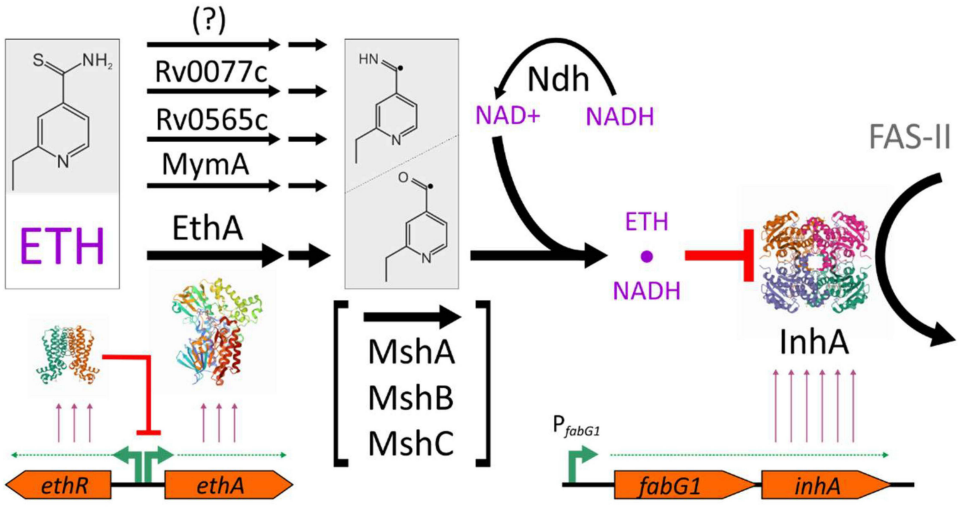

Figure 2 Model of ethionamide action in *M. tuberculosis*: ETH is activated in a multistep process that is largely dependent on the activity of the monooxygenase enzyme EthA. The final product of this activation, the ETH–NADH adduct, inhibits the enzyme InhA. InhA is an acyl-protein-carrying anvil reductase that catalyzes one of the steps of the FAS-II cycle (related to the synthesis of mycolic acid in the cell wall). Transcription of the *ethA* gene is under the control of an inhibitor called EthR. In addition, genes involved in mycothiol biosynthesis, including *mshA, mshB*, and *mshC*, or mycothiol itself, are also involved in ETH activation, although their precise role is not yet fully understood.[57–59]

https://doi.org/10.2147/IDR.S568121

Figure 3 In *M. tuberculosis*, the *inhA* and *fabG1* genes are located in close proximity to each other. Promoter mutations in the *inhA* gene, which is located upstream of the *fabG1* gene, especially the C-15T mutation, can lead to increased expression of the *inhA* gene and thus resistance to drugs such as INH and ETH.

A possible role for an oxidoreductase called Rv0077c in ETH activation has also been discovered. In this pathway, a compound called SMARt-420 binds to the inhibitor Rv0078, leading to expression of the Rv0077c gene and restoring susceptibility to ETH.

In a study, researchers showed that SMARt-420, when bound to the inhibitor Rv0078, increased expression of the Rv0077c gene and restored susceptibility to ETH in resistant strains. These findings indicate that SMARt-420 can be considered as a novel strategy to combat drug resistance in TB.[63]

At least two other metabolic pathways are involved in ETH activation. First, the ratio of NAD^+ to NADH influences the conversion step of ETH-derived radicals to the ETH-NADH adduct.[64] However, in a recent report, *ndh* mutations were found only in combination with *ethA* and *inhA* promoter mutations.[65] The second pathway is the mycothiol biosynthesis pathway: while the exact mechanism of action has not yet been reported, mash- strains have an EthR phenotype.[66] Similar to *ndh, mshA* mutations were detected in only a small percentage of clinical strains, in combination with *ethA* or *inhA* promoter mutations.[65]

In *M. tuberculosis*, the *inhA* and *fabG1* genes are located adjacent to each other and have a partial overlap of 16 nucleotides. Both genes probably function as a polycistronic mRNA in an operon. As shown in Figure 3, the *inhA* gene promoter is located upstream of the *fabG1* gene. Therefore, mutations in the *inhA* gene promoter, especially the PfabG1 c (−15)t mutation, often increase *inhA* gene expression and subsequently increase the number of InhA proteins in the cell, thereby reducing the toxic effect of INH and ETH drugs that target InhA.[57,67]

Previous studies have shown that mutations in the *katG, inhA, fabG1*, and the *oxyR′-ahpC* intergenic region confer resistance to INH.[68,69] Therefore, molecular mutations in the *ethA, inhA*, and *fabG1* promoters are important tools for predicting ETH resistance. Promoter mutations in the *inhA* gene, which is located near the *fabG1* gene, have previously been proposed as markers of ETH resistance.[57,70,71]

Resistance to ETH Arises Mainly in Two Ways

1. Loss of activity of the activating enzyme (EthA)

2. Increased expression of the drug target through mutations in the promoter region of the *inhA* gene

In most cases, simultaneous resistance to INH and ETH is explained by mutations in the promoter region of *inhA* as well as in the *katG, ethA, ethR, mshA, ndh*, and *inhA* genes.[60]

Genetic and Molecular Basis of ETH Resistance

Reduced Activation of ETH by *ethA*

The *ethA* gene (Rv3854c/MT3969) is a non-essential monooxygenase that activates ETH. Its expression is controlled by a TetR-like inhibitor called *ethR* (Rv3855/MT3970). Overexpression of *ethR* or loss of *ethA* function confers resistance to ETH. To date, more than 25 mutations in *ethA* have been identified that are associated with ETH resistance, scattered throughout the gene.[11] The distribution of these mutations throughout the gene suggests that loss of EthA monooxygenase activity does not have a significant biological cost to the bacteria, possibly because there are more than 30 other monooxygenases in the *M. tuberculosis* genome that may play a compensatory role. In contrast to *ethA*, the highly common and resistant S315T mutation in the *katG* gene, although it does not inactivate the *M. tuberculosis* catalase-peroxidase enzyme, loses the enzyme's ability to activate INH.[11,17] In some studies of clinical isolates, mutations in *ethA* have been observed in about 50% of resistant isolates, while the remaining 50% are caused by *inhA* mutations.[17]

However, in other studies, up to 50% of ETH resistance cannot be explained by *ethA* or *inhA* mutations. That is, the cause of resistance in these cases remains unknown.[72]

Also, other mutations in the *ethA-ethR* intergenic region that affect transcription have been associated with a slight increase in MIC, and in one of them, a decrease in *ethA* expression has been experimentally demonstrated. Importantly, this mutation is independently selected by different subclades and synergistically contributes to resistance to ETH with mutations in other regions.[57,73,74]

Genetic and Regulatory Changes in ETH Resistance: *inhA* and *mshA*

According to available studies, the *inhA* gene, which encodes the NADH-dependent enoyl-ACP reductase enzyme and is essential for mycolic acid biosynthesis, is the target of both ETH-NAD and INH-NAD combinations. Among ETH-resistant strains lacking mutations in *ethA*, mutations in the *inhA* promoter account for about two-thirds of the resistance, while promoter combinations with ORF mutations account for about one-third of the resistance. A small number of ETH-resistant strains have only mutations in the *inhA* ORF, usually associated with S94A, S94W, or L11V changes.[11,17,72] Additionally, alterations in the *mshA* gene (Rv0486), which encodes a glycosyltransferase involved in mycothiol biosynthesis, have been identified in ETH-resistant clinical isolates of *M. tuberculosis* and may contribute to reduced drug susceptibility, especially in strains harboring concomitant *ethA* or *inhA* mutations.[57,68]

D-Cycloserine

D-cycloserine (DCS), chemically known as D-4-amino-3-isoxazolidine, is an analog of the amino acid D-Ala and is an oral second-line bacteriostatic anti-TB drug. This broad-spectrum antibiotic is used for MDR-TB because it does not show cross-resistance with other anti-TB drugs.[75]

Studies have shown that DCS is more effective than other anti-TB drugs, including ETH and KAN. However, dose-dependent side effects such as psychosis, depression, and neuropathy have limited its use. The structural analog of CS, Terizidone, also has similar neuropsychiatric toxicities.[76,77]

Mechanism of Action

CS primarily exerts its effect by targeting enzymes critical for peptidoglycan synthesis. Traditionally, it has been regarded as a D-alanine analogue that inhibits alanine racemase (*alr*, Rv3423c/MT3532) and D-alanine-D-alanine ligase (*ddlA*, Rv2981c/MT3059).[75,76] Recent structural and biochemical studies by Batson et al have revealed a more complex mechanism: inside mycobacterial cells, DCS is phosphorylated by cellular kinases to form 3-phosphorylated D-cycloserine.[78] This phosphorylated derivative binds much more tightly to the active site of DdlA, forming a stable phosphonate adduct that irreversibly inactivates the enzyme. This explains the slow, time-dependent inhibition observed with CS and highlights why it can be more potent against DdlA than Alr under certain conditions. Moreover, the necessity of intracellular phosphorylation implies that changes in kinase activity or phosphate metabolism may influence susceptibility in resistant strains.[78] In addition, CS can still inhibit Alr and affect PLP-dependent enzymes, including branched-chain aminotransferases (BCATs), which participate in branched-chain amino acid biosynthesis.[20,79]

Mechanisms of Resistance

Mechanisms of resistance to CS are mainly caused by mutations in genes related to target enzymes or transport and metabolic pathways. Mutations in the *alr* gene, which encodes the enzyme alanine racemase (Alr), can lead to structural changes in this enzyme and reduce the binding of CS, thereby causing resistance. Changes in the *ddl* gene, which produces the enzyme D-alanine-D-alanine ligase (DdlA), may also reduce the sensitivity of the enzyme to CS, resulting in drug resistance. The *cycA* gene, which is responsible for the production of the carrier protein D-alanine, D-serine and glycine, plays a role in the transport of these compounds into the cell. Mutations in *cycA* may reduce the entry of CS into the cell, thus causing resistance.[21] Mutations in the *ald* gene, which encodes the enzyme alanine dehydrogenase, have also been reported as one of the mechanisms of resistance. These mutations can alter the metabolic pathways of the cell and reduce the effectiveness of the drug. In addition, recent studies have shown that mutations in other genes may also be associated with resistance to CS. These genes include those involved in lipid metabolism, stress response, and transport

systems.[21] In general, resistance to CS can arise from mutations in genes encoding target enzymes or other critical pathways in *M. tuberculosis*, reducing the effectiveness of the drug.

Para-Aminosalicylic Acid

Para-aminosalicylic acid, introduced in 1946–1948 shortly after STR, is currently classified by the WHO as a second-line oral agent for drug-resistant TB.[18]

Mechanism of Action of Para-Aminosalicylic Acid

Para-aminosalicylic acid is a structural analogue of para-aminobenzoic acid (PABA) that interferes with the folic acid synthesis pathway in *M. tuberculosis*. Contrary to the initial idea that PAS is similar to sulfonamides and a direct inhibitor of the enzyme dihydroproteoate synthase (DHPS), recent studies have shown that PAS acts as a prodrug. After PAS enters the bacterial cell, DHPS replaces PABA in the folic acid biosynthesis pathway, resulting in the production of abnormal metabolites. These metabolites penetrate downstream steps of the pathway and inhibit critical enzymes such as dihydrofolate reductase (DHFR). This inhibition blocks the synthesis of thymidine, purines, glycine, and methionine, ultimately preventing the synthesis of DNA, RNA, and protein.[80,81]

Mechanisms of Para-Aminosalicylic Acid Resistance

Clinical and genetic studies have shown that several mechanisms are involved in the development of PAS resistance in *M. tuberculosis*, including:

Mutations in the *thyA* Gene

The *thyA* gene (Rv2764c/MT2834), which encodes the enzyme thymidylate synthase, was once considered a PAS resistance gene. However, more extensive studies have shown that the association between mutations in this gene and PAS resistance is weak and inconclusive.[81]

Overexpression of RibD Enzyme

The RibD enzyme (Rv2671/MT2745), which is involved in the riboflavin synthesis pathway, also has dihydrofolate reductase activity. In PAS-resistant clinical isolates, mutations have been shown to increase *ribD* expression, which counteracts the inhibitory effect of PAS.[82]

Reduced Drug Activation and Increased Drug Efflux

PAS must be actively metabolized by enzymes such as FolC to be effective. Mutations in these enzymes can lead to reduced drug activation and thus resistance. Mycobacteria can also reduce intracellular PAS concentrations and become resistant to it by increasing the expression of efflux pumps such as Tap.[19]

In a study, six genes involved in the folate metabolism and thymine nucleotide synthesis pathway, including *thyA, dfrA, folC, folP1, folP2*, and *thyX*, along with three N-acetyltransferase genes [*nhoA, aac(1), aac(2)*], were analyzed to investigate their association with PAS resistance. The results showed that among these genes, mutations in *thyA* and *dfrA* were identified that could result in the production of defective or abnormally functional proteins and thus contribute to drug resistance. In contrast, no specific genetic changes were observed in the other genes examined. These findings suggest that only a portion of PAS resistance can be attributed to changes in these genes and that there may be alternative resistance mechanisms or pathways that have not yet been fully identified.[81]

Clofazimine

Clofazimine (CFZ) is a riminophenazine antibiotic initially developed for leprosy, and is now a key drug for the treatment of MDR-TB and XDR-TB.[83,84]

It is recommended by the WHO in longer MDR-TB regimens and as a substitute in the 6-month BPaLM (bedaquiline (BDQ), pretomanid (Pa), linezolid (LZD), and MOX) / BPaL regimen when BDQ or LZD cannot be used.[85] In recent years, adding CFZ and BDQ to regimens for MDR-TB has significantly improved treatment outcomes and attracted considerable clinical attention due to their strong efficacy.[86] The primary bactericidal mechanism of CFZ involves

targeting mycobacterial type-2 NADH dehydrogenase (NDH-2), causing redox cycling and generation of reactive oxygen species (ROS) that damage bacterial macromolecules. Additional effects may include membrane disruption and inhibition of potassium uptake.[87]

Genetic resistance to CFZ is mainly mediated by mutations in Rv0678, which upregulate the MmpS5/MmpL5 efflux pump, reducing intracellular drug levels.[88] Other resistance-associated mutations occur in *atpE, pepQ*, and Rv1979c, contributing to cross-resistance with BDQ.[86,89] Phenotypic resistance to CFZ remains low globally (2–7% in MDR/XDR-TB isolates), but the prevalence of pre-existing resistance mutations (especially in Rv0678) is increasing.[90]

TBI-166, a novel and optimized analogue of CFZ from the riminophenazine family, has been developed with the aim of increasing efficacy and reducing side effects.[91] In addition to maintaining a similar mechanism of action, this drug also has the ability to inhibit the CYP3A4 enzyme, which may help reduce the toxicity of concomitant drugs such as BDQ. Preclinical results indicate significant efficacy of TBI-166, especially in combination with other drugs, in the treatment of drug-resistant TB.[83,92]

Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics that inhibit protein synthesis by binding to the 50S subunit of the ribosome, and have been used in the treatment of infections caused by Gram-positive bacteria, including resistant *M. tuberculosis*. Linezolid is the first member of this family. Originally developed to combat Gram-positive bacterial infections, LZD has been repurposed for the treatment of TB and has shown promising results in the treatment of MDR-TB. It inhibits the formation of the protein synthesis initiation complex by binding to the 23S rRNA of the large subunit of the bacterial ribosome. LZD is effective due to its good penetration into various tissues, including the lung and cerebrospinal fluid; however, side effects such as bone marrow suppression and mitochondrial toxicity have limited its long-term use. In response to this challenge, extensive research has been conducted to identify novel oxazolidinones with equal or greater efficacy and improved safety in the treatment of TB. These compounds include sutezolid, tedizolid, delpazolid, and TBI-223, which have shown promising preclinical and clinical results.[93]

Mechanisms of LZD Resistance

Mechanisms of LZD resistance are divided into two categories: ribosomal and non-ribosomal mechanisms:

Ribosomal Mechanisms

- Mutations in the *rrl* gene (23S rRNA): Mutations such as G2814T and G2270T in domain V of 23S rRNA alter the structure of the peptidyl transferase center (PTC), which is the binding site of LZD. These changes result in reduced drug efficacy.[94,95]
- Mutations in the *rplC* gene (ribosomal protein L3): The T460C mutation, which results in a change of the amino acid Cys to Arg at position 154, alters the structure of the PTC and increases resistance to LZD. This mutation has been identified in both in vitro-selected LZD-resistant isolates and in a number of clinical isolates resistant to this drug.[96]
- Loss of function of the *tsnR* gene: The *tsnR* gene, which probably encodes a 23S rRNA methylase, increases resistance to LZD when lost in function. The exact mechanism of this phenomenon is not yet fully understood.[94]

Non-Ribosomal Mechanisms

- Increased activity of efflux pumps: Mutations in genes such as Rv0545c, Rv0930, Rv2477, Rv3331, and Rv0890c lead to increased expression of efflux pumps that remove LZD from the cell and reduce its intracellular concentration.[23]
- Mutations in the *fadD32* gene: The C880T (His294Tyr) mutation in the *fadD32* gene, which is involved in mycolic acid synthesis, increases the hydrophobicity of the cell wall. This change prevents the entry of hydrophilic drugs such as LZD into the cell.[97]

β-Lactams

β-Lactam antibiotics inhibit *M. tuberculosis* cell wall synthesis by covalently acylating penicillin-binding proteins (PBPs) and L,D-transpeptidases (LDTs), the enzymes responsible for 4→3 and 3→3 peptidoglycan cross-links, respectively.[98]

The primary mechanism of intrinsic β-lactam resistance in *M. tuberculosis* is the chromosomally encoded extended-spectrum class A β-lactamase BlaC, which rapidly hydrolyzes most penicillins, cephalosporins, and carbapenems.[98,99]

M. tuberculosis peptidoglycan is characterized by a high proportion (up to 80%) of unusual 3→3 cross-links formed predominantly by the L,D-transpeptidases LdtMt1 and LdtMt2, making these enzymes particularly attractive therapeutic targets. Carbapenems, in particular, display high affinity for LdtMt2, and their antimycobacterial activity is dramatically potentiated when BlaC is inhibited.[99]

β-Lactamase inhibitors such as clavulanate, avibactam, relebactam, and vaborbactam irreversibly acylate the active-site serine of BlaC, restoring the activity of partner β-lactams and enabling effective inhibition of both PBPs and LDTs.[99,100] This combination strategy has renewed clinical interest in β-lactams for drug-resistant TB.

Large-scale screening of more than 8,900 β-lactam compounds by an industry–academic consortium identified numerous carbapenems and cephalosporins with intrinsic or inhibitor-potentiated activity against *M. tuberculosis* (MIC ≤ 20 μmol/L), including against non-replicating persisters.[101] Several dual β-lactam combinations (eg, meropenem–faropenem, biapenem–tebipenem) have also shown strong in vitro synergy.[100,101]

Clinically, the combination of meropenem with clavulanate has demonstrated encouraging results when added to background regimens for XDR and pre-XDR-TB, with multiple observational studies and case series reporting high rates of culture conversion and favourable outcomes.[102] These findings, supported by recent mechanistic and screening studies, have repositioned β-lactams—particularly carbapenems combined with potent BlaC inhibitors—as valuable components of salvage and shortened regimens for drug-resistant TB.[103]

Bedaquiline

Bedaquiline (BDQ/TMC207) is a diarylquinoline drug approved in 2012 for the treatment of MDR-TB. BDQ binds to and inhibits the ATP synthase enzyme in *M. tuberculosis*. This enzyme is encoded by the essential *atpE* gene (Rv1305/MT1345).[104] AtpE is part of the membrane complex F0F1 ATP synthase, which provides the energy for ATP production by transporting protons across the membrane. Inhibition of ATP biosynthesis leads to bacterial death in both active and inactive states.[105] Resistance to BDQ has usually been observed through mutations in the *atpE* gene, however, some resistant mutants have been found without any mutations in *atpE* or other ATP synthase-related genes, suggesting the possibility of alternative resistance mechanisms or even other drug targets for BDQ.[22,106] It has recently been shown that verapamil, by inhibiting efflux pumps, can significantly increase bacterial susceptibility to BDQ and CFZ, which emphasizes the possible role of efflux as a resistance mechanism.[107]

Delamanid

Delamanid (DLM), an oral nitro-dihydro-imidazooxazole derivative, represents one of the first new chemical entities approved in over 40 years for the treatment of MDR-TB.[108] Randomized controlled trials and real-world evidence have consistently demonstrated its potent bactericidal and sterilizing activity against *M. tuberculosis*, along with a favorable safety profile characterized primarily by manageable QT-interval prolongation without significant cardiac events.[109,110] Consequently, DLM has been incorporated into WHO-recommended shorter and longer all-oral regimens for rifampicin-resistant and multidrug-resistant TB (RR/MDR-TB) and pre-XDR-TB, including the 6-month BPaL/M-based and BDL (BDQ, DLM, and LZD) regimens.[85]

Mechanism of Action

Delamanid is a prodrug requiring bioactivation by the mycobacterial F_{420}-dependent nitroreductase encoded by the *ddn* (Rv3547) gene.[24] Following activation, it releases reactive nitrogen species, particularly nitric oxide and other intermediates, that irreversibly inhibit methoxy-mycolic and keto-mycolic acid synthesis—essential components of the mycobacterial cell wall—leading to impaired cell-envelope integrity and bacterial death.[24,111] Recent structural and biochemical studies have further identified decaprenylphosphoryl-β-D-ribose 2′-epimerase subunit 2 (DprE2) as an additional target under aerobic conditions, whereby activated DLM competitively antagonizes NADPH binding to

DprE2, thereby blocking the formation of decaprenylphosphoryl-D-arabinose, a critical precursor for arabinogalactan and lipoarabinomannan biosynthesis.[111,112]

Mechanism of Resistance

Resistance to DLM predominantly emerges through loss-of-function mutations in the F_{420}-dependent activation pathway. Non-synonymous mutations in *ddn* are the most frequent mechanism, directly abolishing nitroreductase activity and preventing prodrug activation. Mutations in genes involved in F_{420} coenzyme biosynthesis and recycling—namely *fgd1, fbiA, fbiB, fbiC*, and *fbiD*—reduce intracellular $F_{420}H_2$ levels and similarly impair bioactivation.[24,113] Although rare, alterations affecting redox homeostasis or central carbon metabolism can contribute to low-level, non-specific resistance by modulating cofactor availability.[113] Global surveillance data indicate persistently low prevalence of DLM resistance (<5% in most settings); however, emerging reports from high-burden countries underscore the importance of routine phenotypic and genotypic susceptibility testing to preserve efficacy and limit cross-resistance with the structurally related nitroimidazole pretomanid.[114,115]

Diagnostic Methods for Drug-Resistant *M. tuberculosis*

Rapid and precise detection of resistance to second-line drugs, injectable aminoglycosides, and novel anti-TB therapeutics (eg, BDQ, DLM, LZD) is critically important for managing MDR and XDR *M. tuberculosis* infections. Traditional culture-based drug susceptibility testing (DST) remains the gold standard, but the long turnaround time, biosafety requirements, and limited scalability hinder prompt treatment initiation. Molecular and sequencing-based diagnostic approaches that identify resistance-conferring gene mutations offer a paradigm shift toward rapid, genotypic detection of drug resistance.[116,117]

Reverse hybridization Line Probe Assays (LPAs), such as the GenoType MTBDRsl v2.0 assay enable the detection of key mutations associated with resistance to fluoroquinolones (FQs: *gyrA, gyrB*) and injectable second-line agents (aminoglycosides – AMK, KAN, CAP via *rrs* and *eis* promoter regions).[116] For example, a South African cohort by Gardee et al reported a sensitivity of 100% (95% CI: 95.8–100%) and specificity of 98.9% (95% CI: 96.1–99.9%) for FQs, and a sensitivity of 89.2% (95% CI: 79.1–95.6%) with specificity 98.5% (95% CI: 95.7–99.7%) for SLIDs.[116] LPAs thus provide a rapid alternative to phenotypic DST, although their utility may be limited in samples with low bacillary burden and may not capture rare or novel mutations outside the targeted gene regions.

The Xpert MTB/XDR assay represents a significant advance in nucleic acid amplification tests (NAATs), enabling simultaneous detection of *M. tuberculosis* and resistance to INH, FQs, ETH, and AMK within approximately two hours.[117,118] As highlighted by Maya et al, Xpert MTB/XDR achieved high diagnostic accuracy in a Tanzanian TB reference laboratory. These assays address the limitations of earlier cartridges (eg, Xpert MTB/RIF and Ultra) by expanding beyond RIF resistance alone and aligning with the World Health Organization's "End TB" strategy.[117,118] However, they remain restricted to predefined gene targets and may not detect resistance to novel therapeutics unless explicitly designed to do so.

Targeted Next-Generation Sequencing (tNGS) panels such as the Deeplex Myc-TB assay enable simultaneous deep sequencing of multiple loci implicated in resistance to second-line drugs, injectable agents, and novel therapies (eg, *atpE* for BDQ; *ddn*/*fbi* genes for DLM; *rrl*/*rplC* for LZD).[119–121] A recent Lancet Infectious Diseases article demonstrated that tNGS performed directly on sputum had high sensitivity and specificity for a broad panel of anti-TB drugs, including newer agents.[119] Because tNGS can detect heteroresistance (minor variant populations) and novel mutations, it offers higher resolution than conventional LPAs while retaining faster turnaround than whole-genome sequencing (WGS). Despite this, its implementation still requires optimized workflows and validated interpretation frameworks.[122,123]

WGS offers the most comprehensive genotypic approach, enabling identification of known and novel mutations across the entire *M. tuberculosis* genome, including those linked to injectable second-line agents and novel therapeutics.[124–126] For instance, Walker et al demonstrated that WGS could predict drug susceptibility in *M. tuberculosis* isolates with high concordance to phenotypic DST.[124] Similarly, Gygli et al reported overall sensitivity of 86.8% and specificity of 94.5% for WGS in identifying both first- and second-line drug resistance.[126] Nevertheless,

WGS remains constrained by cost, infrastructure demands, bioinformatics expertise, and the need for curated mutation-to-phenotype databases.

Beyond sequencing, emerging modalities such as Raman spectroscopy combined with machine learning,[127,128] CRISPR-based diagnostics, and nanopore-based targeted sequencing (NTS) are under investigation for rapid, culture-independent detection of drug resistance—including second-line and novel therapy resistance.[129,130] While still in early clinical stages, these technologies may offer low-cost, high-throughput screening suitable for resource-limited settings.

Advantages, Limitations and Implementation Considerations

Molecular diagnostics focused on injectable agents, second-line drugs, and novel therapeutics offer rapid turnaround, high specificity and the ability to detect mixed populations and uncommon mutations.[117,119] LPAs and NAATs are presently suited for decentralized laboratories; tNGS and WGS enable deeper, personalized resistance profiling. Nonetheless, implementation challenges remain: infrastructure cost, need for trained personnel, standardization of mutation interpretation, and accessibility in high-burden, low-resource regions. In addition, panels must be updated continuously as new resistance mechanisms are identified, particularly for novel therapeutics such as BDQ and DLM.

Machine Learning Reveals Polygenic Architecture of Drug Resistance in *M. tuberculosis*

Despite substantial progress in identifying gene mutations that confer drug resistance in *M. tuberculosis*, many resistant isolates remain unexplained by classical models. One reason is that resistance often arises from combinatorial effects, including compensatory mutations and low-frequency variants that do not reside exclusively in canonical resistance genes. Machine-learning analyses of WGS data now reveal dozens to hundreds of potentially novel resistance-associated loci outside the well-known genes. For example, an AI model trained on WGS data recently identified over 100 candidate mutations across 13 anti-TB drugs, including mutations in genes not previously linked to resistance, using a decision tree-based XGBoost classifier to highlight the most influential genomic positions.[131]

Deep learning models like TB-DROP (TuBerculosis Drug Resistance Optimal Prediction), which take genome-wide mutation profiles as input, have further demonstrated high accuracy (mean sensitivity ~ 90% and specificity ~ 87%) for first-line drug resistance without relying solely on prior knowledge of resistance loci.[132] Computational approaches are also being validated on clinical data: for instance, a study on 182 *M. tuberculosis* isolates from Uganda combined SNP profiles and patient metadata to predict resistance to INH, RIF, STR, and EMB with high fidelity.[133]

In addition, refined machine-learning pipelines have begun uncovering cryptic resistance signals in isolates that are phenotypically susceptible: using WGS, researchers found *M. tuberculosis* strains that tested sensitive by culture-based assays but harbored resistance-conferring single nucleotide polymorphisms (SNPs) in genes such as *katG, inhA, rpoB*, and intergenic regions.[134]

These insights mark a shift in our conceptual model of drug resistance: from a narrow view based on a handful of "resistance genes" toward a polygenic, context-dependent architecture, shaped by regulatory variation, compensatory mutations, and evolutionary forces.[135,136] As WGS becomes more routine and machine-learning approaches become increasingly transparent and clinically usable, these "resistance signatures" can be more precisely defined, enabling improved mechanistic understanding and the development of robust algorithm-driven diagnostic tools for drug-resistant TB.[132,133]

Critical Discussion

The present review highlights the expanding complexity of genetic resistance in *M. tuberculosis*. Although classical resistance models emphasize canonical drug-target mutations in genes such as *rrs, rpsL, gyrA*, and *gyrB*, accumulating evidence shows that these mutations explain only a portion of phenotypic drug resistance observed in clinical isolates. This mismatch between genotype and phenotype is well documented in recent molecular studies of aminoglycosides and second-line agents.[32] One of the most significant therapeutic challenges is cross-resistance among aminoglycosides. A single mutation—*rrs* A1401G—can simultaneously cause resistance to AMK, KAN, and CAP.[8] Although *eis* promoter

mutations have been proposed to cause selective KAN resistance, studies show inconsistent predictability in clinical settings, questioning the reliability of single-gene diagnostics.

Furthermore, many resistant isolates lack known mutations, suggesting polygenic mechanisms involving efflux pumps (eg, Rv1258c, MmpL5/MmpS5), transcriptional regulators (*whiB7, ethR*), and metabolic pathways (eg, NADH/NAD^+ shifts during ETH activation). Such noncanonical mechanisms are particularly relevant to resistance against thioamides, LZD, clofazimine, and BDQ.[35,57,137] For FQs, although mutations in *gyrA* codons 90 and 94 remain the primary markers,[50] complementary mechanisms such as *mfpA* DNA mimicry also contribute to drug tolerance.[52] These findings emphasize that resistance is not driven solely by target modifications but often emerges through compensatory pathways and regulatory networks. Modern molecular diagnostic tests, including LPAs, Xpert MTB/XDR, and tNGS panels, significantly improved detection speed; however, they rely on predefined mutation panels and therefore miss rare or novel variants. Whole-genome sequencing has demonstrated superior predictive accuracy,[138,139] but its use is limited by cost, infrastructure, and lack of standardized global mutation databases. A key limitation in resistance research is the insufficient functional validation of newly reported mutations. Many SNPs listed in databases such as ReSeqTB or TBDreamDB lack experimental confirmation, risking overinterpretation and misclassification. Integrating WGS with biochemical assays remains essential to distinguish causal mutations from benign polymorphisms.[140,141]

Finally, the increasing use of novel anti-TB drugs—including BDQ, DLM, pretomanid, and next-generation oxazolidinones—has been accompanied by emerging resistance, primarily driven by mutations in Rv0678, *atpE, ddn*, and *fbi* pathway genes.[121,142] This highlights the urgent need for real-time genomic surveillance and rational drug-combination strategies. Overall, controlling drug-resistant TB requires a shift from single-gene interpretations toward a systems-level understanding of bacterial evolution, metabolic plasticity, and combinatorial resistance networks.

Conclusion

Drug-resistant TB represents a multifactorial challenge shaped by genetic, regulatory, and metabolic mechanisms that enable *M. tuberculosis* to escape both classical and novel therapeutic agents. While mutations in well-known genes remain essential markers, noncanonical and polygenic resistance mechanisms contribute substantially to the complexity observed in clinical isolates.

Advances in molecular diagnostics—especially WGS and machine-learning–based prediction models—are transforming our ability to detect cryptic resistance and design personalized treatment regimens. However, challenges such as limited global access, insufficient functional validation of new variants, and geographic variability in mutation patterns continue to hinder effective TB control.

A comprehensive strategy encompassing rapid genotypic testing, region-specific diagnostic algorithms, rational drug design, and global genomic surveillance will be essential for improving treatment outcomes and achieving WHO End-TB goals. Continued integration of clinical microbiology, structural biology, computational genomics, and population-level data will accelerate progress toward shorter, safer, and more effective therapies for MDR/XDR-TB.

Permission to Reproduce Material from Other Sources

No previously published figures, tables, or text requiring permission for reproduction has been included in this manuscript.

Clinical Trial Registration

This study does not involve a clinical trial and, therefore, does not require registration.

Declaration of AI Assistance

This study used generative AI- and AI-assisted technologies to paraphrase and rewrite certain sections to improve clarity and readability. The content was generated based on the original text, while ensuring accuracy and maintaining scientific integrity.

Data Sharing Statement

This review is based on previously published studies; no new data were generated or analyzed.

Ethics Statement

This study was conducted in accordance with ethical guidelines and approved by the relevant institutional ethics committee. None of the human or animal subjects were included in the experimental procedures.

Funding

This research received no specific grants from any funding agency, commercial entity, or not-for-profit organization.

Disclosure

The author declares no conflict of interest in this work.

References

1. Liu X, Niu H, Guo D, et al. Application value of nucleic acid MALDI-TOF MS in mycobacterial species identification and drug resistance detection in Mycobacterium tuberculosis. *Microbiol Spectrum*. 2025;13(5):e01545–24. doi:10.1128/spectrum.01545-24
2. Khawbung JL, Nath D, Chakraborty S. Drug resistant tuberculosis: a review. *Comp Immunol Microbiol Infect Dis*. 2021;74:101574. doi:10.1016/j.cimid.2020.101574
3. WHO. *Global Tuberculosis Report 2024*; 2024. Vol. 2024:1–69.
4. Salari N, Kanjoori AH, Hosseinian-Far A, Hasheminezhad R, Mansouri K, Mohammadi M. Global prevalence of drug-resistant tuberculosis: a systematic review and meta-analysis. *Infect Dis Poverty*. 2023;12(1):57. doi:10.1186/s40249-023-01107-x
5. Willand N, Flipo M, Villemagne B, Baulard A, Deprez B. Chapter Five - Recent advances in the design of inhibitors of mycobacterial transcriptional regulators to boost thioamides anti-tubercular activity and circumvent acquired-resistance. In: Chibale K, editor. *Annual Reports in Medicinal Chemistry*. Academic Press; 2019:131–152.
6. Furin J, Cox H, Pai M. Tuberculosis. *Lancet*. 2019;393(10181):1642–1656. doi:10.1016/s0140-6736(19)30308-3
7. Bagcchi S. WHO's Global Tuberculosis Report 2022. *Lancet Microbe*. 2023;4(1):e20. doi:10.1016/s2666-5247(22)00359-7
8. Reeves AZ, Campbell PJ, Sultana R, et al. Aminoglycoside cross-resistance in Mycobacterium tuberculosis due to mutations in the 5' untranslated region of whiB7. *Antimicrob Agents Chemother*. 2013;57(4):1857–1865. doi:10.1128/aac.02191-12
9. Allué-Guardia A, García JI, Torrelles JB. Evolution of drug-resistant mycobacterium tuberculosis strains and their adaptation to the human lung environment. *Front Microbiol*. 2021;12:612675. doi:10.3389/fmicb.2021.612675
10. Merza MA, Reza M. Extensively drug-resistant tuberculosis (XDR) and extremely drug-resistant tuberculosis (XXDR): risk factors and molecular perspectives. *Arch Clin Infect Dis*. 2010;5:174–188.
11. Cohen KA, Bishai WR, Pym AS. Molecular basis of drug resistance in Mycobacterium tuberculosis. *Microbiol Spectr*. 2014;2(3). doi:10.1128/microbiolspec.MGM2-0036-2013
12. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. *Int J Tuberc Lung Dis*. 2015;19(11):1276–1289. doi:10.5588/ijtld.15.0389
13. Okamoto S, Tamaru A, Nakajima C, et al. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. *Mol Microbiol*. 2007;63(4):1096–1106. doi:10.1111/j.1365-2958.2006.05585.x
14. Campbell PJ, Morlock GP, Sikes RD, et al. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2011;55(5):2032–2041. doi:10.1128/aac.01550-10
15. Maus CE, Plikaytis BB, Shinnick TM. Mutation of tlyA confers capreomycin resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2005;49(2):571–577. doi:10.1128/aac.49.2.571-577.2005
16. Ginsburg AS, Grosset JH, Bishai WR. Fluoroquinolones, tuberculosis, and resistance. *Lancet Infect Dis*. 2003;3(7):432–442. doi:10.1016/s1473-3099(03)00671-6
17. Morlock GP, Metchock B, Sikes D, Crawford JT, Cooksey RC. ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. *Antimicrob Agents Chemother*. 2003;47(12):3799–3805. doi:10.1128/aac.47.12.3799-3805.2003
18. Lehmann J. Para-aminosalicylic acid in the treatment of tuberculosis. *Lancet*. 1946;247(6384):15–16. doi:10.1016/S0140-6736(46)91185-3
19. Minato Y, Thiede JM, Kordus SL, McKlveen EJ, Turman BJ, Baughn AD. Mycobacterium tuberculosis folate metabolism and the mechanistic basis for para-aminosalicylic acid susceptibility and resistance. *Antimicrob Agents Chemother*. 2015;59(9):5097–5106. doi:10.1128/aac.00647-15
20. Bruning JB, Murillo AC, Chacon O, Barletta RG, Sacchettini JC. Structure of the Mycobacterium tuberculosis D-alanine:D-alanine ligase, a target of the antituberculosis drug D-cycloserine. *Antimicrob Agents Chemother*. 2011;55(1):291–301. doi:10.1128/aac.00558-10
21. Chen J, Zhang S, Cui P, Shi W, Zhang W, Zhang Y. Identification of novel mutations associated with cycloserine resistance in Mycobacterium tuberculosis. *J Antimicrob Chemother*. 2017;72(12):3272–3276. doi:10.1093/jac/dkx316
22. Huitric E, Verhasselt P, Koul A, Andries K, Hoffner S, Andersson DI. Rates and mechanisms of resistance development in Mycobacterium tuberculosis to a novel diarylquinoline ATP synthase inhibitor. *Antimicrob Agents Chemother*. 2010;54(3):1022–1028. doi:10.1128/aac.01611-09
23. Srivastava S, Magombedze G, Koeuth T, et al. Linezolid dose that maximizes sterilizing effect while minimizing toxicity and resistance emergence for tuberculosis. *Antimicrob Agents Chemother*. 2017;61(8). doi:10.1128/aac.00751-17

24. Yan MY, Li H, Qu YM, et al. CRISPR Screening and Comparative LC-MS Analysis Identify Genes Mediating Efficacy of Delamanid and Pretomanid against Mycobacterium tuberculosis. *Adv Sci*. 2024;11(39):e2400176. doi:10.1002/advs.202400176
25. Zhang L, Song J, Kong L, et al. The strategies and techniques of drug discovery from natural products. *Pharmacol Ther*. 2020;216:107686. doi:10.1016/j.pharmthera.2020.107686
26. Singh S, Dhankar N, Garg AK, Molugulu N, Kesharwani P. Chapter 2 - Tuberculosis: introduction, drug regimens, and multidrug-resistance. In: Kesharwani P, editor. *Nanotechnology Based Approaches for Tuberculosis Treatment*. Academic Press; 2020:27–36.
27. Pan Q, Zhao F-L, Ye B-C. Eis, a novel family of arylalkylamine N-acetyltransferase (EC 2.3.1.87). *Sci Rep*. 2018;8(1):2435. doi:10.1038/s41598-018-20802-6
28. Kim KH, An DR, Song J, et al. Mycobacterium tuberculosis Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proc Natl Acad Sci U S A*. 2012;109(20):7729–7734. doi:10.1073/pnas.1120251109
29. Demirci H, Murphy F, Murphy E, Gregory ST, Dahlberg AE, Jogl G. A structural basis for streptomycin-induced misreading of the genetic code. *Nat Commun*. 2013;4(1):1355. doi:10.1038/ncomms2346
30. Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature*. 2000;407(6802):340–348. doi:10.1038/35030019
31. Wang Y, Li Q, Gao H, et al. The roles of rpsL, rrs, and gidB mutations in predicting streptomycin-resistant drugs used on clinical Mycobacterium tuberculosis isolates from Hebei Province, China. *Int J Clin Exp Pathol*. 2019;12(7):2713–2721.
32. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of genetic mutations associated with Mycobacterium tuberculosis resistance to amikacin, kanamycin and capreomycin: a systematic review. *PLoS One*. 2012;7(3):e33275. doi:10.1371/journal.pone.0033275
33. Jnawali HN, Yoo H, Ryoo S, et al. Molecular genetics of Mycobacterium tuberculosis resistant to aminoglycosides and cyclic peptide capreomycin antibiotics in Korea. *World J Microbiol Biotechnol*. 2013;29(6):975–982. doi:10.1007/s11274-013-1256-x
34. Katsukawa C, Tamaru A, Miyata Y, Abe C, Makino M, Suzuki Y. Characterization of the rpsL and rrs genes of streptomycin-resistant clinical isolates of Mycobacterium tuberculosis in Japan. *J Appl Microbiol*. 1997;83(5):634–640. doi:10.1046/j.1365-2672.1997.00279.x
35. Liu J, Shi W, Zhang S, et al. Mutations in efflux pump Rv1258c (Tap) cause resistance to pyrazinamide, isoniazid, and streptomycin in M. tuberculosis. *Front Microbiol*. 2019;10. doi:10.3389/fmicb.2019.00216
36. Zaunbrecher MA, Sikes RD, Metchock B, Shinnick TM, Posey JE. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A*. 2009;106(47):20004–20009. doi:10.1073/pnas.0907925106
37. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2005;49(8):3192–3197. doi:10.1128/aac.49.8.3192-3197.2005
38. Vasava MS, Bhoi MN, Rathwa SK, Borad MA, Nair SG, Patel HD. Drug development against tuberculosis: past, present and future. *Indian J Tuberculosis*. 2017;64(4):252–275. doi:10.1016/j.ijtb.2017.03.002
39. Modolell J, Vázquez D. The inhibition of ribosomal translocation by viomycin. *Eur J Biochem*. 1977;81(3):491–497. doi:10.1111/j.1432-1033.1977.tb11974.x
40. Yamada T, Mizugichi Y, Nierhaus KH, Wittmann HG. Resistance to viomycin conferred by RNA of either ribosomal subunit. *Nature*. 1978;275(5679):460–461. doi:10.1038/275460a0
41. Lin Y, Li Y, Zhu N, et al. The antituberculosis antibiotic capreomycin inhibits protein synthesis by disrupting interaction between ribosomal proteins L12 and L10. *Antimicrob Agents Chemother*. 2014;58(4):2038–2044. doi:10.1128/aac.02394-13
42. Oudghiri A, Karimi H, Chetioui F, et al. Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. *BMC Infect Dis*. 2018;18(1):98. doi:10.1186/s12879-018-3009-9
43. Sowajassatakul A, Prammananan T, Chaiprasert A, Phunpruch S. Molecular characterization of amikacin, kanamycin and capreomycin resistance in M/XDR-TB strains isolated in Thailand. *BMC Microbiol*. 2014;14:165. doi:10.1186/1471-2180-14-165
44. Obrecht D, Bernardini F, Dale G, Dembowsky K. Chapter 15 - Emerging new therapeutics against key gram-negative pathogens. In: Macor JE, editor. *Annual Reports in Medicinal Chemistry*. Academic Press; 2011:245–262.
45. Fedorowicz J, Sączewski J. Modifications of quinolones and fluoroquinolones: hybrid compounds and dual-action molecules. *Monatshefte Für Chemie - Chemical Monthly*. 2018;149(7):1199–1245. doi:10.1007/s00706-018-2215-x
46. Sauberan JB, Bradley JS. 292 - Antimicrobial Agents. In: Long SS, Prober CG, Fischer M, editors. *Principles and Practice of Pediatric Infectious Diseases*. *Fifth Edition)* ed. Elsevier; 2018:1499–1531.e3.
47. Garcia PK, Martinez Borrero R, Annamalai T, et al. Localization of Mycobacterium tuberculosis topoisomerase I C-terminal sequence motif required for inhibition by endogenous toxin MazF4. *Front Microbiol*. 2022;13. doi:10.3389/fmicb.2022.1032320
48. Nagaraja V, Godbole AA, Henderson SR, Maxwell A. DNA topoisomerase I and DNA gyrase as targets for TB therapy. *Drug Discov Today*. 2017;22(3):510–518. doi:10.1016/j.drudis.2016.11.006
49. Mayer C, Takiff H. The molecular genetics of fluoroquinolone resistance in Mycobacterium tuberculosis. *Microbiol Spectrum*. 2014;2(4). doi:10.1128/microbiolspec.mgm2-0009-2013
50. Maruri F, Sterling TR, Kaiga AW, et al. A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. *J Antimicrob Chemother*. 2012;67(4):819–831. doi:10.1093/jac/dkr566
51. Chen J, Chen Z, Li Y, et al. Characterization of gyrA and gyrB mutations and fluoroquinolone resistance in Mycobacterium tuberculosis clinical isolates from Hubei Province, China. *Braz J Infect Dis*. 2012;16(2):136–141. doi:10.1016/s1413-8670(12)70294-5
52. Hegde SS, Vetting MW, Roderick SL, et al. A fluoroquinolone resistance protein from Mycobacterium tuberculosis that mimics DNA. *Science*. 2005;308(5727):1480–1483. doi:10.1126/science.1110699
53. Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. *Ann N Y Acad Sci*. 2015;1354(1):12–31. doi:10.1111/nyas.12830
54. Scardigli A, Caminero JA, Sotgiu G, Centis R, D'Ambrosio L, Migliori GB. Efficacy and tolerability of ethionamide versus prothionamide: a systematic review. *Eur Respir J*. 2016;48(3):946–952. doi:10.1183/13993003.00438-2016
55. Wang F, Langley R, Gulten G, et al. Mechanism of thioamide drug action against tuberculosis and leprosy. *J Exp Med*. 2007;204(1):73–78. doi:10.1084/jem.20062100

56. Thee S, Garcia-Prats AJ, Donald PR, Hesseling AC, Schaaf HS. A review of the use of ethionamide and prothionamide in childhood tuberculosis. *Tuberculosis*. 2016;97:126–136. doi:10.1016/j.tube.2015.09.007
57. Ushtanit A, Kulagina E, Mikhailova Y, Makarova M, Safonova S, Zimenkov D. Molecular determinants of ethionamide resistance in clinical isolates of Mycobacterium tuberculosis. *Antibiotics*. 2022;11(2):133. doi:10.3390/antibiotics11020133
58. Chollet A, Mourey L, Lherbet C, et al. Crystal structure of the enoyl-ACP reductase of Mycobacterium tuberculosis (InhA) in the apo-form and in complex with the active metabolite of isoniazid pre-formed by a biomimetic approach. *J Struct Biol*. 2015;190(3):328–337. doi:10.1016/j.jsb.2015.04.008
59. Tatum NJ, Liebeschuetz JW, Cole JC, et al. New active leads for tuberculosis booster drugs by structure-based drug discovery. *Org Biomol Chem*. 2017;15(48):10245–10255. doi:10.1039/c7ob00910k
60. Rueda J, Realpe T, Mejia GI, et al. Genotypic analysis of genes associated with independent resistance and cross-resistance to isoniazid and ethionamide in Mycobacterium tuberculosis clinical isolates. *Antimicrob Agents Chemother*. 2015;59(12):7805–7810. doi:10.1128/aac.01028-15
61. Bonsor D, Butz SF, Solomons J, et al. Ligation independent cloning (LIC) as a rapid route to families of recombinant biocatalysts from sequenced prokaryotic genomes. *Org Biomol Chem*. 2006;4(7):1252–1260. doi:10.1039/B517338H
62. Grant SS, Wellington S, Kawate T, et al. Baeyer-Villiger monooxygenases EthA and MymA are required for activation of replicating and non-replicating Mycobacterium tuberculosis inhibitors. *Cell Chem Biol*. 2016;23(6):666–677. doi:10.1016/j.chembiol.2016.05.011
63. Blondiaux N, Moune M, Desroses M, et al. Reversion of antibiotic resistance in Mycobacterium tuberculosis by spiroisoxazoline SMARt-420. *Science*. 2017;355(6330):1206–1211. doi:10.1126/science.aag1006
64. Vilcheze C, Weisbrod TR, Chen B, et al. Altered NADH/NAD+ ratio mediates coresistance to isoniazid and ethionamide in mycobacteria. *Antimicrob Agents Chemother*. 2005;49(2):708–720. doi:10.1128/AAC.49.2.708-720.2005
65. Islam MM, Tan Y, Hameed HMA, et al. Detection of novel mutations associated with independent resistance and cross-resistance to isoniazid and prothionamide in Mycobacterium tuberculosis clinical isolates. *Clin Microbiol Infect*. 2019;25(8):1041.e1–1041.e7. doi:10.1016/j.cmi.2018.12.008
66. Xu X, Vilchèze C, Av-Gay Y, Gómez-Velasco A, Jacobs WR. Precise null deletion mutations of the mycothiol synthesis genes reveal their role in isoniazid and ethionamide resistance in Mycobacterium smegmatis. *Antimicrob Agents Chemother*. 2011;55(7):3133–3139. doi:10.1128/aac.00020-11
67. Ushtanit A, Mikhailova Y, Krylova L, et al. Perchlozone resistance in clinical isolates of Mycobacterium tuberculosis. *Antibiotics*. 2023;12(3). doi:10.3390/antibiotics12030590
68. Vilchèze C, Jacobs WR. Resistance to isoniazid and ethionamide in Mycobacterium tuberculosis: genes, mutations, and causalities. *Microbiol Spectr*. 2014;2(4):Mgm2–0014–2013. doi:10.1128/microbiolspec.MGM2-0014-2013
69. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. *PLoS One*. 2015;10(3):e0119628. doi:10.1371/journal.pone.0119628
70. Banerjee A, Dubnau E, Quemard A, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. *Science*. 1994;263(5144):227–230. doi:10.1126/science.8284673
71. Heym B, Honoré N, Truffot-Pernot C, et al. Implications of multidrug resistance for the future of short-course chemotherapy of tuberculosis: a molecular study. *Lancet*. 1994;344(8918):2938. doi:10.1016/s0140-6736(94)91338-2
72. Boonaiam S, Chaiprasert A, Prammananan T, Leechawengwongs M. Genotypic analysis of genes associated with isoniazid and ethionamide resistance in MDR-TB isolates from Thailand. *Clin Microbiol Infect*. 2010;16(4):396–399. doi:10.1111/j.1469-0691.2009.02838.x
73. Farhat MR, Sultana R, Iartchouk O, et al. Genetic determinants of drug resistance in mycobacterium tuberculosis and their diagnostic value. *Am J Respir Crit Care Med*. 2016;194(5):621–630. doi:10.1164/rccm.201510-2091OC
74. Casali N, Nikolayevskyy V, Balabanova Y, et al. Evolution and transmission of drug-resistant tuberculosis in a Russian population. *Nat Genet*. 2014;46(3):279–286. doi:10.1038/ng.2878
75. Mirzayev F, Viney K, Linh NN, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. *Eur Respir J*. 2021;57(6):2003300. doi:10.1183/13993003.03300-2020
76. Tandon VR, Rani N, Gupta R, et al. Cycloserine induced psychosis with hepatic dysfunction. *Indian J Pharmacol*. 2015;47(2):230–231. doi:10.4103/0253-7613.153439
77. Court R, Centner CM, Chirehwa M, et al. Neuropsychiatric toxicity and cycloserine concentrations during treatment for multidrug-resistant tuberculosis. *Int J Infect Dis*. 2021;105:688–694. doi:10.1016/j.ijid.2021.03.001
78. Batson S, de Chiara C, Majce V, et al. Inhibition of D-Ala:D-Ala ligase through a phosphorylated form of the antibiotic D-cycloserine. *Nat Commun*. 2017;8(1):1939. doi:10.1038/s41467-017-02118-7
79. Amorim Franco TM, Favrot L, Vergnolle O, Blanchard JS. Mechanism-based inhibition of the Mycobacterium tuberculosis branched-chain aminotransferase by d- and l-cycloserine. *ACS Chem Biol*. 2017;12(5):1235–1244. doi:10.1021/acschembio.7b00142
80. Chakraborty S, Gruber T, Barry CE, Boshoff HI, Rhee KY. Para-aminosalicylic acid acts as an alternative substrate of folate metabolism in Mycobacterium tuberculosis. *Science*. 2013;339:6115):88–91. doi:10.1126/science.1228980
81. Mathys V, Wintjens R, Lefevre P, et al. Molecular genetics of para-aminosalicylic acid resistance in clinical isolates and spontaneous mutants of Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2009;53(5):2100–2109. doi:10.1128/aac.01197-08
82. Hajian B, Scocchera E, Shoen C, et al. Drugging the folate pathway in Mycobacterium tuberculosis: the role of multi-targeting agents. *Cell Chem Biol*. 2019;26(6):781–791.e6. doi:10.1016/j.chembiol.2019.02.013
83. Stadler JAM, Maartens G, Meintjes G, Wasserman S. Clofazimine for the treatment of tuberculosis. *Front Pharmacol*. 2023;14:1100488. doi:10.3389/fphar.2023.1100488
84. Yano T, Kassovska-Bratinova S, Teh JS, et al. Reduction of clofazimine by mycobacterial type 2 NADH:quinone oxidoreductase: a pathway for the generation of bactericidal levels of reactive oxygen species. *J Biol Chem*. 2011;286(12):10276–10287. doi:10.1074/jbc.M110.200501
85. Pontali E, Raviglione M. Updated treatment guidelines for drug-resistant TB: how safe are clofazimine-based regimens? *IJTLD Open*. 2024;1(11):486–489. doi:10.5588/ijtldopen.24.0490
86. Islam MM, Alam MS, Liu Z, et al. Molecular mechanisms of resistance and treatment efficacy of clofazimine and bedaquiline against Mycobacterium tuberculosis. *Front Med*. 2024. doi:10.3389/fmed.2023.1304857

87. Lamprecht DA, Finin PM, Rahman MA, et al. Turning the respiratory flexibility of Mycobacterium tuberculosis against itself. *Nat Commun*. 2016;7:12393. doi:10.1038/ncomms12393
88. Hartkoorn RC, Uplekar S, Cole ST. Cross-resistance between clofazimine and bedaquiline through upregulation of MmpL5 in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2014;58(5):2979–2981. doi:10.1128/aac.00037-14
89. Liu Y, Shi J, Li L, et al. Spontaneous mutational patterns and novel mutations for delamanid resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2022;66(12):e0053122. doi:10.1128/aac.00531-22
90. Sonnenkalb L, Carter JJ, Spitaleri A, et al. Bedaquiline and clofazimine resistance in Mycobacterium tuberculosis: an in-vitro and in-silico data analysis. *Lancet Microbe*. 2023;4(5):e358–e368. doi:10.1016/s2666-5247(23)00002-2
91. Zhu H, Fu L, Wang B, et al. Activity of clofazimine and TBI-166 against Mycobacterium tuberculosis in different administration intervals in mouse tuberculosis models. *Antimicrob Agents Chemother*. 2021;65(4). doi:10.1128/aac.02164-20
92. Ding Y, Liu H, Wang F, et al. Coadministration of bedaquiline and pyrifazimine reduce exposure to toxic metabolite N-desmethyl bedaquiline. *Front Pharmacol*. 2023;14. doi:10.3389/fphar.2023.1154780
93. Chen RH, Burke A, Cho JG, Alffenaar JW, Davies Forsman L. New oxazolidinones for tuberculosis: are novel treatments on the horizon? *Pharmaceutics*. 2024;16(6):818. doi:10.3390/pharmaceutics16060818
94. Gan WC, Ng HF, Ngeow YF. Mechanisms of linezolid resistance in Mycobacteria. *Pharmaceuticals*. 2023;16(6):784. doi:10.3390/ph16060784
95. Pi R, Liu Q, Jiang Q, Gao Q. Characterization of linezolid-resistance-associated mutations in Mycobacterium tuberculosis through WGS. *J Antimicrob Chemother*. 2019;74(7):1795–1798. doi:10.1093/jac/dkz150
96. Hillemann D, Rüsch-Gerdes S, Richter E. In vitro-selected linezolid-resistant Mycobacterium tuberculosis mutants. *Antimicrob Agents Chemother*. 2008;52(2):800–801. doi:10.1128/aac.01189-07
97. Ng HF, Ngeow YF. Mutations in genes encoding 23S rRNA and FadD32 may be associated with linezolid resistance in Mycobacteroides abscessus. *Microb Drug Resist*. 2023;29(2):41–46. doi:10.1089/mdr.2022.0068
98. Lavollay M, Arthur M, Fourgeaud M, et al. The peptidoglycan of stationary-phase Mycobacterium tuberculosis predominantly contains cross-links generated by L,D-transpeptidation. *J Bacteriol*. 2008;190(12):4360–4366. doi:10.1128/jb.00239-08
99. Ahmad N, Islam Z, Dhar S, Kumar P, Zaidi R. Structure and mechanism basis of β-lactam activity against Mycobacterium tuberculosis: a review of literature. *Indian J Tuberculosis*. 2025;72(3):424–437. doi:10.1016/j.ijtb.2025.01.001
100. Wang F, Cassidy C, Sacchettini JC. Crystal structure and activity studies of the Mycobacterium tuberculosis beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. *Antimicrob Agents Chemother*. 2006;50(8):2762–2771. doi:10.1128/aac.00320-06
101. Gold B, Zhang J, Quezada LL, et al. Identification of β-Lactams active against Mycobacterium tuberculosis by a consortium of pharmaceutical companies and academic institutions. *ACS Infect Dis*. 2022;8(3):557–573. doi:10.1021/acsinfecdis.1c00570
102. Payen MC, De Wit S, Martin C, et al. Clinical use of the meropenem-clavulanate combination for extensively drug-resistant tuberculosis. *Int J Tuberc Lung Dis*. 2012;16(4):558–560. doi:10.5588/ijtld.11.0414
103. Tiberi S, Payen MC, Sotgiu G, et al. Effectiveness and safety of meropenem/clavulanate-containing regimens in the treatment of MDR- and XDR-TB. *Eur Respir J*. 2016;47(4):1235–1243. doi:10.1183/13993003.02146-2015
104. Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. *Science*. 2005;307(5707):223–227. doi:10.1126/science.1106753
105. Koul A, Vranckx L, Dendouga N, et al. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis*. *J Biol Chem*. 2008;283(37):25273–25280. doi:10.1074/jbc.M803899200
106. Biukovic G, Basak S, Manimekalai MS, et al. Variations of subunit varepsilon of the Mycobacterium tuberculosis F1Fo ATP synthase and a novel model for mechanism of action of the tuberculosis drug TMC207. *Antimicrob Agents Chemother*. 2013;57(1):168–176. doi:10.1128/aac.01039-12
107. Gupta S, Cohen KA, Winglee K, Maiga M, Diarra B, Bishai WR. Efflux inhibition with verapamil potentiates bedaquiline in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2014;58(1):574–576. doi:10.1128/aac.01462-13
108. Ryan NJ, Lo JH. Delamanid: first global approval. *Drugs*. 2014;74(9):1041–1045. doi:10.1007/s40265-014-0241-5
109. Ahmed SH, Haider H, Moeed A, et al. Efficacy and safety of bedaquiline and delamanid in the treatment of drug-resistant tuberculosis in adults: a systematic review and meta-analysis. *Indian J Tuberc*. 2024;71(1):79–88. doi:10.1016/j.ijtb.2023.05.005
110. Dawson R, Diacon AH, De Jager V, et al. Safety, pharmacokinetics, and early bactericidal activity of quabodepistat in combination with delamanid, bedaquiline, or both in adults with pulmonary tuberculosis: a randomised, active-controlled, open-label trial. *Lancet Infect Dis*. 2025;25(4):435–444. doi:10.1016/S1473-3099(24)00601-7
111. Abrahams KA, Batt SM, Gurcha SS, Veerapen N, Bashiri G, Besra GS. DprE2 is a molecular target of the anti-tubercular nitroimidazole compounds pretomanid and delamanid. *Nat Commun*. 2023;14(1):3828. doi:10.1038/s41467-023-39300-z
112. Batt SM, Toth S, Rodriguez B, et al. Assay development and inhibition of the Mt-DprE2 essential reductase from Mycobacterium tuberculosis. *Microbiology*. 2023;169(1). doi:10.1099/mic.0.001288
113. Pandey S, Vilchèze C, Werngren J, et al. Loss-of-function mutations in ndh do not confer delamanid, ethionamide, isoniazid, or pretomanid resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2024;68(1):e0109623. doi:10.1128/aac.01096-23
114. Pei S, Song Z, Yang W, et al. The catalogue of Mycobacterium tuberculosis mutations associated with drug resistance to 12 drugs in China from a nationwide survey: a genomic analysis. *Lancet Microbe*. 2024;5(11):100899. doi:10.1016/s2666-5247(24)00131-9
115. Goletti D, Meintjes G, Andrade BB, Zumla A, Shan Lee S. Insights from the 2024 WHO global tuberculosis report - more comprehensive action, innovation, and investments required for achieving WHO end TB goals. *Int J Infect Dis*. 2025;150:107325. doi:10.1016/j.ijid.2024.107325
116. Gardee Y, Dreyer AW, Koornhof HJ, et al. Evaluation of the GenoType MTBDRsl Version 2.0 assay for second-line drug resistance detection of Mycobacterium tuberculosis isolates in South Africa. *J Clin Microbiol*. 2017;55(3):791–800. doi:10.1128/jcm.01865-16
117. Maya T, Wilfred A, Lubinza C, et al. Diagnostic accuracy of the Xpert® MTB/XDR assay for detection of Isoniazid and second-line antituberculosis drugs resistance at central TB reference laboratory in Tanzania. *BMC Infect Dis*. 2024;24(1):672. doi:10.1186/s12879-024-09562-z
118. Pillay S, Steingart KR, Davies GR, et al. Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin. *Cochrane Database Syst Rev*. 2022;5(5):Cd014841. doi:10.1002/14651858.CD014841.pub2

119. Schwab TC, Perrig L, Göller PC, et al. Targeted next-generation sequencing to diagnose drug-resistant tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2024;24(10):1162–1176. doi:10.1016/s1473-3099(24)00263-9
120. Nehru VJ, Brammacharry U, Sri ramkumar SR, et al. Molecular analysis of bedaquiline resistance and genetic variations in clinical isolates of multidrug and fluoroquinolones-resistant Mycobacterium tuberculosis. *J King Saud Univ Sci*. 2024;36(6):103226. doi:10.1016/j.jksus.2024.103226
121. Ahmad Khosravi N, Khosravi AD, Hashemzadeh M, Savari M, Saki M. Investigation of delamanid, bedaquiline, and linezolid resistance rates and related gene mutations in multidrug-resistant Mycobacterium tuberculosis in regional tuberculosis reference laboratories of Iran. *BMC Microbiol*. 2025;25(1):509. doi:10.1186/s12866-025-04213-y
122. Jouet A, Gaudin C, Badalato N, et al. Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. *Eur Respir J*. 2021;57(3):2002338. doi:10.1183/13993003.02338-2020
123. Kcs N, Supply P, Cobelens FGJ, et al. How well do routine molecular diagnostics detect rifampin heteroresistance in Mycobacterium tuberculosis? *J Clin Microbiol*. 2019;57(11). doi:10.1128/jcm.00717-19
124. Walker TM, Kohl TA, Omar SV, et al. Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. *Lancet Infect Dis*. 2015;15(10):1193–1202. doi:10.1016/S1473-3099(15)00062-6
125. Köser CU, Bryant JM, Becq J, et al. Whole-genome sequencing for rapid susceptibility testing of M. tuberculosis. *N Engl J Med*. 2013;369(3):290–292. doi:10.1056/NEJMc1215305
126. Gygli SM, Keller PM, Ballif M, et al. Whole-genome sequencing for drug resistance profile prediction in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2019;63(4). doi:10.1128/aac.02175-18
127. Ogunlade B, Tadesse LF, Li H, et al. Rapid, antibiotic incubation-free determination of tuberculosis drug resistance using machine learning and Raman spectroscopy. *Proc National Acad Sci*. 2024;121:e2315670121. doi:10.1073/pnas.2315670121
128. Wang L, Zhang X-D, Tang J-W, et al. Machine learning analysis of SERS fingerprinting for the rapid determination of Mycobacterium tuberculosis infection and drug resistance. *Comput Struct Biotechnol J*. 2022;20:5364–5377. doi:10.1016/j.csbj.2022.09.031
129. Yang C, Dai G, Guo Y, Wang T, Gao W, Zeng Y. Nanopore-based targeted sequencing (NTS) for drug-resistant tuberculosis: an integrated tool for personalized treatment strategies and guidance for new drug development. *BMC Infect Dis*. 2025;25(1):861. doi:10.1186/s12879-025-11227-4
130. Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F, Collins JJ. CRISPR-based diagnostics. *Nat Biomed Eng*. 2021;5(7):643–656. doi:10.1038/s41551-021-00760-7
131. Paredes-Gutierrez G, Perea-Jacobo R, Acosta-Mesa H-G, et al. Predicting drug resistance in Mycobacterium tuberculosis: a machine learning approach to genomic mutation analysis. *Diagnostics*. 2025;15(3):279. doi:10.3390/diagnostics15030279
132. Wang Y, Jiang Z, Liang P, Liu Z, Cai H, Sun Q. TB-DROP: deep learning-based drug resistance prediction of Mycobacterium tuberculosis utilizing whole genome mutations. *BMC Genomics*. 2024;25(1):167. doi:10.1186/s12864-024-10066-y
133. Babirye SR, Nsubuga M, Mboowa G, Batte C, Galiwango R, Kateete DP. Machine learning-based prediction of antibiotic resistance in Mycobacterium tuberculosis clinical isolates from Uganda. *BMC Infect Dis*. 2024;24(1):1391. doi:10.1186/s12879-024-10282-7
134. Pruthi SS, Billows N, Thorpe J, et al. Leveraging large-scale Mycobacterium tuberculosis whole genome sequence data to characterise drug-resistant mutations using machine learning and statistical approaches. *Sci Rep*. 2024;14(1):27091. doi:10.1038/s41598-024-77947-w
135. Farhat MR, Shapiro BJ, Kieser KJ, et al. Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis. *Nature Genet*. 2013;45(10):1183–1189. doi:10.1038/ng.2747
136. Nimmo C, Millard J, Faulkner V, Monteserin J, Pugh H, Johnson EO. Evolution of Mycobacterium tuberculosis drug resistance in the genomic era. *Front Cell Infect Microbiol*. 2022;12. doi:10.3389/fcimb.2022.954074
137. Briffotaux J, Huang W, Wang X, Gicquel B. MmpS5/MmpL5 as an efflux pump in Mycobacterium species. *Tuberculosis*. 2017;107:13–19. doi:10.1016/j.tube.2017.08.001
138. Laatri S, El Kassimi S, Bentaleb EM, et al. Characterization of drug resistance mutations in Mycobacterium tuberculosis isolates from Moroccan patients using deeplex targeted next-generation sequencing. *Microorganisms*. 2025;13(9):2163. doi:10.3390/microorganisms13092163
139. Carpi G, Seifert M, De la Rossa A, et al. Comparative assessment of line probe assays and targeted next-generation sequencing in drug-resistant tuberculosis diagnosis. *EBioMedicine*. 2025;119:105875. doi:10.1016/j.ebiom.2025.105875
140. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM. Deciphering drug resistance in Mycobacterium tuberculosis using whole-genome sequencing: progress, promise, and challenges. *Genome Med*. 2019;11(1):45. doi:10.1186/s13073-019-0660-8
141. Salamon H, Yamaguchi KD, Cirillo DM, et al. Integration of published information into a resistance-associated mutation database for Mycobacterium tuberculosis. *J Infect Dis*. 2015;211(Suppl 2):S50–7. doi:10.1093/infdis/jiu816
142. Zhu H, Zhou X, Zhuang Z, Li L, Bi J, Mi K. Advances of new drugs bedaquiline and delamanid in the treatment of multi-drug resistant tuberculosis in children. *Front Cell Infect Microbiol*. 2023;13:1183597. doi:10.3389/fcimb.2023.1183597