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An overview of the pattern of first- and second-line anti-tuberculosis drug resistance gene mutations

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Abstract

Tuberculosis is still the most prevalent infectious cause of mortality, and it has a significant medical, societal, and economic impact MDR-TB is a type of tuberculosis (TB) infection produced by bacteria resistant to at least two of the most important first-line anti-TB treatments, isoniazid, and rifampin. Extensively drug-resistant tuberculosis (XDR-TB) is a type of tuberculosis that is resistant to second-line treatments. MDR-TB is a type of tuberculosis (TB) infection produced by bacteria resistant to at least two of the most important first-line anti-TB treatments, isoniazid, and rifampin. Extensively drug-resistant tuberculosis (XDR-TB) is a type of tuberculosis that is resistant to second-line treatments. Because resistant cases have significant morbidity and mortality, multidrug-resistant tuberculosis poses a significant threat to treatment. The most effective anti-tuberculosis drugs are first-line essential anti-tuberculosis drugs, which must be included in any short-term treatment plan. Rifampicin, ethambutol, Isoniazid, streptomycin, and pyrazinamide are among the medications in this group. Ethionamide, amikacin, capreomycin, and para-aminosalicylic acid are Second-line anti-tuberculosis that are clinically ineffective and cause severe responses far more commonly than first-line drugs. Resistance to first-line drugs was connected to mutations in the *pncA, emb, rpsL*, and *rrs* genes, while *rrs, gyrA, eis, tlyA* and *gryB* are associated with second-line drugs.

Keywords: Mutations; Mechanism; Resistance; Tuberculosis; Drug-resistance

1. Introduction

Tb is a group of Mycobacteria that is responsible for tuberculosis (TB). The *M. tuberculosis* complex (MTBC), which can cause tuberculosis, and M. leprae, which causes leprosy, are two types of mycobacteria. Nontuberculous mycobacterium (NTM) contains all other mycobacteria. [1]. NTMs are widely spread in the environment and, in the vast majority of circumstances, are not harmful to humans. Immunologic evidence of present or previous M tuberculosis infection can be found in almost one of the world's populations [2]. There are an estimated 1.7 billion persons infected with *M. tuberculosis* worldwide, yet only around 15% will acquire tuberculosis disease over their lifetime [3]. HIV-positive people, as well as those who are impacted by risk variables such as malnutrition, diabetes, smoking, and alcohol usage, have a substantially increased chance of developing tuberculosis [4, 5].

M. tuberculosis has developed resistance to a variety of anti-TB medications over the years, making infection control challenges. Drug-resistant tuberculosis (DRTB) poses a danger to worldwide TB treatment and prevention, and it constitutes a significant public health concern in several countries, particularly in Sub-Saharan Africa. Because of the advent of drug-resistant TB strains and the high incidence of other infections in Sub-Saharan Africa, the fight against TB

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has become a major problem. While drug resistance is a natural phenomenon, resistance to anti-TB medications is considered man-made in *M. tuberculosis* because it frequently develops from the intentional selection of spontaneous drug-resistant mutations during insufficient or partial therapy [6].

Drug-resistant MTB strains arise at a predictable low frequency from spontaneous chromosomal mutations, but a study found that drug-resistant TB is primarily the result of failed TB prevention strategies due to a lack of proper therapeutic interventions, poor patient compliance, curtailed supply of drugs, and inappropriate treatment drug therapies, all of which result in the emergence of resistant mutants [7]. Emerging anti-TB treatment failure patterns have emerged over time, resulting in an increase in the transmission of MDR and substantially drug-resistant (XDR) strains of Mycobacterium tuberculosis, posing challenges for efficient therapeutic alternatives and infection control [8].

2. Overview of Multidrug Resistance Tuberculosis

Worldwide, an approximated 10.0 million people contracted tuberculosis, with 1.2 million HIV-negative people dying from the disease and 208, 000 HIV-positive people dying from it. Adults made up 88 percent of those with tuberculosis, while children made up 12 %. The WHO regions with the highest number of persons with tuberculosis were South-East Asia (44%), Africa (25%), and Western Asia (18%). India (26 percent), Indonesia (8.5 percent), China (8.4%), the Philippines (6.0 percent), Pakistan (5.7 percent), Nigeria (4.4 percent), Bangladesh (3.6 percent), and South Africa (3.6 percent) contributed for two-thirds of the global total [9].

TB is still a major infectious illness in Nigeria, and the country has long been one of the most TB-affected countries in the world. Nigeria is currently rated 7th in the world among the 30 nations with the highest TB burden, and second in Africa after South Africa, with 429 infections per 1000.000 people [10]. Drug-resistant tuberculosis poses serious threats to global TB control, with the dissemination of drug resistance likely to result from factors such as poor public health infrastructure, which causes delays in drug resistance diagnosis and factual inaccuracies in TB chemotherapy supply, inadequate treatment, which leads to unsupervised therapy, non - compliance, and incorrect anti-TB agent use, ineffective infection prevention and control, and the HIV epidemic.

3. Molecular Mechanisms of Resistance and drug action to First-Line TB Drugs

The emergence of resistance in *M. tuberculosis* arises from a low but consistent frequency of random, single-step spontaneous mutations in large bacterial communities. For rifampicin, the risk of drug resistance mutants is 3.110-8, while for isoniazid and some other regularly used medications [14]. The incidence of mutations in the pathogen's DNA is aided by an environment rich in selective antibiotics [15]. The presence of drug-resistant MTB strains, which have been classified into two categories, is consistent with these findings.

3.1. Rifampicin (RIF) Resistance

Rifampin, rifapentine, and rifabutin have all been utilized as first-line medicines in the treatment of tuberculosis infections in combination with other drugs. It was first used to treat tuberculosis in 1972, and it has strong sterilizing properties [11]. Short therapy courses were established as a result of the use of RIF in combination with INH/ PZA, which decreased routine TB treatment from a year to six months. RIF is effective against low metabolically active bacilli in both the growth and stationary phases.

This is due to its significant sterilizing action in vivo, which correlates with its capacity to reduce TB treatment time. On solid or liquid media, *M. tuberculosis* is sensitive to RIF, with minimum inhibitory concentrations (MIC) ranging from 0.05 to 1 g/ml [12, 13]. RIF is thought to stop bacterial DNA-dependent RNA polymerase from working. RIF inhibits RNA synthesis by attaching to the RNA polymerase subunit, preventing transcription and killing the organism [14, 15].

3.2. Isoniazid (INH) Resistance

Although isoniazid was first developed in the early 1900s, which has been used to treat tuberculosis and related latent infections [16]. The INH medicine works by entering the cell as a pro-drug that is activated by the gene *M. tuberculosis*, which codes for catalase-peroxidase [17]. At relatively low concentrations of 0.02–0.2 g/ml, *M. tuberculosis* is extremely sensitive to INH [18]. The peroxidase activity of this enzyme is required for INH activation and interaction with a range of damaging radical intermediates within bacterial cells.

Oxides, hydroxyl radicals, and organic moieties are common reactive species, which react with nicotinamide adenine dinucleotide (H) to generate the INH-NAD adduct, which deteriorates cell wall components or inhibits cell wall mycolic

acid formation [19, 20]. INH is the most widely used anti-TB medicine, and INH resistance is more widespread among clinical strains than susceptibility to any other agent [21]. In INH-resistant clinical isolates, mutations in the *M. tuberculosis* gene are most prevalent, occurring in 50–80% of infections, limiting the catalase-ability peroxidase to trigger the INH pro-drug [22].

Several gene mutations have been discovered to occur frequently between codons 138 and 328, including the *M. tuberculosis* mutation (S315T), which replaces Ser 315 with Thr [23]. The most prevalent mutation in INH-resistant strains is *M. tuberculosis* S315T, where mutation at Ser315Thr leads to an enzyme that cannot activate INH but retains about half of its catalase-peroxidase activity [24]. As a result, the modified catalase-peroxidase provides excellent INH resistance while still providing adequate oxidative protection to allow the organism to maintain its detoxification activities toward host antibacterial free radicals.

3.3. Pyrazinamide (PZA) Resistance

Pyrazinamide, a nicotinamide analogue, was identified to have anti-TB action in 1952 and targets a fatty-acid production enzyme. Pyrazinamide is a significant first-line medicine for tuberculosis treatment, and it performs a unique role in reducing TB treatment time from 9–12 months to 6 months. PZA has a good sterilization effect on semi-dormant tubercle bacilli because it kills them in an acidic environment, and the minimum inhibitory concentration for PZA is between 16 and 100 g/ml [25]. Pyrazinamide is a prodrug that is metabolized to pyrazine acid (POA) by the intracellularly generated mycobacterial enzyme pyrazinamide or nicotinamidase. The mycobacterial cell's poor efflux mechanism allows enormous accumulation of POA in the cytoplasm, causing membrane potential disturbance [26]. The specific mechanism of PZA resistance has yet to be discovered. PZA-resistant bacterial strains, on the other hand, frequently lose their pyrazinamide activity, and deficient pyrazinamide activity caused by pncA mutations is the most common source of PZA resistance [27].

3.4. Ethambutol (EMB) Resistance

Ethambutol is a first-line antibiotic that is used in combination with INH, RIF, and PZA to avoid mycobacterium-specific antibiotic resistance [28]. Ethambutol is a bacteriostatic antibiotic that works against developing germs but has no effect on bacteria that aren't reproducing. Ethambutol inhibits arabinosyl-transferase, a cell wall biosynthesis enzyme, and interferes with mycobacterial cell wall biosynthesis through a synthetic process [29]. For *M. tuberculosis*, EMB's minimal inhibitory doses vary from 1 to 5 g/ml [25].

Arabinosyl transferase, which is encoded by the limb and is involved in the production of arabinogalactan, has been suggested as an EMB target within the tuberculosis microorganism [30]. In MTB, *embB* is structured into an operon with *embC* and is in the order MB cab; embed, embed, and *embC* are predicted to encode transmembrane proteins that have over 65 percent amino acid identity [31]. *EmbA* and *embB* proteins are involved in the creation of the correct terminal hexaarabinofuranoside motif in arabinogalactan synthesis, while *embC* is essential in lipoarabinomannan synthesis, according to studies [32, 33], *embC* is required for the synthesis of lipoarabinomannans. The most prevalent mutations in *embB* codon 306 have been linked to varying degrees of EMB resistance, suggesting that they can also be n't enough on their own for high-level EMB resistance [34].

3.5. Streptomycin (STR) Resistance

The first medicine to treat tuberculosis was streptomycin (STR), which was discovered in 1943 [35]. It's an aminoglycoside antibiotic that works against MTB as well as a wide range of microorganisms. Streptomycin eliminates actively growing tubercle bacilli at concentrations as low as 2–8 g/ml [25], but it is ineffective towards non-growing or intracellular bacilli [36]. By attaching to the 30S subunit of the bacterial ribosome, the ribosomal enzyme S12 and the 16S rRNA transcribed by the genes *rpsL* and *rrs*, respectively, inhibit the start of translation in protein synthesis, causing misreading of the mRNA message during translation [37]. In 2.29 x 10-8 Mycobacterium TB replications, spontaneous chromosomal mutations caused genetic resistance to streptomycin, according to one study [38].

4. Molecular Mechanisms of Resistance to Second-Line TB Drugs

Second-line medications include aminoglycosides (kanamycin and amikacin), polypeptides (capreomycin, viomycin, and enviomycin), fluoroquinolones (ofloxacin, ciprofloxacin, and gatifloxacin), D-cycloserine, and thioamides, according to the WHO [39] Second-line medications, on the other hand, are fundamentally more harmful and ineffective than the first drugs [39]. The majority of second-line medications are utilized to treat MDR-TB, which lengthens the overall treatment time. The second-line medications are reviewed here based on current knowledge of molecular pathways linked to resistance and drug modes of action.

4.1. Aminoglycosides Resistance

Kanamycin and its metabolite amikacin belong to the aminoglycoside antibiotics family, whereas capreomycin belongs to the cyclic peptide antibiotics family. All of the medications used to treat MDR-TB have almost the same mechanism of action, which is to suppress protein synthesis. Kanamycin and amikacin suppress protein synthesis by modifying ribosomal structures at the 16S rRNA[40], while capreomycin binds to the same location in the ribosome at the interface of the large and small subunits to inhibit protein synthesis [41]. High-level resistance to Kanamycin and Amikacin [13], has been linked to mutations in the 16S rRNA (*rrs*) position 1400, whereas resistance to CPM has been linked to a mutation in the tlyA gene encoding rRNA methyltransferase [42]. For *M. tuberculosis*, the minimum inhibitory values of kanamycin, amikacin, and capreomycin are in the range of 2–4 g/ml [24]. An A1401G mutation in the *rrs* gene, which codes for 16S rRNA, has been linked to the most prevalent mechanistic explanation of drug resistance [43].

Furthermore, isolates tolerant to CPM and KM could have a C1402T or G1484T mutation in the *rrs* gene. In addition, numerous mutations in the *rrs* gene were discovered in a single strain, conferring cross-resistance to various medicines [13, 44]. According to Jugheli and colleagues' research, four types of mutations were found in the 1400 *rrs* region among the 145 isolates sequenced: A1401G, C1402T, C1443G, and T1521C, with the A-to-G substitution at nucleotide 1401 being the most common [45]

4.2. Fluoroquinolones Resistance

Fluoroquinolones are currently used as second-line medications in the treatment of MDR-TB, and they work by blocking the enzyme topoisomerase II [46]. DNA topoisomerases are a broad group of important enzymes that control DNA supercoiling and deactivate tangled nucleic acid strands to meet replicative and transcriptional demands [47]. Chromosome mutations in the quinolone resistance-determining domain of *gyrA* or *gyrB* are the key mechanism through which MTB develops fluoroquinolone resistance [48]. Mutations at positions 90 and 94 of *gyrA* are the most common, but mutations at positions 74, 88, and 91 have also been identified [49]. The quinolone-resistance-determining domain (QRDR) of *gyrA* (320 bp) and *gyrB* (375 bp), a conserved region, has been discovered to be a key component in MTB FQ resistance [50].

In clinical isolates of MTB, mutations in the QRDR of *gyrA* have been found, mostly grouped at codons 90, 91, and 94, with Asp94 being the most common. Codon 88 is a less common site of involvement, and *gyrB* mutations seem to be uncommon in clinical isolates. In general, two *gyrA* mutations or simultaneous *gyrA* and *gyrB* mutations are required to develop greater rates of resistance [25].

4.3. Ethionamide Resistance

Ethionamide is a structurally comparable isonicotinic acid derivative of isoniazid. It's also a prodrug that needs to be activated by the *ethA* gene's monooxygenase [51]. It suppresses the enoyl-ACP reductase enzyme by producing an adduct with NAD, which inhibits mycolic acid production. The transcription repressor *EthR* controls *EthA* [52]. ETH, like INH, is a prodrug activated by *EtaA*/*EthA* (a monooxygenase) and suppresses the same target as INH, the mycolic acid production pathway's *InhA* [53]. For *M. tuberculosis*, the minimum inhibitory concentrations of Ethionamide are 2.5–10 g/ml [25]. *EtaA*, also known as *EthA*, is a flavin adenosine dinucleotide (FAD) that contains an enzyme that oxidizes ETH to the equivalent S-oxide, which is then oxidized further to 2-ethyl-4-aminopyridine, probably via the unstable oxidized sulfinic acid intermediate. Furthermore, mutations in the target gene *InhA* confer ETH and INH resistance. Mutations in *EtaA*/*ethA*, *EthR*, and *InhA*, which induce resistance to both isoniazid and ethionamide, cause resistance to ethionamide [54].

4.4. Cycloserine Resistance

Cycloserine (Cs) is a bacteriostatic medication that is now being utilized to treat MDR-TB patients [55]. It's a cyclic analog of D-alanine, which is one of the key components in the peptidoglycan cross-linking phase [56]. Cycloserine's minimum inhibitory concentrations for *M. tuberculosis* range from 1.5 to 30 g/ml, depending on the culture medium employed [25]. Cycloserine blocks cell wall synthesis by competing with D-alanine for the enzymes D-alanyl-D-alanine synthetase (Ddl) and D-alanine racemase (Alr) thus inhibiting their synthesis [57]. Zhang and Yew (2015) found that M. bovis BCG has a deficiency in the *cycA* gene, which encodes D-serine, L- and D-alanine, and glycine transporters involved in Cs uptake. This could be related to the BCG's inherent resistance to Cs [25]. The mechanism of Cs resistance in *M. tuberculosis*, on the other hand, is yet unknown.

4.5. Treatment of drug-resistant Tuberculosis

Group 1 has the first-line anti-TB medications (pyrazinamide, ethambutol, and rifabutin), while Group 2 contains the injectable therapies (kanamycin, amikacin, and capreomycin). Second-line medications like fluoroquinolones were classed as group 3, and oral bacteriostatic second-line treatments including para-aminosalicylic acid (PAS), cycloserine, terizidone, ethionamide, and protionamide were classified as group 4. Clofazimine, linezolid, amoxicillin/clavulanate, thioacetazone, imipenem/cilastatin, high-dose isoniazid, and clarithromycin were also included in group 5. At least four medications with proven or near-certain efficacy should be included in the therapy regimens [58]. The efficacy and safety of drugs used in the formulation of extended MDR-TB chemotherapy regimens were grouped based on available research. MDR-TB therapy is recommended for all individuals with RR-TB regardless of isoniazid resistance. Patients with RR-TB or MDR-TB who've not previously been treated with second-line drugs and whose resistance to fluoroquinolones and second-line injectable agents has also been excluded or is considered highly unlikely may be treated with a shorter MDR-TB regimen of 9–12 months instead of the longer regimens, according to the guideline [39].

As stated in Table 1, patients with RR-TB or MDR-TB should now be treated with at least five effective anti-TB drugs, including pyrazinamide and four essential second-line TB therapies.

Table 1 Anti-TB drugs recommended for the treatment of RR-TB and MDR-TB

Group classification	Group Name		List of drugs by group
Group A.	Fluoroquinolones		Levofloxacin
			Moxifloxacin
			Gatifloxacin
Group B.	Second-line injectable agents		
			Capreomycin
			Kanamycin
			Streptomycin
Group C.			Ethionamide / prothionamide
	Other core second-line agents		
			Linezolid
			Clofazimine
			Pyrazinamide
		D1	Ethambutol
	Add-on		High-dose isoniazid
Group D.	agents; they		
		D2	Bedaquiline
	are not part of		
			Delamanid
	the core MDR-		
	TB regimen	D3	*p*-aminosalicylic acid
			Imipenem–cilastatind
			Meropenem
			Amoxicillin-clavulanate (Thioacetazone)

It is advised that patients with RR-TB or MDR-TB have their regimen supplemented with elevated isoniazid and/or ethambutol. Patients who have been previously treated with second-line medications for more than one month or who have documented or are anticipated to have strains resistant to drugs in the regimen should not utilize the shorter MDR-TB regimen, according to WHO. Before beginning treatment, resistance to at least fluoroquinolones and the injectable drug employed in the regimen should be ruled out [51].

5. Conclusion

Resistance to first-line drugs was connected to mutations in the rpoB, *M. tuberculosis*, f*abG1-InhA* promoter area, pncA, emb, *rpsL*, and *rrs* genes, while *rrs*, *gyrA*, eis, tlyA and gryB are associated with second-line drugs. In the case of tuberculosis, drug resistance is a man-made problem. It is caused by spontaneous gene alterations in *M. tuberculosis* that make the bacterium resistant to the most regularly used anti-TB medications. Noncompliance with treatment regimens is mentioned as one of the reasons behind this. The typical TB treatment is a six-month regimen of four medications, which is prolonged to 18–24 months in the case of MDR-TB and includes second-line treatments. This makes medication adherence difficult, and non-adherence rates may be substantial, resulting in poor results and the spread of MDR strains.

Compliance with ethical standards

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