International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 4; July 2017; Page No. 14-21

Treatment and advances in tuberculosis research

[1] Amrita Parle, [2] Gurmeet Singh

[1] Associate Professor, Department of Pharmaceutical Chemistry, DIPSAR, New Delhi, India
[2] Department of Pharmaceutical chemistry, DIPSAR, New Delhi, India

Abstract
Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis* causing 1.5 million fatalities around the world each year. Now a days, Mycobacterium is becoming resistant to first line treatment viz isoniazid, rifampicin, ethambutol and ciprofloxacin, developing into more severe and deadly form i.e Multi-drug Resistant Tuberculosis commonly known as 'MDR-Tb'. Different diagnostic tests are there to detect the disease like Smear test, culture test and molecular methods which include reverse hybridization and PCR amplification based detection of *Mycobacterium tuberculosis*. Moreover, different clinical trials are undergoing to check the effectiveness of different new drugs combinations for effective treatment of MDR-Tb and the people having compromised immunity.

Keywords: treatment, tuberculosis

Introduction
Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which mostly affects the lungs. It is transmitted from person to person via droplets released from the throat and lungs of patients with the active respiratory disease [1, 2, 3, 4]. In healthy people, infection with *Mycobacterium tuberculosis* often causes no symptoms, since the person's immune system acts to "wall off" the bacteria.
People infected with TB bacteria have a 10% lifetime risk of falling ill with TB. However, persons with compromised immune systems, such as people living with HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk of falling ill. The symptoms of active TB of the lung are coughing, sometimes with sputum or blood, chest pains, weakness, weight loss, fever and night sweats. When a person develops active TB, the symptoms may be mild for many months. This can lead to delay in seeking care and results in transmission of the bacteria to others. People with active TB can infect 10-15 other people through close contact, over the course of a year. People who are infected with HIV are 20 to 30 times more likely to develop active TB. TB is a leading killer of HIV-positive people. Without proper treatment, about 45% of HIV-negative people with TB and nearly all HIV-positive people with TB die. According to WHO report of 2015[2], 1 in 3 HIV deaths are due to TB.
Use of tobacco greatly increases the risk of TB and associated death. More than 20% of TB cases worldwide are attributable to smoking.

Incidence of Tuberculosis
According to WHO report of 2015[2], 9.6 million people fell ill with TB and 1.5 million died from the disease. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death for women aged 15 to 44. An estimated 1 million children became ill with TB out of which 1, 40 ,000 children died of TB.
TB occurs in every part of the world. In 2014, the largest number of new TB cases occurred in the South-East Asia and Western Pacific Regions, accounting for 58% of new cases globally. However, Africa carried the most severe burden, with 281 cases per 1, 00, 000 population as compared with a global average of 133. About 80% of reported TB cases occurred in 22 countries. The 6 countries that stand out as having the largest number of incident cases were India, Indonesia, Nigeria, Pakistan, People's Republic of China and South Africa.

Historical background of the disease
The frequency of unearthed skeletons with apparent tubercular deformities in ancient Egypt suggests that the disease was common among that population. The discovery of similarly deformed bones in various Neolithic sites in Italy, Denmark and countries in the Middle East also indicates that TB was found throughout the world and can date back to 4,000 years ago. It has been hypothesized that M. bovis, which causes a TB-like disease in cattle, was the hypothetical evolutionary precursor of M. tuberculosis. Europe, with its population explosion in the second millennium A.D. and the growth of large urban centers, became the epicenter for many TB epidemics starting in the 16th and 17th centuries. This disease peaked in Europe in the first half of the 19th century, and it is estimated that one-quarter Europeans died of TB. TB morbidity and mortality rates steadily dropped during the 20th century in the developed world, aided by better public health practices and widespread use of the M. bovis BCG vaccine. But tuberculosis reemerged due to resistance.

Traditional treatment of Tuberculosis
- Tuberculosis is known as "Rajayakshma" in ayurveda. Ayurvedic drug for treatment of TB are Pippali, Vasa, Lasuna and Naradiya mahalakshmi vilasa. Different preparations used for the TB treatment include *Vasantamalati, Kanchanabhra rasa, Rajamriganka rasa, Bhallataka (Semicarpus anacardium)rasayan, Mallasindura* and *Vasa (Adatoda vasica)* [6].

- Bapedi traditional healers in Capricorn, Sekhukhune and Waterberg districts in the Limpopo Province use the following species of plant *Artemisia afra, Eucomis pallidiflora* ssp, *Myrothamnus flabellifolius, Lippia javanica* and *Hypoxis hemerocallidea* to treat TB [7].
- Medical Practitioners in Uganda use plants like *Eucalyptus spp., Warburgia salutaris (G. Bertol.) Chiov., Ocimum suave Wild, Zanthoxylum chalybeum Engl, Momordica foetida Schum, Persea americana Mill.* and *Acacia hockii De Wild*. Plants are used as mixtures or infrequently as mono-preparations in dosage forms of decoctions and infusions [8].

General Characteristics of *Mycobacterium tuberculosis* [9]:

Mycobacterium tuberculosis is non-motile rod shaped bacterium. The rods are 2-4 micrometers in length and 0.2-0.5 micrometers in width. *Mycobacterium tuberculosis* is an obligate aerobe. For this reason, in the classic case of tuberculosis, MTB complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time of 15-20 hours, a physiological characteristic that may contribute to its virulence.

MTB is not classified as either Gram-positive or Gram-negative because it does not have the chemical characteristics of either, although the bacteria do contain Peptidoglycan (murein) in their cell wall. *Mycobacterium* species are classified as acid-fast bacteria due to their impermeability by certain dyes and stains. To visualize the bacilli in sputum sample, an excess of 10,000 organisms per ml are needed with a 100X microscope objective (1000X mag). Appearance of one acid-fast bacillus per slide is regarded as an MTB infection. Mycobacterium tuberculosis bacteria appears as shown in figure:

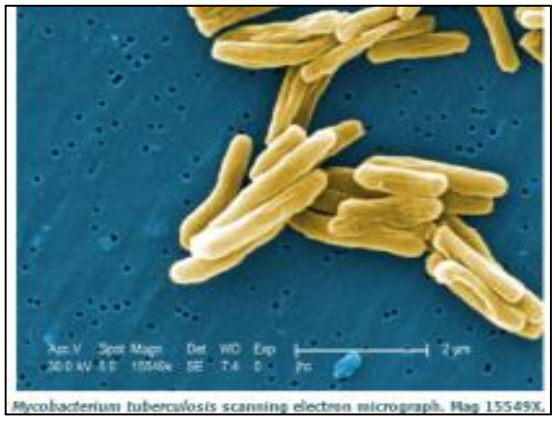

Fig 1

Cell Wall Structure

The cell wall complex contains peptidoglycan. Lipids constitute 60% of the mycobacterial cell wall. The lipid fraction of MTB's cell wall consists of three major components namely mycolic acids, cord factor and wax-D.

Mycolic acids are unique β-branched lipids found in cell walls of *Mycobacterium* and *Corynebacterium.* They make up 50% of the dry weight of the mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic Acids are thought to be a significant determinant of virulence in MTB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule.

Cord Factor is responsible for the serpentine cording. Cord factor is toxic to mammalian cells and is also an inhibitor of Phenotopic migration. Cord factor is most abundantly produced in virulent strains of MTB.

Wax-D in the cell envelope is the major component of Freund's complete adjuvant (CFA) which is used as immunopotentiators. The high concentration of lipids in the cell wall of *Mycobacterium tuberculosis* imparts the properties like impermeability to stains and dyes, resistance to many antibiotics, resistance to acidic and alkaline compounds, resistance to osmotic lysis via complement deposition, resistance to lethal oxidations and helps in survival inside the macrophages.

Mycobacterium bovis is the etiological agent of TB in cows but rarely in humans. Humans can also be infected by mycobacterium bovis due to the consumption of unpasteurized milk. This route of transmission can lead to the development of extra pulmonary TB, exemplified in history by bone infections that led to hunched backs. Other *Mycobacterium* genus includes *Mycobacterium avium* which causes a TB-like disease especially prevalent in AIDS patients, and *Mycobacterium leprae*, the causative agent of leprosy.

Types of Tuberculosis [10]:

1. Classification based on the organ affected

a) **Pulmonary tuberculosis**: It is an infection caused by slow-growing bacteria that grow best in areas of the body that have lots of blood and oxygen. So most often found in the Lungs. 80% of the people are infected with pulmonary tuberculosis. Pulmonary tuberculosis is further divided into two types:
   - **Open" or Pulmonary-positive**: In the case of open tuberculosis an inflammation develops inside the lungs that the immune system is not able control. This enables TB bacteria to be released through the respiratory tract (e.g., through coughing) and this type of tuberculosis infection is contagious.
   - "Closed" or Pulmonary-negative tuberculosis inflammation is on the periphery of the lungs so there is no risk of infection.

b) **Extra pulmonary tuberculosis** is the tuberculosis that can also spread to other parts of the body, most commonly the lymph nodes, but also the bones and joints, spine, intestines, kidneys or brain. Extra pulmonary tuberculosis is not contagious.

2. Classification based on activity

It is divided into latent TB and Active TB. Characteristics of Latent TB and Active TB are described in Table

Table 1

Latent TB in lungs	Active TB in lungs
MTB Present	MTB present
Tuberculin skin test positive	Tuberculin skin test positive
Chest X-ray normal	Chest x-ray usually reveals lesions
Sputum smears and cultures negative	Sputum smears and culture Positive
No symptoms	Symptoms such as cough, fever and Weight loss
Not Infectious	Often infectious before treatment
Not defined as case of TB	Defined as a case of TB

3. Classification based on resistance

Drug-resistant forms of TB have also evolved through misuse or inappropriate use or due to use of poor quality antibiotics.

a) **Multi-drug resistant TB (MDR-TB):** In MDR-TB the bacteria are resistant to at least isoniazid and rifampicin, two of the most effective TB drugs and can only be treated with newer antibiotics over a longer period.
b) **Extensively drug-resistant TB (XDR-TB**): XDR TB (extensively drug resistant TB) is defined as strains resistant to at least rifampicin and isoniazid and resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable TB drugs amikacin, kanamycin or capreomycin.

Development of Tuberculosis [11]:

Five stages are involved in the development of the disease:

Stage 1: Droplet nuclei are generated during coughing, talking and sneezing by infected person. When a person inhales the droplets, most of the larger droplets get lodged in the upper respiratory tract (the nose and throat), where infection is unlikely to develop. However, the smaller droplet nuclei may reach the alveoli, where infection begins. After droplet nuclei are inhaled, the bacteria are nonspecifically taken up by alveolar macrophages. However, the macrophages are not activated and are unable to destroy the intracellular organisms.

Stage 2: This stage begins 7-21 days after initial infection. MTB multiplies virtually unrestricted within unactivated macrophages until the macrophages burst. Other macrophages begin to extravasate from peripheral blood vessels. These macrophages also phagocytose MTB, but they are also unactivated and hence cannot destroy the bacteria.

Stage 3: At this stage lymphocytes begin to infiltrate. The lymphocytes, specifically T-cells recognize, process and present MTB antigen in context of Major Histo-compatibility complex (MHC) molecules. This results in T-cell activation and the liberation of cytokines including γ-interferon (IFN). The liberation of IFN causes the activation of macrophages. These activated macrophages are now capable of destroying MTB. At this stage the individual becomes tuberculin-positive. This positive tuberculin reaction is the result of the host developing a vigorous cell mediated immune (CMI) response. A CMI response must be mounted to control an MTB infection. Activated macrophages and T-cells secrete cytokines that play a role in the development of immune pathology. Tubercle is characterized by "caseation necrosis" meaning it takes on a semi-solid cheesy consistency. MTB cannot multiply within these tubercles because of the low pH and anoxic environment. An antibody mediated immune (AMI) response will not aid in the control of a MTB infection because MTB is intracellular and if exists extracellularly, it is resistant to complement killing due to the high lipid concentration in its cell wall.

Stage 4: Many activated macrophages can be found surrounding the tubercles while many other macrophages remain unactivated or poorly activated. MTB uses these unactivated macrophages to replicate and hence grows. The growing tubercle may invade a bronchus after this MTB infection and can spread to other parts of the lung. Similarly the tubercle may invade an artery or other blood supply line resulting in extra-pulmonary tuberculosis otherwise known as “Milliary tuberculosis”. The name "Milliary" is derived from the fact that metastasizing tubercles are about the same size as that of the millet seed, a grain commonly grown in Africa.

The secondary lesions caused by milliary TB can occur at almost any anatomical location, but usually involve the genitourinary system, bones, joints, lymph nodes and peritoneum. These lesions are of two types First, Exudative lesions resulting from the accumulation of Polymorphonuclear macrophages around MTB. Here the bacteria replicate with virtually no resistance. This situation gives rise to the formation of a "soft tubercle". When the host becomes hypersensitive to tuberculoproteins, granulomatous lesions occur and give rise to the formation of a "hard tubercle".

Stage 5: For unknown reasons, the caseous centers of the tubercles liquefy. This liquid is very conducive to MTB growth, and the organism begins to rapidly multiply extracellularly. After sometime, the large antigen load causes the walls of nearby bronchi to become necrotic and rupture. This results in cavity formation. This also allows MTB to spill into other airways and rapidly spread to other parts of the lung.

Only a very small percent of MTB infections result in disease and still a smaller percentage of MTB infections progress to an advanced stage. When the primary lesion heals, it becomes fibrous and calcifies. When this happens, the lesion is referred to as the Ghon complex. Depending on the size and severity, the Ghon complex may never subside. Typically, the Ghon complex is readily visible upon chest X-ray.

Small metastatic foci containing low numbers of MTB may also calcify. However, in many cases these foci will contain viable organisms. These foci are referred as Simon foci. The Simon foci are also visible upon chest X-ray and are often the site of disease reactivation.

Diagnostic Methods

Timely detection of tuberculosis will help to control the disease in its initial stages and will prevent further development of the disease .Tuberculosis becomes difficult to treat because of

development of drug resistant forms viz. MDR-TB (Multiple drug resistant tuberculosis) and XDR-TB (extremely drug resistant tuberculosis). Different diagnostic techniques for the detection are discussed here.

A) Diagnostic Methods of Tuberculosis (TB) [12-18]

The diagnosis of active disease is usually based on clinical suspicion, chest radiographs, smear for acid-fast bacilli (AFB) and solid/ liquid culture. Different diagnostic tests performed to detect the disease are- Smear test, culture test and molecular methods which include reverse hybridization and PCR amplification based detection of *M. tuberculosis*.

- **Smear Test**: Microscopic examination of smears for acid-fast bacilli is a rapid and inexpensive test, however it is positive in only 34–80% of expectorated sputum samples and is often negative in HIV co-infected TB patients. Moreover, the test does not differentiate TB from infections caused by Non-Tuberculous Mycobacteria (NTM).
- **Culture Test:** The gold standard for TB diagnosis is culture of *M. tuberculosis*. Culture on solid media takes 4–6 weeks while liquid media-based culture systems yield faster (7–12 days) growth of *M. tuberculosis*.
- **Molecular methods**: PCR amplification and reverse hybridization methods have provided rapid diagnosis of TB in smear-negative specimens, particularly when the organism cannot be grown in culture. It rapidly differentiates culture-grown *M. tuberculosis* from Non-tuberculular mycobacterium. These two molecular tests are used for cultured isolates and clinical specimens.

B) Diagnostic methods of MDR-TB and XDR-TB

Rapid detection of drug resistant *M. tuberculosis* and MDR-TB strains, ensures effective treatment of TB patients and limits further development of resistance to additional drugs. The diagnosis of drug-resistant TB and MDR-TB is carried out basically by two methods

- Conventional (phenotypic) methods
- Molecular (genotypic) methods.

1. Conventional (phenotypic) methods

Conventional (phenotypic) methods require culture and detect the growth of *M. tuberculosis* in the presence of anti-TB drugs by proportion, resistance ratio or absolute concentration methods on solid media. Different Detection techniques used in phenotopic methods are Radio-metric system, colorimetric methods and phage based assays.

Radiometric BACTEC 460 TB system (Becton Dickinson) [19]**.**

It is broth- based semi-automated method and regarded as the gold standard for culture and Drug susceptibility testing (DST) of *M. tuberculosis* to both first- and second-line anti-TB drugs. The 460 TB system reports DST results within 4–12 days from primary cultures, while solid media-based methods require nearly 3 weeks.

Colorimetric methods [20]

The methods are based on reduction of redox indicators that are added to culture medium during *in vitro* growth of *M. tuberculosis* For example, tetrazolium salt-based assay utilizes 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) which is yellow in color in its oxidized form which reduces to blue/purple colored compound during growth of *M. tuberculosis*. This simple assay provides rapid, accurate and cost-effective detection of MDR-TB and has performed well with smear-positive sputum samples.

Phage-based assay [21]

In this method mycobacteriophages are used to infect live *M. tuberculosis* in the absence and presence of anti-TB drugs. The growth of the bacilli is dectected using either the phage amplification assay or production of light. The Fast Plaque TB-Response (Biotec Laboratories Ltd.) assay detects drug resistance of *M. tuberculosis* directly in sputum specimens. The method provides rapid (within 2 days) and accurate results when compared with the BACTEC radiometric method.

2. Molecular /Genotypic methods [22, 23]

Molecular (genotypic) Drug susceptibility testing (DST) methods detect resistance associated mutations in target genes of *M.tuberculosis* and provide results within 1–2 days. It can be performed directly on smear-positive sputum and other clinical samples. Although genotypic methods have been developed for all first-line and many second-line drugs, detection of Rifampin resistance is more practical and a priority, since 90–95% Rifampin-resistant strains contain mutations in a small (81-bp) region of a single (*rpoB*) gene. *T*argeted DNA sequencing has been applied for the detection of mutations which cause resistance to RIF, INH and PZA. For other anti-TB drugs, the sensitivity of resistance detection varies more widely due to the number of gene loci involved and the sheer diversity of mutations. Drug-resistant *M. tuberculosis* strains isolated from TB patients of different ethnic background may exhibit few dominant mutations. Genotypic method for Detection of tuberculosis is done following the two techniques namely

i) PCR-restriction fragment length polymorphism (PCR-RFLP) technique

It is a simple, rapid and inexpensive method to detect polymorphism at a single or few codons that are mutated in drug-resistant strains. The DNA sequencing provides unambiguous detection of mutations. Results of resistance detection by other methods have traditionally been confirmed by DNA sequencing of target gene region/codon. Most INH-resistant strains from some geographical locations contain mutations at *katG 315 gene.* However, recent technological advances may lead to rapid, accurate and cost-effective analysis of DNA sequences for detection of MDR *M. tuberculosis* strains.

ii) Reverse hybridization-based line probe technique

This technique is used to detect MDR-TB strains directly in clinical specimens. Since RIF resistance is a surrogate marker for MDR-TB, the positive result also predicts MDR status of ~90% *M. tuberculosis* strains. Although expensive, these assays have performed satisfactorily in high incidence countries for detection of MDR *M. tuberculosis* in clinical samples within one working day. Other hybridization-based assays involve microarrays and have been tested for detecting resistance to RIF, INH, and EMB by using several probes simultaneously.

Treatment of Tuberculosis

WHO recommends Directly Observed Treatment Short course (DOTS) strategy that emphasizes the use of the most effective

standardized, short-course regimen, and fixed-dose drug combinations (FDCs) under medical supervision. This facilitates adherence to treatment and reduces the risk of the development of drug resistance. By doing this, spread of the disease, development of MDR-TB and complications of TB, relapse and death are controlled.

Some of the FIRST LINE fixed dose combinations recommended by WHO are summarized below [24]:

Table 2

Drug	Fixed dose combinations
Ethambutol	Oral liquid: 25 mg/ ml [c]. Tablet: 100 mg to 400 mg (hydrochloride)
Ethambutol + isoniazid	Tablet: 400 mg + 150 mg.
Ethambutol + isoniazid + pyrazinamide + rifampicin	Tablet: 275 mg + 75 mg + 400 mg + 150 mg.
Ethambutol + isoniazid + rifampicin	Tablet: 275 mg + 75 mg + 150 mg.
Isoniazid	Oral liquid: 50 mg/5 ml [c].
	Tablet: 100 mg to 300 mg.
	Tablet (scored): 50 mg.
Isoniazid + pyrazinamide + rifampicin	Tablet: 75 mg + 400 mg + 150 mg.
	150 mg + 500 mg + 150 mg (for intermittent use three times weekly).
Isoniazid + rifampicin	Tablet: 75 mg + 150 mg;
	150 mg + 300 mg
	60 mg + 60 mg (for intermittent use three times weekly).

Isoniazid (INH) [25-27]

It is a pro-drug having the highest activity (MIC <0.05 µg/ml) against actively dividing *M. tuberculosis*. It is activated by catalase–peroxidase encoded by *katG*. The activated INH mainly targets NADH-specific enoyl-acyl carrier protein (ACP) reductase (encoded by *inhA*) and β-ketoacyl ACP synthase (encoded by *kasA*) involved in mycolic acid synthesis which is main constituent of the cell wall of the mycobacterium tuberculosis. Depletion of mycolic acids results in bacterial killing.

Despite the complexity, resistance to INH is mainly due to mutations within the *katG* and *inhA* regulatory region.

Rifampin (RIF)

It is a lipophilic Rifamycin derivative that binds to β-subunit of RNA polymerase (encoded by *rpoB*) and inhibits RNA transcription consequently. This results in inhibition of protein synthesis in *M. tuberculosis*. Mutations in *rpoB* gene causes RIF resistance. Monoresistance to RIF is rare except in HIV-coinfected patients. Rifampin resistance is marker for MDR-TB since ~85–90% RIF-resistant strains are also resistant to INH. Nearly 90–95% of RIF-resistant strains carry missense mutations or small in-frame insertions/deletions mainly involving *rpoB* codons 531, 526 and 516. Resistance in about 5–10% RIF-resistant isolates is due to mutations in N-terminal or other regions of the *rpoB* gene or due to some unknown mechanism. Specific mutations in HSR and N-terminal region also show variations depending upon the ethnicity of TB patient/geographical location from where *M. tuberculosis* strain is isolated.

Mis-sense mutations at *rpoB* codon 511, 518 or 522 cause low-level resistance while those at codon 516, 526 or 531 cause high-level RIF resistance. *M. tuberculosis* isolates with *rpoB* mutations in N-terminal region or at codon 513, 526 or 531 exhibit high-level resistance to both RIF and Rifabutin.

Pyrazinamide (PZA)

It is a pro-drug and highly effective against semi-dormant bacilli in acidic environment (like macrophages). The PZA is activated by pyrazinamidase encoded by *pncA* gene to pyrazinoic acid which, by lowering intracellular pH, inactivates a vital fatty acid synthase. The PZA is highly specific against *M. tuberculosis.* Other mycobacteria are intrinsically resistant to PZA due to lack of an efficient pyrazinamidase. Similar to INH, most PZA-resistant *M. tuberculosis* strains also contain mutations in *pncA* gene that activates the pro-drug.

Ethambutol (EMB)

Ethambutol is used in place of Streptomycin(SM) in combination therapy with INH, RIF and PZA since resistance of *M. tuberculosis* strains to EMB is much less compared to SM. It is observed that ethambutol has less severe side effects than streptomycin. The EMB mainly targets enzymes participating in synthesis and polymerization of cell wall arabinan and interacts with three homologous membrane associated arabinosyl transferases encoded by *embC–embA–embB* genes. It also affects proteins encoded by isoniazid-inducible genes like *iniA* and acyl carrier proteins and other proteins, regulating their expression. Mutations in *embB*, particularly at *embB306*, *embB406* and *embB497* occur more frequently and confer resistance to EMB. The frequency of *embB306* mutations also varies (20–70%) in EMB-resistant strains from different geographical locations

Streptomycin (SM)

It is nearly as effective as EMB. Globally, highest level of resistance is observed for SM. The SM may be used as a first-line or second-line drug for treating patients with failing therapy or MDR-TB, provided the *M. tuberculosis* strain is susceptible to SM. The SM binds to a ribosomal protein and 16S rRNA (encoded by *rpsL* and *rrs*, respectively) causing misreading of mRNA and faulty protein synthesis. Since *M. tuberculosis* genome contains a single *rrs* gene, nearly 30% of SM-resistant *M. tuberculosis* isolates contain mutations in the *rrs* gene. The remaining SM-resistant *M. tuberculosis* strains either contain mutations in *rpsL* (at*rpsL43* or *rpsL88*) or in other genes.

Other aminoglycosides

Kanamycin (KAN) and amikacin (AMI) also inhibit protein synthesis (peptide chain elongation) in *M. tuberculosis* and are used as second-line injectable drugs for actively dividing bacteria. Cross-resistance between KAN/AMI and other injectable agents such as capreomycin (CAP) and viomycin

(VIO) (cyclic peptides) exists. The *rrs* mutation A1401 G is associated with high-level KAN and AMI resistance but usually causes low-level resistance to CAP and no resistance to VIO while C1402 T is associated with high-level CAP and VIO resistance but usually causes low-level resistance to KAN and no resistance to AMI in *M. tuberculosis*. However, *rrs* mutation G1484 T is associated with high-level resistance to CAP, VIO, KAN and AMI. Another mechanism conferring resistance to CAP and VIO involves mutations in the *tlyA* gene.

Drug treatment of MDR-TB

Multi drug resistant (MDR-TB) tuberculosis is the tuberculosis which is resistant to at least isoniazide and rifampicin, the two most powerful first line antitubercular drugs.

Fluoroquinolones (FQs)

Ofloxacin (OFX) and levofloxacin (LFX) are important second-line drugs for treating MDR-TB. The new-generation FQs namely Moxifloxacin (MFX) and gatifloxacin (GFX) have excellent bactericidal activity against *M. tuberculosis*. The FQs inactivate DNA gyrase (composed of two A and two B subunits encoded by *gyrA* and *gyrB* genes, respectively) and inhibit DNA replication. In *M. tuberculosis*, resistance to FQs is mainly associated with mutations at codons 90, 91 and 94 of *gyrA* gene. Some FQ-resistant *M. tuberculosis* strains contain mutations at *gyrB464* or *gyrB495*.

Ethionamide (ETH)

It is structural analog of INH and is used as a second-line drug for MDR-TB. ETH is a pro-drug, however, it is activated by a mono-oxygenase (encoded by e*thA*). The *ethA* catalyses a two step activation of ETH to its active form 4-ethyl-4-amidopyridine. Similar to INH and PZA, majority of ETH-resistant *M. tuberculosis* strains contain mutations in *ethA* that abolish activation of the pro-drug. Similar to INH, the main cellular target of activated ETH is *inhA* and mutations in *inhA* regulatory region that are associated with INH resistance also cause cross-resistance to ETH.

Recommended Doses Of First-Line Anti-tuberculosis Drugs For Adults [24]:

Table 3

Drug	Recommended daily dose range mg/kg body weight
Isoniazid	5
Rifampicin	10
Pyrazinamide	25
Ethambutol	15
Streptomycin	15

Treatment option for XDR

Extensively drug resistant (XDR) TB is MDR-TB, that is resistant to any fluoroquinolone and at least one of the three second line anti tubercular drugs (capreomycin, kanamycin, amikacin). XDR-TB severely reduces the options for treatment. For such cases additional drugs will need to be procured from among the group of agents that are known to have some action against tuberculosis but are not routinely recommended for treatment of MDR-TB.These include clofazimine, linezolid, amoxicillin, thioacetazone, imipenem/cilastatin, clarithromycin and high-dose isoniazid. The efficacy of this treatment is not assured. However toxicity and cost of some of these compounds is high.

Current clinical trials for effectively treating tuberculosis

Clinical trials are in progress to study different dosage regimens of anti-tubercular drugs to increase the therapeutic efficacy of the various drug regimens.

As HIV co-infected tuberculosis is one of the leading cause of deaths, main stress is laid on finding the suitable drug regimen for the treatment of tuberculosis associated with HIV infection. The parameters such as pharmacokinetics, safety of new drugs and new fixed dose combinations are studied in different trials. The brief review of the ongoing clinical trials as well as their progress is summarized in Table: 4.

Table 4

Clinical trial name	Drug	Phase	Reference
Optimal Dosing of 1st Line Antitubercular and Antiretroviral drugs in Children (a Pharmacokinetic Study)	Nevirapine, Lopinavir /Ritonavir	Phase 4	https://ClinicalTrials.gov/show/NCT01637558
Systematic Empirical vs. Test-guided Anti-TB Treatment Impact in Severely Immunosuppressed HIV-infected Adults Initiating ART With CD4 Cell Counts <100/mm^3	ART (Atripla, Truvada, Efavirenz, Combivir) Rifampin,Isoniazid, Pyrazinamide, Ethambutol	Phase 4	https://ClinicalTrials.gov/show/NCT02057796
Pharmacokinetics of Emtricitabine/ Tenofovir/Efavirenz in HIV-infected Patients With Tuberculosis	Emtricitabine/Tenofovir/Efavirenz	Phase 2	https://ClinicalTrials.gov/show/NCT00474435
Pharmacokinetics of Lopinavir/Ritonavir Superboosting in Infants and Young Children Co-infected With HIV and TB	lopinavir with ritonavir in 1:1 ratio or Lopinavir/ritonavir 4:1	Phase 4	https://ClinicalTrials.gov/show/NCT02348177
Pharmacokinetic Study of Super-boosted Lopinavir/Ritonavir Given With Rifampin	Lopinavir/ritonavir and ritonavir	Phase 4	https://ClinicalTrials.gov/show/NCT01700790

Pharmacokinetics and Safety of Rifabutin 150 mg Once Daily Versus Rifabutin 300 mg Thrice Weekly in HIV patients	Lopinavir and Rifabutin	Phase 2	https://ClinicalTrials.gov/show/NCT02415985
Pharmacokinetic Study of Rifampicin Interactions With DMPA and Efaviranz in TB	Medroxyprogesterone acetate depot	Phase 2	https://ClinicalTrials.gov/show/NCT02412436
Raltegravir Versus Efavirenz in Naive HIV-1-infected Patients Receiving Rifampin for Active Tuberculosis	Combination of Tenofovir, lamivudine and raltegravir, Combination of Tenofovir, lamivudine and Efavirenz	Phase 3	https://ClinicalTrials.gov/show/NCT02273765
Open-label Study of Dolutegravir (DTG) or Efavirenz (EFV) for Human Immunodeficiency Virus (HIV) - Tuberculosis (TB) Co-infection	DTG 50 mg or EFV 600 mg	Phase 3	https://ClinicalTrials.gov/show/NCT02178592

Clinical Trials Undergoing In Mdr-Tb Patients

Incomplete treatment of tuberculosis leads to emergence of resistant forms causing MDR-TB and since past few decades, no novel anti-TB drug has surfaced, except for Bedaquiline and Delamanid, which are used for healing pulmonary MDR-TB patients in life threatening conditions. Number of clinical trials are in progress to treat resistant strains of tuberculi bacilli. Some of them are summarized in Table 5

Table 5

Clinical trial name	Drugs	Phase	Reference
A Prospective Patient Registry of Patients Exposed to Bedaquiline	Bedaquilline	Phase 4	https://ClinicalTrials.gov/show/NCT02274389
An Open-label Randomized clinical trial to Evaluate a New Treatment Regimen for Patients With Multi-drug Resistant Tuberculosis	Linezolid, Bedaquiline, Levofloxacin, Pyrazinamide, Isoniazid, Ethionamide, Terizidone, Moxifloxacin and Kanamycin	Phase2/ Phase 3	https://ClinicalTrials.gov/show/NCT02454205
An Exploratory Study of TMC207 in Japanese Participants With Pulmonary Multi-Drug Resistant Tuberculosis (MDR-TB)	TMC207 (bedaquiline) and Background Regimen (BR)	Phase 2	https://ClinicalTrials.gov/show/NCT02365623
Pharmacokinetic Study to Evaluate Anti-mycobacterial Activity of TMC207 in Combination With Background Regimen (BR) of Multidrug Resistant Tuberculosis (MDR-TB) Medications for Treatment of Children/Adolescents Pulmonary MDR-TB	Bedaquiline (TMC207) and Background Regimen (BR)	Phase 2	https://ClinicalTrials.gov/show/NCT02354014
The Evaluation of a Standard Treatment Regimen of Anti-tuberculosis Drugs for Patients With MDR-TB	Regimen A locally-used WHO-approved MDR-TB regimen, Moxifloxacin, Clofazimine, Ethambutol, Pyrazinamide, Isoniazid, Prothionamide, Kanamycin, Levofloxacin and Bedaquiline	Phase 3	https://ClinicalTrials.gov/show/NCT02409290
A 6-Month Safety, Efficacy, and PK Trial of Delamanid in Pediatric Patients With Multidrug Resistant Tuberculosis	Delamanid 100mg, 50 mg Delamanid 50mg, Pediatric Formulation 25 mg Delamanid (Dose to be determined) Pediatric Formulation Delamanid and Optimized Background Regimen (OBR)	Phase 2	https://ClinicalTrials.gov/show/NCT01859923
Pharmacokinetic Study to Evaluate Anti-mycobacterial Activity of TMC207 in Combination With Background Regimen (BR) of Multidrug Resistant Tuberculosis (MDR-TB) Medications for Treatment of Children/Adolescents Pulmonary MDR-TB	Bedaquiline(TMC207) and Background Regimen (BR)	Phase 2	https://ClinicalTrials.gov/show/NCT02354014

Conclusion

Tuberculosis is chasing the mankind from time immmemorable and is one of the leading cause of death. Though the scientists are working to find a newer, effective and safe drug regimen for irradiation of tubercular strains, the ability of the bacillus to modify itself to survive in the presence of the antitubercular drugs, is posing a tough challenge to science fraternity. Moreover, susceptibility of the HIV positive patients incatching the tuberculular infection, is further aggravating the problem. Association of tuberculosis with human immunodeficiency virus (HIV) is the leading cause of death among these patients. Newer drugs with divergent & unique structure with mechanism of action possibly different from that of existing drugs, are urgently required. Efforts are continuously in progress to find out more effective newer drug regimens of the antitubercular drugs, some of which are in the late stages of clinical development. However, the number of these agents/regimens that will actually reach the market as effective, affordable and safe anti-TB drugs/regimens is a still a mystery.

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