Chapter 4

Drug Resistance in *Mycobacterium Tuberculosis*

Microorganisms are one of the oldest inhabitants of the earth. Immense diversity, unique survival skills made them evolve into one of the most successful diverse and prolific living organisms. Simple yet comprehensive biochemical apparatus helped them survive in heterogeneous environments, from extreme arctic temperature to the depths of ocean and to the gut of an animal. During the course of evolution, every life form strengthened the molecular mechanisms to adapt towards external environments to survive during stressful situations like lack of oxygen, nutrients, light and presence of toxic chemicals and immune attack.

Most of the life-threatening microbial infections were rendered curable with the discoveryof antibiotics. Alongside, microbes developed resistance due to

- Single drug therapy
- Inadequate dose
- Discontinued treatment
- Ingestion of wrong antibiotic due to faulty diagnosis
- Inadvertent consumption of antibiotics via food and other means

The jubilations of discovering effective anti-TB drugs like streptomycin, p-aminosalicylic acid and isoniazid evaporated after the occurrence of antibiotic resistance in *Mycobacterium tuberculosis*, almost immediately after the entry of these wonder drugs into clinics. Later, WHO recommended usage of multidrug regimen, DOTS (Directly Observed Treatment-Short course) for better management of this dreadful disease by ensuring patient compliance. This initiative was strictly implemented in many parts of the world and curtailed resistance problem significantly. Eruption of multi drug resistant (MDR) strains during 1993-1995 restored the “global health problem” status to TB. As per latest

reports, *Mycobacterium tuberculosis*, the causal agent for tuberculosis, has claimed 1.5 million lives worldwide during 2013-14, among which 22% are non HIV patients. The incidence of MDR TB increased significantly from 3,10,000 cases in 2012-13 to an estimated 4,80,000 cases in 2013-14, among which 9% belong to Extremely Drug Resistant TB (XDR TB). It is also estimated that MDR TB accounts for 14% of total deaths due to TB.[1] Though effective medicines are available for treating drug-susceptible TB infection, the chances go from bleak to null as we move from MDR to XDR or Totally Drug Resistant TB (TDR TB). Drug resistance remains a major challenge to every section involved in the health care system and is the major driving force for novel antibiotic drug discovery.

- Development of resistance due to medication is generally considered as acquired resistance. It may be due to interruption of the therapy by the patient, prescription of inadequate chemotherapy, and poor drug supply.

- The innate resistance developed in patients without prior treatment with anti-tubercular drugs is called *primary resistance*. The occurrence of primary resistance is a consequence of the level of acquired resistance in the community. The rate of primary resistance is lower than the incidence of acquired one. This resistance is more often to one drug (streptomycin or isoniazid) than to two drugs.

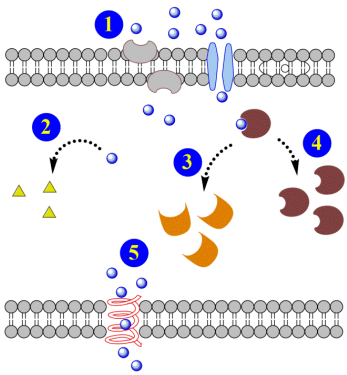
4.1 Major Mechanisms Involved in Thedevelopment of Drug Resistance in Microorganisms

Modulation of Membrane permeability–due to reduced uptake of the antimicrobial agent via modification of transporter proteins.

Degradation or Inactivation of Antibiotic–Expression of an enzyme that inactivates the antimicrobial agent by metabolic modification.

Modification of Target protein structure –

- mutation in the antimicrobial agent's target or post-transcriptional or post-translational modification which reduces the binding of the antimicrobial agent.
- overproduction of the antimicrobial agent'starget.
- expression or suppression of a gene *in vivo* in comparison to the situation *in vitro.*
- presence of an alternative enzyme instead of an enzyme that is inhibited bythe antimicrobial agent.



1. Modification of cell wall and reduced drug intake
2. Metabolic conversion of drug into inactive metabolites
3. Modification of the target protein or active site
4. Over expression of target protein
5. Drug efflux pumps

Fig. 4.1 *Drug resistance mechanisms in Mycobacterium tuberculosis.*

Increased drug clearance via Efflux pumps. These efflux pumps include the pumps of Major Facilitator Superfamily (MFS) family (lfrA, Rv1634 and Rv1258c) and ATP Binding Cassette (ABC) transporters (DrrAB, PstB and Rv2686c-2687c-2688c).[2]

4.1.1 Drug - Resistant Tuberculosis

One among three individuals in the world is infected with dormant TB germs. Only when the bacteria become active then only people become ill with TB. Bacteria become active as a consequence of anything that can decrease the person's immunity, like HIV, advancing age or some medical conditions. TB can usually be treated with a course of four first-line anti-TB drugs. In drug resistant TB, the bacteria are resistant to one or more anti-TB drugs. Essentially, drug-resistance arises in areas with poor TB control programmes.

4.1.2 Multi-Drug Resistant Tuberculosis (MDR-TB)

In MDR-TB, bacteria are resistant to several anti-TB drugs and at least to INH and RIF. It is usually found in patients after failed treatment regimens and represents a significant proportion of tuberculosis patients with acquired resistance. Only exceptionally it is observed in new cases. *Top priority is not the management but the prevention of MDR-TB*. The emergence of MDR-TB has made the scientific community throughout the world to focus on the urgent need for new anti-TB drugs. Resistance has been developed against almost every front-line drug.[3-7]

WHO recommends treatment with at least four drugs in order to reduce resistance burden further. However, unpleasant side effects and relatively long course of treatment remained major road-blocks for its success. The second line drugs used for MDR-TB are more expensive, less effective and more toxic than drugs used in the four standard regimens. It is very important to discover affordable, safer and potent bactericidal anti-TBdrugs to treat MDR-TB and latent infectionsin short treatment period with reduced frequency of doses. It is known that MDR-TB strains are sensitive to other antibiotics like

fluoroquinolones, which inhibit the topoisomerases II and IV as well as DNA gyrases, the essential enzymes to maintain the supercoils in bacterial DNA.[8] Consequently a huge effort has been made by scientists in order to discover new quinolone derivatives endowed with anti-TB activity.[9-12]

4.1.3 Extensive-Drug Resistant Tuberculosis (XDR-TB)

XDR-TB or Extensive Drug Resistant TB (also referred to as Extreme Drug Resistance) is MDR-TB that is resistant to isoniazid, rifampicin, any fluoroquinolone and at least one of the three injectable 2nd line anti-TB drugs (capreomycin, amikacin and kanamycin). XDR-TB can develop when these second-line drugs are also misused or mismanaged and therefore also become ineffective. Because XDR-TB is resistant to first and second-line drugs, treatment options are seriously limited and complicated. The emergence of XDR-TB shows that the development of novel mechanism-based anti-TB agents is necessary.[13]

4.1.4 Basic Concepts in the Development of Drug-Resistant TB

Drug-resistant TB is not a recent phenomenon. *M. tuberculosis* strains that were resistant to streptomycin (SM) appeared soon after the introduction of drug for TB treatment in 1944. Genetic resistance to an anti-TB drug due to spontaneous chromosomal mutations appears at a frequency of 10^{-6} to 10^{-8} mycobacterial replications. The probability of developing bacillary resistance to three drugs used simultaneously becomes 10^{-18} to 10^{-20}. In theory, the chance of drug resistance is thus virtually non-existent when three effective drugs are used in combination for TB treatment. Interestingly, plasmids and transposons mediated resistance is absent in *M. tuberculosis.* Because such mutations resulting in drug resistance are unlinked. Hence development of drug resistance

is largely due to human error including poor patient compliance, ‘monotherapy’ due to irregular drug supply and inappropriate doctor prescription.[14]

Subsequent transmission of resistant *M. tuberculosis* strains from the carrier to others further aggravates the problem. The MDR/XDR phenotype is caused by sequential accumulation of mutations in different genes involved in individual drug resistance. Although the definitions of ‘acquired’ and ‘primary’ drug resistance are conceptually relatively clear, in reality they are often subject to misclassification when previous treatment cannot be readily ascertained. The term ‘initial’ drug resistance is thus often preferred to ‘primary’ drug resistance to include ‘unknown’ or ‘undisclosed’ acquired drug resistance. The matter is currently further simplified by categorizing drug resistance in new cases and previously treated cases of TB.[15] The latter refers to cases with treatment lasting for at least one month.

4.2 Molecular Basis of Drug Action and Resistance

A great deal of progress has been made in our understanding of the molecular basis of drug action and resistance in *M. tuberculosis.* An update on this topic is provided below.

4.2.1 Isoniazid (INH)

INH is the most widely used first-line anti-TB drug. Since its discovery in 1952, INH has been the cornerstone of all effective regimens for treatment of TB, including the latent form. *M. tuberculosis* is highly susceptible to INH (MIC 0.02–0.2 μg/mL) but is virtually not active against non-replicating bacilli or under anaerobic conditions. INH is a prodrug that is activated by the catalase peroxidase enzyme (*Kat*G) encoded by the *kat*G gene [16] to generate a range of

highly reactive species which then attack multiple targets in *M. tuberculosis.*[17] The reactive species generated by *Kat*G-mediated INH activation include both reactive oxygen species such as superoxide, peroxide and hydroxyl radical,[18] nitric oxide [19] and reactive organic species such as isonicotinic-acyl radical or anion [21, 22] and certain electrophilic species.[23] InhA enzyme (enoyl-acyl carrier protein reductase) which is involved in the elongation of fatty acids in mycolic acid synthesis is one of the prime targets of INH.[23] The active species (isonicotinic-acyl radical or anion) derived from KatG-mediated INH activation reacts with NAD(H) (nicotinamide adenine dinucleotide) to form an INH-NAD adduct, and then attacks InhA.[20, 21] A recent study showed that INH-NAD(P) adducts react with other protein targets besides InhA, such as *Dfr*A (an NADPH-dependent dihydrofolatereductase involved in DNA synthesis).[24] Resistance to INH occurs more frequently than for most anti-TB drugs, at a frequency of 1 in 105 bacilli *in vitro*.[25] It is also found that catalase and peroxidase enzymes were absent in the INH-resistant clinical isolates of *M. tuberculosis* [26] encoded by *kat*G, especially in high level resistant strains (MIC > 5 μg/mL).[32] Low level resistant strains (MIC < 1 μg/mL) often still possess catalase activity.[25] Mutation in *kat*G is the main mechanism of INH resistance and KatG S315T mutation is the most common mutation in INH resistant strains, accounting for 50–95% of INH-resistant clinical isolates.[16, 17, 27] Over expression of InhA via mutations in the promoter region of *mab*A/*inh*A operon, or lowering the InhA affinity by mutations at the InhA active site, are also observed in resistant strains.[20, 23] Mutations in *inh*A or its promoter region are usually associated with low-level resistance (MICs = 0.2−1 μg/mL) and are less frequent than *kat*G mutations.[17, 27] Additional mutations in the *katG* conferred higher levels of INH resistance.[28] Mutations in *inh*A was also linked to cross-resistance to the structurally related drug, ethionamide (ETH).[23] About 10–25% of low-level INH-resistant strains does not have mutations in *kat*G or

*inh*A[27], and may be due to new mechanism(s) of resistance. Recently, mutations in another important enzyme *msh*A, encoding an enzyme involved in mycothiol biosynthesis, have been shown to confer INH and ETH resistance in *M. tuberculosis* strains *in vitro*,[29] but its role in clinical resistance remains to be demonstrated.

4.2.2 Rifampicin (RMP)

RMP is an important first-line drug for the treatment of TB. RMP is bactericidal for *M. tuberculosis*, with MICs ranging from 0.05 to 1 μg/mL on solid or liquid media, but the MIC is higher in egg media (MIC = 2.5–10 μg/mL). Strains with MICs < 1 μg/mL in liquid or agar medium or MICs < 40 μg/mL in Löwenstein- Jensen (LJ) medium are considered RMP-susceptible. RMP is active against both growing and latent phase bacilli. The latter activity is related to its high sterilizing activity *in vivo*, correlating with its ability to shorten the 12–18 months TB treatment to 9 months.[30] RMP interferes with RNA synthesis by binding to the β subunit of the RNA polymerase. The RMP-binding site is located upstream of the catalytic centre and physically blocks the elongation of the RNA chain. In *M. tuberculosis*, resistance to RMP occurs at a frequency of 10^{-7} to 10^{-8}. As in other bacteria, mutations in a defined region of the 81 base pair region of the *rpo*B are found in about 96% of RMP-resistant *M. tuberculosis* isolates.[31] RMP-resistant strains are often found to carry mutations at positions 531, 526 and 516. Mutations in *rpo*B generally result in highlevel resistance (MIC > 32 μg/mL) and cross-resistance to all rifamycins. However, specific mutations in codons 511, 516, 518 and 522 are associated with lower level resistance to RMP and rifapentine, but retain susceptibility to rifabutin and rifalazil.[32, 33] The circumstances under which the RMP-dependent strains arise remain unclear, but they often occur as MDR-TB and seem to develop upon repeated treatment with rifamycins in patients with repetitive treatments.

4.2.3 Pyrazinamide (PZA)

PZA is an important first-line drug used along with INH and RMP. PZA plays a unique role in shortening the previous 9–12 months TB treatment to 6 months because it kills a population of persistent bacilli in acidic pH environment in the lesions that are not killed by other drugs.[30] PZA is an unconventional and paradoxical anti-TB drug that has high sterilizing activity *in vivo* [34] but no activity against tubercle bacilli at normal culture conditions near neutral pH.[35] PZA is only active against *M. tuberculosis* at acid pH (e.g., 5.5).[36] Even at acid pH (5.5), PZA activity is rather reduced, with MICs in the range of 6.25–50 μg/mL. PZA activity is enhanced under low oxygen or anaerobic conditions [37] and by agents that compromise the membrane energy status, such as weak acids [38] and energy inhibitors such as DCC (dicyclohexylcarbodiimide), azide and rotenone.[39] PZA is a prodrug that requires conversion to its active form pyrazinoic acid (POA) by the pyrazinamidase/ nicotinamidase enzyme encoded by the *pnc*A gene of *M. tuberculosis*.[40] The POA produced intracellularly, reaches the cell surface through passive diffusion and a defective efflux.[41] The extracellular acid pH facilitates the formation of uncharged protonated POA, which then permeates through the membrane and causes accumulation of POA and disrupts membrane potential in *M. tuberculosis*.[39] The protonated POA brings protons into the cell and could eventually cause cytoplasmic acidification and de-energize the membrane by collapsing the proton motive force, which affects membrane transport. The target of PZA is related to membrane energy metabolism although the specific target remains to be identified. Fas-I was proposed as a target for PZA [42] but its validity is questioned.[43] PZA-resistant *M. tuberculosis* strains lose pyrazinamidase/ nicotinamidase activity.[44] Using a cloned *M. tuberculosis pnc*A, scientists have shown that defective pyrazinamidase activity due to *pnc*A mutations is the major cause of PZA resistance. Most PZA-resistant *M. tuberculosis* strains (72–97%) have mutations

in *pnc*A,[45-52] however, some resistant strains do not have *pnc*A mutations. The lower percentage of PZA-resistant strains with *pnc A*mutations (e.g., 72%) [47] reported in some studies could be caused by false resistance due to well-known problems with PZA susceptibility. PZA is active only against *M. tuberculosis* complex organisms (*M. tuberculosis*, *M. bovis* from *M. microti*), but not *M. bovis*, due to a characteristic mutation in its *pnc*A gene [40]. Strains of *M. bovis*, including BCG, are naturally resistant to PZA and lack pyrazinamidase; these features are commonly used to distinguish *M. Bovis* from *M. tuberculosis.* A single point mutation of 'C' to 'G' at nucleotide position 169 of the *pnc*A gene compared with the *M. tuberculosis pnc*A sequence, causing amino acid substitution at position 57 of the *pnc*A sequence is said to be responsible for the natural resistance to PZA in *M. bovis*. However, the correlation between pyrazinamidase activity and PZA susceptibility is not true for other naturally PZA-resistant mycobacterial species whose intrinsic PZA resistance is most likely due to their highly active POA efflux mechanism.[41]

4.2.4 Ethambutol (EMB)

EMB is an indispensable ingredient in all anti TB regimen containing other first line drugs INH, RMP and PZA. It is a bacteriostatic (MIC 0.5–2 μg/mL) and shows its activity chiefly on replicating bacilli by interfering with the biosynthesis of cell wall arabinogalactan.[53] It inhibits the polymerization of cell-wall arabinan of arabinogalactan and of lipoarabinomannan. Further, it induces the accumulation of D-arabinofuranosyl-P-decaprenol, an intermediate in arabinan biosynthesis.[53, 54] Arabinosyl-transferase (embB), a critical enzyme involved in the arabinogalactan synthesis has been proposed as the target of EMB in *M. tuberculosis* and *M. avium.* Strains resistant to EMB have MICs > 7.5 μg/mL. Mutation to EMB resistance occurs at a frequency of 10^{-5}. Resistance to EMB arise mostly due to mutations in the *emb*B geneand

occasionally *emb*C.[55] It was found that mutations leading to certain amino acid changes are indeed causing EMB resistance while other amino acid substitutions have little effect on EMB resistance.[56] However, about 35% of EMB-resistant strains (MIC < 10 μg/mL) do not have *emb*B mutations,[57] suggesting that there may be other mechanisms of EMB resistance. Further studies are needed to identify potential new mechanisms of EMB resistance.

4.2.5 Aminoglycosides (Streptomycin (SM)/Kanamycin (KM)/ Amikacin (AMK)/Capreomycin CPM)

SM is an aminoglycoside antibiotic that is active against a variety of bacterial species, including *M. tuberculosis.* SM kills actively growing tubercle bacilli with MICs of 2–4 μg/mL, inactive against non-growing or intracellular bacilli.[30] SM inhibits protein synthesis by binding to the 30S subunit of bacterial ribosome, causing misreading of the mRNA message during translation.[58] The site of action of SM is the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA. Resistance to SM is caused by mutations in the S12 protein encoded by *rps*L gene and 16S rRNA encoded by *rrs* gene.[59] Mutations in *rps*L and *rrs* are the major mechanisms of SM resistance, accounting for respectively about 50% and 20% of SM-resistant strains.[59-61] The most common mutation in *rps*L is a substitution in codon 43 from lysine to arginine, causing high-level resistance to SM. Mutation in codon 88 is also common. However, about 20–30% of SM-resistant strains with a low level of resistance (MIC < 32 μg/mL) do not have mutations in *rps*L or *rrs*,[62] which indicates other mechanism(s) of resistance. Recently, low-level SM resistance in 33% of resistant *M. tuberculosis* isolates [63] were found to carry a mutation in *gid*B, encoding a conserved 7-methylguanosine- methyltransferase specific for 16S rRNA. In addition, some low-level SM resistance seems to be caused by increased efflux.[64] KM and its derivative AMK are also inhibitors of protein synthesis through modification of

ribosomal structures at the 16S rRNA. Mutations at 16S rRNA (*rrs*) position 1400 are associated with high-level resistance to KM and AMK.[65, 66] CPM is a polypeptide antibiotic. A gene called *tly*A encoding rRNA methyltransferase was shown to be involved in resistance to CPM.[67] The rRNA methyltransferase modifies nucleotide C1409 in helix 44 of 16S rRNA and nucleotide C1920 in helix 69 of 23S rRNA.[68] SM resistant strains are usually still susceptible to KM and AMK.

4.2.6 Fluoroquinolones (FQ)

DNA topoisomerases are a diverse set of essential enzymes responsible for maintaining chromosomes in an appropriate topological state. In the cell, topoisomerases regulate DNA supercoiling and unlink tangled nucleic acid strands to meet replicative and transcriptional needs.[69] In most bacterial species, FQs inhibit DNA gyrase (topoisomerase II) and topoisomerase IV, resulting in microbial death. DNA gyrase is a tetrameric A2B2 protein. Between the two subunits, A subunit carries the breakage-reunion active site, but the B subunit promotes adenosine triphosphate hydrolysis. *M. tuberculosis* has *gyr*A and *gyr*B correspondingly encoding the A and B subunits.[70] A conserved region, the quinolone-resistance-determining region (QRDR) of *gyr*A (320 bp) and *gyr*B (375 bp), has been found to be the most important area involved in the exhibition of FQ resistance in *M. tuberculosis*.[76] Mutations within the QRDR of *gyr*A have been found in clinical and laboratory-selected isolates of *M. tuberculosis*, basically clustered at codons [56, 68-74] with Asp94 as the pretty common one.[72, 75] For clinical isolates, *gyr*B mutations appear to be of much rarer occurrence.[73, 74, 77] Generally, two mutations in *gyr*A or concomitant mutations in *gyr*A plus *gyr*B are required for the development of higher levels of resistance.[70, 78] Recently, a new mechanism of quinolone resistance mediated by MfpA was identified.[79] MfpA is a member of the penta peptide repeat family of proteins from *M. tuberculosis*,

whose expression causes resistance to FQ drugs. MfpA binds to DNA gyrase and inhibits its activity in the form of a DNA mimicry, which explains its inhibitory effect on DNA gyrase and quinolone resistance.[79] The *M. tuberculosis Rv2686c-Rv2687c-Rv2688c* operon, encoding an ATP-binding cassette transporter, has been shown to confer resistance to ciprofloxacin and to a lesser extent norfloxacin, moxifloxacin and sparfloxacin in *M. smegmatis*.[80] The resistance level was found to decrease in the presence of efflux pump inhibitors such as reserpine and verapamil. However, it remains to be determined if clinical strains elaborate MfpA or the *Rv2686c-Rv2687c-Rv2688c* operon to develop clinical resistance to quinolones. Furthermore, it has been suggested that, regarding *M. tuberculosis* resistance to FQs, the underlying genetic mutations can show substantial disparity among different geographic regions.[74]

4.2.7 Ethionamide (ETH)/Prothionamide (PTH) and Thioamides

ETH (2-ethylisonicotinamide) is a derivative of isonicotinic acid and is bactericidal only against *M. tuberculosis, M. avium-intracellulare* and *M. leprae.* Like INH, ETH is also a prodrug that is activated by EtaA/EthA (a monooxygenase) [81, 82] and inhibits the similar target as INH, the InhA of the mycolic acid synthesis pathway. Prothionamide (PTH, 2-ethyl-4-pyridine-carbothioamide) shares structure and activity almost identical to that of ETH. EtaA or EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidizes ETH to the corresponding S-oxide, which is further oxidized to 2-ethyl-4-amidopyridine, presumably via the unstable oxidized sulfinicacid intermediate. EtaA also activates thiacetazone, thiocarlide, thiobenzamide and perhaps other thioamide drugs,[83] which explains the cross-resistance between ETH and thiacetazone, thiocarlide and other thioamides and thioureas.[84] Mutations in the drug-activating enzyme EtaA/EthA [81, 82] cause resistance to ETH and other thioamides. In addition, mutations in the target InhA confer

resistance to both ETH and INH.

4.2.8 Oxazolidinones

Oxazolidinones are a very important group of synthetic antibacterial agents. Linezolid, an approved drug in this category elicits bactericidal activity by binding to the ribosomal 50S subunit and blocking an early step in the protein synthesis.[85] In view of its potential anti-TB activity,[86] a new oxazolidinone PNU100480 was developed, which showed good anti-TB activity and better pharmacokinetic profile than linezolid in a murine model.[87] Further, this compound was also found to be active against drug-resistant *M. tuberculosis* isolates. When compared to other anti-TB drugs, resistance to linezolid in *M. tuberculosisis* relatively rare (1.9% among 210 MDR strains). However, in the linezolid resistant strains mutations were largely observed G2061T and G2576T mutations inthe 23S rRNA gene.[88] In another recent study mutations were also observed at T460C in rplC, encoding the 50S ribosomal L3 protein.[89] It is also possible that involvement of efflux pumps or other non-ribosomal alterations may also play important role in linezolid resistance in *M. smegmatis*.[90]

4.2.9 Cycloserine

D-cycloserine is one of the oldest anti-TB drug that inhibits the synthesis of peptidoglycan by competing with D-alanine and blocking the action of D-alanine: D-alanine ligase (Ddl). This drug was also found to inhibit alanine racemase (AlrA) which converts L-alanine to D-alanine.[91] Resistance to this drug is mainly due to reduced drug permeation and over production of AlrAenzyme.[92]

4.3 New Drugs, New Targets and New Resistance Mechanisms

Quite a lot of new drugs are being projected as candidates for the treatment of TB. They exert anti TB activity by interacting with diverse targets, which are in many cases different from the classical targets of other anti-TB drugs. Surprisingly, new mechanisms of resistance have already been identified, even before these drugs have been put into clinical use.

4.3.1 Nitroimidazoles

Bicyclic nitroimidazole derivatives, PA-824 and OPC-67683 are very important candidates for treating MDR TB.[93] They are highly potent, relatively safe and possess novel mechanism of action. Nitroimidazole derivatives are prodrugs and are activated via, deazaflavin (cofactor F420) dependent nitroreducase (Ddn) mediated bioreduction. In this the aromatic nitro group is reduced to a reactive nitro radical anion intermediate within the cell.[94] This process alsoreleases NO gas inside the bacteria, causing severe damage to its respiratory apparatus.[95] The reduction preferably takes place in anaerobic environment; hence well oxygenated host cells are spared. Resistance to bicyclic nitroimidazole compounds was found to be associated most commonly with the lack of drug specific nitroreductase enzymes or its deazaflavin cofactor. More recently, a nitroimidazo-oxazine specific protein causing minor structural changes in the drug has also been identified. The resistant strains most commonly showed mutations in the gene Rv3547, a protein with high structural specificity for these drugs.[96] The total frequency of resistance by any mechanism to PA-824 was determined by fluctuation analysis in MTB strain H37Rv to be 9.0×10^{-7}, slightly less than that of 1.3×10^{-6} for INH.

4.3.2 SQ109

SQ109 is a highly potent analogue of Ethambutol, which acts synergistically with every first-line anti-TB agent including EMB. Even though the mode of action of SQ109 is not very much known, but it is understood that it affects mycobacterial cell wall synthesis in a manner unlike to that exercised by ethambutol.[97]

SQ109 inhibits biosynthesis of trehalosedimycolate (TDM) and other cell wall mycolates (methoxy, keto and alpha) chiefly by blocking transport of trehalosemonomycolate (TMM) across cell membrane via inhibiting MmpL3 transporter (Rv0206c protein).[98] Interestingly, development of resistance to SQ109 appears to be feeble as it targets a protein critical for survival of the microbe. All the resistant strains observed *in vitro*, has shown mutations in the Mmpl3 gene. In strains resistant to isoniazid, ethambutol and SQ109, it was established that there is an up-regulation of *ahp*C; which signifiesits possible role in the development of resistance to this drug.[99] Induction of the efflux pump viatranscription of the iniBAC operon required was also indicated in the SQ-109 resistance in MTB.[100]

4.3.3 Bedaquiline (TMC207, R207910, Sirturo®)

Bedaquilineis a diarylquinoline antibiotic with excellent bactericidal activity against *M. tuberculosis*. In combination with other anti-TB drugs it achieved significant sputum conversion rates in MDRTB. Several studies confirmed that bedaquiline selectively inhibits mycobacterial ATP synthase. The *in vitro* generated resistant species showed A63P and I66M mutations in the *atp*E gene which encodes Cpart in the F0 subunit of the ATP synthase.[101] The other study reveals that six distinct mutations, Asp28 → Gly, Asp28 →Ala, Leu59 →Val,

Glu61 →Asp, Ala63 →Pro, and Ile66 →Met, have been identified in the subunit forming a C ring in the ATP synthase.[102] It was also found that *atp*E gene is highly conserved in *Mycobacterium* species. One exception is *M. xenopi*, in which residue Ala63 in the *atp*E protein is replaced by Met, rendering it naturally resistant to bedaquiline. The reasons underlying exceptional specificity of this drug to mycobacterial *atp*E proteins are yet to come to light. In another recent study it was found that 55% (32 out of 58) of the *in vitro* generated resistant species showed no mutation in *atp*E gene hinting at alternate mechanism for development of resistance or even its bactericidal action.[103]

4.3.4 Benzothiazinones

Benzothiazinonesare a relatively new class of anti-TB antibiotics with excellent bactericidal activity against Mtb clinical isolates (MIC 0.75–30 ng/mL). Benzothiazinone irreversibly inhibits DprE1 subunit of the enzyme and thus inhibits epimerization of decaprenyl-phosphoryl-β-O-ribosetodecaprenyl - phosphorylarabinose, a chief component in mycobacterial cell wall assemblage. Resistance to benzothiazinones is mainly due to mutation of the dpeE1 gene, in which Cys387 codon was replaced by Ser or Gly.[104] *M. avium*, which is naturally resistant to benzothiazinones had the codon Cys387 replaced by an Ala. *M. smegmatis* is less susceptible to benzothiazinones (MIC 4ng/mL) and showed overexpression of nitroreductaseNfnB, which inactivates the critically needed nitro group to an amino group.[105] *M. tuberculosis*, however, seems to lack nitroreductases and unable to inactivate these drugs.

4.4 Conclusions

Drug resistance in Mtb is a major hurdle for the effective disease management and chemotherapy. It increases both financial and pill burden on the patient. In

MDR or XDR cases the treatment options are severely limited, available drugs are more toxic and thetreatment period often goes beyond 18 months. Successful implementation of DOTS and improving patient compliance significantly reduced the resistance problem in many parts of the world. Strict policies and legislations are to be made and implemented to avoid accidental or unnecessary exposure to antibiotics. Though drug resistant Mtb strains often carry a prominent and functional mutated gene, there are many cases of resistant strains with no trace of these predictable mutations. This intriguing complexity in the molecular mechanisms of drug resistance needed inquiry to further for knowledge which can be of immense help during development of newer drugs or biologicals.

Unlike in many diseases, TB diagnosis is a challenging task. Detection of MDR strain is even harder. Classical drug susceptibility tests take more than three weeks' time and it is probably more important to have diagnostic tools that are easy to use, inexpensive and provide rapid results of drug susceptibility or resistance of a strain.

Microbes seem to develop resistance to virtually any antibiotic. It would be wise to look for drugs to reverse drug resistance. Recently verapamil, a calcium channel blocker, increased drug susceptibility of a MDR TB strain.[106] Piperine, a natural product also showed similar activity in MRSA. Blocking p-glycoprotein mediated efflux pump was suggested as the mechanism for this activity.[107] Thioridazine, an antipsychotic drug was recently found to have excellent anti-TB activity. Activating pulmonary macrophages is one of the mechanisms ascribed to thioridazine's anti-TB activity.[108] So far no resistance was reported for thioridazine. With more and more new drugs filling the clinical trials pipeline, future appears more hopeful now than at any other time in the recent past.

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