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Molecular mechanisms of action and resistance to the first-line drugs against *Mycobacterium tuberculosis*

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Abstract

Tuberculosis, caused by the infectious pathogenic bacteria *Mycobacterium tuberculosis*, is one of the top 10 infectious agents (above AIDS/HIV) that cause death globally, and a large number of people contract the disease every year. Significantly, the four first-line drugs (rifampicin, isoniazid, pyrazinamide, and ethambutol) that make up the foundation of treatment regimens throughout the first six to nine months of treatment are delivered in various combinations when administering TB treatments. It is very important to continuously update information on molecular mechanisms of action and resistance to the anti-tuberculosis drugs against *M. tuberculosis* due the global rises in 558 000 new cases of rifampicin-resistant/ multidrug-resistant tuberculosis recently.In many countries and regions, even more severe cases of drug resistance have been documented in recent years. The aim of this review is to provide an overview of the latest report on molecular mechanisms of action and resistance to the first-line drugs against *M. tuberculosis*.A better knowledge of the mechanisms of action and resistance of anti-tuberculosis drugs would be very helpful for efficient tuberculosis therapy and clinical care.

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Introduction

Tuberculosis is a communicable disease and one of the top ten causes of death worldwide from a single infectious agent (above AIDS/HIV) and a lot of people fall sick with the TB disease each year (Bu *et al.,* 2022; Islam *et al.,*2023). The COVID-19 pandemic is still having a negative effect on the burden of tuberculosis disease and the availability of tuberculosis diagnosis and treatment. Global tuberculosis targets are not being met, and progress gained in the years leading up to 2019 has slowed, halted, or reversed. Globally, there is an estimate that 10.6 million people have developed tuberculosis disease by the year 2021, a rise of 4.5% from 10.1 million in 2020 (WHO, 2022). According to estimates, the number of new cases of drug-resistant tuberculosis increased from 2020 to 2021, accounting for 450 000 cases of rifampicin-resistant tuberculosis in 2021. Geographically, the Western Pacific (18%), Africa (23%) and South-East Asia (45%) areas of the World Health Organization (WHO) had the highest percentage of tuberculosis cases in 2021 (18%), followed by the Eastern Mediterranean (8.1%), the Americas (2.9%) and Europe (2.2%). 87% of the estimated incident cases worldwide were from the 30 countries with the highest tuberculosis burdens (WHO, 2022). More than two thirds of the world's total came from eight of these nations, including Nigeria (4.4%), Bangladesh (3.6%), Pakistan (5.7%), the Philippines (7.0%), and the Democratic Republic of the Congo (2.9%).

Drug-resistant tuberculosis is still a danger issue to public health (Boshoff *et al.,*2023). Almost all existing antibiotics are no longer as effective against *M. tuberculosis* due to the rising incidence of drug resistance in this disease, which makes efforts to stop its spread worldwide more difficult (Waller *et al.,*2023). The major worry is resistance to rifampicin, the most efficient first-line treatment (Prasad *et al.,*2018). Multidrug-resistant tuberculosis is described as having resistance to rifampicin and isoniazid. Rifampicin-resistant tuberculosis and multidrug-resistant tuberculosis both need to be treated with second-line drugs. Between 2015 and 2020, the predicted annual number of people who developed multidrug-resistant tuberculosis or rifampicin-resistant tuberculosis was largely steady, but it increased in 2021 (WHO, 2022). A projected 450 000 incident cases were reported in 2021, an increase of 3.1% from the 437 000 reported in 2020. Multidrug-/rifampicin-resistant tuberculosis were expected to account for 3.6% of new cases and 18% of those that had previously been treated in tuberculosis in 2021; in 2015, the corresponding numbers were 3.9% and 20%. 26% of cases worldwide in 2021 were in India, 8.5% were in the Russian Federation, and 7.9% were in Pakistan (WHO, 2022). These three nations accounted for 42% of all cases worldwide. The COVID-19 pandemic's effects on tuberculosis detection are thought to be the primary reason for the overall rise in tuberculosis incidence between 2020 and 2021, which is the key explanation for the rise (WHO, 2022).

Immune system of human body can't stop growing of bacteria, when *M. tuberculosis* becomes active form, this is called tuberculosis disease (Miggiano*et al.,*2020). It is very significant that persons who have tuberculosis disease are treated by several drugs for six to nine months. More than twenty drugs have been introduced for the treatment of tuberculosis until now. These drugs have been divided into first-line drugs and second line drugs, and have been categorized into five different groups bythe WHO.

The anti-tuberculosis drugs isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide are collectively called the first-line drugs. In general, the first-line drugs have the significant activity against drug-susceptible tuberculosis (Dartois and Rubin, 2022). These drugs are the core component of tuberculosis control problem worldwide. In fact, isoniazid and rifampicin drugs are still the cornerstones for treating in drug-susceptible tuberculosis (Stemkens *et al.,*2023). Both drugs are available and cheap worldwide. The aim of this review is to provide an overview of the latest report on molecular mechanisms of action and resistance to the first-line drugs against *M. tuberculosis*.

Mechanisms of action and resistance to first-line drugs

Drug sensitive disease is treated effectively by using first-line drugs: isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin. However, the continuation phase of the tuberculosis treatment is very important to kill slow growing strains of *M. tuberculosis*. The WHO recommends that drug sensitive tuberculosis patience has to take tuberculosis drug treatment more than six months including a two month intensive treatment phase followed by a continuation phase of four or seven months. Sometimes, the first-line drugs treatment can fail to cure tuberculosis for several factors. Anti-tuberculosis drugs emerges resistance arise primarily due to inappropriate treatment regimens, previous use of anti-tuberculosis drugs, primary infection with drug-resistant strains, and poor adherence to regulated treatment (Gandhi *et al.,* 2010; Dheda*et al.,*2014). Mechanisms of drug resistance of first- and second-line drugs are presented in Table 1. First-line anti-tuberculosis drugs areisoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin and second-line anti-tuberculosis drugs areofloxacin, levofloxacin, moxifloxacin, ciprofloxacin, kanamycin, amikacin, capreomycin, ethionamide/prothionamide, cycloserine/terizidone, p-aminosalicylic acid.

Isoniazid

One of the most important first-line drugs for treating both the conventional treatment regimen and drug sensitive *M. tuberculosis* strains is isoniazid (Mitchison, 1985). In 1952, isoniazid was first offered as an anti-tuberculosis drug (Fox, 1952). Isoniazid does not work against non-growing bacilli; it is only effective against *M. tuberculosis* that is growing (Zhang *et al.,* 1992). Like other tuberculosis drugs, pyrazinamide, ethionamide, and prothionamide, isoniazid is a pro-drug that needs to be activated by the catalase/peroxidase enzyme that is encoded by the *katG* gene (Zhang *et al.,* 1992). A hypothetical isonicotinoyl anion or radical is formed when the catalase/peroxidase enzyme activates INH (Zhang *et al.,* 1992; Lei *et al.,*2000). When this substance combines with NAD+, an INH-NAD adduct is created that binds. There are two main ways that the fundamental mechanisms of isoniazid resistance might occur. First, mutations in the *katG* gene or in the regulator region can be used to block the activation of the isoniazid medication (Vilchèze and Jacobs, 2014). For example, up to 97.5% of clinical isolates of *M. tuberculosis* that is resistant to isoniazid had the *katG* gene mutation S315 (Kiepiela*et al.,*2000). Worldwide, mutations in the *katG* gene, primarily the S315T alteration, have been most commonly associated with INH resistance. Second, mutations in the *inhA* gene or its promoter region can circumvent the inhibition of *InhA* by the isoniazid-NAD adduct (Vilchèze and Jacobs, 2014). The most common mutations after *katG* gene mutations are found in the *mabA-inhA*regulatory region. Significantly, two of the most prevalent variants are *inhA*-15, which results in low-level INH resistance, and *katG* 315, which results in high-level INH resistance (Zenteno-Cuevas *et al.,* 2019; Hsu *et al.,* 2023; Khan *et al.,*2023). However, it has not yet been shown that mutations in the *oxyR-ahpC* area directly confer INH resistance (Kandler *et al.,* 2018; Hsu *et al.,*2023).

Rifampicin

One of the most effective first-line anti-tuberculosis drugs is rifampicin, which also acts as a surrogate marker for the identification of multidrug-resistant tuberculosis (Sinha *et al.,*2020). Rifampicin has very good sterilizing properties and was first commercialized in 1972 as an anti-tuberculosis antibiotic. Rifampicin inhibits bacterial transcription activity by binding to the β-subunit of RNA polymerase (*rpoB*) (Ramaswamy and Musser, 1998), the enzyme responsible for mycobacterial gene transcription and expression. This ultimately leads to the organism's death. Rifampicin's ability to effectively inhibit both actively developing and slowly metabolizing (non-growing) bacilli is a crucial feature (Mitchison, 1979). Adverse responses to RIF are comparatively rare. It might irritate someone's stomach. Compared to the administration of isoniazid, hepatotoxicity is less common.Treating tuberculosis is hampered by the rapid identification

of genes linked to rifampicin resistance in *Mtb* strains. The primary cause of Mtb resistance to rifampicin drug is mutations of the *rpoB* gene (Lai *et al.,*2002; Zaczek *et al.,*2009). Research has demonstrated that rifampicin resistance emerges infrequently in comparison to other anti-tuberculosis drugs, and approximately 90% of cases resistant to rifampicin drug are also resistant to isoniazid drug (Farooqi *et al.,*2012). Genetic alterations in the *rpoB* gene are present in over 90% of clinical cases of rifampicin-resistant tuberculosis, and over 80% of these cases are multidrug-resistant tuberculosis (Huang *et al.,* 2018; Jagielski *et al.,*2018).Of note, mutations in an 81-bp segment of the *rpoB* gene, which is situated in *Mtb* between *rpoB* codons 426 and 452, are mostly linked to rifampicin resistance (Jagielski *et al.,* 2018; Zaw *et al.,*2018). Another recent study reported that 98.06% of the rifampicin-resistant tuberculosis cases contained the *rpoB* gene alterations, and 47.57% of them were resistant to both rifampicin and isoniazid at the same time (Zeng*et al.,*2021). Though the resistance mechanism for the remaining 5% of rifampicin-resistant isolates is still unknown, it's possible that there are alternative mechanisms at play, such as increased efflux pump activity or decreased cell wall permeability (Xu *et al.,*2021).

Streptomycin

In 1942, the first utilized as first-line drug to treat tuberculosis cases was streptomycin, an aminocyclitol drug, along with the four other drugs in the regimen includes rifampicin, isoniazid, ethambutol and pyrazinamide. Following the discovery of the streptomycin drug, it was used as tuberculosis mono-therapy, which quickly caused streptomycin-resistant strains to become resistant. While streptomycin is inactive against non-growing tubercle bacilli, it destroys those that grow slowly. Mutations in the *rpsL* gene (Rv0682) and the rrs gene (Rvnr01), which are encoding for the 16S rRNA and the ribosomal protein S12, respectively, have been linked to streptomycin resistance (Nahid *et al.,* 2019; Cohen *et al.,* 2020; Jia *et al.,* 2021; Shafipour*et al.,*2022). The majority of chromosomal alterations are that cause streptomycin resistance. Intermediate or higher levels of streptomycin resistance have been associated with mutations in the *rpsL* or rrs genes (Nahid *et al.,* 2019; Cohen *et al.,* 2020; Jia *et al.,*2021). Depending on the population and geographic area, different STR resistance related mutations have different types and frequencies. Nonetheless, a number of studies revealed that the prevalence of mutations varies from 37.7% to 94.6% depending on the nation (Cohen *et al.,* 2020; Jia *et al.,*2021; Shafipour*et al.,* 2022). In STR-resistant *M. tuberculosis* isolates, the most notable alterations are located in the rpsL gene at codons 43 and 88 (Nahid*et al.,* 2019; Cohen *et al.,* 2020; Jia *et al.,* 2021; Shafipour*et al.,* 2022). In STR-resistant *M. tuberculosis* isolates, the most prevalent mutations in the rrs gene are found at many locations, including 513, 514, 517, 905, 906, 907, 908, and 1401 (Nahid *et al.,*2019).

The *gidB* (*Rv3919c*) gene has been shown to encode a 7-methylguanosine (m7G) methyltransferase that is dependent on S-adenosylmethionine (SAM) and that methylates the G527 in the 530 loop of the 16S rRNA. High-performance liquid chromatography analysis of 16S rRNA revealed that the *gidB* mutant lacked an m7G alteration, which was linked to resistance to STR (Rodríguez-García *et al,*2021). Interestingly, non-synonymous mutations in gidB typically correspond to low resistance values (Jia *et al.,*2021; Shafipour*et al.,*2022).

Pyrazinamide

One of the cornerstone drugs for the treatment of multidrug-resistant tuberculosis is pyrazinamide, a significant first-line anti-tuberculosis drug used in short-course chemotherapy (Mitchison, 1985).PZA, a prodrug and structural analogue of nicotinamide, must be transformed by the non-essential enzyme pyrazinamidase, which is encoded by the *pncA* gene, into pyrazinoic acid (POA).Both the function of membrane transporters and membrane energetics are disrupted by pyrazinoic acid. Numerous genes, including *pncA*, *panD*, *rpsA*, *clpC*, and the putative efflux pumps *Rv3756c*, *Rv0191*, *Rv1667c*, and *Rv3008* have been associated to PZA resistance;

nevertheless, *pncA* mutations are highly associated with PZA resistance and account for the majority of PZA resistance cases (70–97%) throughout the entire coding region of *pncA* gene. Up to 20% of non-multi-drug resistant tuberculosis patients have PZA resistance, indicating that resistance has been greatly underestimated despite the critical role PZA plays in clinical outcomes (Juma *et al.*,2019).

Table 1. Genes involved in mechanisms of action and resistance to the first-line and second-line drugs in *M.tuberculosis*.

Drugs	Gene involved in resistance	Role of gene product	Resistance mechanism of action	Reference
Isoniazid	*katG*	Catalase-peroxidase	Inhibits of mycolic acid synthesis and other effects	Banerjee *et al.*,1994; Zhang *et al.*, 1992; Wilson *et al.*, 1996; Mdlulie*t al.*, 1998 ; Parish *et al.*, 2007
	inhA	Enoyl ACP reductase		
	ahpC	Alkyl hydroperoxide reductase		
	fabG1	3-Oxoacyl (acyl-carrier protein) reductases		
	iniA	Efflux pump associated		
	fadE24	Involved in fatty acid β-oxidation		
	kasA	Ketoacyl acyl carrier protein synthase		
	ndh	NADH dehydrogenase		
Rifampicin	*rpoB*	β-subunit of RNA polymerase	Inhibits of RNA synthesis	Telentie*t al.*,1993; Comas *et al.*, 2012
	rpoA	α-subunit of RNA polymerase		
	rpoC	β´-subunit of RNA polymerase		
Pyrazinamide	*pncA*	PZase	Inhibits of trans-translation and pantothenate and CoA synthesis	Scorpio and Zhang, 1996; Zhang *et al.*, 2013
	rpsA	Ribosomal S1 protein		
	panD	Aspartate decarboxylase		
Streptomycin	*rpsL*	S12 ribosomal protein	Inhibits of protein synthesis	Nair *et al.*,1993; Okamoto *et al.*, 2007
	rrs	16S rRNA		
	gidB	7-methylguanosine methyltransferase		
Ethambutol	*embB*	Arabinosyl transferase	Inhibits of arabinogalactan synthesis	Telentie*t al.*,1997; Safi *et al.*, 2013
	embA	Arabinosyl transferase		
	embC	Arabinosyl transferase		
	embR	Regulator of embCAB operon expression		
	rmlD	dTDP-4-dehydrorhamnose reductase		
	ubiA	DPPR synthase		
Fluoroquinolone	*gyrA*	DNA gyrase subunit A	Inhibition of DNA synthesis	Ginsburg *et al.* 2003 ; Zhang *et al.* 2005
	gyrB	DNA gyrase subunit B		
kanamycin / amikacin	*rrs*	16S rRNA	Inhibits of protein synthesis	Alangadene*t al.*,1998;Reeves *et al.*, 2013
	eis	Aminoglycoside acetyltransferase		
	whiB7	Transcriptional regulator		
Capreomycin / viomycin	*tlyA*	rRNA methyltransferase	Inhibits of protein synthesis	Maus *et al.*,2005; Johansene*t al.*, 2006
	rrs	16S rRNA		
Ethionamide/ Prothionamide	*inhA*	Fatty acid enoyl acyl carrier protein reductase A	Disrupts cell wall biosynthesis	Baulard*et al.*, 2000
	ethA	Flavin monooxygenase		
	ethR	Transcriptional repressor		
Para-aminosalicylic acid	*thyA*	Thymidylate synthase A	Inhibits of folic acid and thymine nucleotide metabolism	Rengarajan *et al.*, 2004
	folC	Dihydrofolate synthase		
	dfrA	Dihydrofolate reductase		
	ribD	Riboflavin biosynthesis		
D-cycloserine	*alr*	D-alanine racemase	Inhibits the synthesis of peptidoglycan in the cell wall	Caceres *et al.*,1997;Bruninge*t al.*, 2011; Saier*et al.*, 2009
	ddl	D-alanine: D-alanine ligase		
	ald	L-alanine dehydrogenase		
	CycA	D-serine proton symporter		
Clofazimine	*Rv0678*	Transcription repressor for efflux pump MmpL5	Produces of reactive oxygen species, inhibits of energy production	Milano *et al.*, 2009 ; Zhang *et al.*, 2015
	rv1979c	Unknown		
	rv2535c	Unknown		

ACP = acyl-carrier-protein; NADH = Nicotinamide adenine dinucleotide; CoA = coenzyme A; DPPR = 5-phospho-a -d-ribose-1-diphosphate: decaprenyl-phosphate 5-phosphoribosyltransferase.

The most frequent causes of pyrazinamide resistance in *M. tuberculosis* are mutations in the *pncA* gene or its promoter region, which lowers PZase activity (Mok *et al.*,2021). It is very significant to note that whole genome sequencing may help identify pyrazinamide resistance because it will be difficult to build a quick molecular drug susceptibility screening due to the distributed nature of mutations throughout the entire

pncA gene.A very recent study reported that PZA resistance has increased recently among MDR-TB cases (55% to 58%), which emphasizes the need for the development of both conventional and innovative treatment regimens (Wang *et al.*,2023).

Ethambutol

One crucial first-line anti-TB drug for treating drug-susceptible tuberculosis and halting the development of treatment resistance is EMB. Because of the strong synergistic benefits of ethambutol when combined with other drugs, it is also frequently utilized to create regimens for drug-resistant tuberculosis (Zhu *et al.,* 2018; Wang *et al.*,2020). In the intense phase of tuberculosis treatment, the drug is usually prescribed as part of a four-drug regimen that also includes isoniazid, rifampicin, and pyrazinamide. It is alarming to note that more than 4 percent of clinical isolates of *M. tuberculosis* have been shown to be resistant to ethambutol. The *embCAB* gene locus, which is involved in the synthesis of the cell wall components arabinogalactan and lipoarabinomannan, is the main target of ethambutol (Telenti*et al.*,1997). Most often, mutations in the *embB* gene-particularly the classic mutations at codons 306, 406, and 497—are linked to the emergence of ethambutol resistance (Zhao *et al.*,2015). Changes in the *embB* gene, specifically in *embB* codon 306, also known as the ethambutol resistance determining region (ERDR), have been frequently linked to resistance to ethambutol. It has been thought that ERDR sequence analysis can quickly screen for ethambutol resistance. Nevertheless, isolates of *M. tuberculosis* that are sensitive to ethambutol have also been found to have mutations at these codons (Plinke*et al.*,2006). Concerns are raised regarding the clinical importance of ethambutol mutations for the development of ethambutol resistance due to the inconsistent results between phenotypic and genotypic resistance tests. Because of this, the molecular diagnostics for ethambutol susceptibility are considerably behind those for other anti-tuberculosis drugs, which present a significant obstacle to the development of an appropriate treatment strategy.

Conclusion

Treatment for tuberculosis clinical strains resistant to first-line tuberculosis regimens is more challenging than for drug-susceptible strains; it requires prolonged chemotherapy (up to two years of treatment), costly and toxic medications, and a higher risk of treatment failure and mortality. In conclusion, our review article contributes to the evaluation of the current knowledge regarding mutations linked to the drug-resistant *M. tuberculosis* complex.

Therefore, early identification of first-line drugs resistance in *M. tuberculosis* clinical isolates will have clear benefits for patients as well as the general public's health issues, as it will allow for early access to the right effective treatment and the prevention of drug-resistant strains of *M. tuberculosis* strains.

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