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Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

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The challenges in combating multidrug-resistant *Mycobacterium tuberculosis*

Review article

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DOI: 10.21608/jmals.2024.372569

Abstract

The essential aim of the current investigation is to focus on how Mycobacterium TB, the bacteria that causes tuberculosis, becomes resistant to treatment. This review highlights the following crucial points: Introduction to the incidence of TB and its relevance as a worldwide health concern, particularly with the introduction of multidrug-resistant forms. *Mycobacterium tuberculosis* has various mechanisms that allow it to resist anti-TB medications. These include an impermeable cell wall, a slow metabolism, and the presence of drug excretion pumps. The transmission of the infection and the risk factors that increase the likelihood of developing tuberculosis are detailed. An in-depth examination into how molecules develop resistance to some of the most used TB medications, such as isoniazid, rifampicin, pyrazinamide, ethambutol, fluoroquinolones, and aminoglycosides, as well as genetic alterations associated with resistance. Discuss the need to use more than one medicine to treat tuberculosis so that resistance does not develop. This refers to the present tendency in research to discover novel compounds capable of killing drug-resistant strains.

In summary, this review covers molecular and cellular drug resistance mechanisms in M. tuberculosis, focusing on the most important tuberculosis drugs and novel treatment methods.

Keywords: *Mycobacterium tuberculosis*, multidrug-resistant, tuberculosis, anti-TB medications.

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1. Introduction:

M. tuberculosis bacteria, which results in tuberculosis, has resistance to drugs due to various characteristics. This bacteria impacts different parts of the body such as the kidney, spine, and brain in addition to the lungs. Those who are infected with TB do not always get ill. Thus, latent tuberculosis infection (LTBI) and tuberculosis sickness are the two conditions associated with TB. TB may lead to death if it is not treated (1,2). Several research have been performed to clarify the drug resistance strategies in this organism. Impenetrable cell wall and gene changes, that are related to *M. tuberculosis'* drug resistance, are taken into consideration in this research. It is indicated that various repeated mutations in different drug-resistance genes are responsible for M. tuberculosis. In addition, the reduction of metabolism in the extended dormant period leads to a significant increase in drug resistance. Efflux pumps and the waxy impermeable cell wall are necessary elements for drug resistance. Thus, understanding the molecular mechanisms that TB employs for acquiring antibiotic resistance is fundamental (3).

As is shown by the World Health Organization (WHO), TB represents the main infectious reason of death worldwide, especially, among those who suffer from HIV. Concerning the new TB cases,

Received: April 3, 2024. Accepted: July 14, 2024. Published: August 8, 2024

there were 10 million novel cases worldwide in 2018, 1.5 million cases of deaths, 484 thousand of rifampin-resistant TB, and 78% of multidrug-resistant TB cases **(1)**. Moreover, Iraq represents one of the countries in the World Health Organization's Eastern Mediterranean Region (WHO-EMR) which has over 38 million inhabitants and a TB prevalence of 42 cases per 100,000 people (4). Accordingly, Iraq is considered one of the top seven countries in the WHO-EMR where 3% of total TB cases occur **(5)**. Many investigations are done to study the different drug resistance processes finding new chemicals for effective TB treatment. This is done to maintain control over drug resistance (3). Various natural materials have been shown to exhibit efficacy against distinct resistant strains of Mycobacterium TB; however, alkaloids are particularly noteworthy in this regard **(6).** In 2009, Mo et al. discovered that Ambiguine produced from Fischerella ambigua effectively suppresses M. tuberculosis H37RV **(7).** Cryptolepine, an indoloquinoline alkaloid isolated from Cryptolepis sanguinolenta, has shown activity against M. fortuitum **(8).** Various species of mycobacteria are susceptible to the antibiotic effects of other alkaloids, such as indoles, pyrroles, carbazoles, indolo quinolines, manzamines, quinolines, and isoquinolines **(6).** One group of these compounds consists of quinolizidine alkaloids, which have exhibited anti-TB properties **(9).** According to Wink *et al.* (1984), sparteine, in particular, has shown bacteriostatic action against Mycobacterium phlei **(10).** Antimicrobial resistance develops naturally and irreversibly as a consequence of specific chromosomal alterations brought on by each drug **(2).**

2. How does TB spread? (11).

The TB microbes are transmitted from one individual to another via the air. By coughing, speaking, or singing, an individual with tuberculosis of the lungs or larynx can release TB bacteria into the atmosphere. People in the vicinity may become infected by inhaling these microorganisms. TB is not transmitted by:

- shaking someone's hand
- sharing food or drink
- touching bed linens or toilet seats
- sharing toothbrushes
- kissing **(11).**

The risk of tuberculosis infection increases if the bacteria are inhaled and then lodged in the lungs. They can also be moved through the blood to the kidney, spine, brain, throat, lungs, and some other organs. This means the bacteria may spread to other people. Although tuberculosis in other parts of the body, including the kidneys or spine, is usually not infectious, people who have the disease are more likely to spread it to those they are in close contact with every day. Members of the family, friends, coworkers, and classmates are included in this category **(12).**

3. Risk of TB infection:

Diseases may spread more easily in certain homes or places of employment than in others. These conditions increase the risk of tuberculosis infection **(13).**

- Living in or traveling through a nation that has a high prevalence of tuberculosis, which includes some countries in Latin America, Africa, Asia, and the Pacific Countries.
- Living or working in environments where individuals are confined to close quarters, such as jails, nursing homes, and shelters for homeless people, are examples of such environments.
- Living in an area that has been found to have a high risk of tuberculosis.
- Being employed in the medical field and serving patients who are at a high risk of contracting tuberculosis.

4. Causes of drug resistance in *M. tuberculosis* Resistance

M. tuberculosis has two main drug resistance types: primary, which is transmitted, and secondary, which is acquired. Primary resistance occurs in TB patients who have either never used antituberculosis drugs or have taken them for a very brief duration. There is a hypothesis that these individuals initially acquired drug-resistant bacteria, which would be considered new cases. Acquired resistance to anti-tuberculosis drugs occurs during treatment or in persons receiving therapy for at least one month **(14).** TB drug resistance is not new. Streptomycin-resistant *M. tuberculosis* emerged immediately after the antibiotic was licensed for therapy in 1944 for several reasons **(14).**

4.1. Impermeable Cell Wall

Antibiotics progressively accumulate surrounding the cell as a result of this impermeability. Different cellular components or the release of enzymes progressively detoxify the medications accumulated around the cell wall **(15).** Additionally, *M. tuberculosis* and *M. bovis* include the membrane channel protein CpnT, which serves the twin functions of selective sensitivity to antibacterial drugs and nutrition absorption. Research states that M. bovis's CpnT mutant is more resistant to most antitubercular drugs, including bactericidal nitric oxide, which treats mice with M. TB. **(16)**. According to Erik et al. (2015), M. tuberculosis cell wall comprises three primary components: mycolic acids, wax-D, and cord factors. To prevent hydrophobic substances from penetrating the cell wall, the hydrophilic arabinogalactan layer prevents this from happening. The hydrophobic mycolic acids that make up this layer also greatly restrict the movement of hydrophilic molecules **(16).**

4.2. Slow Metabolism Mechanism:

Even though a negative correlation between medication resistance and the organism's generation time has been shown. Nonetheless, it has been noted that *M. tuberculosis's* sluggish growth rate contributes significantly to its treatment resistance; for instance, unstable medicines like carbapenems lose their effectiveness more quickly than the mycobacterial growth rate **(17)**. Antibiotics work best against metabolically active, quickly replicating bacteria rather than those with slower metabolism and longer generation times **(18).**

4.3. Numerous Efflux Pumps:

It has also been shown that efflux pumps in *M. tuberculosis* may adapt to medication resistance. Via multidrug efflux pumps, drugs can exit cells by passing through both the inner and outer membranes (Knutson *et al*., 2004). It has been demonstrated that *M. tuberculosis* drug efflux pumps comprise regulatory protein systems. By regulating the formation of efflux pump regulatory protein systems, these systems are capable of influencing drug resistance **(19).**

5. Drug resistance of *M. tuberculosis*

5.1. Isoniazid

INH has been the mainstay of all successful treatment plans for latent infection and tuberculosis. INH has a high degree of susceptibility to *M. tuberculosis* (minimum inhibitory concentration [MIC] 0.02–0.2 µg/ml). INH only works against tubercle bacilli that are growing **(20).** The katG gene encodes the catalase-peroxidase enzyme, which activates the prodrug INH **(21)(22).** This enzyme generates some highly reactive compounds that assault different sites in *M. tuberculosis* **(23).** KatG and inhA mutations are found in most isoniazid-resistant Mtb strains, although the mechanism of action is unclear **(24)**. Isoniazid-resistant strains of KatG had a higher frequency of S315T mutations. So, the synthesis of isoniazid products with low affinity will be increased with the mentioned mutation **(25).** Furthermore, the changes in inhA's active site prevent it from binding the drugs effectively. For instance, Ethionamide is the drug that employed the inhA as their target site **(26).**

Moreover, there are two essential drugs for TB treatment isoniazid and rifampicin. Although isoniazid is inert, it will be pharmacologically active when it is subjected to metabolic processes in the body **(20).**

5.2. Rifampicin (RMP)

For treating TB, RMP is regarded as one of the important first-line drugs. The RMP with minimum inhibitory concentration against *M. tuberculosis* is measured in liquid and solid medium ranging from 0.05 to 1 µg/ml. another advantage of RMP is that its sterilizing activity in vivo influences the treatment of TB by reducing it from 12-18 months to 9 months **(27).** RMP can bind the beta subunit of RNA polymerase and obstruct the synthesis of RNA. Together with the βsubunit, the RNA polymerase's core enzyme, an oligomer consisting of four α2Hββ′ chains, accurately initiates transcription from promoters **(28).** The elongation of the RNA chain is physically inhibited by the RMP-binding site, which is situated upstream of the catalytic center. Resistance to RMP is observed in *M. tuberculosis* at a rate of $10^{-7}$to 10^{-8} **(29).** It has been shown that rifampicin resistance in *M. tuberculosis* is caused by mutations in RNA polymerase's rpoB, which delays the drug's affinity **(30).** Certain investigations have successfully identified particular codons in which mutations can lead to the development of rifampicin resistance **(31)(32).** The presence of RMP-dependent *M. tuberculosis* strains in clinical settings is fascinating and concerning.59,60 Despite growing poorly on egg-based media, some strains developed better on RMP. There are some similarities between RMP-dependent strains and L-form bacteria **(33)(34).**

5.3. Pyrazinamide (PZA)

PZA is a first-line medication like INH and RMP. PZA's unique ability to remove a particular population of persister bacilli in the lesions' acidic pH environment reduces TB therapy from 9-12 months to 6 months **(35)(36).** PZA is an unusual and paradoxical antituberculosis medication that, according to Zhang and Mitchison (2003), has a significant sterilizing action in vivo but, in normal culture conditions, is inert against tubercle bacilli at pH values close to neutral **(37).** Only at acid pH levels, such as 5.5, is PZA effective against *M. tuberculosis* **(38).** With MICs ranging from 6.25 to 50 µg/ml, PZA activity is very low, even at an acid pH of 5.5 **(39).** The activity of PZA is enhanced in anaerobic or low-oxygen environments **(40).** Additionally, medications that interfere with the energy status of the plasma membrane, including weak acids, energy inhibitors (DCCD), azide, and rotenone **(41)**, and rotenone, are capable of disrupting PZA activity. The pncA gene of Mycobacterium tuberculosis encodes the pyrazinamidase/nicotinamidase enzyme, which is necessary for the conversion of the prodrug PZA to the active form POA **(36).** Extracellular acid pH encourages the production of uncharged protonated POA in *M. tuberculosis*, which in turn disturbs membrane potential by penetrating the membrane **(42).**

5.4. Ethambutol (EMB):

To prevent drug resistance, EMB [(S, S′)-2,2′(ethylenediamine)di-1-butanol] is used first-line with INH, RMP, and PZA. The bacteriostatic EMB develops bacilli but does not affect non-replicating ones. EMB prevents cell wall arabinogalactan production **(43).** It stops the polymerization of arabinan in cell walls, which are made up of arabinogalactan and lipoarabinomannan. It also causes D-arabinofuranosyl-P-decaprenol to build up, a step in producing arabinan **(44)(45).** Ethambutol exerts its mechanism of action by impeding the development of cell walls in *M. tuberculosis.* One potential alternative explanation for the mechanism of action of ethambutol is its ability to impede the synthesis of spermidine **(46).** Argyrou et al. (2006) found that RNA metabolism is disrupted when mycolic acid transport to the cell membrane is blocked, and phospholipid synthesis is

suppressed **(47)(48).** According to Safi et al. (2008), a significant increase in ethambutol resistance was caused by a mutation in ubiA (Rv3806c). Deretic et al. found ubiA mutations in every Mtb they studied that was resistant to ethambutol in 1995. On the other hand, Kapata et al. (2016) found that the geographic location affects the ubiA mutations in *M. tuberculosis* **(49).**

5.5. Fluoroquinolones (FQ)

To fulfill the demands of transcription and replication, topoisomerases in cells control DNA supercoiling and unlink knotted nucleic acid strands. FQs cause microbial mortality in the majority of bacterial species by inhibiting DNA gyrase (topoisomerase II) and topoisomerase IV. The protein DNA gyrase is a tetrameric A2B2 **(50).** Findings indicate that the development of FQ resistance in *M. tuberculosis* relies on a conserved area known as the quinolone-resistance-determining region (QRDR) of gyrA and gyrB **(51).** It has also been shown that the frequency of mutations conferring FQ resistance in *M. tuberculosis* and the distribution of resistance alleles selected may be affected by the concentration of FQ. Selection at low FQ concentrations resulted in the development of several mutants with modest levels of resistance **(52).** It was shown that high doses of FQ restricted the variation to a few kinds; the mutant prevention concentration is the concentration at which no mutant was created **(53).**

5.6. Aminoglycosides (SM)

Aminoglycosides impede the synthesis of new proteins by impeding the translation of the mRNA message through their binding to the 30S subunit of the bacterial ribosome **(54).** SM operates at the 16S ribosomal RNA and the ribosomal protein S12-composed 30S subunit of the ribosome. Alterations in the rpsL gene encode the S12 protein, whereas the rrs gene encodes the 16S rRNA.SM resistance is mostly caused by changes in rpsL and rrs, which make up about 50% and 20% of SM-resistant strains, respectively **(5).** Both kanamycin (KM) and its derivative amikacin (AMK) may inhibit protein synthesis by altering ribosomal structures in the 16S ribosomal region. Mutations at 16S rRNA (rrs) position 1400 provide considerable KM and AMK resistance **(55).**

This has traditionally been true for every new anti-tuberculosis drug introduced in monotherapy, to prevent the development of resistance. A way to solve this problem was to use combination therapy with drugs that targeted different genetic regions. Nevertheless, some patients developed resistance to isoniazid when any drug was administered in conjunction with it, contingent on the drug's capacity to inhibit bacterial proliferation and, consequently, the development of isoniazid-resistant variants; the effectiveness of drugs could be ranked according to this capability. The absence of treatment failure and total inhibition of bacterial growth were observed when the efficacy of the drugs was enhanced through the use of combinations consisting of three to four drugs. Consequently, even potent new agents must be incorporated into a combination regimen to avert treatment failure and resistance development **(56).** Avermectins and other broad-spectrum antihelminthic drugs have demonstrated encouraging in vitro antimicrobial efficacy against *M. tuberculosis* **(57).**

The intracellular *M. tuberculosis* was effectively inhibited by benzohydroxamate in conjunction with transition metals like Cu2+ and Co2+.

Nevertheless, to rule out harmful effects on human cells and comprehend how they work, more study is necessary **(58).** As the efficacy of repurposing anti-infectives to target the pathogen has been constrained, efforts are being directed toward the evaluation of antitubercular therapies utilizing pharmaceuticals not previously employed for infection treatment, including anticancer and antipsychotic medications **(59).** This study has already found many potential antituberculosis drugs. Prominent antimicrobial agents such as eltrombopag

and fluvastatin exhibit significant antimicrobial properties both in vitro and during infection. This is likely attributed to the inhibition of the Zmp1 and PDF proteins of *M. tuberculosis* **(60)**. Furthermore, significant adverse effects accompany the antimicrobial activity of many antipsychotic medications, which have been observed exclusively at high concentrations. Nonetheless, many phenothiazine derivatives that are non-neuroleptic have shown antibacterial effectiveness against various pathogens, such as *M. tuberculosis*, both in vitro and in vivo, without producing unfavorable side effects **(61)**.

Conclusion

Mycobacterium tuberculosis drug resistance threatens global tuberculosis control efforts. This review shows that bacteria's impermeable cell wall, slow metabolism, and various drug excretion pumps contribute to their complicated resistance mechanisms. Resistance is mostly caused by gene changes that impact medication action sites. Therefore, numerous medicines must be used to treat tuberculosis to avoid resistance. We need more research to find effective chemicals, especially against extremely resistant strains of several medications that threaten public health. Understanding resistance pathways is essential to creating better TB treatment and control tactics.

Conflicts of Interest:

The authors declare no conflict of interest.

Fund:

No funds, grants, or other support were received.

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