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Gene Mutations Associated with Drug Resistance in *Mycobacterium tuberculosis*

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Abstract
Worldwide, tuberculosis (TB) continues to be a serious health issue. The spread of drug-resistant *Mycobacterium tuberculosis* strains is increasing, making it more difficult to control the disease. New molecular biology tools and the deciphering of the *M. tuberculosis* genome have made it possible to identify functionally relevant changes in nucleic acid sequences now take into account these mutations. The conventional methods for identifying strains based on cultures are time-consuming, laborious, and performed by specialized technicians. The result of the test is not generated until two months after the samples are received. Patients with TB are not given the right treatment during this time, and resistant strains may spread to the rest of the population. This review provides the most important mutations in *M. tuberculosis* genes that are associated with drug resistance, how gene expression analysis was used to find new markers of drug resistance strains, and how new anti-tuberculosis drugs were developed to fight drug-resistant strains.

Keywords
Mycobacterium tuberculosis, INH, Tuberculosis (TB), Multidrug-Resistant TB (MDR TB), mutations.

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INTRODUCTION:
Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis* a species of pathogenic bacteria in the family Mycobacteriaceae. The bacteria usually attack the lungs, but TB bacteria can damage any part of the body such as the kidney, spine, and brain. Not everyone infected with TB bacteria becomes ill. [1] *M. tuberculosis* has an unusual, waxy coating on the surface of its cell primarily due to the presence of mycolic acid. This coating makes the cells resistant to Gram staining, and as a result, M. tuberculosis can appear weakly Gram-positive.[2] Tuberculosis regiment treatment includes the first line drugs rifampicin, isoniazid, ethambutol and pyrazinamide and strains that develop resistance to the two more effective antituberculosis drugs, isoniazid, and rifampicin, named multidrug resistance strains.[3][4]

Mutations that confer resistance in *Mycobacterium tuberculosis*
There two types of resistance that are observed in *M. tuberculosis*, one is the genetic resistance where the mutations in genomic regions, on target genes, confer the capacity to avoid the drug effect; the second is the phenotypic resistance, where epigenomic modifications, including alteration of protein structures, generate resistance to drugs without showing the mutation on DNA.[5]
Many works have revealed, using microbiological and clinical data, mutational events in clinical isolates from patients with tuberculosis. Multidrug resistance appears to result from the sequential acquisition of mutations. Possible reasons for the acquisition of mutations include inadequate prescription and delivery of chemotherapy, poor

compliance, or an insufficient number of active drugs in the treatment regimen.[6]

Drugs	MIC (µg/mL)	Drug mode of action	Gene	Target enzyme	Frequency of mutations (%) associated with resistance
Isoniazide	0.02–0.2	Inhibits mycolic acid synthesis and other multiple effects	*katG*	Catalase peroxidase	30–60
			InhA	Fatty acid enoyl acyl carrier protein reductase A	70–80
			ahpC	Alkyl hydroperoxidase reductase	Not known
			kasA	β-ketoacyl-ACP synthase	Not known
			ndh	NADH dehydrogenase	9.5
Rifampicin	0.05–1	Inhibits RNA synthesis	*rpoB*	β subunit of RNA polymerase	95
Streptomycin	2–8	Inhibits protein synthesis	*rpsL*	Ribosomal protein S12	65–67
			rrs	16S rRNA	
			gidB	7-Methylguanosine methyltransferase	33
Ethambutol	1–5	Inhibits arabinogalactan synthesis	*embCAB*	Arabinosyl transferase	70–90
Pyrazinamide	16–100	Disrupts plasmamembrane and energy metabolism (inhibits pantothenate and CoA synthesis)	*pncA*	Pyrazinamidase	>70
			IS6110 insertion		Not known
Fluoroquinolones	0.5–2.5	Introduces negative supercoils in DNA molecules	*gyrA*	DNA gyrase	42-85
			gyrB		
Kanamycin/Amikacin	2–4	Inhibits protein synthesis	*rrs*	16S rRNA	>60

Table: 1 List of gene mutation resulting in drug resistance against *Mycobactreium tuberculosis*

Searching for new markers to identify drug resistance of *Mycobactreium tuberculosis.*

In the comprehension and linking-up of hereditary relationship with the medication obstruction aggregate in *M. tuberculosis*, changes in unambiguous qualities have been the most widely recognized relationship as recently portrayed; however, not all resistant *M. tuberculosis* strains possess the associated mutations that have been reported, indicating that other mechanisms may be at work [7]. The level of some gene's expression has been examined for this purpose. Efflux pump genes are one of them. These genes are important components that help drugs escape from cells and give *M. tuberculosis* resistance to drugs [10]. Super families of efflux pumps have been established: resistance nodulation division (RND), small multidrug resistance (SMR), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC) [8]. The efflux pumps in *M. tuberculosis* include 12 RND-type mycobacterial large membrane proteins (MmpL) [9], 37 ABC transporters-26 complete and 11 incomplete-of which 21 are putative exporters-including antibiotic transporters that make up 2.5% of the genome [11, 12] and 16 putative MFS drug efflux pumps [14]. In some studies [13, 14, 15, 16], efflux pump genes were found to be over expressed in drug-resistant *M. tuberculosis* strains. The significance of efflux pumps in drug resistance has prompted the investigation of the use of efflux pump inhibitors and anti-tuberculosis drugs as part of a combined treatment.

Development of new drug resistance *Mycobacterium tuberculosis* strains

M.tuberculosis drug resistance makes the disease more difficult to control. There were an estimated 480,000 cases of MDR tuberculosis in 2015 and another 100,000 cases were added that were resistant to rifampicin. These cases are more likely to develop multi-drug resistance. Once drug susceptibility testing results are available, new therapeutic regimens involving second-line medications have been implemented in response to drug-resistant tuberculosis [17, 18]. Treatment for drug resistance tuberculosis can last up to 20 months and includes a fluoroquinolone, an injectable aminoglycoside, and an oral bacteriostatic second line drug and a first line drug. Drug therapy for a

patient infected with a susceptible strain of *M. tuberculosis* lasts six months with a variety of combinations of the first-line drugs. The search for new drugs continues because the problem of resistant tuberculosis is getting worse. This includes finding drugs that are more effective against latent TB and drug-resistant strains as well as shortening the duration of treatment. The following stages are involved in the creation of new anti-tuberculosis medications: basic research, the creation of new anti-tuberculosis compounds or drugs, preclinical and clinical studies, phases I, II, and III, before reaching technology transfer; All of these processes take a long time [19]. Numerous natural, semi-synthetic, and synthetic compounds have been tested in vivo and in vitro as part of the ongoing search for better anti tuberculosis medications. We will specify a few new medications that depend on the design of first line drugs, among which a few analogs have been depicted with action against delicate and drug safe *M. tuberculosis* strains. SQ109 and analogues based on carbamate prodrugs [20], S2824 and analogues with a homopiperazine ring [21], 1, 2 diamines [22], ferrocenyl compounds [23] and dihydrosphingosineethambutol analogues [24] are examples of novel compounds based on ethambutol. POEs (pyrazinoic acid esters) and 5Cl-substituted pyrazinoic acid derivatives have been described within pyrazinamide analogues [25]. However, adverse effects of these compounds must be taken into account. There have been reports of aromatic and heterocyclic aldehydes with electron-donating or electron-withdrawing groups for isoniazide-based compounds [26], and rifampicin has been described in the rifamycins alongside rifabutin, rifapnetine, rifalazil and rifametane[27].

First line anti tuberculosis drugs

Rifampicin

Rifampicin is a rifamycin derivative that was first introduced as an anti-tuberculosis medication in 1972. Together with isoniazid, it forms the foundation of the multidrug treatment plan for TB. It is one of the most effective antibiotics for TB. Rifampicin is dynamic against developing and non-developing (slow using) bacilli [28]. Rifampicin inhibits the elongation of messenger RNA in M. tuberculosis by binding to the -subunit of the RNA polymerase [29]. The rpoB gene, which is responsible for coding for the -subunit of the RNA polymerase, is mutated in the majority of clinical isolates of *M. tuberculosis* that are resistant to rifampicin. Conformational changes occur as a result, decreasing the drug's affinity and leading to the development of resistance [30].

Isoniazid

Isoniazid and rifampicin continue to serve as the foundation for TB treatment, having been introduced in 1952 as an anti-TB medication. Isoniazid, in contrast to rifampicin, only inhibits metabolically active replicating bacilli. Isoniazid, which is also known as isonicotinic acid hydrazide, is a pro-drug that works by activating the catalase / peroxidase enzyme KatG, which is encoded by the katG gene [31]. Through the inhA-encoded NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, isoniazid inhibits the synthesis of mycolic acids [32]. Despite the drug's straightforward structure, mutations in several genes, including katG, inhA, ahpC, kasA, and NDH, have been linked to resistance.

Ethambutol

Ethambutol was first introduced in the treatment of TB in 1966 and is part of the current first-line regimen to treat the disease. Ethambutol is bacteriostatic against multiplying bacilli interfering with the biosynthesis of arabinogalactan in the cell wall [33]. In M. tuberculosis, the genes embCAB, organized as an operon, code for arabinosyl transferase, which is involved in the synthesis of arabinogalactan, producing the accumulation of the intermediate d-arabinofuranosyl-P-decaprenol [34].

Streptomycin

Originally isolated from the soil microorganism Streptomyces griseus, streptomycin was the first antibiotic to be successfully used against TB. Unfortunately, as soon as it was prescribed, resistance to it emerged, a result of being administered as monotherapy [35]. Streptomycin is an aminocyclitol glycoside active against actively growing bacilli and its mode of action is by inhibiting the initiation of the translation in the protein synthesis [36]. More specifically, streptomycin acts at the level of the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA coded by the genes rpsL and rrs, respectively [37].

Second line anti tuberculosis drugs

Flouroquinolones

In the treatment of MDR-TB, fluoroquinolones are currently used as a second-line medication. As a by-product of the antimalarial chloroquine, ciprofloxacin and ofloxacin were discovered to be synthetic derivatives of the parent compound, nalidixic acid [38]. Clinical trials are evaluating newer-generation quinolones like moxifloxacin and gatifloxacin, which have been proposed as first-line antibiotics with the goal of reducing the duration of treatment for tuberculosis [39, 40].

Kanamycin, Capreomycin, Amikacin, Viomycin

Capreomycin and viomycin are cyclic peptide antibiotics, whereas kanamycin and amikacin are aminoglycosides, their inhibition of protein synthesis

is the same as that of these four antibiotics. In the treatment of MDR-TB, all four drugs are used as second-line treatments. By altering 16S rRNA, kanamycin and amikacin inhibit protein synthesis. The most well-known transformations found in kanamycin-safe strains are at position 1400 and 1401 of the rrs quality, presenting undeniable level protection from kanamycin and amikacin. However, there have also been reports of mutations at position 1483 [41, 42]. Contrary to what was previously believed, kanamycin and amikacin do not fully cross-resist each other. A few investigations have shown variable levels and examples of opposition recommending that different systems of obstruction may be conceivable [43]. Mutations in the promoter region of the eis gene, which encodes an aminoglycoside acetyltransferase, have been linked to low-level resistance to kanamycin [88]. Over expression of the protein and low levels of resistance to kanamycin were caused by mutations at positions 10 and 35 of the eis promoter, but not amikacin. Up to 80% of clinical isolates with low kanamycin resistance were found to have these mutations [44, 45].

Ethionamide

Ethionamide is a structurally similar isoniazid-like isonicotinic acid derivative. Additionally, it is a pro-drug that needs to be activated by an ethA-encoded monooxygenase. By forming an adduct with NAD that prevents the enzyme enoyl-ACP reductase from operating, it hinders the production of mycolic acid. The transcriptional repressor EthR controls EthA [46]. Mutations in etaA/ethA, ethR, and inhA-mutations that are resistant to both isoniazid and ethionamide-cause ethionamide resistance [47, 48]. In addition, spontaneous *M. tuberculosis* isoniazid- and ethionamide-resistant mutants were found to map to mshA, an enzyme necessary formycothiol biosynthesis [49].

Para-Amino Salicylic Acid

Para-amino salicylic acid, or PAS, was one of the first anti tuberculosis drugs used to treat the disease. Along with streptomycin and isoniazid, PAS is now considered a second-line drug for MDR-TB treatment. Its mechanism of action was not completely understood until recently. As an analog of para-amino benzoic acid, it is thought to compete with it for dihydropteroate synthase, interfering with folate synthesis. Transposon mutagenesis was used to find mutations in the thyA gene that were linked to PAS resistance and were also found in clinical isolates that were resistant to PAS [50]. In laboratory isolates of *M. tuberculosis*, several missense mutations in folC, which encodes dihydrofolate synthase, conferred resistance to PAS [51]. Five PAS-resistant isolates were found in a panel of 85 clinical MDR-TB isolates with folC mutations. However, the fact that less than 40% of PAS-resistant strains contained mutations in thyA suggests the existence of additional drug resistance mechanisms [52].

New anti-tuberculosis drugs

Bedaquiline

Bedaquiline, which was previously known as TMC207 or R207910, is a new antibiotic in the diarylquinoline class that has specific activity against *M. tuberculosis* and has also demonstrated in vitro activity against other non-tuberculous mycobacteria [53]. Bedaquiline inhibits *M. tuberculosis* ATP synthase, a novel anti mycobacterial target. By examining bedaquiline-resistant *M. tuberculosis* and M. smegmatis mutants, this mode of action was discovered. The only mutation identified by sequencing these mutants' genomes and comparing them to those of the susceptible strains was in the atpE gene, which codes for the c portion of the F0 subunit of the ATP synthase [54]. Bedaquiline is more specific than mitochondrial ATP synthase for this complex structure, which produces the ATP that the mycobacterial cell requires [55].

Delamanid

Delamanid, previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against *M. tuberculosis* that acts by inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III trial. Delamanid, previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against *M. tuberculosis* that acts by inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III trial [56].

Benzothiozinones

Recently, the 1,3-benzothiazin-4-one, or benzothiazinone (BTZ), class of drugs with anti-mycobacterial properties was described. BTZ043's mode of action was initially identified at the level of cell wall biogenesis through transcriptome analysis. The drug's target was identified at the level of the gene rv3790, which, along with rv3791, encodes proteins that catalyse the epimerization of decaprenyl phosphoryl ribose (DPR) to decaprenyl phosphoryl arabinose (DPA), a precursor for arabinan synthesis that is necessary for the bacterial cell wall [57]. This was accomplished through additional genetic analysis that made use of mutants that were generated in vitro These two important enzymes have been given the names DprE1 and DprE2 [58].

Types of drug resistance tuberculosis

Multidrug-Resistant TB (MDR TB)

Multidrug-resistant TB (MDR TB) is caused by TB bacteria that are resistant to at least isoniazid and rifampin, the two most potent TB drugs. These drugs

are used to treat all persons with TB disease. TB experts should be consulted in the treatment of MDR TB.[59]

Pre-extensively drug-resistant TB (pre-XDR TB)

Pre-Extensively Drug-resistant TB (pre-XDR TB) is a type of MDR TB caused by TB bacteria that are resistant to isoniazid, rifampin, and a fluroquinolone OR by TB bacteria that are resistant to isoniazid, rifampin, and a second-line injectable (amikacin, capreomycin, and kanamycin).[59]

Extensively drug-resistant TB (XDR TB)

TB bacteria that are resistant to isoniazid, rifampin, a fluroquinolone, and a second-line injectable (amikacin, capreomycin, and kanamycin) OR TB bacteria that are resistant to isoniazid, rifampin, a fluroquinolone, and bedaquiline or linezolid are responsible for the rare form of MDR TB known as extensively drug-resistant TB (XDR). Patients are left with treatment options that are significantly less effective because XDR TB is resistant to the most potent TB medications. People with HIV infection or other conditions that can weaken the immune system are particularly at risk from XDR tuberculosis. Once infected, these individuals are more likely to develop TB disease and are also more likely to die from it. When treating XDR TB, experts in the field should be consulted.[59]

Causes of drug resistance TB

Drug-resistant TB can occur when the drugs used to treat TB are misused or mismanaged. Examples of misuse or mismanagement include.

- ✓ People do not complete a full course of TB treatment.
- ✓ Health care providers prescribe the wrong treatment (the wrong dose or length of time)
- ✓ Drugs for proper treatment are not available.
- ✓ Drugs are of poor quality.

Drug-resistant TB is more common in people who.

- ✓ Do not take their TB drugs regularly.
- ✓ Do not take all of their TB drugs.
- ✓ Develop TB disease again, after being treated for TB disease in the past
- ✓ Come from areas of the world where drug-resistant TB is common
- ✓ Have spent time with someone known to have drug-resistant TB disease[59]

Prevention of drug resistance TB

The main method for forestalling the spread of medication safe TB is to take all TB sedates precisely as recommended by the medical services supplier. It is not recommended to skip doses or stop treatment early. If someone is receiving treatment for TB disease and is having difficulty taking the medication, they should tell their doctor. By quickly diagnosing cases, adhering to recommended treatment guidelines, monitoring patients' responses to treatment, and ensuring that therapy is completed, healthcare providers can aid in the prevention of drug-resistant tuberculosis.

Avoiding contact with known drug-resistant TB patients in closed or crowded settings like hospitals, prisons, or homeless shelters is another way to avoid contracting the disease. Infection control and occupational health professionals should be consulted by those who work in healthcare facilities or hospitals where TB patients are likely to be seen.[59]

Treatment of drug resistance in TB

TB bacteria that are resistant to at least one first-line anti-TB drug cause drug-resistant TB. Multidrug-resistant tuberculosis, or MDR TB, is resistant to at least isoniazid (INH) and rifampin (RIF), as well as multiple anti-TB medications. Extensively drug-resistant tuberculosis (XDR TB) is a rare form of MDR tuberculosis that is resistant to isoniazid, rifampin any fluoroquinolone, and at least one of three injectable second-line drugs.

It is difficult to treat and cure drug-resistant tuberculosis. Life-threatening consequences can result from inappropriate management. A specialist in the disease should be consulted or used to manage drug-resistant TB [59]. The US Food and Drug Administration (FDA) has issued a safety announcement regarding fluoroquinolone antibiotics. Due to the potential for side effects, the FDA has advised limiting the use of fluoroquinolone antibiotics for certain mild infections. In particular, the FDA stated that patients with sinusitis, bronchitis, and uncomplicated urinary tract infections who have other treatment options generally outweigh the benefits of fluoroquinolone antibacterial medications.

Fluoroquinolone antibacterial medications are absolutely necessary for some patients who have drug-resistant TB disease, drug-resistant latent TB infection, or who are unable to tolerate first-line TB medications. Patients who are taking fluoroquinolone antibacterial medications for tuberculosis also have a chance of experiencing the side effects that have been noted by the FDA. Due to the fact that tuberculosis is a potentially fatal or debilitating infection, there are no better treatments for these patients and the benefits of fluoroquinolone antibacterial medications outweigh the risks [59, 60].

CONCLUSION:

A significant obstacle to TB treatment and global public health is the alarming rise in TB incidence and drug resistance in *Mycobacterium tuberculosis*. The

blends of inborn and obtained drug obstruction instruments which incorporate hereditary transformations, chromosomal adjustments, impermeability of cell envelope, drug efflux, drug debasement and change, target modification and target mimicry render the Mycobacterum cells impervious to most classes of antituberculosis agents. By acquiring compensatory mutations, MDR strains regain fitness and further stabilize transmissibility. As a result, MDR and XDR strains continue to pose a threat even after new anti-TB medications are introduced to the market. Mtb's transition from a metabolically active, replicating form to a dormant, non-replicating form results in phenotypic drug tolerance and is a recalcitrant component of TB treatment. This is in addition to Mycobacterium inherent and acquired drug resistance. Our understanding of the remarkable diversity of Mycobacterium antimicrobial resistance mechanisms has significantly increased in recent years as a result of advancements in genomics and biology. The molecular basis of antimicrobial resistance in this group of bacteria still needs to be better understood, despite significant progress. Both the production of new anti-TB medications and the use of currently available antibiotics in therapy are hampered by these mechanisms of drug resistance and drug tolerance. To further enhance the therapeutic outcomes of TB patients, a deeper comprehension of the precise mechanisms of antimicrobial resistance in Mtb is the best hope for the future. The advancements in anti-tubercular agents—whether they are already in use, are being developed for the first time, or have been repurposed—their mode of action, and the mechanisms by which M. tuberculosis drugs become resistant are summarized in this review. It is intended to facilitate a better understanding of drug resistance for effective TB treatment and clinical management and is based on recent literature and WHO guidelines.

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