Antibiotic Resistance in Mycobacterium Tuberculosis and Non-Tuberculous Mycobacteria

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ABSTRACT

Mycobacterium tuberculosis (MTB) and non-tuberculous mycobacteria (NTM) antibiotic resistance presents an important challenge to the treatment of mycobacterial infections. The therapeutic approaches are complicated by the resistance of both MTB and NTM to a variety of antibiotics. Resistance to first-line drugs such as isoniazid, rifampicin, ethambutol, and streptomycin has been consistently increasing in MTB, underscoring the necessity of effective treatment strategies. Conversely, the necessity of species-specific treatment regimens is underscored by the high resistance rates of NTM species, such as Mycobacterium avium complex, M. kansasii, and M. abscessus complex, to commonly used anti-tuberculosis pharmaceuticals. A combination of intrinsic and acquired factors are involved in the mechanisms of antibiotic resistance in these mycobacteria. Features such as biofilm formation, thick cell walls, and reduced drug uptake are responsible for intrinsic resistance in NTM, whereas acquired resistance can develop as a result of protracted antibiotic exposure. Understanding these resistance mechanisms is essential for the development of new therapies and the prevention of the increasing prevalence of drug resistance in mycobacterial infections. The significance of continuous surveillance, species-specific treatment protocols, and the development of novel antimicrobial agents to effectively manage mycobacterial diseases is emphasized by the prevalence of antibiotic resistance in MTB and NTM. This review article focuses on the molecular mechanisms that have resulted in the development of resistance in both MTB and NTMs, as well as the extent to which various classes of antimycobacterial drugs act.

Keywords: Antibiotic Resistance, Mycobacterium Tuberculosis, Mycobacterial Infections

I. INTRODUCTION

Antibiotic resistance has emerged as a critical global challenge in the management of infectious diseases, impacting both bacterial and non-bacterial pathogens [1]. Among these, *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB), and various non-tuberculous mycobacteria (NTMs) species have garnered increasing attention due to their ability to develop resistance to multiple antibiotics [2,3]. The growing threat of antibiotic resistance in MTB and NTM has raised concerns about the efficacy of current treatment regimens, patient outcomes, and the broader public health implications.

Tuberculosis remains a leading cause of morbidity and mortality worldwide, despite extensive efforts to control its spread (4). The emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) has severely compromised treatment options, leading to prolonged and less effective therapeutic courses (5). These resistant forms of MTB are often associated with treatment failures, increased healthcare costs, and higher mortality rates. The alarming progression from drug-susceptible to drug-resistant TB underscores the need for vigilant surveillance, prompt diagnosis, and innovative treatment strategies to curb the spread of resistance.

Non-tuberculous mycobacteria, on the other hand, represent a diverse group of mycobacterial species that are ubiquitous in the environment[6]. Although they are generally considered opportunistic pathogens, NTM infections have been on the rise, particularly among immunocompromised individuals. Antibiotic resistance in NTM is a complex phenomenon influenced by factors such as exposure to antibiotics, underlying diseases, and the specific species involved [3,7]. The emergence of resistance in NTM poses a significant challenge to clinicians, as these infections are notoriously difficult to treat and can lead to chronic and debilitating conditions.

The mechanisms underlying antibiotic resistance in both MTB and NTM are multifaceted [8]. These include the acquisition of mutations in drug target genes, alteration of cell wall permeability, efflux pump activation, and the formation of drug-resistant persisters [9,10]. The intricate interplay between genetic changes, selective pressures, and the host environment contributes to the evolution and dissemination of drug-resistant strains. As these mycobacteria continue to adapt and develop resistance to an expanding range of antibiotics, the urgency to address this issue

becomes more apparent [11]. Therefore, there is need to conduct a detailed survey on the extend of antibiotic resistance with a particular focus on MTB and NTB.

II. FINDINGS & DISCUSSION

2.1 Modes of Action of Antimycobacterial Drugs

Antimycobacterial drugs are medications mainly used to treat infections caused by MTB. By extension, these drugs act broadly and are often prescribed in treatment of NTMs. These drugs act through various modes of action to inhibit the growth and survival of the bacteria. One mode of action of antimycobacterial drugs is the disruption of cell wall integrity. Drugs such as isoniazid, ethambutol, ethionamide, and cycloserine target components involved in cell wall synthesis, leading to the inhibition of bacterial growth [12]. These drugs interfere with the biosynthesis of mycolic acids, which are essential components of the mycobacterial cell wall [13].

Another mode of action is the limitation of energy available for cellular processes. Pyrazinamide and bedaquiline are examples of drugs that target the energy metabolism of Mycobacterium tuberculosis [14]. Pyrazinamide disrupts the energy production in the bacteria, while bedaquiline inhibits the ATP synthase enzyme, which is involved in energy production [14]. Antimycobacterial drugs can also inhibit normal cellular functionality by interfering with the biosynthesis of essential macromolecules, cofactors, and metabolites. Rifamycin, fluoroquinolone, aminoglycoside, and oxazolidinone class antibiotics, as well as para-aminosalicylic acid, belong to this category of drugs [15]. These drugs target various cellular processes, such as protein synthesis, DNA replication, and folate metabolism, leading to the inhibition of bacterial growth.

In recent years, there has been a focus on the discovery of new antimycobacterial drugs with novel mechanisms of action. Quinoline-based compounds have shown promise as potential antimycobacterial agents [16]. TMC207, a quinoline derivative, has been approved by the U.S. Food and Drug Administration for the treatment of multidrug-resistant TB [17]. These compounds act through unique mechanisms, making them valuable additions to the arsenal of antimycobacterial drugs. Efflux pumps, such as the P55 pump, play a role in drug resistance in *Mycobacterium tuberculosis* [18]. These pumps actively remove drugs from the bacterial cell, reducing their effectiveness. Understanding the mechanisms of action of these pumps can help in the development of strategies to overcome drug resistance.

2.2 First-Line Antimycobacterial Drugs

Treating infections brought on by MTB, the bacterium that causes tuberculosis (TB), is the cornerstone of first-line antimycobacterial medication therapy. The typical short-course chemotherapy regimens that are advised by international health organisations are based on these medications [19]. Certain medications aid in the eradication and containment of the illness by going straight after the mycobacterial cells. Achieving a full recovery with the least amount of drug resistance is the main goal of first-line antimycobacterial medication use [20]. Among the first-line antimycobacterial medications are:

Isoniazid (INH) stops the production of mycolic acids, which are vital parts of the cell wall of mycobacteria[21,22]. One of the best medications for treating *M. tuberculosis*, it can be taken throughout both the start and maintenance of treatment. RNA synthesis is inhibited by Rifampicin (RIF), a compound that targets bacterial RNA polymerase [23]. When used against both dormant and actively proliferating *M. tuberculosis* cells, it is incredibly effective. The severe stage of tuberculosis treatment requires rifampicin. The bacterium becomes more vulnerable to the acidic environment inside macrophages when pyrazinamide (PZA) interferes with the membrane transport activities of mycobacteria[24,25]. PZA is applied during the first intense phase in order to quickly lower the bacterial load. Mycobacterial cell wall formation is aided by arabinosyl transferases, which are inhibited by ethambutol (EMB)(26). To increase efficacy and prevent drug resistance, it is frequently used in combination with other first-line medications.

When these first-line medications are taken in tandem, they can more effectively and completely cure tuberculosis by targeting its different stages [27]. The typical regimen for treating tuberculosis consists of an intense phase that lasts for the first four first-line medications to be given, and a continuation phase that uses a mix of INH and RIF. The direct targeting of dormant and actively dividing *M. tuberculosis* populations is guaranteed by this combination therapy [28]. There are still issues even though first-line antimycobacterial medications have significantly improved TB patients' prognosis. To stop the formation of drug-resistant bacteria, strict adherence to therapy is essential. Potential problems in the management process include side effects, pharmacological interactions, and insufficient treatment.

2.3 Second-Line Antimycobacterial Drugs

Drug-resistant tuberculosis and complicated NTM infection cases are treated with second-line antimycobacterial medications [29]. These medications are used when infections brought on by NTM or MTB show resistance to first-line antibiotics, requiring a more specialised and aggressive therapeutic strategy.

When it comes to TB, second-line antimycobacterial medications are used to treat extensively drug-resistant (XDR-TB) and multidrug-resistant (MDR-TB) infections [5]. Isoniazid and rifampicin, two of the most effective first-line anti-TB medications, are at least partially resistant in MDR-TB patients. By adding further resistance to any fluoroquinolone and at least one injectable second-line medication (kanamycin, amikacin, or capreomycin), XDR-TB goes beyond resistance. A more complex treatment plan is required for these resistant strains of *M. tuberculosis*, which frequently combines second-line antimycobacterial medications[30]. Important second-line antibiotics against mycobacteria include:

Aminoglycosides: These medications, which attach to the bacterial ribosome, prevent the synthesis of proteins (Amikacin, Kanamycin, Capreomycin)[31]. In order to improve therapy efficacy for MDR-TB and XDR-TB, aminoglycosides are frequently included in combination regimens.

Fluoroquinolone antibiotics (Levofloxacin, Moxifloxacin): These medicines work by specifically targeting bacterial topoisomerases to prevent DNA replication[32]. In combination therapy for drug-resistant tuberculosis, fluoroquinolones are effective second-line medications.

Ethionamide and Prothionamide: These medications prevent the synthesis of mycolic acids, which interferes with the formation of the cell walls of mycobacteria[12]. They are frequently incorporated into MDR-TB treatment plans.

Terizidone and cyclopenerine: These medications block the enzymes that assemble peptidoglycans, hence preventing the formation of cell walls[33]. They are frequently a component of MDR-TB combo therapy.

Para-aminosalicylic acid (PAS): PAS prevents bacteria from synthesising folic acid, which stops the bacteria from growing [34]. It is a component of combination therapy used to treat MDR-TB.

Thioamides (Ethionamide, Protionamide): These medications prevent the creation of mycolic acid, which prevents the formation of mycobacterial cell walls [12]. They frequently work in tandem with other second-line medications.

Clofazimine: Despite being categorised as a third-line medication, clofazimine is a valuable choice in drug-resistant tuberculosis cases due to its effectiveness against M. tuberculosis, especially for patients with few other treatment options [35].

The main characteristics of second-line antimycobacterial medications are their potency, specificity, and side effect potential. Because there is a chance of toxicities and drug interactions, their administration needs to be closely watched. To guarantee both therapy efficacy and patient safety, appropriate dose, patient education, and routine clinical evaluations are crucial.

2.4 Third-Line Antimycobacterial Drugs

Drug-resistant TB and complicated NTM infection cases are treated with third-line antimycobacterial medications. These medications are used when infections brought on by NTM or MTB show resistance to both first- and second-line antibiotics, making treatment effectively difficult.

When treating patients with XDR and MDR-TB, the use of third-line antimycobacterial medications becomes crucial[5]. Isoniazid and rifampicin, two of the most effective first-line anti-TB medications, are at least partially resistant in MDR-TB patients. This resistance profile is further elevated by XDR-TB to include resistance to any fluoroquinolone, at least one injectable second-line antibiotic (kanamycin, amikacin, or capreomycin), and resistance to both first-line medications. Treatment for these resistant strains of *M. tuberculosis* is challenging and requires combinations of third-line antimycobacterial medications for effective results.

Among the most important third-line antimycobacterial medications are:

Clofazimine: Initially created to treat leprosy, clofazimine has proven effective against types of tuberculosis that are resistant to drugs[36]. Its distinct mode of action involves inhibiting RNA synthesis and interacting with bacterial DNA[37]. When treating MDR and XDR TB, clofazimine is frequently utilised as part of an all-encompassing regimen.

One more recent drug to be added to the arsenal of anti-TB medications is delamanid. It stops the mycobacterial cell wall's ability to synthesise mycolic acids, which stops the growth of the bacterium[38]. Delamanid is frequently utilised when other treatment choices are restricted since it has demonstrated efficacy against MDR-TB.

Since it works against mycobacteria, linezolid—which was first created as an antibiotic for Gram-positive bacteria—has been repurposed. By focusing on the 23S ribosomal RNA, it prevents the creation of proteins [39].

Although possible adverse effects and drug combinations frequently restrict its use, linezolid is used to treat drug-resistant tuberculosis.

Bedaquiline is a brand-new medication that stops the mycobacterial ATP synthase enzyme, which is necessary for the synthesis of energy[40]. It is seen to be an essential part of regimens for patients with drug-resistant tuberculosis and is especially approved for the treatment of MDR-TB.[41]

Pretomanid: Another relatively new weapon in the arsenal against tuberculosis is pretomanid. By preventing the formation of mycolic acid, it compromises the integrity of the cell wall [41]. It is frequently used to treat MDR and XDR TB in conjunction with bedaquiline and linezolid.

Although third-line antimycobacterial medications provide promise for the treatment of difficult NTM infections and drug-resistant tuberculosis, using them is not without difficulties. Adverse effects, possible drug interactions, and administration-related logistical challenges are possible concerns with these treatments. As a result, using them calls for cautious thought, close patient observation, and coordination with specialists.

2.5 Fourth-Line Antimycobacterial Drugs

A fourth-line antimycobacterial drug regimen is an advanced and specialized treatment approach utilized in cases of XDR-TB, where the bacterium MTB exhibits resistance not only to first-line and second-line drugs but also to some of the potent drugs typically used to manage drug-resistant TB [42]. The implementation of a fourth-line regimen becomes necessary when standard treatment options are exhausted, and the infection poses a significant challenge to effective management.

In a fourth-line regimen, a combination of fourth-line antimycobacterial drugs is employed to target XDR-TB. These drugs are selected based on their activity against the resistant strain, with the aim of achieving successful treatment outcomes. Fourth-line antimycobacterial drugs are typically used in conjunction with third-line drugs, including those like clofazimine, delamanid, bedaquiline, and linezolid, which are specifically approved or repurposed for the treatment of drug-resistant TB.

III. METHODOLOGY

This review aims to explore the current landscape of antibiotic resistance in Mycobacterium tuberculosis and non-tuberculous mycobacteria, highlighting the challenges posed by drug-resistant strains, the underlying mechanisms of resistance, and the implications for patient management and public health. By examining the similarities and differences between these two groups of mycobacteria, we can gain insights into potential strategies to combat antibiotic resistance and ensure the effective treatment of mycobacterial infections.

IV. FINDINGS & DISCUSSION

4.1 Gene Mutations and Antimycobacterial Drug Resistance

Gene mutations play a pivotal role in the emergence of antimycobacterial drug resistance, impacting both MTB and NTM. These mutations occur in response to selective pressures from antimicrobial agents and contribute to the ability of mycobacteria to survive and proliferate in the presence of these drugs. Understanding the mechanisms behind these mutations is crucial for effective management and containment of drug-resistant mycobacterial infections.

4.1.1 Mycobacterium Tuberculosis (MTB*)*

Spontaneous Mutations: MTB exhibits a relatively slow replication rate, but its propensity for spontaneous mutations during DNA replication leads to genetic diversity[43]. Some of these mutations confer resistance to antimycobacterial drugs. For example, mutations in the *rpoB* gene lead to rifampicin resistance, while mutations in the *katG* or *inhA* genes result in isoniazid resistance.

Target Site Alterations: Mutations often occur in genes encoding the targets of antimycobacterial drugs [44]. For instance, mutations in the *gyrA* and *gyrB* genes contribute to fluoroquinolone resistance, while mutations in the *embB* gene can lead to ethambutol resistance.

Efflux Pump Activation: Mutations in genes encoding efflux pumps can lead to increased drug efflux, reducing drug concentrations within the bacterial cell [45]. This contributes to multidrug resistance by decreasing the intracellular drug levels.

4.1.2 Non-Tuberculous Mycobacteria (NTM)

Intrinsic Resistance Mechanisms: NTM naturally possess mechanisms that confer resistance to certain antimycobacterial drugs [7]. For example, the intrinsic impermeability of the cell wall of some NTM species can limit the entry of drugs.

Spontaneous Mutations: Similar to MTB, NTM can develop resistance-associated mutations spontaneously during DNA replication [46]. These mutations can occur in various genes, leading to drug resistance.

Altered Drug Targets: Mutations in genes encoding drug targets can alter the binding affinity of the drug, rendering it less effective[47]. For example, mutations in the 16S rRNA gene can lead to aminoglycoside resistance in some NTM species.

Biofilm Formation: Some NTM species are known to form biofilms, protective structures that shield bacteria from antimicrobial agents and the host immune response[48]. Biofilm formation can contribute to drug tolerance and resistance.

Understanding the genetic basis of antimycobacterial drug resistance has led to the development of molecular diagnostic tools that can identify resistance-associated mutations quickly. These tools aid in making informed treatment decisions, improving patient outcomes, and preventing the spread of drug-resistant strains.

4.2 Antibiotic Resistance in MTB

TB is on the rise in developing and underdeveloped countries, despite the availability of antibiotics. This is in accordance with the ongoing emergence of MDR-TB strains that are resistant to fluroquinolones, aminoglycosides, and the first-line drugs: isoniazid, rifampicin, ethambutol, and pyrazinamide. This is similar to the cases of extensively drug-resistant TB (XDR TB) and totally-drug-resistant TB strains, respectively [49,50]. Persistence is a significant characteristic of MTB cells, as a small number of persistent cells can evade antibiotics at concentrations that exceed the minimum inhibitory concentration [51]. Furthermore, persistent MTB cells can maintain the MTB population, thereby maintaining the disease status as active. Persistence is also associated with cellular dormancy, which is characterised by elevated levels of transcription activator protein and triacylglycerol [52,53]. The mechanisms of action of MTB resistance appear to be dependent on pre-transcriptional mutations, and peptidome studies have enabled researchers to gain a more comprehensive understanding of these mechanisms [54]. The MTB adaptive response to antibiotic treatment is mediated by alarmones, which are constituted of pentaphosphate guanosine and tetraphosphate guanosine, as well as ppGpp [55]. Alarmones facilitate the survival of MTB by influencing biofilm formation, antibiotic resistance, persistence, and virulence, thereby illustrating the "stringent response." The enzyme Rel, which is encoded by the gene rv2583c, regulates the alarmones in Mycobacteria [56]. In particular, the MTB stringent response is dependent on the down-regulation of rRNA and ribosomal protein synthesis, which is followed by the up-regulation of amino acid biosynthetic operons. This process provides the amino acids necessary for MTB survival [57,58]. In the context of a granuloma, MTB must surmount oxidative, nitrosative, and nutrient challenges among other major stresses before infecting macrophages and surviving for years [59,60]. Furthermore, the PE-PGRS proteins of MTB facilitate its interaction with host cells and its survival in the granuloma [61]. The survival of MTB is either impacted or its proliferation is inhibited by the deletion of Rel [62]. In reality, the H37Rv delta-Rel- MTB induces a mild form of lung tuberculosis, characterised by the preservation of organ architecture and the presence of a limited number of granulomas, in contrast to the parental strain, H37rv, which induces severe pulmonary injury in mice [63]. These data indicate that MTB is unable to induce a chronic TB infection in the absence of a stringent response. It is intriguing that Rel inhibitors destroy MTB and increase the susceptibility of the bacteria to antibiotics, thereby improving the microbicidal activity of isoniazid [64].

Finally, MTB is shielded from antibiotics by biofilms that contain a matrix of extracellular DNA, carbohydrates, lipids, and proteins [65,66](Basaraba & Ojha, 2017; Chakraborty & Kumar, 2019). The expression of genes involved in glycopeptidolipid synthesis, which are essential for biofilm formation, is regulated by the Rel-induced stringent response in the double knockout deltaRel /deltaRel Z Mycobacterium smegmatis strain [67]. Such evidence has been presented.

4.3 Antibiotic Resistance in NTM

Despite NTMs being less widespread pathogens for humans than *M. tuberculosis*, they are an emerging threat to not only immunocompromised population but also in immunocompetent group [3]. Due to lack of proper diagnosis, is not possible to readily identify NTM-pulmonary disease (NTMPD) using basic mycobacteriology, clinical history, radiologic imaging and the tuberculin skin test [68]. *Mycobacterium abscessus* which is one of the NTM species causing NTMPD reported to be multidrug-resistant, and it's emerging as an important global threat to individuals with cystic fibrosis [69]. Consequently, multidrug-resistant NTM are potentially emerging to be causing relapse and reinfection [70].

Currently, the treatment for almost all NTM infections is based on macrolide-based antibiotics, such as clarithromycin or azithromycin, [3]. However, for Slow Growing group, for example *Mycobacterium avium* (MAC), *Mycobacterium intracellularae* (MI), *M. szulgai* (MZ), *M. kansasii* (MK) and M. *smiae* (MS), their regimen also includes ethambutol and rifampicin [71]. While for rapid growers Mycobacterium (RGM) e.g. M. *fortuitum, M. chelonae,* and *M. abscessus*, their regimen includes an aminoglycoside and either cefoxitin, imipenem or tigecycline [72,73]. These treatments are largely empirical and can last for as long as 18 months, are costly, and are often associated with toxicities and side effects. Furthermore, a major bottleneck is the low susceptibility of NTMs to most antibiotics, including the ones used against MTB [74]. This is worrying since we may finally have very high rate of NTM drug resistance that will leave little or no option drugs for NTM treatment. Resistance can be either intrinsic (natural) or acquired; intrinsic resistance is where an organism possesses a set of special features that allows it to tolerate a particular drug or survive in an otherwise hostile chemical environment [75]. Mechanisms by which NTMs are intrinsically resistant to antibiotics include their thick, impermeable cell walls or their presence in biofilms and granulomas, these effectively decrease drug uptake, as well as the expression of proteins that specifically target clinically used antibacterial compounds [9]. On the other hand, acquired resistance is where a resistant strain emerges from a population that was previously drug-sensitive [75]. These events are usually related to the prolonged antibiotic treatments required to cure NTM infections. The acquired resistance is particularly severe for NTMs that only have a single copy of genes encoding common target proteins such as ribosomes, thus increasing the risk of acquiring protective mutations with single-drug treatments [76].

There dearth information concerning NTM treatment protocols, however research has shown that antimycobacterial susceptibility testing (AST) is not routinely done before treating NTM except in non-responsive disease due to SGM (*M. avium* complex, *M. kansasii*) or infection due to RGM and this could result to drug resistance [6]. NTM treatment is given for 12 months after sputum culture conversion and the treatment response in NTMPD is variable and depends on isolated NTM species and severity of the underlying NTMPD [6]. Incidence of pulmonary diseases caused by NTM is increasing at an alarming rate, to an extent that, it's surpassing tuberculosis in many countries [74]. Furthermore, current chemotherapies require long treatment times and the clinical outcomes are often disappointing. Therefore, there is an urgent need to initiate new drug models and developments to help in accelerating the drug discovery process which will be more efficacious anti-NTM drugs and lesser toxicity than the available ones.

V. CONCLUSIONS & RECOMMENDATIONS

The critical need for innovative strategies to effectively manage mycobacterial infections is emphasised by the increasing prevalence of antibiotic resistance in both MTB and NTM. It is important to develop more effective treatment approaches, as the resistance of MTB to first-line drugs such as isoniazid, rifampicin, ethambutol, and streptomycin has consistently increased. In the same vein, the necessity for species-specific treatment regimens is underscored by the high resistance rates of NTM species to commonly used anti-tuberculosis medications. In addition to acquired resistance resulting from protracted antibiotic exposure, the mechanisms driving this resistance are a combination of intrinsic factors, such as biofilm formation in NTM and thick cell walls. The development of novel antimicrobial agents, continuous surveillance, and enhanced diagnostic capabilities are essential for combating the increasing prevalence of antibiotic resistance in mycobacterial infections. The design of more effective therapies and the prevention of the further spread of drug-resistant strains will be significantly influenced by the advancement of our understanding of the specific resistance mechanisms in both MTB and NTM. In order to enhance patient outcomes and public health, a multifaceted approach that incorporates clinical, microbiological, and pharmacological expertise is necessary to address the challenge of antibiotic resistance in mycobacteria.

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