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Review Article

Drug-target genes and their spontaneous mutations associated with resistance to first-line, second-line, third-line, novel and repurposed anti-tuberculosis drugs in Mycobacterium tuberculosis resistant strains

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ABSTRACT

Drug-resistant tuberculosis is a threat to the control of tuberculosis globally, it develops mainly due to mutations in target genes of *Mycobacterium tuberculosis* (MTB). Mutations in the rpoB gene confer resistance to rifampicin (RIF). The most frequent mutations conferring resistance to RIF include; Ser531Leu, Asp516Val, and His526Asp. Isoniazid resistance (INHr) occur most frequently due to mutations in the *katG, inhA* and its promoter. Most frequent mutation in *katG* is Ser315Thr 1, while in inhA include; Thr8Cys, Ala16Gly, and Cys15Thr. Mutations in *embA, embB, embC, embR, ubiA, aftA*, and *iniA* genes confer resistance to ethambutol. 70% of mutations in the *embB* gene occur in codon 306, 406, or 497 and include; Met306Leu, Gly43Cys, and Ser412Pro. Mutations in the *pncA, panD, clpC1*, and *Rv2783c* genes mediate resistance to pyrazinamide. Frequent mutations in *pnc*A include; Tyr64Ser, Phe94Ala, and Trp68Gly. MTB resistance to streptomycin (STR) occur due to mutations in the *rrs*, *gid*B, and *rps*L genes. Mutations *rrs* (Ala80Pro), and *rps*L (Lys43Arg) confer resistance to STR. Fluoroquinolone resistance is mediated via mutations in the *gyr*A and *gyr*B genes. The most common mutations in the *gyr*A gene include; Gly88Cys, Ala90Val, and Ser91Pro. While those in the gyrB gene include; Glu540Val, and Asn538Asp. Mutations in the rrs and *eis* promoter region cause resistance to the kanamycin and amikacin. While mutations in the *rrs* and *tly*A cause resistance to capreomycin and viomycin. Common mutations in *rrs* include; Cys1402Thr, Ala1401Gly, and Gly1484Thr. While mutations in the *eis* include; Cys12Thr, Gly10Ala, and Gly37Thr. Detection of drug-target genes and their mutations has therapeutic and diagnostic value.

1. Introduction

Tuberculosis (TB) is a global public health emergency.[1,2] It causes high mortality and morbidity rates, it kills one person every 21 seconds.[3,4] TB is caused by species of the *Mycobacterium tuberculosis* complex (MTBC), some of which are adapted to humans (*Mycobacterium tuberculosis* [MTB] and *Mycobacterium africanum*), while others to animals (*Mycobacterium bovis*, *Mycobacterium*

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microti, Mycobacterium pinnipedii, Mycobacterium mungi, and *Mycobacterium caprae*), and others are smooth bacilli (*Mycobacterium canettii*).[5–7] MTB is the major causative pathogen for human TB among the species of MTBC, as it causes 97-99% of all TB cases globally.[3]

TB is the second leading cause of death from a single infectious agent, after coronavirus disease 2019 (COVID-19), a highly infectious disease caused by a virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[8,9] The current TB morality is severely impacted by COVID-19 and not HIV/AIDS. COVID-19 has reversed years of progress made in the fight against TB. Globally TB targets have gone off-track and many years of progress reversed.[9] The burden of TB is driven by the emergence and spreading of drug-resistant MTB strains.[2,4,10] TB-HIV co-infection has led to an increase in different types of drug-resistant TB.[11]

The latest World Health Organization Global TB Report of 2021 revealed that 132,222 cases of multidrug-resistant/rifampicin resistant tuberculosis (MDR/RR-TB) and 25,681 of pre-extensively drug-resistant/extensively drug-resistant tuberculosis (pre-XDR/XDR-TB) were detected in 2020.[9] The global prevalence of MDR/RR-TB stood at 3.5% and 18% among the new TB cases and previously treated cases respectively.[9] In 2019, 206,030 cases of MDR/RR-TB were detected and notified globally.[12] This meant the global prevalence of MDR/RR-TB was 3.3% among the new TB cases and 17.7% among the previously treated cases.[12,13] In 2018, 186,772 cases of MDR/RR-TB were detected and notified globally.[14] The global prevalence of MDR/RR-TB was 3.4% and 18% among the new TB cases and previously treated TB cases respectively.[14] In 2017, 160,684 cases of MDR/RR-TB were detected and notified globally.[15] The global prevalence of MDR/RR-TB was 3.5% among the new TB cases and 18% among the previously treated TB cases, in 2017.[15] An estimated 8.5% of MDR-TB cases were estimated to have XDR-TB in 2017. In 2016, 153,119 cases of MDR/RR-TB were detected and notified globally.[16] The global prevalence of MDR/RR-TB was 4.1% and 19% among the new TB cases and previously treated TB cases respectively, in 2016.[16]

Drug-resistant tuberculosis (DR-TB) is jeopardizing efforts in the control and prevention of TB.[2] DR-TB can be transmitted through the air from one person to another or can develop when MTB strains become resistant to anti-TB drugs due to a number of factors which include: poor adherence, poor compliance, poor selection of regimens, low efficacy anti-TB drugs, late diagnosis, interrupted supply, stock-outs, spontaneous mutations, and chromosomal replication errors.[3,10,17–21] Different categories of DR-TB have been defined and they include; multidrug-resistant tuberculosis (MDR-TB), pre-extensively drug-resistant tuberculosis (Pre-XDR-TB), extensively drug-resistant tuberculosis (XDR-TB), extremely drug-resistant tuberculosis (XXDR-TB), and totally drug-resistant tuberculosis (TDR-TB).[4,5,22]

MDR-TB is TB resistant to at least rifampicin (RIF) and isoniazid (INH).[23,24] Pre-XDR TB is TB resistant to RIF, INH, plus any one fluoroquinolone (FLQ) (levofloxacin, moxifloxacin, ofloxacin, or gatifloxacin), or any one of the second-line injectable drugs (SLIDs) (kanamycin, capreomycin, or amikacin).[3] XDR-TB is TB resistant to RIF, INH, plus any FLQs, plus any SLIDs.[10] XXDR-TB is a type of TB characterized by the presence of MTB isolates that show in-vitro resistance to all first- and second-line anti-TB drugs tested.[4,25,26] TDR-TB is TB characterized by MTB isolates that show resistance to all tested antibiotics plus some that are currently in the discovery pipeline.[4,27]

Mutations in target genes or their promoter regions of genes are associated with resistance to anti-TB drugs.[22] Resistance to first-line anti-TB drugs is mainly caused by mutations in the following genes: *rpo*B gene for resistance to rifampicin; *inh*A, *kas*A, *ahp*C, *kat*G and *ndh* genes for resistance to isoniazid; *emb*B gene for resistance to ethambutol; and finally, the *pnc*A, *pan*D, *rps*A, *clpC*1, and *Rv*2783*c* genes for resistance to pyrazinamide.[28,29] While resistance to second-line anti-TB drugs is mainly caused by mutations in the following genes: *rpsL, rrs* and *gid*B genes for resistance to streptomycin; *gyr*A and *gyr*B genes for resistance to the fluoroquinolones; *rrs, eis* and *tly*A genes for resistance to the aminoglycosides and cyclic polypeptide antibiotics.[29,30]

The aim of this comprehensive mini-review was to highlight molecular targets and their mutations associated with drug-resistance to first-line, second-line, novel and repurposed anti-tuberculosis drugs in resistant MTB clinical isolates. The identification of drug-target genes with their spontaneous mutations helps in designing new drugs or improving the efficacy of the available drugs as well as help in providing vital information for designing new molecular diagnostic assays for rapid detection of DR-TB.

2. Mechanisms for Drug-Resistance to First-Line Anti-Tuberculosis Drugs

First-line anti-TB drugs used are rifampicin, isoniazid, pyrazinamide and ethambutol.[31] MTB resistance to each one of the first-line anti-TB drugs has been detected in TB patients. MTB resistance is attributed to a number of factors some of which include: the mycolic acid, lipid layer of the cell wall, presence of β-lactamase enzymes, presence of efflux pumps, and the development of mutations in the target genes of MTB.[32] The mycolic acid, lipid layer of MTB makes the cell wall to become less permeable to a number of anti-TB drugs.[33] The efflux pumps play a role of pumping several antimicrobial agents out of the cells of MTB.[33,34] The β-lactamase enzyme of MTB inactivates the β-lactam antibiotics thus causes resistance to this class

of antibiotics.[33] The development of mutations in the target genes of MTB is a major mechanism through which resistance to anti-TB drugs occurs.[35,36]

3. Drug Target Genes and Mutations Conferring Resistance to Isoniazid

Isoniazid resistance is brought about by mutations in several genes of *M. tuberculosis* such as the *kat*G, *inh*A, *ahp*C, *kas*A, *oxy*R, furA, fabG1-inhA, and *ndh* genes.[3,34,37] However, Current research has shown that resistance to isoniazid can also be caused by an upregulation of efflux pumps or isoniazid inactivators.[38,39] Mutations in the *katG, inhA* and its promoter, and the *oxy*R-*ahp*C intergenic region frequently confer resistance to INH.[3,22,29] While mutations in the following genes *oxy*R, *fur*A, *ndh, ahp*C, *kas*A, and fabG1-inhA infrequently confer resistance to INH.[3,34] Recent studies have also found that mutations in the *dfr*A gene cause resistance to isoniazid.[29] Mutations in the *inh*A gene cause resistance to both isoniazid and ethionamide which share the same binding site on the promoter region.[40] The most frequently identified mutation in the *katG* gene is the Ser315Thr1, which confer a high-level resistance to INH.[3,23,29] While that in the inhA gene is the C-15T, which confer low-level resistance to INH.[3] The two mutations *katG* MUT (Ser315Thr1) and *inh*A MUT (C-15T) account for 80% of resistance to INH.[34,41] The four most frequently identified mutations in the *inh*A gene that are associated with resistance to INH are Cys15Thr, Thr8Cys, Thr8Ala, and Ala16Gly.[3,42] Isoniazid resistance that is associated with mutations in the *katG* gene occurs before rifampicin resistance.[29] Mutations in the *katG* gene can therefore, serve as a key marker for pre-MDR TB.[43]

4. Drug Target Genes and Mutations Conferring Resistance to Rifampicin

Mutations within a hypervariable region of the *rpoB* gene, which codes for the β-subunit RNA polymerase confer resistance to RIF in 95% of MTB clinical isolates.[22,44–47] About 96% of RIF- resistance occurs within the rifampicin resistance determining region (RRDR) which is also called the "hot-spot region" (HSR-rpoB), covering codons 507-533 of the *rpoB* gene.[22] Mutations at codons 531, 526, and 516 in the *rpoB* gene are the most commonly identified in RIF-resistant MTB isolates.[22] Mutations at codons 529, 526, 518, and 516 confer low-level resistance to RIF, whereas mutations at codons 526-531 show the highest frequency and are associated with a high-level resistance to RIF.[22,23] Mutations at codon 531 is associated with cross-resistance to rifabutin.[22] The most frequent mutations associated with resistance to RIF in the *rpoB* gene are Ser531Leu, His526Asp, His526Tyr, and Asp516Val.[23,42] These point mutations involve changes in the positions of amino acids, and can be an insertion, deletion, or missense.[45,48]

5. Drug Target Genes and Mutations Conferring Resistance to Ethambutol

Mutations in genes that confer resistance to ethambutol (EMB), occur in specific regions known as ethambutol resistance-determining regions (ERDR) or "hot-spot regions" (HSR).[49] EMB resistance in M. tuberculosis is caused by mutations in the following genes: *embA, embB, embC, embR, UbiA, aftA*, and *ini*A genes.[5,22,29,50] The most common mechanism for resistance to ethambutol is mutation in the *emb*B gene, occurring at codon 306.[29] Mutations at codon 406 and 497 within the *emb*B gene have also been detected.[51] For example the mutations Met306Leu and Met306Val at codon 306 in the *emb*B gene are associated with resistance to EMB.[22] Novel mutations *emb*B Gly43Cys, *emb*B Gly554Asn, and embB Ser412Pro in the *emb*B gene also confer resistance to EMB.[5] Mutations in the *ubi*A gene in-conjunction with mutations in the embB gene have been found to cause a high-level resistance to ethambutol.[22,52] Studies have also shown that the high-level resistance to ethambutol develop via a stepwise acquisition of mutations in the *emb*B, *ubi*A, and *emb*C genes.[53] From the total of 98% of mutations that occur in the *emb*B CAB locus of the *emb*B gene in resistant MTB isolates, 70% are found in codon 306, 406, or 497, while 13% of the mutations are found out side of the three regions between condons 296 and 426, and 15% are in the *emb*C-*emb*A intergenic region.[22] The *emb*CAB operon comprises of three genes *emb*A, *emb*B, and *emb*C. Therefore, mutations in this operon are associated with resistance to EMB.[51]

6. Drug Target Genes and Mutations Conferring Resistance to Pyrazinamide

Mutations in the *pnc*A, *pan*D, *rps*A, *clp*C1(Rv3596c), and Rv2783c genes of MTB confer resistance to pyranamide (PZA).[22,29,40,54] However, mutations in the *pnc*A and its promoter region are the most frequently identified as they account for 72-99% of resistance to PZA.[22,54] The most frequent mutations in the *pnc*A gene are: Asp49Asn, Tyr64Ser, Trp68Gly, and Phe94Ala.[54] Studies have also shown that PZA resistance is strongly associated with rifampicin resistance. This finding confirms that the burden of PZA resistance is in patients who have rifampicin resistance.[29]

7. Drug Target Genes and Mutations Conferring Resistance to Second-Line Anti-Tuberculosis Drugs

The second-line drugs are key in the management of drug-resistant TB, and they include: fluoroquinolones, aminoglycosides, streptomycin, cycloserine, ethionamide,

prothionamide, para-amino salicylic acid, and cyclic polypeptides.[29,37] Drug-resistance to all anti-TB drugs has been reported in some countries.[55] MTB resistance is mainly caused by mutations in the target genes.[56] Mutations in the *rpsL, rrs* and *gid* genes cause resistance to streptomycin, while mutations in the *gyr*A and *gyr*B genes cause resistance to the fluoroquinolones. Mutations in the *rrs, eis* and *tly*A genes cause resistance to the aminoglycosides and cyclic polypeptide antibiotics.[30]

8. Drug Target Genes and Mutations Conferring Resistance to Streptomycin

Streptomycin (STR) resistance by *M. tuberculosis* is caused by mutations in the *rpsL, rrs*, and *gid*B genes.[22,23,40] The following mutations confer resistance to STR, *rps*L (Lys43Arg, Lys88Gln, Lys88Arg, Cys117Thr), *rrs* (Cys517Thr, Ala514Cys, Ala906Gly, Ala907Cys), *gid*B (Ala183Val, Gly71Arg, Tyr22His, Gly37Arg, Pro75Ser, Gly76Asp, Ile81Thr, Phe100Leu, Val124Gly, Ala134Gly, Ala138Pro, Ser149Arg, Leu152Ser, and Gly157Arg).[57] Mutations in the *rps*L and *rrs* genes are the major mechanisms that confer resistance to STR in *M. tuberculosis*, they account for 60-70% of resistance to STR.[29] Recent studies have revealed that mutations in the *gid*B gene cause low-level resistance and accounts for 33% of resistance to STR in clinical *M. tuberculosis* isolates.[29,37,40] The most frequently identified mutation in the rpsL gene is the replacement of lysine with arginine at position 43 and 88.[22] While in the *rrs* gene is the mutation Ala80Pro.[22] MTB strains that are resistant to STR confer cross-resistance to amikacin and kanamycin.[22]

9. Drug Target Genes and Mutations Conferring Resistance to Aminoglycosides and Cyclic Poly-Peptide Antibiotics

Aminoglycosides and the cyclic polypeptide antibiotics are second-line drugs that are used in the treatment of drug-resistant TB.[29] The two key aminoglycosides are kanamycin (KAN) and amikacin (AMK), while capreomycin (CAP) and viomycin (VIO) are key cyclic polypeptide antibiotics.[37] The three drugs kanamycin, amikacin, and capreomycin are called second-line injectable drugs.[29]

Mutations in the *rrs, eis, tly*A genes result in resistance to the second-line injectable drugs[37] Mutations in the rrs gene cause resistance to all the three second-line injectable drugs, and is the most common molecular mechanism for resistance to this class of anti-TB drugs.[29] Mutations in the *rrs* gene, specifically at positions 1400, 1401, and 1483 base pair (bp) are associated with a high-level resistance to both AMK and KAN in KAN-resistant MTB strains.[22] The mutation Ala1401Gly in the *rrs* gene confer a high-level resistance to AMK and KAN along with cross-resistance to CAP. While the mutation Cys1402Thr or Gly1484Thr is associated with resistance to CAP and a cross-resistance to KAN or VIO.[22] Mutations in the *rrs* gene are also associated with resistance to CAP and VIO.[34] Mutations in the *eis* gene confer low-level resistance to KAN.[22] Mutations in the *rrs* gene accounts for about 70-80% resistance to CAP and AMK as well as 60% resistance to KAN in resistant *M. tuberculosis* isolates worldwide.[29] Mutations in the eis gene cause about 80% low-level resistance to KAN but not to AMK.[29] While mutations in the *tly*A gene cause about 3% resistance to CAP.[29,37]Cross-resistance to streptomycin and KAN occur due to mutations in the whiB7 gene of *M. tuberculosis*.[58] Mutations in the *tly*A gene also results in resistance in both CAP and VIO.[37]

10. Drug Target Genes and Mutations Conferring Resistance to Fluoroquinolones

Fluoroquinolones (FLQs) are second-line anti-TB drugs, examples of those used in the treatment of drug-resistant TB include; levofloxacin, ofloxacin, gatifloxacin and moxifloxacin.[59–61]

Mutations in the quinolone resistance determining region (QRDR) of both gyrA and gyrB genes of MTB cause resistance to FLQs.[3,22,29,34,40] Mutations in the *gyr*A gene cause a high-level resistance to FLQs, while mutations in the *gyr*B gene cause a low-level resistance to FLQs. However, combined mutations in both the *gyr*A and *gyr*B genes result in a high-level resistance to FLQs.[58] The most common mutations in the *gyr*A gene are: Gly88Cys, Gly88Ala, Ala90Val, Ser91Pro, Asp94Gly, Asp94Ala, Asp94His, Asp94Asn and Asp94Tyr. While those in the gyrB gene are: Glu540Val, and Asn538Asp.[3,22] Mutations in the gyrB gene are not frequently found among MTB clinical isolates.[22] Mutations in both *gyr*A and *gyr*B genes, such as Asn538Ile (gyrB)-Asp94Ala (*gyr*A) and Ala543Val (*gyr*B)-Asp94Asn/Asp94Gly(gyrA) cause very high-resistance to FLQs.[22,58] Cross-resistance among the FLQs occurs.[37] Resistance to ofloxacin causes resistance to other FLQs.[61] FLQ resistance is one of the most important criteria that is used for defining extensively drug-resistant TB.[62]

The second mechanism through which *M. tuberculosis* develops resistance to FLQs is the use of efflux pumps.[34,59] These biological pumps remove the fluoroquinolone drugs out of the mycobacterial cells.[59]

11. Drug Target Genes and Mutations Conferring Resistance to Cycloserine

D-cycloserine (DCS) is a second-line drug used in the treatment of MDR-TB and XDR-TB.[22,63]

Resistance to cycloserine by MTB is due to mutations in the *alr*, *ddl*A, ald (Rv2780) and *cyc*A genes.[22,64,65] Mutations in the alr gene are the main cause for resistance to cycloserine and involve three major mutations: alr $_{mtb}$

Y364D, alr $_{mtb}$ R373L,and alr $_{mtb}$ M319T.[63] The point mutation in the cycA gene of M. bovis confer resistance to DCS.[22] While the over expression of alrA cause resistance to DCS in recombinant mutant of M. smegmatis.[22]

12. Drug Target Genes and Mutations Conferring Resistance to Ethionamide

Ethionamide (ETH) is a second-line drug used in the treatment of MDR-TB.[66] Mutations in the *eth*R, *inh*A, *ndh*, *msh*A, and *eta*A/*eth*A genes of MTB result in resistance to ethionamide.[22,29,66] Mutations in the *inh*A gene result in co-resistance to ETH and INH, because the two drugs are structural analogues of each other, share the same target and mechanism of action.[29,66] Mutations in the *eth*A and *eth*R genes confer resistance to ETH and prothionamide.[22] Mutations in *eth*A/*eth*R genes, coupled with mutations in the *inh*A or its promoter region confer resistance to both ETH and INH.[22] The mutation C-15T confer a low-level resistance to INH and a cross-resistance to ETH.[67,68] The mutations Ala95Thr and Phe110Leu in the *eth*R gene confer resistance to ETH.[69] While the mutations Ile95Pro, Ser94Ala, and Ile21Thr in the *inh*A gene confer resistance to both INH and ETH in MTB resistant clinical isolates.[70]

13. Drug Target Genes and Mutations Conferring Resistance to Prothionamide

Prothionamide (PTH) is a second-line drug used in the treatment of drug-susceptible TB meningitis, miliary TB and MDR-TB.[71] PTH is a structural analogue of INH. The two drugs have a common target, which is *inh*A gene.[69] Mutations in the *ethA, ethR, mshA, ndh*, *katG, inhA* and/or its promoter region result in resistance to PTH.[69,71,72] PTH resistance is most commonly caused by mutations in the *eth*A gene of MTB.[71] Mutations in the inhA and ndh genes result in cross-resistance to PTH, ETH, and INH.[29,66,71,72] Mutations in both the *ethA* and *mshA* genes result in cross-resistance to PTH and ETH.[66,71,72] The mutations Val152Met and Arg216Cys in the ethR gene confer resistance to PTH.[69] Mutations in the *katG* and ethA confer resistance to both PTH and INH, for instance *katG* (Ser315Th, Ala264Val, Thr275Ala), and ethA (Cys137Arg, Ser266Arg, Pro334Ala).[69]

14. Drug Target Genes and Mutations Conferring Resistance to Para-Amino Salicylic Acid

Para-amino salicylic acid (PAS) is a structural analogue of para-amino benzoic acid (PABA), it is a second-line drug used in the treatment of MDR-TB.[29,73] PAS improves the cure rate and reduces the emergence of drug-resistant TB.[74] Mutations in the thyA, dfrA, folC, folP1, folP2, and ribD genes of *M. tuberculosis* confer resistance to PAS.[22,29,75,76] The following mutations in the *thy*A gene confer resistance to PAS Arg127Leu, Leu143Pro, Leu172Pro, Cys146Arg, Ala182Pro, and Val261Gly.[76] A study done at the Central Laboratory, Public Health Medical Centre, Chongqing, in South-western China found that resistance to PAS in MTB was mainly caused by mutations in the thyA, ribD and folC genes, with mutations in the folC gene being the most frequent.[73]

15. Drug Target Genes and Mutations Conferring Resistance to Novel and Repurposed Anti-Tuberculosis Drugs

The emergence of new resistant mechanisms by *M. tuberculosis* has led to the development of new anti-TB drugs by different pharmaceutical companies to treat drug-resistant TB cases.[77] Examples of new anti-TB drugs that have recently been developed include: bedaquiline (BDQ), linezolid (LZD), delamanid (DLM), pretomanid (PTM), and clofazimine (CFZ). However, drug-resistance has already been reported even to some of these newly developed drugs.[29]

16. Drug Target Genes and Mutations Conferring Resistance to Bedaquiline

Bedaquiline (BDQ) is used in combination with other anti-TB drugs for the treatment of MDR-TB.[78] Mutations in the *atp*E (Rv1305), *pep*Q (Rv2535c), and *mmp*R (Rv0678), Rv1979c genes of MTB confer resistance to BDQ.[29,79] Mutations in the *atp*E gene cause a high-level resistance to BDQ with the most frequently identified mutations being Ala63Pro and Ile66Met.[22,29,34,80] The other mutations occurring in *atp*E include Asp28Ala, Ala63Val, and Ile66Val.[81] Mutations in the Rv0678 gene include Gly66Glu, Met1Ala, Trp42Arg, Ser53Leu, Ser63Arg, and Ser63Gly.[82] Mutations in the Rv0678 gene cause an upregulation of MmpL5, a multi-substrate efflux pump, resulting to resistance not only to BDQ but also to clofazimine (CFZ).[22] Mutations in the Rv0678 gene cause a low-level cross resistance between BDQ and CFZ.[79] Similarly, mutations in the Rv2535c cause a low-level resistance to BDQ and CFZ.[79] Mutations in the pepQ gene confer cross-resistance between CLO and BDQ.[22]

17. Drug Target Genes and Mutations Conferring Resistance to Pretomanid and Delamanid

Mutations in the *ddn, fgd1, fbiA, fbiB, fbiC, fbiD,* and MmpS5-MmpL5genes genes result in resistance to pretomanid (PTM) or delamanid (DLM).[22,29,81,83,84] Mutations in the fbiA and fgd1 genes of *M. tuberculosis* result in resistance to DLM.[29] Mutations in the fgd1 occur between codons 43 and 230, for Pro43Arg example.[81] Cross-resistance between the drugs, PTM and DLM has been reported. This cross-resistance is inevitable

because the two drugs have a similar chemical structure.[80] The following mutations ddn (Asp113Asn, Arg72Trp, Gly34Arg, Gly81Ser, Pro131Leu, Pro45Leu, Met1Thr, Trp88Arg, Tyr65Ser, Leu49Pro, Leu107Pro, Gly81Asp, and Gly53Asp), fgd1 (Thr960Cys, Arg64Ser, Gln88Glu, Lys270Met, Lys296Glu, Lys296Arg, Arg247Trp, and Met93Ile), *fbi*A (Lys2Glu, Ile280Val, Ile209Val, Ser126Pro, Arg304Gln, Arg175His, Asp49Tyr, Gly139Arg, Ala178Thr, and Asp49Thr), *fbi*B (Lys448Arg, Ala31Thr, Asp90Asn, Arg265Gln, Val348Ile, Gly325Ser, Pro182Leu, Pro361Ala, and Leu326Phe), *fbi*C (Val318Ile, Cys105Arg, Leu228Phe, Leu377Pro, Ala856Pro, Ala835Val, Ser762Asn, Ala416Val, Trp678Gly, Thr273Ala, Trp678Gly, Ile128Val, Gly655Ser, and Thr455Ala) confer resistance to DLM or PTM.[81,84,85] The three non-synonymous single nucleotide polymorphisms (SNPs), Gly84Val, Ala175Thr, and Met221Arg in the *ndh* gene of *Mycobacterium smegmatis* confer resistance to DLM, but not in *M. tuberculosis.*[84]

18. Drug Target Genes and Mutations Conferring Resistance to Linezolid

Linezolid (LZD) is used in combination with other anti-TB drugs for the treatment of complicated cases of MDR-TB and XDR-TB.[86] Mutations in the *rrl* and *rpl*C genes of *M. tuberculosis* confer resistance to both LZD and sutezolid (SZD).[22,29,87] The following mutations *rrl* (Ala2810Cys, Gly2299Thr, Gly2814Thr, Gly2270Thr, Gly2270Cys, and Gly2746Ala), and *rpl*C (Cys154Arg, Thr460Cys, Ala328Gly, and Cys154Arg) confer resistance to LZD.[22,81,88,89] Mutations in the *rpl*C gene are associated with higher minimum inhibitory concentration (MIC) values to LZD, while those in *rrl* gene are associated with lower MIC values.[89] Mutations in the *rrl* gene cause a high-level resistance to LZD.[29,87]

19. Drug Target Genes and Mutations Conferring Resistance to Clofazimine

Clofazimine (CFZ) is used in combination with other anti-TB drugs for the treatment of drug-resistant TB.[90] Resistance to CFZ by *M. tuberculosis* is attributed to mutations in the Rv0678, Rv1979c, Rv2535c, *ndh* and *pep*Q genes.[29,88] The following mutations Rv0678 (Val85Phe, Arg31Ser, Gly65Ala, Ala86Val, Arg109Pro, Gln131His, Val20Phe, Cys46Tyr, Ala36Val, Thr33Asn, Leu43Arg, Gln51Arg, Ser68Gly, Ala59Val, Ser53Leu, Gly65Glu, Ser63Asn, Ala84Glu, Glu66Val, Arg90Pro, Arg89Leu, Ala102Val, Leu114Pro, Ala102Thr, Leu122Pro, and Val351Ala), pepQ (Leu145Ile), Rv1979c (Val351Ala), and Rv2535 (Glu89*- a stop codon mutation) confer resistance to CFZ.[56,91] The major mechanism for resistance to CFZ is due to mutations in the Rv0678 gene.[91] Cross-resistance between the drugs CFZ and bedaquiline has been reported and is actually due to mutations in the Rv0678 and *pep*Q genes as well as to an up regulation in the MmpL5 efflux pump in *M. tuberculosis*.[29,92]

20. Drug Target Genes and Mutations Conferring Resistance to Ethylenediamine

The drug 1,2-ethylenediamine (SQ-109) is derived from ethambutol pharmacophore. SQ-109 inhibits cell wall biosynthesis by blocking MmpL3 (Mycobacterial membrane proteins large 3).[93] It is effective against MTB clinical isolates that are resistant to ethambutol.[93] MmpL3 performs a vital role in cell wall biosynthesis in MTB, as it helps in transporting trehalose mono-mycolates (TMMs) across the cell envelop/inner membrane for subsequent incorporation into trehalose di-mycolates (TDMs) or arabinogalactan during cell wall biosynthesis in MTB.[82,94] Mutations in the MmpL3 gene of resistant MTB clinical isolates cause resistance SQ-109. While an up-regulation of the *ahp*C gene causes resistance to SQ-109, EMB, and INH.[22] Mutations in the MmpL3 gene include Val285Ala, Leu567Pro, Val646Met, Met649Thr, Ala700Thr, Leu567Pro, and Gln40Arg.[82,94]

21. Conclusion

Drug-resistant TB (MDR-TB, pre-XDR-TB, XDR-TB, XXDR-TB, and TDR-TB) is one of the major public health crises, causing high mortality rates globally, and is hampering efforts in the control of TB. DR-TB has worsened due to the COVID-19 pandemic, which has reversed years of progress made in the fight against TB. DR-TB is mainly caused by spontaneous mutations in genes that code for drug converting enzymes or drug-targets. Identification of drug-target genes with their spontaneous mutations helps in designing new drugs or improving the efficacy of the available drugs as well as help in providing vital information for designing new molecular diagnostic assays for rapid detection of DR-TB. Indeed, drug-target genes with their mutations offer therapeutic and diagnostic value.

22. Conflict of Interest

The authors declare no conflict of interest with regards to the publication of this research review article.

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