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VARIOUS MECHANISMS OF DRUG RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS – A COMPREHENSIVE REVIEW

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ABSTRACT

Globally, tuberculosis (TB) is a severe public health issue. The presence of multidrug resistant (MDR) strains of *Mycobacterium tuberculosis*, the disease's causative agent, exacerbates its condition. Even more severe cases of medication resistance have been documented in recent years. Improved methods for quickly detecting drug resistance in *M. tuberculosis* and the exploration of novel targets for therapeutic activity and development will result from a deeper understanding of the molecular processes behind drug resistance in this pathogen. The methods of action of anti-tuberculosis medications and the molecular causes of treatment resistance in *M. tuberculosis* are covered in this study.

KEYWORDS: Drug resistance, Molecular mechanisms, *Mycobacterium tuberculosis.*

INTRODUCTION

Globally, tuberculosis (TB) continues to be a major infectious disease and public health problem. In 2012, there were an estimated 8.6 million incident cases of tuberculosis (TB), and the illness was responsible for 1.3 million fatalities, according to the most recent World Health Organisation (WHO) study. There have been almost 500000 cases in children and 320000 fatalities linked to HIV infection.[1] But the rise in medication resistance is much more concerning. There were an estimated 450000 instances of multidrug resistant (MDR) tuberculosis in 2012, and the disease was the cause of 170000 fatalities. *Mycobacterium tuberculosis* strains that are resistant to isoniazid and rifampicin, two essential medications for treating the illness, are the source of multidrug-resistant tuberculosis, or MDR-TB. More resistant *M. tuberculosis* strains, known as extensively drug-resistant (XDR)-TB strains, have been identified since 2006.[2,3,4] Apart from being multidrug resistant, these isolates exhibit resistance against all fluoroquinolones and at least one injectable second-line antibiotic, such as amikacin, capreomycin, or kanamycin. More recently, strains of *M. tuberculosis* have been described that are fully drug resistant (TDR)-TB, meaning they are resistant to every antibiotic.[5,6,7] This is a more concerning condition. Reducing and controlling the spread of these resistant strains of tuberculosis (TB) requires early diagnosis of all kinds of treatment resistance. Finding novel therapeutic targets and improving methods of drug resistance detection will be made possible by a greater understanding of the mechanisms of action of anti-TB medications and the emergence of drug resistance. The primary anti-TB medications' methods of action and resistance will be examined in the next sections, along with newly discovered medications that have been shown to have anti-TB activity.

FIRST LINE ANTI TB DRUGS

Rifampicin - A rifamycin derivative called rifampicin was first used as an antituberculosis medication in 1972. It is one of the most potent anti-TB medicines and forms the cornerstone of the multidrug therapy regimen for tuberculosis, along with isoniazid. Both growing and non-growing (slow metabolising) bacilli are susceptible to the effects of rifampicin.[8] Rifampicin inhibits the elongation of messenger RNA in *M. tuberculosis* via binding to the β-subunit of the RNA polymerase.[9] Most clinical isolates of *M. tuberculosis* that are resistant to rifampicin include mutations in the rpoB gene, which produces the β-subunit of RNA polymerase. Conformational alterations therefore take place, lowering the drug's affinity and fostering the emergence of resistance.[10] The so-called "hot-spot region," which is an 81-bp region encompassing codons 507–533 of the rpoB gene, has mutations that account for around 96% of *M. tuberculosis* isolates that are resistant to rifampicin. The rifampicin resistance-determining region is another

name for this area.[11] In most investigations, rifampicin resistance is most frequently linked to mutations in codons 516, 526, and 531.[12,13] A few papers have also mentioned the occurrence of mutations outside of rpoB's hot-spot area, but less often.[14,15] There may be cross-resistance to other rifamycins. Some codons (e.g., 518 or 529) have been linked to low-level rifampicin resistance, but these mutations have not affected susceptibility to other rifamycins, including rifabutin or rifalazil.[16,17] This is crucial for tuberculosis patients who require antiretroviral treatment since rifabutin induces the cytochrome P450 CYP3A oxidative enzyme less effectively than other metabolites.[18] However, monoresistance to rifampicin is extremely uncommon, and practically all strains that are resistant to the antibiotic also exhibit resistance to other medications, most notably isoniazid. Rifampicin resistance is regarded as a proxy marker for MDR-TB because of this.[19] Current genome sequencing investigations have revealed that rifampicin-resistant bacteria harbouring mutations in rpoB have acquired compensatory mutations in rpoA and rpoC, which encode the α and β' subunits of RNA polymerase.[20] These compensatory mutations have been linked to a higher transmissibility in certain contexts and would be responsible for restoring the fitness of these strains in vivo.[21,22]

Isoniazid: Introduced in 1952 as an anti-tuberculosis drug, isoniazid continues to be the cornerstone of TB therapy, along with rifampicin. Isoniazid, in contrast to rifampicin, only works against bacilli that replicate metabolically. Isonazid, also referred to as isonicotinic acid hydrazide, is a pro-drug that, in order to work has to be activated by the catalase/peroxidase enzyme KatG, which is represented by the katG gene.[23] Isoniazid works by blocking the NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, which is encoded by inhA, from synthesising mycolic acids.[24] Notwithstanding its straightforward structure, resistance to this medication has been linked to mutations in a number of genes, including NDH, katG, inhA, ahpC, and kasA. Isoniazid resistance is primarily caused by two molecular mechanisms: gene mutations in the promoter regions of katG and inhA. Indeed, these two genes have been identified to be most often mutated in cases of isoniazid resistance in multiple investigations.[25,26] Of them, S315T in katG has been shown to be the most common gene mutation. This mutation results in an isoniazid product that is unable to generate the isoniazid-NAD adduct necessary for it to have antimicrobial action.[27,28] This mutation is more common in MDR strains[26], and it has been repeatedly linked to high-level resistance (MIC > 1 µg/mL) to isoniazid.[29] The second most common mutation causes an overexpression of InhA and, less commonly, a mutation in its active site that lowers its affinity for the isoniazid-NAD adduct.[28] This mutation occurs in the promoter region of InhA. Position −15C/T has been identified as the most common mutation, and it is more frequently linked to low level isoniazid resistance (MIC < 1 µg/mL). Mutations in inhA result in resistance to both isoniazid and ethionamide, a structurally similar medication with the same target.[30,31] According to a recent research, cross-resistance to ethionamide and high-level isoniazid resistance were caused by a mutation in the inhA coding area and a mutation in the inhA regulatory region.[32] Dihydrofolate reductase (DfrA) in *M. tuberculosis* is inhibited by the 4R isomer of the isoniazid-NADP adduct, according to a recent intriguing discovery.[33] This finding raises the possibility that mutations in dfrA contribute to isoniazid resistance. In addition, 16 other proteins, including InhA and DfrA, were shown to be highly affinity bound by these adducts during an examination of the proteome of isoniazid targets in *M. tuberculosis.*[34] These findings may indicate further, as of yet unidentified effects of isoniazid on the bacterium. However, no mutation in dfrA linked to isoniazid resistance has been found in two recent investigations.[35,36] The alkyl hydroperoxidase reductase that *M. tuberculosis* encodes is linked to resistance to reactive oxygen intermediates. Originally, it was suggested that mutations in the ahpC promoter may serve as stand-in markers for isoniazid resistance.[37] It is now better established that mutations in the ahpC promoter do not induce isoniazid resistance; rather, they are compensatory alterations for the loss of catalase/peroxidase function.[38] Furthermore, isoniazid resistance is not conferred by overexpressing AhpC.[39] Single nucleotide polymorphisms in additional genes, such as kasA and the oxyR-ahpC and furA-katG intergenic areas, have been discovered in a number of investigations involving isoniazid-resistant clinical isolates of *M. tuberculosis.*[26,40,41] Their direct contribution to isoniazid resistance, however, has not yet been adequately shown. However, it has been shown unequivocally that mutations in ndh in M. smegmatis and M. bovis BCG induce co-resistance to isoniazid and ethionamide by changing the NADH/NAD ratios inside the cell and competitively inhibiting the INH-NAD adduct.[42,43] Additionally, a recent study discovered that *M. tuberculosis* had increased inhA expression, which in turn provided isoniazid resistance due to a silent mutation in mabA.[44]

Ethambutol: Since its introduction in 1966, ethambutol has been a part of the first-line therapy regimen for tuberculosis. Ethambutol inhibits the growth of bacteria by interfering with their ability to synthesise arabinogalactan in the cell wall.[45] The arabinosyl transferase gene, encoded by the operon embCAB in *M. tuberculosis*, is involved in the synthesis of arabinogalactan and contributes to the buildup of the intermediate d-arabinofuranosyl-P-decaprenol.[46] Mutations in the gene embB have been identified as the recognised mechanism of resistance to ethambutol; in the majority of the conducted investigations, mutations at position embB306 have been shown to be the most common.[47,48] However, some research has also shown mutations in embB306 in isolates that are sensitive to ethambutol.[49] Furthermore, mutations in embB306 were linked to a propensity to become resistant to an

increasing number of medicines and to spread, rather than a direct correlation with ethambutol resistance, according to a research involving a sizable number of *M. tuberculosis* isolates.[50] Allelic exchange experiments have really demonstrated that ethambutol resistance was actually caused by specific mutations resulting in certain amino acid substitutions, but ethambutol resistance was mostly unaffected by other amino acid changes.[51] More recently, the same authors reported that mutations in embB and embC, as well as in the genes that encode the decaprenylphosphoryl-B-d-arabinose (DPA) biosynthesis and utilisation pathway, Rv3806c and Rv3792, accumulate to produce a range of MICs for ethambutol, depending on the type and number of mutations.[52] These results may affect how well existing molecular techniques identify ethambutol resistance. Thus, mutations in embB306 result in varying degrees of ethambutol resistance and are necessary but insufficient to provide high levels of ethambutol resistance. About 30% of ethambutol-resistant strains still lack an embB mutation, highlighting the necessity to find additional potential drug resistance pathways.

Pyrazinamide: Since its introduction to TB treatment in the early 1950s, pyrazinamide has been a conventional first-line regimen for the illness's management. The introduction of pyrazinamide, an analogue of nicotinamide, allowed for a six-month treatment duration reduction. Its property is that it inhibits semi-dormant bacilli that live in acidic settings, such the lesions caused by tuberculosis.[53] Pyrazinamide, being a pro-drug, requires the enzyme pyrazinamidase/nicotinamidase, which is encoded by the pncA gene, to transform it into its active form, pyrazinoic acid.[54,55] According to the suggested mechanism of action, pyrazinamide is converted to pyrazinoic acid, which alters the energetics of the bacterial membrane and prevents membrane transfer. Passive diffusion allows pyrazinamide to enter the bacterial cell, where it transforms into pyrazinoic acid, which is then eliminated by a weak efflux pump. An ineffective efflux pump would cause the protonated pyrazinoic acid to be reabsorbed into the cell and accumulate within in an acidic environment, causing harm to the cell.[56] Additionally, pyrazinoic acid and its n-propyl ester have been shown in one research to be able to block *M. tuberculosis* bacilli's fatty acid synthase type I during replication.[57,58] The prior hypothesis has been questioned by a recent research, which suggests that pyrazinoic acid inhibits trans-translation, a ribosome-sparing mechanism in *M. tuberculosis*.[59] The ribosomal protein 1 (RpsA) was suggested as the focus of the investigation, which was conducted in pyrazinamide-resistant strains with mutations in rpsA but not in pncA. Increased resistance to pyrazinamide was seen upon overexpression of RpsA, and it was shown that pyrazinoic acid was bound to RpsA.[59] Although this is a very interesting theory for pyrazinamide's target, it is impossible to assume that rpsA mutations are the actual target of pyrazinamide because allelic transfers were not performed in this work. The most frequent discovery in strains resistant to pyrazinamide is still mutations in the pncA gene. Although these mutations are dispersed across the gene, the majority of them happen in either an 82-bp region of the putative promoter or a 561-bp region of the open reading frame.[60,61] A small number of investigations have shown the presence of pyrazinamide-resistant strains in the absence of pncA mutations, suggesting that the resistance may result from mutations in an additional regulatory gene that has not yet been found.[62] The information available now suggests that rpsA mutations only partially contribute to pyrazinamide resistance.[63,64,65]

Streptomycin: The first antibiotic to be effectively used against tuberculosis (TB) was streptomycin, which was initially identified from the soil bacterium *Streptomyces griseus*. Unfortunately, because it was given as monotherapy, resistance to it developed as soon as it was recommended.[66] Streptomycin is an aminocyclitol glycoside that works by preventing the start of protein synthesis's translation, making it effective against bacilli that are actively proliferating.[67] More precisely, streptomycin functions at the level of the 16S rRNA, which is coded by the genes rpsL and rrs, respectively, and the 30S component of the ribosome at the ribosomal protein S12.[68] Therefore, 60%–70% of the resistance identified is attributed to mutations in rpsL and rrs, which are the main mechanisms of resistance to streptomycin.[69] The most often reported mutation in rpsL to far be a swap of arginine for lysine in codon 43. High levels of streptomycin resistance are caused by this mutation. The most frequent mutations in rrs are between nucleotides 530 and 915. Despite the absence of mutations in either of these two genes, a significant proportion of strains resistant to streptomycin still exist, indicating the existence of other resistance mechanisms. Recent reports have also indicated that low-level resistance to streptomycin is conferred by mutations in gidB, a gene encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA.[70,71]

SECOND LINE ANTI TB DRUGS

Fluoroquinolones: As of right now, fluoroquinolones are used as second-line medications to treat MDR-TB. The synthetic derivatives of nalidixic acid, which was found as a by-product of the antimalarial drug chloroquine, are ciprofloxacin and ofloxacin.[72] To reduce the duration of therapy for tuberculosis, newer-generation quinolones such gatifloxacin and moxifloxacin are being assessed in clinical studies and suggested as first-line medications.[73,74] Fluoroquinolones work by blocking two essential enzymes for bacterial survival, topoisomerase II (DNA gyrase) and topoisomerase IV. The genes gyrA, gyrB, parC, and parE, respectively, encode these proteins.[75] Since type II topoisomerase, also known as DNA gyrase, is the sole enzyme found in *M. tuberculosis*, it is the only target of fluoroquinolone action.[76] DNA supercoiling is catalysed by type II topoisomerase, a tetramer made up of two α and β subunits that are encoded by gyrA and

gyrB, respectively.[77] Chromosome mutations in the gyrA or gyrB region that determines quinolone resistance are the primary mechanism responsible for the development of fluoroquinolone resistance in *M. tuberculosis*. While mutations at positions 74, 88, and 91 of gyrA have also been observed, the most common mutations discovered are at positions 90 and 94.[78,79] Published recently[80] is a systematic review of gyrase mutations linked to fluoroquinolone resistance in *M. tuberculosis*. The occurrence of a naturally occurring polymorphism at position 95 in gyrA in *M. tuberculosis* is an intriguing discovery. This polymorphism is unrelated to fluoroquinolone resistance, as it is also present in strains that are sensitive to the drug.[81] The discovery that the combined presence of the gyrA mutations T80A and A90G resulted in hypersusceptibility to multiple quinolones is another intriguing discovery.[82] This discovery may indicate that the issue of fluoroquinolone resistance in *M. tuberculosis* is more complicated than first believed. Although isolated reports have recognised the existence of isolates resistant to gatifloxacin and moxifloxacin that were still susceptible to ofloxacin, cross-resistance across fluoroquinolones is believed to occur.[83] Furthermore, it has been proposed that *M. tuberculosis* may be resistant to fluoroquinolones due to the involvement of efflux systems.[84]

Kanamycin, Capreomycin, Amikacin, Viomycin: All four of these antibiotics work by preventing the production of new proteins. However, capreomycin and viomycin are cyclic peptide antibiotics, and kanamycin and amikacin are aminoglycosides. The four medications are all second-line treatments for MDR-TB. Amikacin and kanamycin modify 16S rRNA to prevent the creation of proteins. The rrs gene's positions 1400 and 1401 are most often mutated in kanamycin-resistant strains, resulting in high-level resistance to both kanamycin and amikacin. But there have also been reports of mutations at location 1483.[85,86] Contrary to popular belief, kanamycin and amikacin do not fully exhibit cross-resistance. Variable degrees and patterns of resistance have been seen in certain investigations, indicating the possibility of additional resistance mechanisms.[87] Accordingly, mutations in the eis gene's promoter region, which codes for an aminoglycoside acetyltransferase, have been linked to low-level kanamycin resistance.[88] Amplification of the eis promoter at positions −10 and −35 resulted in low-level resistance to amikacin but overexpression of the protein against kanamycin. Up to 80% of clinical isolates with low-level kanamycin resistance were reported to have these mutations.[88,89] On the other hand, capreomycin and viomycin share a structure and bind to the ribosome at the point where the small and large subunits meet.[90] According to earlier research, they exhibit complete cross-resistance.[91] Resistance to viomycin and capreomycin has also been linked to mutations in the tlyA gene. An rRNA methyltransferase that is specialised for ribose 2'-O-methylation in rRNA is called TlyA. The lack of methylation activity is determined by mutations in tlyA.[92] A recent meta-analysis assessing the relationship between genetic variants and resistance to second-line medications has verified the occurrence of tlyA mutations in addition to rrs and eis mutations, despite the fact that other studies did not identify this correlation.[93]

Ethionamide: Isonicotinic acid derivative ethionamide shares structural similarities with isoniazid. Additionally, it is a pro-drug that needs the ethA gene's monooxygenase to activate it. By creating an adduct with NAD that inhibits the enoyl-ACP reductase enzyme, it obstructs the formation of mycolic acid. The transcriptional repressor EthR controls the expression of EthA.[94] Mutations in etaA/ethA, ethR, and inhA can result in resistance to ethionamide and isoniazid.[95,96] These mutations also produce resistance to ethionamide. Furthermore, research on spontaneously occurring isoniazid- and ethionamide-resistant mutants of Mycothiosis has revealed that these mutants map to mshA, which encodes an enzyme necessary for the manufacture of mycothiol.[97]

Para-Amino Salicylic Acid: Para-amino salicylic acid, or PAS, was among the first anti-tuberculosis medications used to treat the illness, along with isoniazid and streptomycin. However, it is currently regarded as a second-line medication used in the treatment of multidrug-resistant tuberculosis (MDR-TB). Its exact mode of action remained unknown until recently. Since it is an analogue of para-amino benzoic acid, it is hypothesised that it will compete with it for dihydropteroate synthase, obstructing the production of folate. Alterations in the thyA gene linked to PAS resistance were found in a transposon mutagenesis study, and these alterations were also found in clinical isolates resistant to PAS.[98] Numerous missense variants in the dihydrofolate synthase gene, folC have also been found recently to confer resistance to PAS in laboratory isolates of *Mycobacterium TB.*[99] Five of the 85 clinical MDR-TB isolates in the panel had mutations in folC, making them resistant to PAS. However, thyA mutations were found in fewer than 40% of PAS-resistant strains, suggesting the possibility of other drug resistance pathways.[100]

Cycloserine: In MDR-TB treatment regimens, cyclorine is an oral bacteriostatic second-line anti-tuberculosis medication. It is an analogue of d-alanine that prevents peptidoglycan production by obstructing d-alanine's activity: d-alanine ligase. Additionally, it has the ability to block AlrA, the enzyme that converts l-alanine to d-alanine.[101] While the precise function of cycloserine in *Mycobacterium TB* remains unclear, prior research in *Mycobacterium smegmatis* shown that overexpression of alrA resulted in recombinant mutants' resistance to cycloserine.[102] More recently, it has also been demonstrated that *M. bovis* BCG's resistance to cycloserine was partly caused by a single mutation in cycA, which encodes a d-alanine transporter.[103]

Thioacetazone: Due to its favourable in vitro activity against *M. tuberculosis* and its extremely cheap cost, thioacetazone is an old medication that was used in the treatment of tuberculosis (TB). However, it has toxicity issues, particularly in individuals who are also HIV-positive. It works by preventing the formation of mycolic acid and is included in the WHO's group 5 of medications.[104]

Macrolides: Because macrolides have little effect against *M. tuberculosis*, they are more often used for the treatment of other mycobacterial diseases. Among these, clarithromycin is regarded by the WHO as belonging to medication group 5. Low cell wall permeability and the expression of emr37, a gene that codes for a methylase at a particular location in the 23S rRNA and prevents the antibiotic from binding, have been linked to intrinsic resistance to macrolides. It was discovered that this intrinsic resistance may be induced with sub-inhibitory doses of clarithromycin in trials using *Mycobacterium microti* and *M. tuberculosis*, resulting in a four- to eight-fold rise in MIC values.[105] Furthermore, sub-inhibitory amounts of ethambutol restored resistance to clarithromycin in trials conducted with clinical isolates of *M. tuberculosis*, indicating a permeability barrier as the source of intrinsic resistance to the macrolide.[106]

Clofazimine: A riminophenazine molecule called clofazimine was long thought to have anti-TB properties.[107] Owing to the presence of alternative potent anti-TB medications during that era and certain unfavourable consequences, such skin pigmentation, its application was restricted to the management of leprosy.[108] It is now regarded as one of the WHO's group 5 medications for the treatment of MDR-TB. The precise mechanism of action of this antibiotic remained unclear until recently. However, recent research has suggested that clofazimine may target the outer membrane.[109] According to a different research, clofazimine in *M. tuberculosis* is reduced by NADH dehydrogenase and then releases bactericidal quantities of reactive oxygen species (ROS) by spontaneous reoxidation.[110] Although clofazimine resistance has not yet been thoroughly studied, a recent study discovered that mutations in the transcriptional regulator Rv0678 in spontaneous mutants of the reference strain H37Rv led to an upregulation of MmpL5, a multisubstrate efflux pump, which resulted in resistance to bedaquiline as well as clofazimine.[111]

Linezolid: Linezolid, an oxazolidinone initially licensed for clinical use in the treatment of skin infections and nosocomial pneumonia caused by Gram-positive bacteria, is also a member of category 5 medications of second-line anti-TB therapies.[112] Linezolid works by attaching to the 50S ribosomal subunit and inhibiting an early stage of protein synthesis.[101] While 1.9% of MDR strains were found to be resistant to linezolid in a research analysing 210 strains, resistance to the drug is still uncommon in *M. tuberculosis.*[113] Subsequent investigation of in vitro chosen linezolid-resistant mutants revealed that bacteria exhibiting 23S rRNA mutations had MICs of 16–32 µg/mL, whereas susceptible strains or strains with MICs of 4–8 µg/mL had no mutations.[114] In vitro-selected mutants and clinical isolates of *M. tuberculosis* resistant to linezolid has been discovered to carry the mutation T460C in rplC, which codes for the 50S ribosomal L3 protein.[115] This finding is also the result of a more recent investigation that used next-generation sequencing. Prior research has also revealed indications that efflux pumps may play a role in *M. tuberculosis*' resistance to linezolid.[84]

Table 1: First and second line anti TB drugs.

Drug	Gene	Mechanism Involved	References
Isoniazid	katG, inhA	Catalase/peroxidase; enoyl reductase	[26]
Rifampicin	rpoB	RNA polymerase	[11,12,13]
Pyrazinamide	pncA, rpsA	Pyrazinamidase; ribosomal protein 1	
Ethambutol	embB	Arabinosyl transferase	[40,41]
Streptomycin	rpsL, rrs, gidB	S12 ribosomal protein, 16A rRNA, 7-methylguanosine methyltransferase	[59,60,61]
Quinolones	gyrA, gyrB	DNA gyrase	[67,68]
Capreomycin	rrs, tlyA	16S rRNA, rRNA methyltransferase	[80,81]
Kanamycin/Amikacin	rrs	16S rRNA	[83]
Ethionamide	ethA	Enoyl-ACP reductase	[85,86]
Para-aminosalicylic acid	thyA, folC	Thymidylate synthase A	[87,88,89]

NEW ANTI TB DRUGS

Despite the pharmaceutical industry's purported lack of interest in developing novel antibiotics, a number of anti-tuberculosis medications are currently being developed, and some of them are being tested in novel combinations and clinical trials to shorten the duration of TB treatment.

Bedaquiline: Bedaquiline, formerly known as TMC207 or R207910, is a novel antibiotic of the diarylquinoline family that specifically targets *M. tuberculosis*. It has also demonstrated action against other non-tuberculous mycobacteria in vitro.[116] Using Mycobacterium smegmatis in a whole-cell test, a high-throughput screening of hundreds of chemicals led to the discovery of bedaquiline.[117] The medication was evaluated clinically for drug susceptibility and multidrug-resistant

tuberculosis after demonstrating action against *M. tuberculosis* both in vitro and in vivo.[74,118,119] Bedaquiline, marketed as Sirturo, has just been granted conditional clearance for the treatment of multidrug-resistant tuberculosis (MDR-TB), based on the outcomes of two phase II clinical trials. However, this authorisation comes with a "black box" warning because of the reported inexplicable fatalities and lengthening of QT interval. This novel medication has recently been reviewed and evaluated in several publications.[120,121] Although it was supposed to commence in 2013, a phase III clinical study hasn't yet begun. In an effort to shorten treatment duration, bedaquiline is also being assessed in novel combination regimens.[122] Bedaquiline works by blocking *M. tuberculosis*'s ATP synthase, which was an entirely novel target of action for an antimycobacterial medication. By examining *M. tuberculosis* and *M. smegmatis* mutants resistant to bedaquiline, this route of action was found. The atpE gene, which codes for the c portion of the F0 subunit of ATP synthase, was the only mutation discovered by sequencing the genomes of these mutants and comparing them to those of the susceptible strains.[117] The mycobacterial cell requires this intricate structure to produce ATP[123], for which bedaquiline has a preferred selectivity in comparison to mitochondrial ATP synthase.[124] Although I66M has also been discovered, A63P is the most common mutation in the atpE gene reported in bedaquiline-resistant mutants. The latter brings about a change that prevents bedaquiline from properly attaching to its target.[125,126] However, only 15 out of 53 resistant mutants in a research to examine the causes of bedaquiline resistance in *M. tuberculosis* exhibited mutations in atpE. The absence of mutations in the F0 or F1 operons, or in atpE, in the remaining 38 strains raises the possibility of other resistance mechanisms.[127]

Delamanid: Delamanid, formerly known as OPC-67683, is a nitro-dihydro-imidazooxazole derivative that inhibits the formation of mycolic acid to exert its anti *M. tuberculosis* action. A phase III trial is now conducting clinical assessment of the drug.[74] Delamanid has been demonstrated to exhibit strong early bactericidal action similar to that of rifampicin[129], as well as extremely good in vitro and in vivo activity against drug-susceptible and drug-resistant *M. tuberculosis.*[128] More lately, a clinical study for MDR-TB has demonstrated the safety and effectiveness of delamanid.[130] Delamanid works specifically by inhibiting the production of mycolic acid; however, it varies from isoniazid in that it only inhibits the synthesis of methoxy- and keto-mycolic acid, whereas isoniazid also inhibits the synthesis of α-mycolic acid.[128] For delamanid to function, *M. tuberculosis* must also reductively activate it. A mutation in the Rv3547 gene was discovered in mycobacteria resistant to delamanid in an experimental setting, indicating that this gene plays a part in the drug's activation.[128]

PA-824: Nitroimidazole's bicyclic derivative PA-824 shown targeted efficacy against *M. tuberculosis.*[131] This small-molecule drug demonstrated excellent safety and well-tolerated properties in addition to demonstrating excellent in vitro and in vivo efficacy in animal models.[132,133] Additional clinical assessments of PA-824 are also being conducted.[122,134] The production of proteins and cell wall lipids is inhibited by PA-824, which requires a nitroreductase to activate it.[131] It has been demonstrated that depletion of a certain glucose-6-phosphate dehydrogenase (FGD1) or the dezaflavin cofactor F420 is most frequently linked to the mechanism of resistance to PA-824. More recently, a protein unique to nitroimidazo-oxazine has also been shown to cause slight structural alterations in the medication.[135]

SQ-109: Synthetic ethambutol analogue Compound SQ-109 has demonstrated action against drug-susceptible and drug-resistant *Mycobacterium TB* both in vitro and in vivo.[136] Additionally, it has demonstrated synergistic in vitro action when paired with first-line medications and, surprisingly, with bedaquiline and the oxazolidinone PNU-10048.[137,138,139] A phase II clinical study is presently being conducted to assess SQ-109.[74] Trehalose monomycolate, a precursor to trehalose dimycolate, accumulates as a result of SQ-109's interference with the assembly of mycolic acids into the bacterial cell wall core. According to transcriptional studies, SQ-109 stimulates the transcription of the iniBAC operon, which is necessary for the efflux pump to operate, just as other cell wall inhibitors such isoniazid and ethambutol.[140] Furthermore, changes in the mmpL3 gene were discovered by whole-genome sequencing and the production of spontaneously produced resistance mutants to SQ-109 analogues. This suggests that MmpL3 is the target of SQ-109 and signals MmpL3 as a transporter of trehalose monomycolate.[141]

Benzothiazinones: Recently, benzothiazinone (BTZ), also known as 1,3-benzothiazin-4-one, was characterised as a novel type of medication having antimycobacterial action.[142] 2-[2-S-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl], the principal compound,6-(trifluoromethyl)-nitro-8It was discovered that -4H-1,3-benzothiazin-4-one (BTZ043) had anti *M. tuberculosis* action in vitro, ex vivo, and in vivo. It was also shown to be effective against MDR clinical isolates of *M. tuberculosis* and drug-susceptible isolates.[143] Through transcriptome research, BTZ043's method of action was first identified at the level of cell wall biosynthesis. The drug's target was found to be the gene rv3790, which, along with rv3791, encodes proteins that catalyse the epimerization of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA), a precursor for the synthesis of arabinan required for the bacterial cell wall.[144] This information was obtained through additional genetic analysis using in vitro generated mutants. These two important enzymes were suggested to be called DprE1 and DprE2.[142] A recent research has provided a more accurate description of BTZ043's mode

of action, demonstrating that the drug works by first reducing an important nitro group to a nitroso derivative, which then reacts with a cysteine residue in DprE1 to activate the drug in the bacterium.[145] An alternate mechanism of resistance has been proposed in investigations involving *M. smegmatis*. The medication was rendered inactive due to the reduction of an important nitro group to an amino group caused by the overexpression of the nitroreductase NfnB.[146] Even though *M. tuberculosis* doesn't seem to have any nitroreductases that can lower the medication, this discovery may be crucial for the creation of novel BTZ analogues with increased efficacy. There have been reports of BTZs containing piperazine quite lately. The main drug, PBTZ169, has demonstrated in vitro synergy with bedaquiline and has increased activity, tolerability, and effectiveness in animal models, making it an appealing novel option for additional clinical development.[147]

DISCUSSION AND CONCLUSION

Drug resistance in tuberculosis is still a man-made problem. It appears when *M. tuberculosis* undergoes spontaneous gene alterations that make the germs resistant to the majority of anti-TB medications. The foremost cause of this is indicated to be the non-compliance with the treatment regimens. In order to treat multidrug resistant tuberculosis (MDR-TB), the typical six-month regimen of four medications is prolonged to 18 - 24 months, during which time second-line treatments are used. This makes following the treatment plans extremely difficult, and the likelihood of non-adherence may be considerable. This might lead to unfavourable results and the spread of MDR strains. Even while medication resistance in *M. tuberculosis* is definitely linked to changes in a number of genes, resistant strains of the disease frequently lack any known alterations. For instance, a new study that used whole-genome sequencing found additional genes and intergenic areas linked to drug resistance and its development.[148] This suggests that drug resistance in tuberculosis is a more complicated issue than previously thought. Further elucidation is required on the correlation between certain gene mutations and the emergence of MDR-TB or XDR-TB, as well as the relationship between drug resistance and bacterial fitness. Further research is needed to understand the connection between efflux pump mechanisms and the emergence of clinical drug resistance, as well as the potential impact of porins on intrinsic resistance to certain antibiotics. Therefore, it is crucial that we learn more about the other pathways of drug resistance to the current anti-TB medications. This may significantly affect the dynamics of tuberculosis transmission as well as the search for and creation of novel anti-tuberculosis medications.

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