Journal of Advances in Microbiology

Volume 24, Issue 6, Page 12-29, 2024; Article no.JAMB.118291
ISSN: 2456-7116

Navigating the Challenge of Tuberculosis Drug Resistance: Unveiling Intrinsic and Acquired Pathways to Overcome the Threat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamb/2024/v24i6829

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/118291

Review Article

Received: 05/04/2024
Accepted: 09/06/2024
Published: 13/06/2024

ABSTRACT

Tuberculosis (TB), caused by the bacteria Mycobacterium *tuberculosis* (MTB), continues to be a persistent worldwide health problem, made worse by the appearance of strains that are resistant to drugs. This review thoroughly examines the complex phenomenon of drug resistance in TB, investigating both the intrinsic characteristics and acquired changes that contribute to the bacterial ability to withstand treatment. It explores the progression of tuberculosis treatment, starting with the

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Cite as: *Aslam, Hafiz Muhammad, Muhammad Umar Sharif, Amna Uroos, Mahnoor Rana, and Waqas Farooq. 2024. "Navigating the Challenge of Tuberculosis Drug Resistance: Unveiling Intrinsic and Acquired Pathways to Overcome the Threat". Journal of Advances in Microbiology 24 (6):12-29. https://doi.org/10.9734/jamb/2024/v24i6829.*

basic medications that target cell wall formation, then moving on to more advanced tactics that overcome resistance. It also discusses the new third-line therapies, providing insights into the dynamic landscape of TB treatment. Considering the increasing prevalence of multidrug-resistant and extensively drug-resistant strains, it is crucial to adopt innovative strategies. These may involve altering existing antibiotics, investigating new chemicals, and gaining a more comprehensive understanding of new targets. This review emphasizes the need of knowing MTB medication resistance mechanisms that will enhance our understanding of MTB medication resistance and prepares us for future study, providing insights and approaches to address this global health issue more effectively.

Keywords: Tuberculosis; mycobacterium tuberculosis; antimicrobial resistance; drug resistance; genetic pathways.

1. INTRODUCTION

The bacterial infection known as tuberculosis (TB), which mostly affects the respiratory system, is caused by Mycobacterium tuberculosis (MTB). While mostly affecting the lungs, it can also cause harm to other tissues. Approximately 90% of MTB cases do not acquire active TB illness over their lives because the infection is well controlled and contained [1]. According to the World Health Organization (WHO), TB still remains the most prevalent contagious disease that causes death globally [2,3]. Its causative agent MTB is to blame for about two-million fatalities every year and quietly invades a quarter of the world's population. Latency is a distinctive aspect of TB infection, where MTB establishes a long-lasting dynamic balance with the host's immune system, showing no signs or symptoms of the disease. TB, as both an acute and persistent infection, remains undefeated and poses a significant social, medical, and biological challenge on a global scale. According to the WHO's 2022 report on TB, in 2021, around 10.6 million individuals have developed TB. TB incidence rate experienced a 3.6% upswing from 2020 to 2021 [4]. Individuals with HIV or those dealing with other immune-weakening conditions, such as diabetes, face heightened vulnerability, significantly increasing their chances of contracting the disease. Additionally, children are susceptible due to their underdeveloped immune systems [5]. WHO has set lofty targets to end the TB pandemic, aiming for a 90% alleviation of the disease by 2035. In addition to addressing latent TB in individuals who are vulnerable to it, the supporting End TB Strategy emphasizes early diagnosis and treatment for those who exhibit detectable signs [3].

Drug-resistant MTB strains have emerged, posing a serious threat to the management of TB infections. This development is driven by a complex interaction between acquired and inherent mechanisms. This intensifying problem poses a serious threat to the efficacy of conventional treatment approaches and poses a significant obstacle to global efforts to combat TB. The management of drug resistance requires the careful selection of medication combinations, attentive patient monitoring, and patient commitment to treatment [6]. One of the biggest challenges in managing TB is combating the resistant strains of MTB [137]. This problem is driven by a complex interaction between acquired and innate mechanisms, endangering the efficacy of standard treatment approaches and posing a significant barrier to worldwide efforts to eliminate the infection. Strict commitment to therapy, careful patient monitoring, and the deliberate mixture of drugs are essential components in combating drug resistance [1].

It is essential to comprehend the mechanism of action of previously available mycobacterial medications as well as the evolution of drug-resistance to methodically create novel anti-TB drugs. Modern approaches, including systems biology, metabolomics, next-generation sequencing for thorough whole-genome transcriptional analysis, genetics research, and metabolomics, have completely changed our knowledge of novel therapeutic targets and the mechanisms underlying drug resistance. As such, the mechanism of action of first-, second-, and third-line medications is examined in this article. Additionally, it examines the mechanisms of intrinsic and extrinsic drug resistance, providing insight into how MTB has evolved to exhibit these resistances.

2. EVOLUTION OF MTB

MTB emerged as a human infectious agent in Africa. Subsequently, it disseminated beyond the

continent through human migrations [7, 8]. MTB causes human TB, while *M. bovis, M. pinnipedii,* and *M. africanum* afflict certain African locales. *M. caprae* causes mammalian TB. M. *microti* alone causes vole TB [9]. Most researchers believe that archaic MTB variants evolved from environmental mycobacteria, notably smooth tubercle *bacillus*. In some East African countries, these microbes can still be recovered from people with compromised immune systems. Importantly, these strains do not have the ability to cause chronically persistent infection in hosts with weaken immune and are not transmissible between people. These primitive MTB variants came up with time due to genetic constraints, resulting in disease reactivation even years of latent infection [10]. Following the process of domestication, humans acquired the ability to propagate the infectious agents to animals, resulting in M. *bovis* emergence as a infectious agent affecting both domestic and feral animals [11]. Due to the development of the agricultural sector, civilizations, and urbanization, more people live in metropolitan regions worldwide and more MTB variants with enhanced transmissibility and virulence, known as contemporary MTB strains, were evolved. These modern strains disseminated globally, giving rise to TB epidemics that afflicted humanity for centuries. Notably, these strains continue to be held accountable for the majority of all instances of TB today [9].

3. PATHOGENICITY OF MTB

Comprehending the Pathogenicity of MTB require understanding the bacterium's metabolic capabilities during infection and identifying particular virulence factors that aid its entry into human cells, particularly macrophages. The structural elements of the MTB's cell wall, envelope, and other pertinent structures are well understood. These Structures are essential for its survival during infection within macrophages, likely during periods of persistence, and are essential for the bacterium's ability to be phagocytosed by a mechanism that shields it from reactive oxygen intermediates [12].

MTB typically enters the alveolar passages through aerosol droplets, making its initial contact with resident macrophages. However, there's also the possibility that the bacteria may be initially ingested by type II alveolar cells. These cells outnumber macrophages in the alveoli, and MTB has been observed to infect and proliferate in these pneumocytes under laboratory conditions. Surfactant protein A can improve MTB binding and absorption by stimulating mannose receptor function. Surfactant protein-D found in alveoli, inhibits MTB phagocytosis by inhibiting mannosyl oligosaccharide residue on the microbial cell wall. This interference is hypothesized to inhibit MTB from interacting with mannose receptors on the macrophages' surface [13-16].

Infection with MTB occurs when a small number of MTB distributed in the air by the individuals with active pulmonary TB contact the host's alveoli. In this environment, professional alveolar macrophages promptly phagocytize the MTB. Typically, these macrophages are capable of eliminating the invading bacteria through the innate immune response [17]. If the bacilli manage to survive the initial defense mounted by macrophages, they initiate active replication within these immune cells. Within a few weeks of augmented growth rate the bacteria disseminate to surrounding cells, particularly endothelial and epithelial cells, resulting in a high bacterial load. in the early stages of infection MTB has the capacity to propagate other organs via lymphatics and bloodstream, allowing it to infect various tissues throughout the body [18, 19]. Subsequently, once the adaptive immune response gets activated, lymphocytes and neutrophils along with other immune cells head to the infection site. Neutrophils, lymphocytes, and other immune cells head to the infection site once the adaptive immune response gets going. As a result, a cellular infiltration occurs, which eventually develops into a granuloma's distinctive shape [20]. Fibrous elements surround the granuloma, leading to calcification, creating a structure where the bacilli are encapsulated and shielded by the host immune response. This initial lesion, conventionally referred to as the Ghon complex, was historically considered the "sanctuary" of MTB during latent TB, with the bacteria enduring in a quiescent, non-metabolically active condition for prolonged durations spanning from years to decades or, more frequently, throughout a lifetime. This hypothesis states that TB reactivates when bacilli replicate in the initial lesion for unknown reasons during latent infection, causing active disease. This idea has major pathophysiological and clinical consequences, suggesting that TB reactivation begins at the infection site. Thus, this review analyzes first-, second-, and third-line pharmacological mechanisms. It also examines intrinsic and extrinsic drug resistance mechanisms in MTB and their evolution [21].

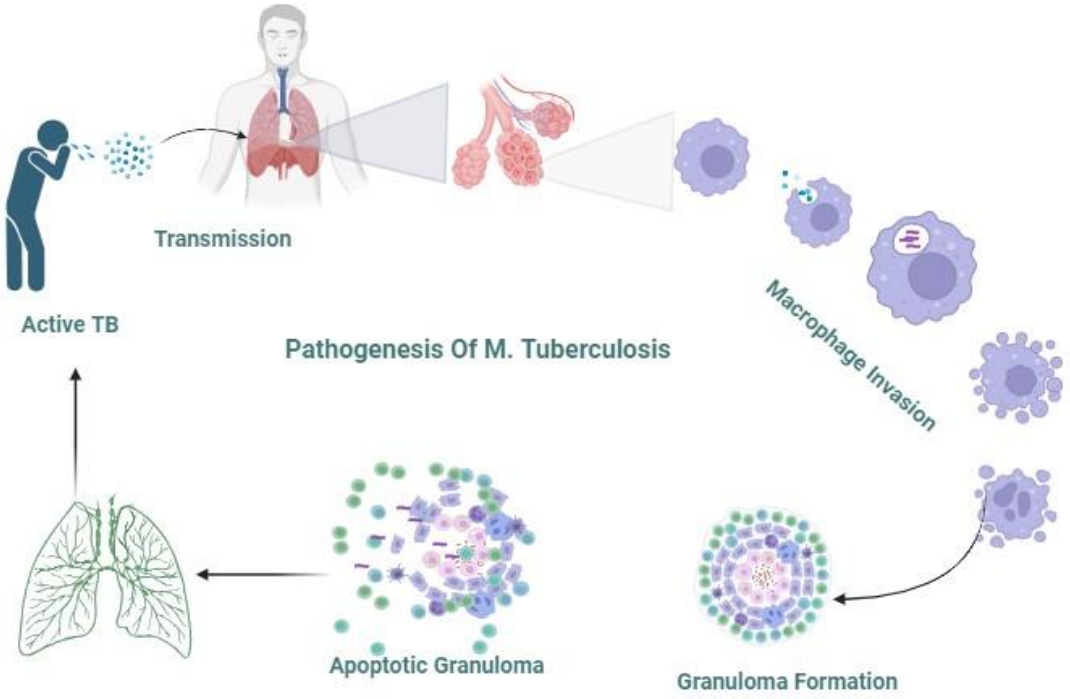

Fig. 1. Pathogenesis of Mycobacterium *Tuberculosis*

Animal models of TB have shown that MTB remains in active metabolic state and multiplies in host during quiescent TB, even without symptoms [22, 23]. A single TB-affected individual had many lesions. Sterile lesions, cytotoxic or damaged hypoxic lesions with a variety of microorganisms, and liquid cavities with significant bacterial loads were present. In pulmonary TB, many similar lesions were present at once. These lesions responded differently to chemotherapy, suggesting they may be separate subsets of MTB in different microenvironments [9, 24]. A recent paradigm shows that most MTBs remain dormant or inactive during latent infection, reproducing less. Actively multiplying bacilli, called "scouts," are treated and removed by immune systems of the host, activating many peripheral blood MTB-targeting memory T-cells. Dormant bacteria, which are efficiently eliminated from the host, replenish the pool of latent, actively replicating bacilli. If the immune system fails to handle scouts, bacteria multiply exponentially, causing sickness and active TB [9,25,26].

4. DRUG RESISTANCE TUBERCULOSIS

Varying responses to pharmacological therapy by a bacterial population might result from drug resistance, tolerance, or persistence mechanisms believed to be pertinent in TB. Balaban et al. provided a paradigm for distinguishing these pathways based on the minimum duration of killing (MDK) and minimum inhibitory concentrations. The MIC99 is the minimal concentration needed to eradicate 99% of microbial population, whereas the MDK99 represents the minimal time allowed to cause a 99% reduction in the population [27].

Drug resistance: This is heritable and typically results by a mutation in the enzyme that activates the prodrug or in the gene that codes for the drug target. It leads to a net decrease in drug effectiveness, observable as an increase in MIC99.

Drug Tolerance: Cells able to transiently survive otherwise lethal antibiotic concentrations acquired phenotypically drug-tolerant state. Cells that can withstand otherwise fatal antibiotic doses over short periods of time exhibit phenotypic drug tolerance. This can occur in a variety of ways, including mitigated growth. Drug tolerant strains have MICs similar to susceptible groups, but their MDK99 values are significantly higher.

Persistence: Persistence, like drug tolerance, describes transitory surviving in inhibitory antimicrobial concentrations; however, only a tiny fraction of the population exhibits this characteristic. Persistence is distinguished through biphasic or multiphasic cell death pathways, in which a significant proportion of population is eradicated, but persisters are eliminated more gradually. In this case, the MIC-99 and MDK-99 levels are quite near to those of a sensitive population, but the MDK-99 number is significantly higher.

5. MECHANISM OF DRUG RESISTANCE

MTB develops drug resistance through a variety of processes, including impervious cell wall, drug efflux pumps, modification, and degradation, altered target, and simulating or mimicking the target. There are two types of resistance mechanisms: intrinsic or innate resistance mechanisms and acquired resistance mechanisms.

Intrinsic or Innate Resistance mechanism: MTB's capacity to counter antibiotic cytotoxicity is attributed to the built-in or inherent resistance mechanisms. The factors that contribute to intrinsic resistance have been described here.

Impermeability of Cell Wall: The cell wall of MTB has three unusual lipids: mycolic acid, cord factor, and wax D. Arabinogalactan is found in the outer mycomembrane of cell wall, while peptidoglycan resides in the inner. Phospholipid, glycopeptidolipids, lipoglycans, and trehalose mycolate make up the outside leaflet of the cell membrane, whereas mycolic acid chains make up the interior leaflet. [28]. The hydrophilic impediment of Arabinogalactan causes hydrophilic substance tolerance. This causes antibiotic accumulation near the cell. Cellular components, including enzymes like β-lactamase, are generated and released from cells to purify or break down drugs [29]. Porins, which are proteins that form channels, are found in the outermost layer of the cell wall [30]. Porins help hydrophilic compounds like antibiotics enter cells. Thus, porin reduction and cell wall fatty composition impede the inflow of hydrophilic molecules [31]. Antibacterial medications target bacteria via the outer membrane transport protein CpnT. A cpnT mutation in MTB strains has been linked to increased resistance [30].

Metabolic slowdown: Since most antibiotics target metabolically active MTB, a drug-resistant state can be efficiently induced by reducing its metabolic rate under the influence of drug pressure [32-34]. In MTB, several interrelated processes that control lipid, carbon, and energy metabolism are coordinated to cause a metabolic slowdown. When treatment is initiated, genes that regulate aerobic respiration, ATP production, and the Krebs cycle, essential processes in energy metabolism, are impaired in response to antibiotic stress [35].

Up Regulation of Efflux Pump Activity: Efflux pumps are protein transport channels that actively expel many substances, mainly antimicrobial agents, from the cell [36]. Perpetual resistance to various anti-TB agents has been associated with mutations in efflux genes [37]. Furthermore, in genetically susceptible MTB variants, an upsurge in efflux pump expression acts as a form of adaptation that can be initiated under drug pressure. The efflux pumps have conditional, reversible, and transitory expression [38]. The broad target range of efflux pumps can lead MTB to be less susceptible to multiple TB drugs, like how a particular mutation for a particular drug might create "cross-resistance" to another [39, 40].

Several efflux pumps have been found to be increased due to antibiotic stress, whether from a certain or a fusion of structurally distinct drugs. Wang *et al*. [41] reported five drug efflux pump super-families in MTB. These belong to:

(i) MATE Superfamily: Engaged in the extrusion of multidrug and toxic compounds, this protein superfamily facilitates the removal of diverse substances from cells.
(ii) RND Superfamily: The superfamily of resistance nodulation cell division plays an essential role in the expulsion of drugs and toxic compounds from bacterial cells, contributing to mechanisms of resistance.
(iii) SMR Superfamily: Within this superfamily, small-multidrug resistance proteins participate in expelling small, hydrophobic drugs from bacterial cells.
(iv) MFS Superfamily: Encompassing a variety of membrane transport proteins, the major facilitator superfamily aids in transporting diverse substrates across cell membranes.
(v) ABC Superfamily: The ATP-binding-cassette superfamily consists of primary transporter pumps that utilize ATP for actively transporting a broad range of substances across cellular membranes.

ABC pumps are ATP-dependent main transporter pumps, on the other hand others are proton driven secondary transporter pumps.

Modifications in the target of drug: MTB can produce several enzymes that alter or decay numerous antibiotics, such as aminoglycosides, β-lactams, and macrolides. β-lactams, for example, primarily target microbial transpeptidase, causes cell death by denaturing the cell wall. MTB generates the class A ambler blaC gene-coded β-lactamase, causing resistance to β-lactams [42]. Inactivation and alterations of drugs in MTB are facilitated by modification enzymes like methyltransferases and acetyltransferases. These enzymes change drug structures, blocking their identification and activity. Enhanced MTB-expressed intracellular persistence proteins acetylate aminoglycosides. [43]. This response causes Capreomycin, kanamycin, gentamicin, and tobramycin resistance [44]. The eis gene encodes an intracellular protein that acetylates antibiotics and alters aminoglycoside and cyclic peptide chemistry. Modification of medication by MTB is a notable example of enzymatic degradation. Through acetylation, the eis gene deactivates second-line aminoglycoside antibiotics like Kanamycin A and cyclic peptide antibiotics like Capreomycin. By helping macrophages survive, eis protects MTBs from the immune system. Thus, eis is essential for MTB pathogenicity and antibiotic resistance [45].

In some instances, alterations are made to the targets instead of the medications themselves. Drugs exhibit selectivity in their affinity for binding. As a result, alterations in the target impede the drug's binding to a particular site, leading to resistance against the drug. Rifampin, streptomycin, lincosamides, and macrolides face resistance due to modifications in the target site within MTB. This altered target prevents the reversible binding of these drugs, which, in turn, inhibits the translocation of peptidyl tRNA translocation and protein synthesis. Additionally, the growth of Mycobacteria is impeded by the reversible binding of these drugs to certain location on the larger rRNA subunit [46].

Target Mimicry: To resist antibiotic therapy, MTB integrates a variety of strategies, such as molecular mimicry. Fluoroquinolone binding to DNA gyrases or topoisomerases impairs DNA replication, transcription, repair, and degradation, eventually leading to cell death. MTB features the presence of MfpA, identified as Mycobacterial Fluoroquinolone Resistance Protein A. Remarkably, MfpA mimics the size and structure of DNA B-form, letting it to interact with DNA gyrase and obstruct the interaction of fluoroquinolones to DNA gyrase [47].

Latency: Another avenue for drug resistance involves bacterial cells enters a state of latency, persistence, or dormancy. Bacteria are assumed to have little metabolic activity at this stage, resulting in little or no synthesis of prospective therapeutic targets. This method favors microorganisms to become resistant to antibiotics. According to one study, peptidoglycan rearrangement during latency makes bacteria more resistant to antimicrobial agents, implying that L and D-transpeptidases might be suitable therapeutic targets for such cases. Several transcription factors and genes are active during latency allowing MTB to thrive in the latent stage. The activation process includes crucial genes responsible for virulence, proliferation, oxidative imbalance, membrane efflux, nutrient deprivation, and respiration. For instance, the regulation of MTB's resistance phenotype involves distinct mechanisms through the expression of various heat shock genes (e.g., α-crystallin), sigma factors, and transcription factors like CarD, RbpA, and Whib7 [31,44,48,49].

DNA damage repair system: An essential factor contributing to antibiotic resistance is bacteria's flawed DNA Damage Repairing system. This deficit grants bacteria an additional advantage, making it easier for mutations that confer resistance to accumulate as part of genome. Suboptimal doses of specific drugs or antibiotics as fluoroquinolones, cause mutations that affect the bacterial DNA damage repair mechanism [50].

Similarly isoniazid, moxifloxacin, and rifampicin have shown to activate the stress response at inadequate levels, potentially raising genome-wide mutation rate. [51]. Moreover, it has been observed that anti-mutator genes contain point mutations within their sequences, potentially representing another factor contributing to deficiencies in the DNA repair system [52].

Acquired Resistance: Anti-mycobacterial agents exhibit high-affinity binding to their targets, disrupting the normal activity of these targets. Resistance can arise from mutations in target structure, impeding effective antibiotic

interaction. In contrast to other bacterial species where drug resistance is generally acquired through horizontal gene transfer which is facilitated by transposable DNA fragments. MTB acquires resistance primarily through random mutations occurring spontaneously in the genomes responsible for encoding drug targets.

Abolition of Prodrug Activation: Drug resistance in mycobacteria can emerge when antimycobacterial medications exist in a prodrug form without undergoing activation. Isoniazid, delamanid, pyrazinamide, pretomanid, ethionamide, and para aminosalicylic acid are all examples of drugs falling within this category. Prodrug inactivation may be attributed to diverse substitutions in gene sequence and whole chromosome deletion and insertion. For instance, the mycobacterial enzyme pyrazinamidase, which is expressed in the pncA gene, catalyzes the conversion of pyrazinamide, a prodrug that functions as a biochemically inactive substance, into the bioactive form pyrazinoic acid. Resistance to pyrazinamide results from a deficiency in pyrazinamidase activity, its increased expression makes it more susceptible [53].

Activation of a transcriptional regulator: At transcriptional level MTB actively regulated stress biochemically by sigma factors and WhiB transcription factors. The inherent resistance to antimicrobial drugs is intricately shaped by a dynamic biochemical network that encompasses regulatory proteins, effector proteins, and inducers [54].

WhiB genes encode transcription regulators that regulate cell division, disease progression, and stress responses like antibiotic exposure [55]. Among the seven whiB-like genes discovered in MTB, whiB3 and whiB7 have been associated with treatment resistance [49, 55, 56]. Antibiotic pressure promotes whiB7 expression, according to research. In vitro, whiB7-null mutants are more antibiotic-sensitive. These mutants are also more sensitive to heat shock, iron shortage, and stationary phase entrance [49, 57]. In response to drug assault, the transcriptional regulator whiB7 alters cell processes and increases efflux pump synthesis, such as Rv1258c, to induce tolerance. [58, 59]. WhiB7 regulates the eis gene, which is essential for macrophage survival [58]. WhiB3 regulates drug sensitivity via balancing redox and bioenergetic balance, which affect TB drug responsiveness [60]. A condition-specific model shows that changing transcription factor expression levels may create different MTB phenotypes. Overexpressing the transcription factor whiB4 with isoniazid and ethionamide was predicted to produce specific results [61].

MTB possesses multiple sigma factors, including SigF, that contribute to multidrug-resistant phenotypes together with WhiB genes. Sigma factors aid bacterial transcription by binding with RNA polymerase. Anti-sigma factors and protein kinases mediate posttranslational modifications like phosphorylation and acetylation in the complex sigma factor regulatory network. MTB contains 13 sigma factors (SigA–SigM). SigA expression decreases and SigB, Sig-E, Sig-F, Sig-G, Sig-H, Sig-I, and Sig-J expression increases under drug pressure. MTB may adapt to antibiotic stress via SigA, SigB, SigE, and SigF, according to studies [62-64].

Bypassing metabolic pathways: In TB, "metabolic bypass" means MTB avoids antibiotic-targeted metabolic pathways. These processes, whether acquired or intrinsic, are essential for bacterial growth and survival. However, changes in particular cellular processes or enzymes may allow MTB strains to resist antibiotics [65]. The shift of carbon flux from energy-generating pathways to energy storage pathways has stopped MTB development and reduced drug sensitivity [66]. Investigating gene expression informs metabolic reprogramming research. Under drug-induced stress, MTB can switch from Krebs-cycle carbon metabolism to fatty acid storage. Overexpression of triacylglycerol synthase gene tgs1 aids this transformation [67]. Alpha-ketoglutarate synthesis is bypassed by glyoxylate bypass enzyme genes. This alternate pathway restricts amino acid synthesis, slowing development and metabolism. Bacteria like MTB can use metabolic escape routes to sustain energy production, biosynthesis, and food uptake even in the presence of antimicrobials. This metabolic flexibility makes the disease persistent and causes drug-resistant forms to fail treatment [66].

Drug Specific Mechanism of Resistance: Mycobacteria depend on their quick response to stimuli for survival. Existing evidence shows that different interrelated biological processes create and consolidate drug tolerance driven by therapeutic efficacy. Several mechanisms are universal and occur across drug categories, whereas others are drug-specific and only occur after exposure. Drug pressure rapidly affects the

expression of several MTB genes, resulting in transcriptional and posttranscriptional modifications that help MTBs develop a tolerant phenotype [68]. TB drug resistance and the mechanism of action will be explored in the following sections.

Resistance to first-line TB drugs: First-line TB medications include isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin. TB strains resistant to isoniazid and rifampicin are multidrug-resistant.

Rifampicin: Rifampicin, which was first released in 1972, is a crucial component in the treatment of TB. It is effective against both actively growing and sluggish metabolizing bacteria. The mode of action of rifampicin entails binding to the β-subunit of RNA-polymerase, obstructing the elongation of mRNA. The majority of clinical isolates that are resistant to rifampicin in MTB commonly showcase mutations in the rpoB gene, responsible for coding the β-subunit of RNA polymerase. These mutations induce conformational changes that diminish the drug's affinity, leading to the development of resistance [69].

In rifampicin resistant strains that have preexisting mutations in rpoB, genomic investigations have revealed the establishment of compensatory mutations in rpoA and rpoC as well. These genes encode the α and β subunits of RNA polymerase, respectivelyThese compensatory mutations are hypothesized to play a role in reinstating the agility of such strains, particularly in an in vivo experiment. Additionally, in specific contexts, these compensatory mutations have been linked to heightened transmissibility [70]. The presence of compensatory mutations implies that while mutations in rpoB confer resistance to rifampicin, they may also compromise the bacterium's overall fitness. Compensatory mutations can subsequently arise to counterbalance this fitness cost, enabling the bacteria to survive and propagate more efficiently. Understanding these compensatory dynamics of mutations is essential to understanding of emergence and spread of MTB drug resistance [71,72]

Isoniazid: Isoniazid, first in 1952, and rifampicin are key TB treatments. Isoniazid targets reproducing, active-metabolizing bacteria, unlike rifampicin. As a pro-drug, isoniazid blocks mycolic acid production, a vital component of the mycobacterial cell wall, only when the katG gene activates the KatG enzyme. During activation, the inhA gene-encoded NADH-dependent enoyl acyl carrier protein (ACP) reductase impairs cell wall synthesis and stunts bacterial growth [73].

Isoniazid resistance of MTB has caused changes in kasA, katG, NDH, ahpC, and inhA genes, demonstrating the bacterium's complex mechanisms to resist the medicine [69, 74]. Mutant katG genes, especially in the catalase-peroxidase enzyme active site, cause isoniazid resistance. In addition, isoniazid resistance is often linked to inhA gene and promoter alterations. Research has shown that mutations in both genes are most often associated with isoniazid resistance, with the katG S315T gene mutation being the more common [75,76].

This mutation produces an isoniazid product that cannot form the necessary isoniazid-NAD adduct, which is essential for antibacterial activity [76, 77]. Additionally, this genetic change is linked to higher isoniazid resistance (MIC > 1 µg/mL) and is more prevalent in MDR strains [78]. InhA overexpression results from the second most common mutation in the promoter region. A rare active site mutation reduces its affinity for the isoniazid-NAD adduct [79]. The most common mutation, −15C/T, is linked to low-level isoniazid resistance. Mutations in inhA cause isoniazid resistance and affect the structurally related ethionamide, which targets the same pathway [80, 81].

Ethambutol: Ethambutol, a key MTB medication, inhibits embCAB operon arabinosyl transferases to kill microbes. This operon produces arabinogalactan in the mycobacterial cell wall, and its mutations, particularly in the embB gene, provide ethambutol resistanceThese genetic changes have been regularly found to affect the arabinosyl transferase enzyme's structure and function, reducing binding affinity of ethambutol. The intermediate D-arabinofuranosyl-P-decaprenol accumulates due to this change. This resistance mechanism alters cell wall composition, preventing ethambutol's effects and allowing the bacterium to survive [82]. Further, ethambutol resistance genes go beyond the embCAB operon. Clinical isolates have revealed a spectrum of mutations in embB, embA, and embC, highlighting the genetic variability of ethambutol resistance [83]. Ethambutol resistance is usually caused by embB gene mutations at embB306 in several

studies [84]. A secondary method of resistance involving katG gene mutations has been proposed, showing how resistance mechanisms are interrelated but physiological mechanism of resistance is still needing further trials [85].

Pyrazinamide (PZA): Pyrazinamide targets acidic semi-dormant bacteria as an anti-TB prodrug since 1952. When activated by bacterial pyrazinamidase or nicotinamidase, it produces pyrazinoic acid, affecting cell membrane potential at acidic pH and ATP generation. PZA kills low-energy semi-dormant bacteria by reducing cellular energy. PZA reduces uracil and methionine absorption, inhibiting RNA and protein synthesis. Beyond pncA, mutations in the rpsA and panD genes have been linked to PZA resistance, complicating the genetics of resistance. MTB also possesses PZA resistance due to the ATP-dependent ATPase gene clpC1. The presence of several genes makes PZA resistance difficult, requiring a thorough genetic understanding to design tailored therapy techniques and surveillance measures against resistant strains. Research is still being conducted to better understand the molecular details of PZA resistance, which could lead to solutions for reducing the difficulties associated with medication resistance in the treatment of TB [86-89].

Streptomycin: Since 1948, streptomycin has been used to treat TB. As an aminocyclitol glycoside, it works against actively proliferating bacteria but not latent or host cell bacteria [90]. Streptomycin works in stages to perform antibacterial action. Initially, it establishes ionic connections with bacterial cell surfaces to enter the periplasmic region. It enters the cytoplasm via membrane channels using proton motive forces. After entering the 30S ribosomal subunit, streptomycin binds to the 16S rRNA and K45 residue in the S12 protein. This binding impairs protein synthesis elongation, initiation, and codon reading precision. Since translational proofreading is disrupted, bacterial cell protein synthesis is reduced, resulting in bactericide. Understanding these molecular interactions optimizes Streptomycin's therapeutic use and helps build effective bacterial infection protection measures [91, 92]. In resistant strains, a deletion produced a frameshift mutation in the ORF sequence of whiB7, a gene that activates efflux pumps. [31]. TA (toxin-antitoxin) systems are involved in streptomycin tolerance, which parallels hunger. MTB may use numerous basic stress pathways to address various pressures [93].

Resistance to second line TB drugs: Second-line medications have been launched due to rifampicin and isoniazid resistance. Fluoroquinolones, aminoglycosides, para-aminosalicylic acid, cycloserine/terizidone.

Fluoroquinolones: Fluoroquinolones, including ciprofloxacin and ofloxacin, are used as second line drugs for treating MDR-TB. These compounds, synthesized from nalidixic acid, a by-product of the antimalarial chloroquine, exert their antibacterial effects by inhibiting the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV in bacteria. When just type II topoisomerase is found in MTB, it turns out to be the potential target for fluoroquinolones. Fluoroquinolone resistance in MTB is typically caused by chromosomal mutations in the quinolone sensitivity-determining region of gyrA or gyrB. The most prevalent gyrA mutations have been identified at positions 90 and 94, however mutations at locations 74, 88, and 91 have been identified as well [94].

Aminoglycosides: Aminoglycosides (AGs) disrupt bacterial protein synthesis by binding to ribosomal RNA. Prolonged exposure to AGs prompts the overexpression of the regulatory gene whiB7 in MTB. This, in turn, upregulates genes associated with drug efflux (like tap) and ribosomal protection (including eis). Consequently, the heightened expression of these genes diminishes the sensitivity of TB bacteria to commonly used AGs such as amikacin, kanamycin, capreomycin, and streptomycin [44, 95, 96]. SigA demonstrates a potential role within MTB by forming a complex with WhiB7 at the SigA promoter and engaging with the DevR/DosR dormancy regulon. Particularly evident under hypoxic and chemical stress conditions, this interaction instigates distinct physiological responses. These responses include metabolic rate reduction, growth pausing, and metabolic pathway alterations. These alterations impair drug response. The specificity of these effects under hypoxic and chemical stress settings shows SigA's complex role in bacterial response to difficult environments. This intricate control by SigA may affect antibiotic susceptibility of the bacterium [49,97,98].

P-Aminosalicylic Acid (PAS): Para aminosalicylic acid (PAS), launched in 1946 as

an anti-TB medication, was used alongside isoniazid and streptomycin but discontinued due to extreme toxicity and the availability of more effective and less toxic treatments. PAS, a prodrug, targeting the folate-dependent thymidylate synthase enzyme in thymine biosynthesis. When activated, it competes with natural hydroxyl dihydrofolate, affecting folate metabolism. This interference inhibits cell iron intake, killing bacteria [99].

Many studies have found that the thyA gene, which encodes thymidylate synthase needed to generate dTTP, is the most frequently altered gene linked to para-aminosalicylic acid resistance [100-102]. The most common para-aminosalicylic acid resistance mutation in the thyA gene is 202ACC to GCC. Mutations V261G, V263I, and R127L were found in catalytic site of the enzyme [103]. MTB isolates from labs have missense mutations in the 1464-bp folC gene, which encodes FolC-dihydrofolate synthase/folylpolyglutamate synthase and is related with para-aminosalicylic acid resistance [104]. Bifunctional enzyme FolC transforms folates into polyglutamate derivatives needed for bacterial biosynthesis. Five of 85 MDR-TB clinical isolates resistant to para-aminosalicylic acid had folC mutations [105]. Furthermore, increased expression of the folate metabolism-related enzyme dihydrofolate reductase (DHFR) has been connected to PAS resistance [99].

Ethionamide: Like isoniazid, ethionamide functions as a prodrug in the treatment of TB, particularly MDR-TB. It is a second-line antibiotic. It inhibits the InhA enzyme, which is essential for the formation of mycolic acid in the MTB cell wall, but only after intracellular activation. The disruption of mycolic acid synthesis weakens the structural integrity of cell wall, which eventually leads to the death of the bacterium. Ethionamide is frequently used as a component of a combination therapy for the efficient management of MDR-TB in order to maximize efficacy and reduce the risk of resistance [106]. Treatment difficulties may arise from ethionamide resistance. Resistance is caused by mutations in important genes such inhA, which codes for the target enzyme, and ethA, which activates ethionamide. Because mutations in the katG gene, which are associated to isoniazid resistance, can also confer resistance to ethionamide, cross-resistance with isoniazid may arise [107].

Cycloserine/Terizidone: Since the late 1950s, cyclopenrine, a cyclic analog of D-alanine, has been used in TB therapy. It is a component of the Terizidone formulation and works by inhibiting the formation of bacterial cell walls by specifically targeting enzymes that are essential for peptidoglycan synthesis, such as alanine racemase (AlrA) and D-alanine ligase. The medication prevents bacteria from manufacturing their cell walls by interfering with these mechanisms [108-110]. Additionally, cyclorine inhibits the activities of alanine permease and D-alanyl-D-alanine synthetase, weakening the bacterial cell wall and causing bacterial cell death [111]. Reactions with cycloserine are caused by mutations in the alr gene, specifically D344N. There have been other reports of mutations in related genes associated to lipid metabolism, transport systems, and stress response. Analogous to furanose sugar, cyclopentrine enters cells via the furanose transporter, which is represented by the sugI gene. Resistance results from sugI mutations that prevent cycloserine entry, such as Q6stop [112]. A potential reason for resistance to cycloserine and its equivalent terizidone is the potential loss of function associated with the ald gene, which is responsible for encoding alanine dehydrogenase [113].

Resistance to third-line TB drugs: When treating patients with extensively drug-resistant tuberculosis (XDR-TB), a combination of third-line anti-TB medications is used that is described as follows.

Bedaquiline: Bedaquiline, previously TMC207/R207910, is a new diarylquinoline antibiotic with selective MTB action and in vitro efficacy against non-tuberculous mycobacteria. Bedaquiline reduces mouse organ bacterial burden in tests, showing its potential as a standalone treatment and in conjunction with RIF, PZA, and isoniazid. Such combinations have been shown to abbreviate treatment to two months, making bedaquiline a promising TB medication [114]. It targets ATP synthase of MTB, affecting the F-ATP synthase enzyme and reducing bacterial cell ATP levels [115]. Studying drug-resistant mutants of MTB and M. smegmatis revealed a single mutation in the atpE gene, which encodes the c portion of the F0 subunit of ATP synthase. This mutation revealed mechanism of resistance of bedaquiline. High-level Bedaquiline resistance is linked to mutations in the atpE gene, notably A63P and I66M, which encodes the C subunit of ATP

synthase. Mutations raise the minimal inhibitory concentration by 16–128 times [116]. Mutations in genes like Rv0678 and pepQ can cause bedaquiline resistance through non-target-based mechanisms, increasing MICs by 2 to 8 times [117]. MmpL5 and MmpS5 are negatively regulated by transcriptional regulator Rv0678. Mutations in Rv0678 upregulate MmpL5 and MmpS5, causing drug resistance and bedaquiline resistance [118].

Nitroimidazoles: Nitroimidazoles delamanid and pretomanid were originally known as OPC67683 and PA-824. Being prodrugs triggered by MTB's reductive metabolism, Delamanid and pretomanid work against MTB strains that are susceptible or resistant to traditional anti-TB drugs. Mycobacterial F420-dependent reductase coenzyme system generates active free radicals [119]. Delamanid inhibits methoxy and keto-mycolic acid production in MTB cell walls. These acids are essential for cell wall structure. Delamanid targets this biosynthetic process and has powerful antimycobacterial activity, making it a useful TB therapy [120]. Activating pretomanid reduces mycolic acid formation, making it hazardous to bacteria. Disrupting protein and lipid synthesis kills bacteria. Pretomanid produces reactive nitrogen species (RNS), which destroy bacterial cells [121,122].

Lack of a glucose-6-phosphate dehydrogenase enzyme or mutations in critical genes like ddn (which encodes the F420-dependent nitroreductase enzyme), fgd1, fbiA, fbiB, and fbiC can cause pretomanid and delamanid resistance [117, 123]. Delamanid-resistant genes also cause pretomanid resistance. Animal research found mutations in Rv2983 that cause pretomanid resistance [124].

Linezolid: Linezolid is a synthetic oxazolidinone antibiotic first licensed in 2000 for drug-resistant gram-positive bacterial infections, has shown effectiveness in laboratory and animal trials against drug-resistant strains of the MTB complex. Now categorized as a Group A drug, it is included in modern short-course regimens for most patients. However, its use is often constrained by associated toxicities, particularly hematologic and neurologic side effects [125]. Linezolid suppresses bacterial protein synthesis by preventing the synthesis of 70S initiation complex by binding to the 50S ribosomal subunit at its contact with the 30S unit. The anticipated development of linezolid resistance is gradual and sluggish [126].

Linezolid resistance is frequently associated with particular mutations, such as T460C in rplC and G2576T, G2447T, and G2061T in 23S rRNA, which contribute to resistance [127]. Ribosomal protein L3 (rplC) gene mutations like C154R are prevalent. Rare rrl and rplD gene mutations have been found [128]. Linezolid is aggressively removed from bacterial cells by efflux pumps, making XDR-TB isolates resistant to it. These molecular transporters remove antibiotics before they can work. This process allows bacteria to avoid inhibitory effects of linezolid, reducing medication efficacy and causing resistance [126].

Clofazimine: Clofazimine (CFZ), a fat-soluble riminophenazine dye, affects ion transporters and the respiratory chain by targeting outer membranes of bacteria. As an artificial electron acceptor, CFZ auto-oxidation oxidizes NADH and reduces ATP while creating ROS that kill bacterial cells. Interferon-gamma and CFZ synergistically boost anti-TB effects. CFZ may kill MTB with PZA and CLM. CFZ also reduces MTB-derived components' inhibitory influence on phagocyte intracellular killing pathways, potentially enhancing MTB killing. Recent data reveal that CFZ, PZA, and CLM may kill MTB synergistically [129, 130].

Mutations in the rv0678 gene and enhanced MmpL5 expression cause clofazimine resistance. Some patients feature rv1979c or rv2535c gene mutations [131,132]. A large majority of clofazimine-resistant mutants have rv0678 gene mutations. Rv0678 regulates the multi-substrate efflux pumps MmpS5 and MmpL5 by encoding a transcriptional repressor [131-134]. Mutations in rv0678 give Mycobacterium TB isolates clofazimine and bedaquiline resistance [135]. Even in strains without the rv0678 mutation, clofazimine resistance is linked to rv1979c and rv2535c mutations. The roles of rv1979c, perhaps amino acid transporters with permease activity, and Rv2535c, a peptidase, are unclear. Further research is needed to determine how rv1979c and rv2535c contribute to clofazimine resistance, highlighting the complex chemical pathways involved [132]. Standard short-course chemotherapy, which uses economical and safe first-line medications, is likely to prevent resistance in a population of patients, most of whom have drug-sensitive TB [136].

Table 1. List of First-Line Drugs

First-Line Drugs		
Pharmacological Agent	**Mechanism of Action**	**Resistance Targeting Genes**
Isoniazid	Inhibits mycolic acid synthesis	katG, inhA, kasA, NDH, ahpC,
Rifampicin	Inhibits RNA synthesis	rpoB, rpoA, rpoC
Pyrazinamide	Disrupts membrane potential	pncA, rpsA, panD, clpC1
Ethambutol	Inhibits arabinogalactan biosynthesis	embB, embA, embC, katG
	Second-Line Drugs	
Aminoglycosides	Inhibits protein synthesis	whiB7, eis, SigA
Fluoroquinolones	Inhibits DNA gyrase and topoisomerase	gyrA, gyrB
P-Aminosalicylic Acid	Inhibit biosynthesis of thymine, disrupting folate metabolism	thyA, folC
Ethionamide	Inhibits mycolic acid synthesis, disrupts membrane potential	ethA, InhA, katG
Cycloserine/Terizidone	Disrupts bacterial cell wall synthesis	alr, sugI, ald
	Third-Line Drugs	
Bedaquiline	Inhibits ATP synthesis	atpE, pepQ, Rv0678
Delamanid	Inhibits ATP synthesis	ddn, fgd1, fbiA, fbiB, fbiC
Pretomanid	Inhibits mycolic acid synthesis, produces RNS	ddn, fgd1, fbiA, fbiB, fbiC
Linezolid	Inhibits protein synthesis	rplC, rrl, rplD
Clofazimine	Targets bacterial cell membrane and respiratory chain	rv0678, rv1979c, rv2535c

6. CONCLUSION AND FUTURE DIRECTION

The review covers the molecular and clinical aspects of first, second, and third-line TB treatment resistance. It shows how MTB overcomes therapeutic difficulties, from first-line cell wall synthesis medicines to emerging third-line therapy. It shows how TB bacteria resist current treatments and the difficulty of TB treatment. Effective treatments are needed due to the rise of drug-resistant MTB strains and low treatment success rates worldwide. New anti-mycobacterial drugs are scarcer and drug-resistant isolates have emerged, ending the "antibiotics era." Mycobacterium species, including MTB, have distinct intrinsic and acquired drug resistance mechanisms, requiring new treatments.

Adapting antibiotics and finding new sources from undiscovered ecosystems that target novel pathways and counter resistance mechanisms may help treat drug-resistant Mycobacterium species. Recent advances in genetics and pulmonary biology have increased our understanding of Mycobacteria antibiotic resistance mechanisms. To completely understand antibiotic resistance in context of molecular mechanisms in these bacteria, more research is needed. Designing drugs that target several drug-resistant genes or pathways may help fight drug resistance. Personalized therapies using phytochemicals alone or with antibiotics are possible. Due to the growing diversity of Mycobacterium TB strains, new immune system-boosting techniques are needed. Emerging immunotherapies and gene manipulation methods are needed for direct bacterial targeting, medication efficacy, and immune response enhancement.

Nanoparticle-targeted drug-gene co-delivery to host cells may help fight resistant strains. Polymeric nanoparticles may become a preferred non-viral gene delivery technology for microbial infections. Precision medicine, customized to individual resistance profiles, could develop medications that directly target drug resistance mutations. Researchers are also studying host-directed treatments to improve TB immune system regulation. Understanding resistance mechanisms is a cornerstone for effective and sustained TB control. Based on this understanding, preventive, early identification, and individualized treatment techniques must be created. Our only hope of stopping drug-resistant TB, improving patient outcomes, and ending this long-standing disease is this method.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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