Chapter

Challenges in Drug Discovery against Tuberculosis

Manish Dwivedi and Priya Giri

Abstract

Tuberculosis (TB) is one of the deadly diseases in the present era caused by *Mycobacterium tuberculosis*. Principally, this bacterium attacks the lungs, however, MTB Has been observed affecting any part of the human body including the kidney, spine, and brain. Drug-resistant progression and other associated properties of MTB become a major hurdle in drug discovery to fight against tuberculosis. Moreover, some of the challenging situations such as the low range of chemical agents, the time-consuming process of drug development, the shortage of predictive animal models, and inadequate information of the physicochemical evidence required for effective bacterial penetration, are additional hindrances for the pharmaceutical scientist. In the current chapter, we focus on challenges encountered during drug discovery and need to be overcome as *M. tuberculosis* has a substantial barrier in its lipid-containing cell wall to inhibit the influx of drugs which is the initial requirement of the drug to show its therapeutic effect. There is also an immediate need for efficient vaccine development which may show its effect on adolescents and adults along with infants. Investigation on key bacterial targets has been troublesome, in light of the vulnerability around the microenvironments found in vivo and subsequently, the importance of exceptional metabolic pathways. The manuscript is prepared after the extensive literature survey to explore the vigorous approaches in novel drug designing and in proposing potent drug targets. The re-engineering and repositioning of prominent antitubercular drugs are required to attain viable control.

Keywords: *Mycobacterium tuberculosis*, drug, challenges, bacterial targets

1. Introduction

Tuberculosis (TB), one of the most common deadly disease is caused by a bacterium called *Mycobacterium tuberculosis*. Robert Koch in 1882, isolated the mammalian strain and proved that the *Mycobacterium tuberculosis* plays a causative role in Tuberculosis. As per the latest WHO report approximately one-fourth of the world's population are infected with *Mycobacterium tuberculosis* (Mtb), whereas 5–10% of the total will develop TB disease during their lifetime [1, 2]. The WHO estimated that in 2018, about 10 million people were affected due to TB worldwide and 1.5 million people suffering from the ailment, including 2,51,000 people who additionally had HIV [3, 4]. In the past, TB was a major reason for death around the globe [5, 6]. In industrialized nations, TB is getting slow due to vast development and improvements in drugs and new antibiotics [5, 7].

TB may exist in two forms, active (dynamic) TB and Latent TB. Dynamic tuberculosis is a condition where MTB causes contamination; regularly, in the lungs, albeit numerous frameworks can be included. Dynamic TB is a multiorgan illness brought about by essential disease or as reactivation of inert tuberculosis. As need be, dynamic tuberculosis could be essential tuberculosis or reactivation tuberculosis.

Latent TB happens when an individual has the TB microscopic organisms inside their body, however, the microbes are available in tiny numbers. They are monitored by the body's safe framework and do not bring on any indications. Individuals with idle TB do not feel wiped out and are not irresistible. They cannot give the TB microscopic organisms to others. Moreover, they will generally have an ordinary chest x-ray and a negative sputum test. It is regularly just realized that somebody has latent TB since they have had a TB test, for example, the TB skin test. There are two kinds of test that can be utilized. These are the TB skin test (TST) and the fresher IGRA blood test. In nations where there is a significant degree of TB, (for example, the high weight TB nations) most individuals may have latent TB.

Fortunately, most of the TB patients have latent infection i.e., bacteria are present in the body but is not causing active disease. Hence at any one time, there are about 10 million people across the world with active tuberculosis infection and that causes deaths in about 10% of them. So, approximately there are 1 million deaths per year due to tuberculosis [8, 9]. The Mycobacteria principally target the lungs, moreover, it has been observed that *M. tuberculosis* may also reach and affect other parts of the body, such as the kidney, spine, and brain. A few people get tuberculosis ailment long after getting contaminated, even before their immune system can battle against the TB bacteria. Others may get the ailment years after the fact when their immune system gets frail for some other reason [8, 10].

Tuberculosis possesses a genuine risk to human wellbeing and one of the main reasons for significant human demise on the planet. Moreover, the emergence of drug resistance and its relationship with HIV infections have intensified worldwide circumstances. Unfortunately, despite advanced modalities for diagnosis and treatment of TB, people are still suffering a lot. There are specific properties associated with MTB that has presented vast challenges to develop an efficient drug against Tuberculosis [11]. The major obstacles in TB treatment like screening of compounds with anti-tubercular activity, the long duration medication, the lack of predictive animal models, and insufficient information on the physico-synthetic properties required for successful bacterial penetration [12], are being encountered by the pharmaceutical scientist.

The danger of creating dynamic (active) Tuberculosis ascends to 30% in diabetes victims. Usually, 80% - 90% of the patient having an infection of drug-resistant tuberculosis are relieved by taking concentrated anti-toxin treatment [2]. However, treatment by antibiotics is dependent on a load of drug-resistant *M. tuberculosis* in the patient [13]. Therapy of anti-drug or multidrug-resistant Tuberculosis (MDRTB: impervious to isoniazid and rifampin) is increasingly perplexing and takes nearly 2 years of chemotherapy amalgamation [14, 15]. Thus, progressively viable medicines are necessary to avoid or the emergence of tuberculosis. Treatment of the significant levels of drug-resistant *Mycobacterium tuberculosis* contamination, which incorporate rifampin-resistant (Rif-TB), MDR-TB, and extensively resistant TB (XDR-TB) requires new medications method and approaches to combat [5, 10]. The development of new methods of treatment is a complex process as anti-tuberculosis drugs are mostly given in combination to inhibit the further emergence of drug-resistant TB [14]. Moreover, dormant TB has also been observed in many people in which TB is not in a dynamic position and do not show any symptoms in a patient [16] whereas dynamic TB happens when the body cannot possess the

TB pathogen but at this condition, the bacteria can reproduce and cause wanted symptoms and people with dynamic TB can spread the contamination [9, 15]. In certain condition, some MTB strains are not affected by the treatment method and hard to treat tuberculosis [17, 18].

2. Need of research on new TB vaccine

In recent decades, advanced diagnosis and treatment method of TB has reduced the mortality rate up to significant level but TB still exists in world population causing extensive human suffering, economic burden led to global inequity. There are neonatal BCG vaccines that can prevent infants and young children from severe forms of TB but this vaccine is unable to show its effect in adolescents and adults who are crucial in TB transmission. We need to develop new efficient vaccines which could work in all age group people that may assist to fulfill the WHO end TB strategy that aims to reduce the TB mortality and TB incidences by 95% and 90% respectively worldwide.

Now, WHO is putting much efforts to produce TB vaccines and the Product Development for Vaccines Advisory Committee (PDVAC) is asking to develop a WHO preferred Product Characteristics (PPC) for new TB vaccines. The WHO's PPC data was established to document the crucial and priority requirements for vaccines which may show better safety and efficacy compared to BCG vaccine which is given to neonates and infants against pulmonary TB in adults, and new TB vaccines.

The major vaccine platforms like whole-cell vaccines, adjuvanted proteins, and recombinant subunit vector vaccines, are being considered in the pipeline of TB vaccine development. Now focus is on TB treatment in adolescents and adults by developing an effective candidate vaccine that may also replace the BCG in early life immunization. Many other aspects are in consideration in vaccine development, such as BCG boosters, reduction of treatment period using immunotherapeutic adjuncts and vaccine to prevent diseases reoccurrence in TB patient.

In recent developments, as per WHO report, there is TB vaccine candidate ($M72/AS01_E$) developed by the pharmaceutical company GlaxoSmithKline, in partnership with AERAS and was observed substantially effective against Tuberculosis disease and these results came out in a Phase IIb trial carried out in Kenya, South Africa and Zambia in patients having latent tuberculosis. This vaccine was found with 50% efficacy over about 3 years of continuous monitoring.

3. Globally situation of tuberculosis

According to the report of WHO, a sum of 1.4 million individuals passed on from TB in 2019 (counting 208,000 individuals with HIV). Around the world, TB is one of the top 10 reasons for death and the main source from a solitary irresistible specialist (above HIV/AIDS). In 2019, an expected 10 million individuals became sick with tuberculosis (TB) around the world. 5.6 million men, 3.2 million ladies and 1.2 million youngsters. In 2019, 1.2 million kids became sick with TB worldwide. The youngster and juvenile TB is frequently ignored by wellbeing suppliers and can be hard to analyze and treat. In 2019, the 30 high TB trouble nations represented 87% of new TB cases. Eight nations represent 66% of the aggregate, with India driving the tally, trailed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa. Multidrug-safe TB (MDR-TB) stays a general wellbeing emergency and a wellbeing security danger. A worldwide all out of 206 030 individuals with multidrug-or rifampicin-safe TB (MDR/RR-TB) were identified and told in 2019, a 10% expansion

from 186 883 out of 2018. Internationally, the TB rate is falling at about 2% each year and somewhere in the range of 2015 and 2019, the combined decrease was 9%. This was not exactly most of the way to the End TB Strategy achievement of a 20% decrease somewhere in the range of 2015 and 2020. An expected 60 million lives were saved through TB analysis and treatment somewhere in the range of 2000 and 2019. Finishing the TB plague by 2030 is among the wellbeing focuses of the United Nations Sustainable Development Goals (SDGs). Tuberculosis generally influences grown-ups in their most gainful years. Nonetheless, all age bunches are in danger. More than 95% of cases and passings are in non-industrial nations. Multidrug-resistant tuberculosis (MDR-TB) is a type of TB brought about by microbes that do not react to isoniazid and rifampicin, the 2 best first-line hostile to TB drugs. MDR-TB is treatable and reparable by utilizing second-line drugs. Nonetheless, second-line treatment choices are restricted and require broad chemotherapy (as long as 2 years of treatment) with meds that are costly and poisonous.

Sometimes, more serious medication opposition can create. TB brought about by microbes that do not react to the best second-line hostile to TB medications can leave patients with no further treatment alternatives.

In 2019, MDR-TB stays a general wellbeing emergency and a wellbeing security danger. A worldwide total of 206 030 individuals with multidrug-or rifampicin-safe TB (MDR/RR-TB) were identified and advised in 2019, a 10% increment from 186 883 out of 2018. About portion of the worldwide weight of MDR-TB is in 3 nations – India, China and the Russian Federation.

Around the world, just 57% of MDR-TB patients are presently effectively treated. In 2020, WHO suggested another more limited (9–11 months) and completely oral routine for patients with MDB-TB. This exploration has shown that patients think that it's simpler to finish the routine, contrasted and the more drawn-out regimens that last as long as 20 months. Protection from fluoroquinolones ought to be rejected preceding the commencement of treatment with this routine.

As per WHO rules, the discovery of MDR/RR-TB requires the bacteriological affirmation of TB and testing for drug obstruction utilizing quick sub-atomic tests, culture strategies or sequencing advancements. Treatment requires a course of second-line drugs for at any rate 9 months and as long as 20 months, upheld by advising and checking for unfavorable occasions. WHO prescribes extended admittance to every single oral routine. Before the finish of 2019, 89 nations began utilizing more limited MDR-TB regimens and 109 had imported or begun utilizing bedaquiline, with an end goal to improve the viability of MDR-TB treatment.

4. The course of events in *Mycobacterium tuberculosis*

Mycobacterium tuberculosis basically passes through the 5 stages during its life cycle. At the first stage, the bacteria are inhaled through the air and typically engulfed by alveolar macrophages, further proceed to the symbiosis stage and causing the caseous necrosis in later stages. Eventually spread to other cells and causing rapid spread of diseases. The whole cycle is presented in detail in **Figure 1** and as a flow chart in **Figure 2**. The Mycobacterium gets entry into the lungs and resides in the alveoli of the lungs while it begins its primary infection. If the immune system fails to eliminate it then there are three cases observed with the mycobacterium in the alveoli. The first case could be the elimination phase, in which the immune system completely eliminates the infection. The next one retention phase where the immune system suppresses the infection but the bacteria remain viable and, in this case, the infection is known as Latent Tuberculosis which is the most asymptomatic Tuberculosis. And the third phase may involve Active infection, which makes the

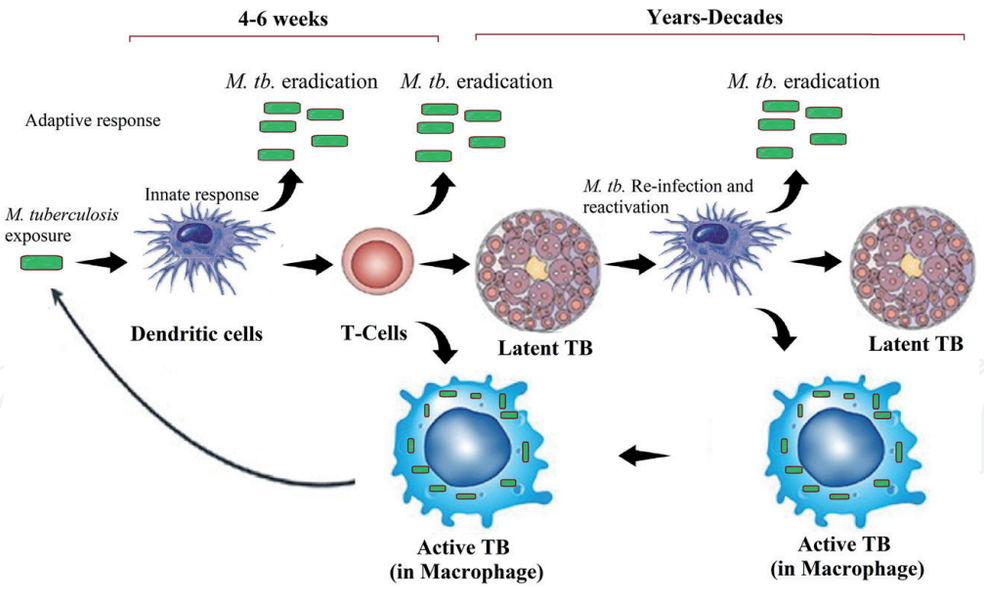

Figure 1.
Life cycle of Mycobacterium tuberculosis. This presentation is influenced with the figure available at online resource on study of the tuberculosis. (https://sites.google.com/site/mycobacteriumtbstudy/home/life-cycle-of-organism).

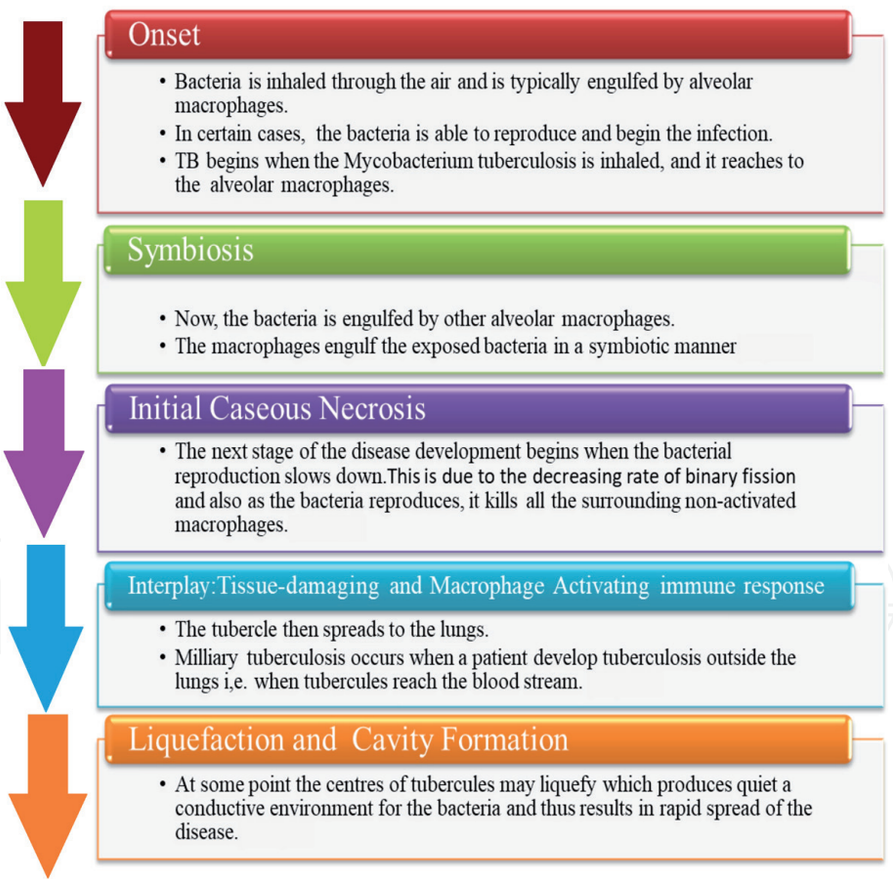

Figure 2.
Flow chart presentation of life cycle of Mycobacterium tuberculosis.

mycobacterium capable of evades the immune response and separates the infection in the lung tissue and at this point of active infection it is known as Active Tuberculosis [19–21].

M. tuberculosis has 5 stages in its life cycle as mentioned in **Figure 2** as flow chart [1, 2, 7, 22].

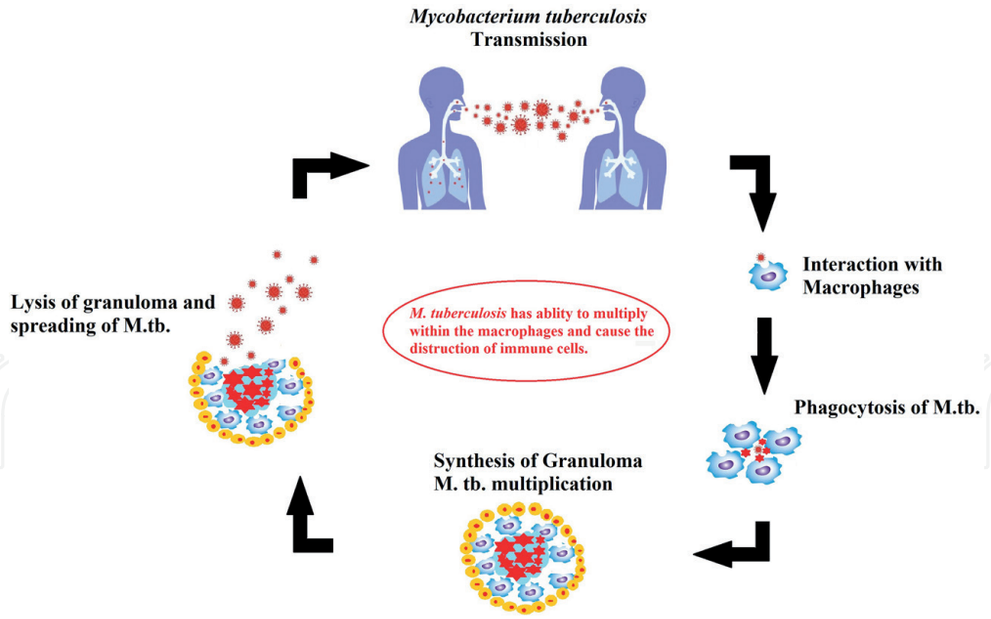

Figure 3.
Transmission of Mycobacterium tuberculosis. The representation is influenced with figure available in online resource. (https://www.istockphoto.com/in/vector/tuberculosis-life-cycle-of-mycobacterium-tuberculos-gm1200338165-343779875).

5. Pathogenesis and transmission of *Mycobacterium tuberculosis*

If somebody has active lung disease with TB they will cough and, in the cough, there would be infected droplets carrying the bacteria that could be inhaled by somebody else [8, 15]. Once the bacteria is inhaled it goes into the lungs and then it invades the normal mechanism for protecting lungs against bacterial infection which are the alveolar macrophages. It actively seeks out and invades these macrophages because it can prevent the normal macrophage killing mechanism. So, it diverts the normal figure lysosome pathways and that allows it to survive in the macrophage and it can be latent in that macrophage for decades [3]. Also, Macrophages because they move will allow the bacterium to spread bull RAC across the body and this is one of the reasons why sites of immune functions such as the lymph nodes often get infected with Tuberculosis and long-term persistence within the macrophages is led to latent diseases [18]. Besides, there is a certain inflammatory response to this infection which causes a very distinctive histologic appearance called granulomas and that is one of the hallmarks of Tuberculosis infection. Our closest infection is the presence of granulomas in the infected tissue [5, 6]. This transmission process is represented in **Figure 3**.

6. Mechanism of drug-resistant TB

This has been observed that various mechanism of drug resistance in M. tuberculosis is involved.

6.1 Presence of cell wall

The basic property leading to passive resistance to antibiotics in M. tuberculosis is because of its impervious cell wall [23]. The hydrophilic layer of arabinogalactan ensures the impervious nature of the cell wall to the surrounding hydrophobic substances. This layer is also present in hydrophobic mycolic acids which significantly

prevents the entry of hydrophilic molecules [24]. This impervious nature of the cell wall results in the deposition of antibiotics throughout the cell wall, the accumulated antibiotics near the cell wall are removed steadily by the release of enzyme & with the involvement of several cellular components [25]. It is demonstrated that β-lactams, which act as inhibitors to the inclusion of peptidoglycan (responsible for maintaining the rigidity of the cell wall) into the cell wall, are degraded by the mycobacteria due to the presence of β-lactamases, which are the enzyme responsible for degradation of β-lactam antibiotics. Danilchanka et al. [24], reported the presence of CpnT channel protein in the outer membrane of both M. tuberculosis and *M. bovis*, which plays a dual role in nutrient absorption and selective sensitivity to antibacterial agents.

6.2 Slow metabolism mechanism

Bacteria that have long-generation time & undergo metabolic processes with a slower rate are estimated to be challenging targets for most of the antibiotics i.e., bacteria that are metabolically active and rapidly replicating act as a good target for antibiotics [26]. However, in M. tuberculosis, it is still unclear whether the long generation time confirms its resistance to drugs. However, it is been reported that the slow growth rate of M. tuberculosis plays a crucial role in drug resistance. For example, antibiotics such as carbapenems lose their activity comparatively at a faster rate than the growth rate of M. tuberculosis [27]. It is seen that certain specific genes which are involved in the production of triacyl-glycerol permit the growth of M. tuberculosis even in oxygen-deprived conditions. Triacylglycerol decline in the metabolic processes of M. tuberculosis.

6.3 Possession of numerous efflux pumps

These protein channels play a vital role in the regulation of normal metabolism and the physiology of the organism such as toxins, signaling molecules through the cell wall, residues, and nutrient transport [28]. Efflux pumps have shown adaptation to drug resistance in M. tuberculosis. Multi-drug efflux pumps serve as an outlet for cell antibiotics and usually pass through both the inner and outer membranes of the cell [29]. Regulatory protein systems are present in Drug-efflux proteins which are responsible for controlling the expression of the efflux pump and thus helps in specializing them for drug resistance roles [28].

6.4 Mutation in genetic materials

It has been shown that the acquisition of antibiotics resistance in M. tuberculosis is the result of spontaneous mutation in several chromosomal genes. This frequent mutation has been found to cause a deliberate alteration to the required interaction between each drug against tuberculosis and its specified target.

M. tuberculosis shows resistance to rifampicin due to mutation in rpoB of RNA polymerase, decelerating its affinity for rifampicin [30]. It has been identified in certain studies that specific codons can cause resistance to rifampicin only with the onset of mutation in them [31, 32]. Resistance to pyrazinamide is due to mutation in the pncA gene [33, 34]. The mutations in pncA gene account for the large number of resistance cases reported in Mycobacterial tuberculosis.

The mode of action of isoniazid resistance is complex and remains unclear, however, most strains of Mtb resistant to isoniazid are associated with a mutation in KatG and inhA [35, 36]. S315T of KatG mutation is more common in isoniazid-resistant strains. Mutation at this phase results in the formation of isoniazid product with a low affinity for isoniazid adduct [37].

Mutations in embB497 and embB406, codon 306 in embB and Polymorphism in embA, embC, are all involved in ethambutol resistance [38]. In 2013, Safi et al. proposed that the mutation in ubiA (Rv3806c) showed a high level of ethambutol resistance [39]. Some investigators have reported that the mutations in tlyA gene play a vital role in the resistance of Viomycin and Capreomycin [40, 41].

7. Extrapulmonary tuberculosis (EPTB)

TB as a rule influences the lungs, however, it can likewise influence different pieces of the body, like the brain, the kidneys, or the spine. An individual with TB can pass on if they do not get treatment. TB influencing any piece of the body other than lung parenchyma including different structure inside the chest like the pleura, pericardium and perihilar lymph hubs, alluded as extra aspiratory tuberculosis. EPTB incorporates tuberculosis meningitis, stomach tuberculosis (for the most part with ascites), skeletal tuberculosis, Pott's infection (spine), scrofula (lymph-adenitis), and genitourinary (renal) tuberculosis. Scattered, or miliary tuberculosis regularly incorporates aspiratory and extrapulmonary locales. It is assessed that extrapulmonary tuberculosis (EPTB) represents 15–25% of all instances of TB. HIV patients, particularly with low CD4 tallies, have higher paces of EPTB. Youngsters are bound to have skeletal TB than grown-ups [42]. Approximately 10% of all TB cases have both pulmonary and extrapulmonary TB, and an additional 20% have EPTB without pulmonary involvement [2, 43].

8. Major limitations and considerations to work with *M. Tuberculosis*

Mycobacterium tuberculosis is a gradually developing bacteria which must be handled cautiously under exacting containment to minimize the hazard to research centre individual [4]. The bacterium can reproduce inside the macrophage and kill the immune cell. Another limitation presented by the bacteria in the innovative work of new drugs is the idea of its cell wall which is wealthy in lipids and ultimately makes the development of homogenous and single-cell culture and troublesome [2]. *M. tuberculosis* can evade the immune response and recreate inside macrophages coming about because of several bacterial variables which along these lines can modulate the immune reaction [4, 5]. Although *M. tuberculosis* is Gram-positive bacteria its cell wall resembles the external membrane of Gram-negative bacteria since it is composed of an asymmetric bi-layer containing particular mycolic acids, along with glyco-lipids, lipo-glycans, and proteins [3, 9]. Therefore, novel drugs with viability and quicker acting mechanism which can most likely work in the shorter-term and along these lines give better outcomes in the treatment are desperately required [7].

9. Possible opinion regarding the challenges of new drug discovery for tuberculosis

Besides, the development of XDR strains of M. tuberculosis, 5.4% of MDR-TB cases are discovered to be XDR-TB (World Health Organization, 2010, Ref. [3]). Multidrug and Extensive Drug-Resistant Tuberculosis: 2010 Global Report on Surveillance and Response (World Health Organization, 2010, Ref. [4]) is testing TB treatment programs in a few nations and even raises the chance of a re-visitation of a circumstance much the same as the pre-anti-microbial TB time [1]. As

of now, MDR-TB is treated by a blend of eight to ten medications with treatments enduring up to 18 two years; just four of these medications were really evolved to treat TB5. Such imperfect treatment prompts practically 30% of MDR-TB patients to encounter treatment disappointment [44]. The treatment alternatives for XDR-TB are exceptionally restricted as XDR-TB bacilli are safe not exclusively to isoniazid and rifampicin, yet in addition to fluoroquinolones and injectables, for example, aminoglycosides. Furthermore, there are not kidding results with most MDR-TB and XDR-TB drugs, incorporating nephrotoxicity and ototoxicity with aminoglycosides, hepatotoxicity with ethionamide and dysglycaemia with gatifloxacin [45]. In this manner, the current circumstance requires the prompt distinguishing proof of new frameworks that can address arising opposition and furthermore requests the direct of suitable clinical preliminaries as verifiably not very many clinical examinations have been performed to assess the adequacy of medications in MDR-TB or XDR-TB patient gatherings. Improving the diagnostics with more extensive inclusion of medication vulnerability testing will likewise assist with tending to the high mortality of MDR/XDR-TB and control the development of obstruction.

Critical difficulties exist in TB drug revelation because of the idea of the causative bacterium. The absence of prescient models for compound section into mycobacteria is likewise a restricting variable since the direct trial proof is arduous to get. Creating essential guidelines around compound passage and efflux could help with improving hits from biochemical screens which need entire cell action, just as adjusting the synthetic properties needed for great pharmacokinetic properties [8].

10. Existing and upcoming tuberculosis drug regime

The present routine of medication for drug-sensitive Tuberculosis treatment was set up during the 1980s. This treatment process encompasses four levels of medications, isonicotinic acid hydrazide, rifampin, Ethambutol dihydrochloride and Pyrazinoic acid amide for six months of treatment (**Table 1**). The essential focus of Tuberculosis drugs is cell wall biogenesis, deoxyribonucleotide replication, ribonucleotide transcription, and protein synthesis [15, 46].

Treatment of drug-resistant or multidrug-resistant (MDR) tuberculosis is substantially further unpredictable [8]. The success of the treatment process relies upon the patient record and drug affectability. MDR-Tuberculosis needs therapy for a long time with a combination of 5 other medications. These second-line drugs will in general be progressively costly and incorporate Sirturo, 2-ethylthioisonicotinamide, Seromycin, Moxifloxacino, and Streptomycine, just like cutting edge medications rifampin systemic and Myambutol [5, 46]. For MDR tuberculosis therapy, we need to go through at least 6 months long treatment process including various vaccinations. Some have been observed to show adverse effects like heart electrophysiology dysfunction and ototoxicity [10, 13].

11. Drug combination trials and standardization of TB regimens

The WHO-recommended formulations of anti-TB drugs and fixed-dose combinations (FDCs) of drugs appear in the *WHO Model List of Essential Medicines* (available at www.who.int/medicines/publications/essentialmedicines/en). The formulations and combinations of anti-TB drugs available in each country should conform to this list.

Drug	Drug property	Acting pH	Site of action
Isoniazid (H)	Bactericidal after 24 hrs with a high potency. Kills more than 90% of bacilli in first few days of treatment.	Both alkaline and acidic medium	Both intracellular and extracellular
Rifampicin (R)	Bactericidal within 1 hrs with high potency.	Both alkaline and acidic medium	Both intracellular and extracellular
Pyrazinamide (Z)	Bactericidal with a low potency	Acidic medium	Intracellular bacilli
Ethambutol (E)	Bacteriostatic with a low potency. Minimizes the emergence of drug resistance	Both alkaline and acidic medium	Both intracellular and extracellular
Streptomycin (S)	Bactericidal with a low potency	Alkaline medium	Extracellular bacilli

Table 1.
Current drugs and their property.

Normalized treatment implies that all patients in a characterized bunch get a similar treatment routine. Standard regimens have the accompanying benefits over the individualized solution of medications:

i. errors in remedy – and in this way the danger of advancement of medication opposition – are decreased;

ii. estimating drug needs, buying, circulation and checking are encouraged;

iii. staff preparing is encouraged;

iv. costs are decreased;

v. maintaining a regular drug supply when patients move to start with one region then onto the next is made simpler;

vi. outcome assessment is helpful and results are tantamount.

12. Pharmaco-kinetic and pharmaco-dynamic contemplations for tuberculosis medications

Pharmacokinetic (PK) and pharmacodynamics (PD) properties of a medicinal drug play a substantial role to propose its feasibility for medicinal purpose *In vivo* [47, 48]. Along with the PK/PD of any anti-tubercular drugs, medication also considers other factors like comorbid conditions, safety profile, oral bioavailability and metabolic strength [4, 10]. Oral administration is mostly preferred for advanced Tuberculosis medication whereas, oral bioavailability is critical to treat Tuberculosis [4, 46]. Solubility and gastrointestinal permeability are the two major factors that affect oral bioavailability. At present. Generally, the bioavailability of tablets Tuberculosis ranges from 40–90% and new drugs must show such property of bioavailability [2, 7]. The smaller successive dosing of drugs is suggested to improve the adhesion and recommend to have daily doses. An ideal TB medicine must transmit to the lungs, the site of the primary infection, and should have the

ability to infiltrate the granuloma to reach, such as intracellular and extracellular bacilli in the centre of hypoxia and undoubtedly necrotic region [9]. Preferably, the adhesion of drugs compounds in the target tissue must be maintained at a chosen site at minimal inhibitory conditions [49, 50]. This approach is used to avoid the phenomenon of drug binding to plasma protein, inhibition of tissue diffusion and improving the half-life of medicine. Lipophilic drugs have a major portion in anti-tubercular drugs. PK/PD and mode of action determines the dose of drugs for the treatment [5, 6].

In terms of drug safety, an ideal drug for Tuberculosis should not show any acute toxicity or long duration for the treatment [47, 51]. Because of the global nature of Tuberculosis therapy, an excellent drug must not show drug–drug interaction with other chemically or biologically active TB drugs within the regime [7, 22].

13. Target identification

With the entire genomic sequence available for *Mycobacterium tuberculosis*, the potentiality to explore new targets for the development of antibiotic throughout the *M. tuberculosis* genome became convenient [9, 10]. Novel chemical entities & targets are expected to avoid resistance to existing drugs and therefore improve current treatments. An ideal target for the development of antibiotic must necessarily be in vivo, vulnerable to medicines and drug-effective [6].

Genetic screens trials are the preliminary step in manifesting which genetic products might be targeted at chemotherapy against tuberculosis. Howsoever, all the necessary genes are not equally vulnerable to pharmacological action [20]. Besides, the target should also be available for competitive or chemical inhibition. That is, the target must have the ability to bind with another molecule rather than its substrate [10, 52, 53]. The inhibition or initiation of the protein function with a possible concentration of the low molecular weight compounds results in cellular breakdowns, such as cell death leading to apoptosis or attenuated growth [14, 46]. Besides being susceptible to chemical inhibition, an anti-target screen inhibitor should also produce drug-like compounds with specificity to affect target function in the absence of interference with any host orthologs [5, 54].

14. Current status of tuberculosis drug discovery

Various strategies have been developed by researchers and investigators and they proposed combined drugs for clinical trials after screening. All these drugs have a specific mode of actions but at the same time, they also showed some side effect which is a challenging task for investigators (**Table 2**). Currently, about 7 new combinations of drugs are under clinical trials. These lead combinations have been recognized by several methods and differential screening [10]. Few screening methodologies are as follows:

14.1 SQ109

A combinatorial library entirely based on 1,2-ethylenediamines such as Ethambutol was examined on two high-throughput in-vitro analysis. The first evaluation involves dilution of bouillon to calculate minimal inhibitory concentration (MIC) contrary to *Mycobacterium tuberculosis* [55]. The subsequent measurement is based on iniBAC promoter, inhibition of cell wall and bioluminescent assays for high-throughput screening [56]. SQ109 was determined on this screen. But the

Mode of therapy	Implemented Drugs	Possible adverse effects
First line oral agents (Ref. 33, 34)	Isoniazid	Hepatotoxicity, dermatological, gastrointestinal, hypersensitivity
	Rifampicin	Heartburn, epigastric distress, Thrombocytopenia, Leukopenia, hemolytic anemia, Menstrual disturbances etc.
	Ethambutol	Retrobulbar neuritis, gastrointestinal disturbance.
	Pyrazinamide	GI disturbances, Thrombocytopenia, sideroblastic anemia, Mild arthralgia myalgia etc.
Injectable anti-TB drugs	Streptomycin	burning, crawling, itching, numbness, prickling etc.
	Kanamicin	pain or irritation
	Amikacin	diarrhea, hearing loss, spinning sensation (vertigo), numbness etc.
Fluroquinol drugs	Ofloxacin	Nausea, diarrhea, constipation, gas, vomiting etc.
	Levofloxacin	low blood sugar, headache, hunger, sweating, irritability etc.
	Gatifloxacin	red, irritated, itchy, or teary eyes, blurred vision, eye pain etc.
Second line oral drugs	Ethionamide	Nausea, vomiting, diarrhea, abdominal/stomach pain etc.
	Prothionamide	depression and hallucinations
	Cycloserine	Headache, drowsiness, dizziness, or shaking etc.
	p-Aminosalicylic acid	persistent nausea, vomiting and diarrhea etc.
Anti-TB drugs with long term safety	Linezolid	severe diarrhea or diarrhea that is watery or bloody, fungal infections, low platelet counts etc.
	Redaquiline	Nausea / Vomiting, Dizziness, Headache, Hemoptysis etc.
	Clofazimine	diarrhea, nausea, vomiting, gastrointestinal intolerance etc.
	Amoxicillin	severe skin rash, itching, hives, difficulty breathing or swallowing etc.
	High dose Isoniazid	increased blood levels of liver enzymes and numbness etc.

Table 2.
Current mode of therapy and therapeutic drugs for tuberculosis.

mode of action and efficacy of SQ109 differ widely from ethambutol [57, 58]. SQ109 is bactericidal in nature and works by targeting a transmembrane transport protein MmpL3 which is responsible for transmitting trehalose monomicolate during cell wall synthesis [59, 60]. It acts against extracellular as well as intracellular bacilli and works on acute and chronic mouse models of tuberculosis infection [61]. SQ109 improved the pharmacological efficacy of the present four available first-line drugs against tuberculosis and represents synergy with Sirturo. It is presently under phase 2 clinical studies [5, 15].

14.2 Q203

It is an amide compound of imidazopyridine and was recognized by the whole-cell screening of infected macrophages [17]. Q203 prevents ATP synthesis via causing an

interruption in the electron transport chain and thus also inhibits the cytochrome bc1 complex involved in the electron transport mechanism. Q203 possess an exceptional Pharmacokinetic profile and prevents bacterial replication [2, 20].

14.3 TBA-7371

A member of a series of 1,4-azaindole which was recognized by a strategy of transformation of scaffolds preceded by a program of optimization of lead of a compound imidazopyridine [62]. TBA-7371 inhibits DprE1 non-covalently, a decaprenyl phosphoryl-β-Dribose2′-epimerase, in cell wall Arabian biosynthetic pathway. TBA-7371 is bactericidal and is working against both acute and chronic mouse models of tuberculosis infection. It is under phase 1 clinical studies [3, 46, 57].

14.4 OPC-167832

It is a derivative of 3,4-dihydrocarostyril. OPC-167832 is bactericidal and works by targeting DprE1, leading to the prevention of mycobacterial infection. It represents improved performance when in combination with delamide. Presently it falls under the category of phase 1 clinical studies [10, 15].

14.5 GSK-070

It targets leucyl-tRNA synthetase and is an oxaborole derivative. Oxaborols block leucyl-t-RNA synthesis and ultimately results in blocking protein synthesis by constructing an adduct with t-RNA. It is active against both acute and chronic tuberculosis infection [10, 63].

14.6 PBTZ-169 & BTZ-043

They belong to benzothiazinones and were diagnosed from a broth dilution evaluation in vitro for the detection of antibacterial and antifungal activities. Benzothiazinones basically prevents the formation of arabinose involved in the biosynthesis of cell wall by covalently targeting DprE1. Both PBTZ-169 and BTZ-043 are bactericidal thus prevents bacterial replication and multidrug-resistant tuberculosis infection. They represent almost equal potency against isoniazid and rifampin in the mouse models of recurrent tuberculosis infection. PBTZ-169 is under phase 1 scientific studies [7, 9, 63].

15. Risk factors associated with tuberculosis treatments

Recent emigration makes Tuberculosis very likely to reactivate. Vitamin D deficiency has the same effect because vitamin D is an immune modulator and deficiency of that weakens the immune system, thus protecting against tuberculosis [3, 9]. Another factor, HIV infection, which is present in 8% of patient cases of tuberculosis and this problem of HIV allowing TB to be reactive and become a problem is actually before the patient has become heavily immune-suppressed [64]. Smoking, diabetes and the elderly are all examples where the immune system has been weakened to a degree and allows the potential infection to take hold and cause a problem [22, 63]. Homelessness drug abuse, alcoholism and other immune suppression steroids after transplantation to mention corrosive tumor necrosis factor treatment, all make an individual more likely to reactivate latent disease, like tuberculosis [6]. The antibiotics being used for the TB treatment have also shown

some of the side effects and the present major challenge to researchers to overcome these drawbacks of antibiotics (**Table 2**).

16. Recent developments in diagnostic approaches for tuberculosis

It is not easy to conduct a clinal diagnosis of tuberculosis very frequently as confirmed diagnosis requires culturing the bacteria *M. tuberculosis* in a sample from the patient [5] and which is very slow-growing. For lung diseases, we take morning sputum for culture purpose and microscopic studies. We also have to do Biopsies of the affected tissues, because that will provide us with a sample for culture and also for looking histologically for the characteristic presence of granulomas [53]. Mycobacterial culture confirms the presence of mycobacterium in given samples by microscopy analysis, and we may also draw the resistance profile i.e., whether the present strain belongs to the sensitive *M. tuberculosis* group or has resistance to some drugs that can be used to treat it. The major obstacles in culture MTB are that it is slow-growing bacteria and may take 3–4 weeks in liquid culture media [51]. The acid-fast bacilli of mycobacterial infection are detected by the microscopy analysis whereas latent tuberculosis disease is identified by immunological responses to tuberculosis antigens, i.e., i) Heaf test / Montoux: cofounded by BCG ii) Interferon Gamma Release Assays (IGRA) [48, 49]. There are some tools developed recently for the detection of drug-resistant MTB that facilitates early detection too, such GeneXpert, line prob. assay, LAMP assay etc.

16.1 GeneXpert

GeneXpert can detect mutations that cause resistance against Rifampicin. The test is a molecular TB test that detects the DNA of *Mycobacterium tuberculosis*. It uses a sputum sample and thus provides result in less than 2 hours. It can also detect the genetic mutations which are associated with drug Rifampicin resistance [65]. WHO recommended that this test should be used as the primitive diagnosis test in individuals suspected of having Multi-drug resistant TB, or HIV associated TB.

16.2 Line probe assay (LPA)

This technique also helps to detects mutations causing resistance against Rifampicin. Moreover, this assay can also detect mutations related to drug isoniazid [66]. The line probe assay (LPA), is typically based on strip technology and thus is used in the diagnosis of TB. It also detects RIF as well as Isoniazid (INH) resistance caused due to mutations in *rpo*β, and both *inhA* and *katG* genes.

16.3 Loop-mediated isothermal amplification (LAMP) assay

WHO has recommended the TB-LAMP (loop-mediated isothermal amplification) test that requires minimal laboratory infrastructure and has been evaluated as an alternative to sputum smear microscopy, which remains the most widespread test used in resource-limited environments. TB-LAMP is a unique temperature-independent way to amplify the DNA of tuberculosis patients. It is a manual test that takes less than one hour and results can be visualized with the naked eye under UV light. The potent TB-LAMP instrument can be used at the level of the peripheral health center where microscopy is often performed. (https://www.who.int/tb/features_archive/TB_LAMP/en/).

17. Available treatment

At present treatment of tuberculosis requires more than one antibiotic with prolonged combination therapies to eradicate the infection and prevent resistance [58] and the standard therapy may include 4 antibiotics i.e., Isoniazid & Rifampicin (most effective drugs and these are given for six months and thus these two helps in killing the bacteria), Pyrazinamide & Ethambutol (given for first two months only) [67, 68]. During treatment antibiotics are required for a long period, the minimum treatment period is six months and if the patient is having CNS or bone disease it often goes on for at least 12 months [69]. The patient is asked to take four drugs for two months and then followed by two drugs for four months, and the actual dose given to the patient is decided by their body weight such as if a patient is lower than 50 kg, they get a lower dose while if the patient is above 50 kg, they get a higher dose [13].

Corticosteroids are given to patients with CNS or pericardial disease because this reduces the further chances of having long term brain damage. All the cases need to be monitored and notified so that there can be a screening process of the patient's close contacts as well [14, 17].

18. Conclusion

M. tuberculosis is a difficult pathogen to combat and the frontline drugs currently in use are between 40 and 60 years of age. There is an urgent need of novel tuberculosis drugs, but the time to identify, develop and ultimately advance new drug regimens on the market has been extremely slow in the past decade. Organic biochemistry remains to be performed to know the mechanism of activity, to empower lead advancement, and to ensure in vivo effectiveness [20]. Current efforts to develop drugs against tuberculosis are not enough to end the global tuberculosis epidemic. Due to the diversification and complexity of the infection for *M tuberculosis*, no model can completely define the in-vivo conditions in which mycobacteria are found in Tuberculosis patients and there is no sole standard detection condition for generating successful compounds for tuberculosis drug development. Recent efforts have focused on the development of whole-cell screening trials because objective-based biochemical screens of inhibitors over the past two decades have not provided new tuberculosis drugs [68]. There are significant challenges in the discovery of anti-tuberculosis drugs due to the nature of the causative bacteria. The lack of predictive models for the entry of compounds into mycobacteria is also a limiting factor. Several additional barriers in the development of tuberculosis drugs include: there are no well-established (PK)– (PD) paradigms, lack of validation and human-like pathology of animal models currently available for drug discovery, lack of clinical laboratories suitable for clinical trials, and the lack of adequate research funds. The biggest challenge in the development of anti-tuberculosis drugs is to reduce the duration of treatment for patients with drug-sensitive tuberculosis [18]. Noval drugs are needed to achieve this and overcome drug resistance. In addition, it should be possible to use new drugs for patients with HIV/AIDS co-infection. The present condition of tuberculosis drug development is far better than what was seen past 10–15 years ago. Howsoever, the development is still lacking behind because of the significant challenges in the drug discovery against drug-resistant tuberculosis and the shorter duration of the treatment required for tuberculosis prevention [12, 13].

We need to identify essential Tuberculosis targets based on better knowledge of the disease pathogen and physiology, develop sharp screening trials, and prepare compounds specifically designed to provide better clues for antibacterial activities [11].

Recent granuloma models are based on a single cell type to imitate the aggregate complex that is formed. Biomedical engineering methods can produce further diversified but still organized multicellular structures that clearly defines the organization of human granulomas. The challenge is that the need is urgent, but the process of discovery and development requires an excessive number of resources and time. The search for more effective vaccines should continue to provide long-term solutions to tuberculosis. At the same time, the development of drugs and regimes must be accelerated with a clearer approach [1, 9].

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Conflict of interest

The authors declare no conflict of interest.

Author details

Manish Dwivedi* and Priya Giri
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow, India

*Address all correspondence to: mdwivedi@lko.amity.edu

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